

Effects of salinity in the form of simulated sea-spray (NaCl or NaCl + H₃BO₃ solution) on growth and photosynthetic performance of sage (*Salvia officinalis*)**Olga Nasta¹, Anastasia Akoumianaki-Ioannidou^{1*}, Georgios Liakopoulos² and Aimilia-Eleni Nikolopoulou²**¹Laboratory of Floriculture and Landscape Architecture, Department of Crop Science, Agricultural University of Athens, Athens, Greece²Laboratory of Plant Physiology and Morphology, Department of Crop Science, Agricultural University of Athens, Athens, Greece***Corresponding author: akouman@aua.gr****Abstract**

Plants in coastal areas are frequently subjected to salinity stress as a result of the deposition of droplets of sea water due to wind. Herein, we studied the effects of simulated sea spray [spraying solution of 410 mM NaCl (S treatments) or 410 mM NaCl + 420 μM H₃BO₃ (SB treatments)], applied once (S1 and SB1 treatments) or twice per week (S2 and SB2 treatments), on growth and photosynthesis of the aromatic and medicinal semi-shrub perennial *Salvia officinalis* L. Twenty days after the beginning of treatments, photosynthetic parameters showed significant decline due to salinity. The most affected plants were those of the S2 treatment followed by those of the S1 treatment. Interestingly, photosynthesis of SB2 and SB1 plants was much less affected indicating that boron alleviated the negative effects of NaCl. However, after 76 days, SB1 plants were affected similarly to S1 and S2 plants while SB2 plants showed the lower photosynthetic performance, indicating that the effect of boron was not beneficial any longer possibly due to its accumulation at toxic levels together with NaCl. We showed, in accordance with previous studies, that boron may alleviate the negative effects of salinity. Additionally, we state that this effect was observed during saline spraying, suggesting that the interaction between the two components was not attributed to changes in root function. We stress that saline spray and perhaps also root salinity, can yield different results when boron is included.

Keywords: boric acid; chlorophyll fluorescence; interaction; leaf surface.**Abbreviations:** A_n_photosynthetic rate, ANOVA_analysis of variance, C_control plants (not sprayed), DW_dry weight, E_transpiration rate, ETR_electron transport rate, F_chlorophyll fluorescence yield, FW_fresh weight, F_m_maximal fluorescence yield in the dark-adapted state, F_m'_maximum fluorescence yield in the light-adapted state, F_o_minimum fluorescence yield in the dark-adapted state, F_s_steady-state fluorescence yield in the light-adapted state, g_s_stomatal conductance, LAI_leaf area index, LSD_least significant difference, NPQ_non-photochemical quenching, PAR_photosynthetically active radiation, PFD_photon flux density, PSII_photosystem II, RWC_relative water content, S1_plants sprayed with NaCl solution once per week, S2_plants sprayed with NaCl solution twice per week, SB1_plants sprayed with NaCl+H₃BO₃ solution once per week, SB2_plants sprayed with NaCl+H₃BO₃ solution twice per week, SW_water saturated weight, W_plants sprayed with deionized water twice per week, Φ_{PSII}_effective quantum yield of PSII photochemistry, Φ_{PSIIo}_maximum (intrinsic) quantum yield of PSII photochemistry.**Introduction**

Salinity is one of the major problems of modern agriculture which limits plant growth and reduces agricultural production (Plaut et al., 2013). In the majority of cases where salinity affects plants, high concentrations of toxic ions, primarily Na⁺ as well as the co-occurring Cl⁻, accumulate in the soil due to unfavorable environmental conditions, inappropriate cultural practices, marginal quality of irrigation water or combinations of the above factors. Often, salts are delivered to the root zone secondarily through upward water movement from salt-rich shallow aquifers or through seepage to lower fields which become saline. Another cause of soil salinization is the seawater intrusion into coastal soils and channel water or airborne saline droplets from sea (Tezara et al., 2003; Plaut et al., 2013). A plethora of studies have addressed the effects of salinity – i.e. increased concentrations of NaCl in the growth substrate – on growth and physiological parameters of plants (Parida and Das, 2005; Parvaiz and Satyawati, 2008 and references therein) while other studies examined the

interactive effect of NaCl and boron because, apart from the physiological importance of the interaction between the two components, boron often co-occurs in irrigation water, especially in arid and semiarid environments where the use of desalinated water or reclaimed wastewater is expanding (Wu and Guo, 2006; Del Carmen Rodríguez-Hernández et al., 2013) or in areas which are proximal to the sea. Studies on the combination between substrate salinity and boron have frequently shown inconsistent results (Yermiyahu et al., 2008; Masood et al., 2012; Wimmer and Goldbach, 2012 and references therein) and it seems that the interaction between the two components is considerably complex (Smith et al., 2013). Salinity stress can also occur when air-borne saline droplets, also called sea-spray, arrive on aerial plant parts (Tezara et al., 2003; De Vos et al., 2010). Such stress occurs only in plants grown in coastal areas that are additionally subjected to frequent winds. Plants may exhibit higher sensitivity to saline spray compared to the equal

concentration of salt in the root zone (Wu et al., 2001; Wu and Guo, 2006) although the opposite may also be observed (De Vos et al., 2010). Tolerance to salt spray seems to depend on different adaptive mechanisms than tolerance to root-zone salinity (Ashraf et al., 1986) possibly due to the limitations in leaf penetration in the former and the involvement of root uptake mechanisms in the latter. Despite the fact that plant cuticle, and in some cases also epidermal appendages – predominantly hairs, reduce the wettability of plant surfaces and restrict the penetration of hydrophilic solutes such as Na^+ and Cl^- ions, it has been unequivocally shown that polar solutes do penetrate through cuticles (even astomatous ones) and accumulate in the leaf tissues (Fernández and Eichert, 2009). In intact leaves, ions may penetrate through the cuticle (either by dis-solution / diffusion, via supposed aqueous pores (Schreiber, 2005)), through stomata (Fernández and Eichert, 2009) or through leaf surface injuries (Boyce, 1954). There is limited number of studies on the effect of sea spray on plants (De Vos et al., 2010; Gonthier et al., 2010; Mereu et al., 2011). In these studies, the spraying solution is either natural (Gonthier et al., 2010) or artificial sea water (Mereu et al., 2011), or NaCl solution with a concentration equal (De Vos et al., 2010) to that of sea water or lower (Wu and Guo, 2006). Boron concentration in sea water is present in potentially toxic levels (the average boron concentration in sea water is ca. 416 μM). In this study, we included boron in the spraying solution and compared its effects with a solution containing only NaCl to investigate if a specific interaction between these two components exists when both are applied in the aerial parts. All concentrations were the average sea water concentrations. The aim of this study was to investigate (i) the effects of saline droplets (simulating sea-spray) on growth and photosynthetic performance of sage (*Salvia officinalis*) and (ii) whether a synergistic or antagonistic effect between NaCl and boron occurs when these components are applied together directly on the above-ground plant parts in the form of spray. Selecting native plants for various exploitations such as cultivation of aromatic and medicinal plants for commercial use or landscaping in urban and suburban areas becomes increasingly prevalent. *Salvia officinalis* (a native species of the Mediterranean region) was chosen in the present study for its medicinal and culinary value as well the ornamental value of its foliage and flowers. To our knowledge, there are no previous studies concerning salt tolerance of this species.

Results

Growth and morphological parameters, relative water content and concentration of chlorophylls

Before the initiation of treatments, all plant groups had approximately the same height while, just one week after the treatments, all plant groups had approximately the same leaf area index (Table 1). Plant growth in terms of the above two parameters was reduced due to simulated sea spraying (NaCl and $\text{NaCl}+\text{H}_3\text{BO}_3$) when was applied twice per week (S2 and SB2 plants), 22 days and also 76 days (for plant height) and 96 days (for LAI) after the initiation of treatments. Nearly in all cases, the height and LAI of plants sprayed once per week (S1 and SB1 plants) were not affected by salinity indicating that spraying frequency was the primary factor affecting plant growth. However, when comparing S and SB treatments, it was obvious that, at both spraying frequencies, NaCl spray affected plant growth more severely than $\text{NaCl}+\text{H}_3\text{BO}_3$ spray albeit differences remained in general non-significant (Table

1). No statistically significant differences in leaf thickness, RWC and concentration of chlorophylls were detected. However, concerning relative water content, plants that were sprayed twice per week (regardless of the composition of the spraying solution) tended to show lower values compared to those that were sprayed once per week and the control plants. Regarding the concentration of chlorophylls, all sea-spray treated plants tended to have lower values compared to the control plants after 26 days. After 83 days S2 and SB2 plants tended to have lower values in comparison with S1 and SB1 plants. Additionally, S2 and SB2 plants showed a tendency for thicker (i.e. more pachymorphic) leaves 83 days after the initiation of treatments (Table 1).

Gas exchange parameters

Salt spray treatments (S1, S2 and SB2) significantly reduced photosynthetic rate (A_n) compared to the control plants after ca. 20 days (Fig 1A). On the other hand, $\text{NaCl}+\text{H}_3\text{BO}_3$ spray applied once per week (SB1 treatment) caused a much less significant decrease in A_n compared to the control plants (in fact, in almost all light intensity levels, A_n of SB1 treatment was similar to that of the control plants) and it was significantly higher than that of S2 treatment (the treatment which showed the lower A_n , Fig 1A). At high PFD (but not at the highest), A_n of SB1 plants was also significantly higher than that of S1 plants, indicating that the addition of H_3BO_3 in the spraying solution partially amended the detrimental effects of NaCl. On the other hand, no differences were observed between S2 and SB2 (although A_n of SB2 plants was always higher than that of S2 plants), indicating that the beneficial effect of boron was not able to counterbalance the salt stress at high spraying frequency (Fig 1A). Stomatal conductance to CO_2 (g_s) (Fig 1B) showed no significant differences between treatments but, as evident by the plotted data, S2 plants recorded the lowest values of that parameter as well. Transpiration rate (E) was severely affected by the salt treatments but not in the case of SB1 plants where E was either similar to control plants or relatively higher (Fig 1C). S1 treatment as well as SB2 and S2 treatments even more intensely, showed significantly lower E compared to C, W and SB1 treatments indicating that the spraying frequency and, partially, the absence of boron from the spraying solution, were the key factors for the reduction of E (Fig 1C). Fig 2 shows that ca. 76 days after the initiation of treatments, the effects of salinity on gas exchange parameters were quite different than those after ca. 20 days. In particular, A_n and E of SB2 and S2 plants were significantly lower compared to the control plants. SB1 treatment also exhibited reduced A_n and E (albeit it was not statistically significant) compared to the control plants. Among all treatments, SB2 plants showed the lowest values of A_n and E (Figs 2A and 2C) which were not observed after ca. 20 days after the initiation of treatments (compare Fig 2A with 1A, also 2C with 1C). There was no clear evidence of the effects of the different treatments on g_s (Fig 2B). The above results, concerning the different effects of the treatments between the two periods of measurements (i.e. 20 and 76 days after the initiation of treatments), indicate that the early beneficial effect of boron was probably gradually diminished during the course of the experiment. This was evident both for the SB1 treatment and, especially, for the SB2 treatment.

Chlorophyll fluorescence parameters

According to Table 2, maximum (intrinsic) quantum yield of PSII photochemistry (Φ_{PSII_0}) was not significantly affected

Table 1. Growth and morphological parameters, relative water content and chlorophyll concentration in leaves of *Salvia officinalis*. Treatments are as follows: C: control plants (not sprayed); W: plants sprayed with deionized water twice per week; S1: plants sprayed with NaCl solution once per week; S2: plants sprayed with NaCl solution twice per week; SB1: plants sprayed with NaCl+H₃BO₃ solution once per week; SB2: plants sprayed with NaCl+H₃BO₃ solution twice per week. Date is date of sampling for each measurement and in parenthesis is the number of days before (-) or after the initiation of treatments. Numbers are means of five replicates \pm standard error of the mean. Different letters denote statistically significant differences between means across treatments ($P \leq 0.05$).

Plant height (cm)						
Date	C	W	S1	S2	SB1	SB2
11/6 (-10)	25 \pm 1 ^a	25 \pm 1 ^a	24 \pm 1 ^a	23 \pm 1 ^a	27 \pm 1 ^a	25 \pm 1 ^a
13/7 (22)	28 \pm 1 ^{ab}	27 \pm 1 ^{bc}	27 \pm 1 ^{bc}	25 \pm 1 ^c	30 \pm 0 ^a	25 \pm 1 ^{bc}
5/9 (76)	31 \pm 1 ^a	31 \pm 2 ^{ab}	29 \pm 1 ^{ab}	25 \pm 1 ^c	32 \pm 1 ^a	28 \pm 1 ^{bc}
Leaf area index (m ² m ⁻²)						
Date	C	W	S1	S2	SB1	SB2
28/6 (7)	0.7 \pm 0.1 ^a	0.7 \pm 0.1 ^a	0.7 \pm 0.1 ^a	0.9 \pm 0.1 ^a	0.8 \pm 0.2 ^a	0.6 \pm 0.1 ^a
25/9 (96)	1.5 \pm 0.2 ^a	1.1 \pm 0.1 ^b	1.0 \pm 0.1 ^{bc}	0.7 \pm 0.1 ^c	1.1 \pm 0.1 ^b	0.8 \pm 0.1 ^c
Leaf thickness (μ m)						
Date	C	W	S1	S2	SB1	SB2
17/7 (26)	0.44 \pm 0.04 ^a	0.38 \pm 0.02 ^a	0.41 \pm 0.02 ^a	0.41 \pm 0.03 ^a	0.45 \pm 0.02 ^a	0.42 \pm 0.04 ^a
12/9 (83)	0.48 \pm 0.04 ^a	0.49 \pm 0.02 ^a	0.46 \pm 0.02 ^a	0.51 \pm 0.05 ^a	0.49 \pm 0.01 ^a	0.54 \pm 0.03 ^a
Relative water content (%)						
Date	C	W	S1	S2	SB1	SB2
17/7 (26)	72 \pm 5 ^a	74 \pm 3 ^a	70 \pm 2 ^a	71 \pm 5 ^a	73 \pm 2 ^a	72 \pm 2 ^a
12/9 (83)	62 \pm 8 ^a	53 \pm 7 ^a	63 \pm 2 ^a	52 \pm 8 ^a	54 \pm 4 ^a	50 \pm 10 ^a
Concentration of chlorophylls (μ g cm ⁻²)						
Date	C	W	S1	S2	SB1	SB2
12/6 (-9)					15 \pm 3	
17/7 (26)	15 \pm 4 ^a	15 \pm 3 ^a	12 \pm 2 ^a	10 \pm 1 ^a	10 \pm 3 ^a	9 \pm 2 ^a
12/9 (83)	15 \pm 3 ^a	10 \pm 3 ^a	13 \pm 2 ^a	10 \pm 2 ^a	11 \pm 2 ^a	9 \pm 2 ^a

ca. 20 days after the initiation of the saline treatments. On the other hand, all the examined operative parameters of PSII functionality were affected by the salt spray (Fig 3). The examination of effective quantum yield of PSII photochemistry (Φ_{PSII} , Fig 3A) and electron transport rate (ETR, Fig 3B) indicated that, at all actinic light intensity levels, the salt spray treatments S1 and S2 were much more affected (and in that order, all statistically significant compared to the control plants) than the combined NaCl+H₃BO₃ treatments (SB1 and SB2). It is also worth noticing that no statistically significant differences were recorded between SB and control plants. However, at some PFD levels, and depending on the spraying frequency, SB treatments were statistically different from S1 and S2 treatments. Spraying frequency itself had a weak (SB treatments) or relatively stronger (S treatments) effect on the Φ_{PSII} and ETR parameters. The above results indicate that, similarly to A_n , the quantum yield of PSII was severely affected by the NaCl spraying treatments whereas the effect of the combined NaCl+H₃BO₃ treatments was not significant, demonstrating the amending effect of boron after ca. 20 days of spray applications. The inability of the PSII reaction centers to efficiently exploit captured light energy was evident from the non-photochemical quenching parameter (NPQ, Fig 3C). A strong engagement of photoprotective mechanisms in S1 and S2 plants resulting in depression of the maximum fluorescence yield under light conditions (F_m') was evident by the higher values of the NPQ parameter in comparison with the control plants as well as SB treatments (since the F_m values, the second factor involved in the calculation of NPQ, did not differ among treatments; data not shown), especially under moderate light levels (Fig 3C). After ca. 76 days from the beginning of the spray applications, Φ_{PSIIo} was significantly affected by the saline treatments, i.e. lower values were measured for S2 and SB2 treatments (Table 2). The effects of saline treatments on operative photochemical parameters (Fig 4) were quite similar to those observed for gas exchange parameters (Fig 2). In particular, S2 and SB2 treatments showed the lower

values in photochemical efficiency (Φ_{PSII} and ETR, Figs 4A and 4B) and almost the highest values for NPQ (Fig 4C) indicating that the high spraying frequency was a key determinant of the progression of plant stress. On the other hand, plants of SB1 treatment showed the highest Φ_{PSII} and ETR values (just slightly lower than those of the unsprayed control, C) and the lowest values of the NPQ parameter (Fig4). These results indicate that while boron in SB1 was capable of ameliorating the negative effects of NaCl, this was not evident for the high spraying frequency SB2 treatment which showed the worse photosynthetic performance, even worse than that of S1 and S2 treatments (albeit no statistically significant differences were found in these datasets in particular, excluding the differences between SB1 and SB2 in NPQ under moderate light, Fig 4C). According to correlations between photosynthetic parameters ca. 20 days after the initiation of treatments (Table 3, upper half), the major determinants for A_n were, as expected, E and Φ_{PSII} , which were also strongly correlated. Φ_{PSII} was also negatively correlated with NPQ (Table 3, upper half). Contrary to the above sampling period, ca. 76 days after the initiation of treatments, photosynthetic rate was primarily depended on the Φ_{PSII} and Φ_{PSIIo} parameters (Table 3, lower half). Moreover, Φ_{PSII} was strongly correlated with Φ_{PSIIo} while no correlation was observed either with E or NPQ (Table 3, lower half).

Discussion

Sea spray derived salinity stress is common in plants cultivated in coastal regions. Many regions in Mediterranean type-climates (The Mediterranean basin, parts of North and South America and Australia) but also additional coastal areas of the temperate climatic zone may be appropriate for the cultivation of high-added value plants such as sage (sage, for example, has been naturalized in many places throughout the world). This study focused on the effects of saline (410 mM NaCl) spray (with variations in spraying frequency) on the photosynthetic performance of sage plants. The calculated amount of NaCl applied (based on the mean total leaf surface

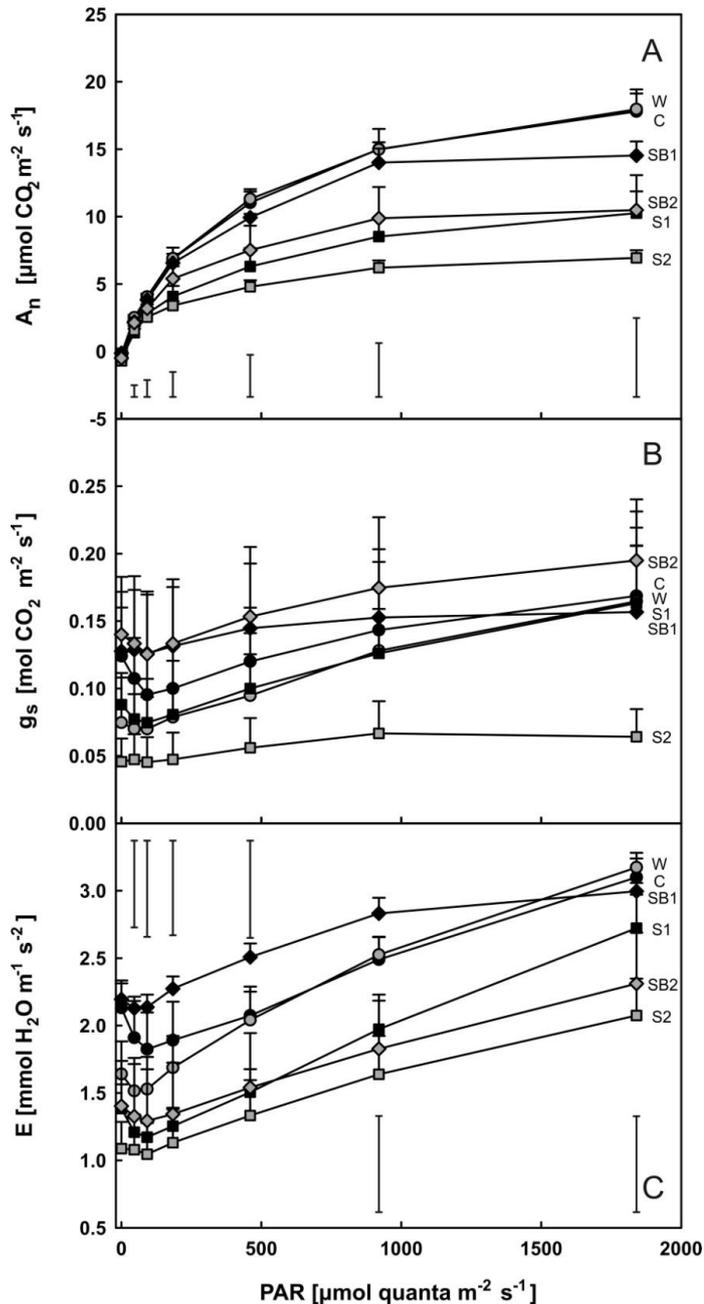


Fig 1. Light response curves of photosynthetic rate (A), stomatal conductance to CO₂ (B) and transpiration rate (C) of *Salvia officinalis* recorded ca. 20 days after the initiation of treatments. Treatments are as follows: C: control plants (not sprayed), black circles; W: plants sprayed with deionized water twice per week, gray circles; S1: plants sprayed with NaCl solution once per week, black squares; S2: plants sprayed with NaCl solution twice per week, gray squares; SB1: plants sprayed with NaCl+H₃BO₃ solution once per week, black diamonds; SB2: plants sprayed with NaCl+H₃BO₃ solution twice per week, gray diamonds. Numbers are means of five replicates \pm standard error of the mean. Vertical bars in each light intensity denote LSD values for significant differences between means across treatments ($P \leq 0.05$). For clarity, statistics are annotated only from the fourth intensity level and upwards.

per plant at the beginning of the experiment and the volume of spraying) is ca. 36 mg NaCl dm⁻² leaf area per application (applied once or twice per week, thus equals ca. 5-10 mg NaCl dm⁻² day⁻¹), which is in the lower end of the range of values appearing in the literature (concerning naturally occurring sea-spray), typically ca. 10-50 mg NaCl dm⁻² day⁻¹; De Vos et al., (2010). The effect of boric acid, also at seawater concentration, was examined together with NaCl aiming at the evaluation of the ameliorating capacity of boric acid against the negative effects of salinity, hence testing whether the two components act in an antagonistic way, as previously reported for root-zone salinity conditions (Yermiyahu et al., 2008; Díaz et al., 2011). The addition of boric acid is interesting inasmuch as the two components not only show various types of interaction but also they often co-occur in irrigation water or are contained in high concentrations in naturally occurring sea-spray. According to the results of the present study, on the short-term, saline spray negatively affected all photosynthetic parameters except chlorophylls concentration despite that this particular parameter is considered as a sensitive indicator of salt tolerance (Cuin et al., 2010). The plants mostly affected were those of S2 treatment followed by those of S1 treatment. Interestingly, photosynthesis of SB2 and SB1 plants was much less affected indicating that boron alleviated the negative effects of NaCl. Correlation between E and Φ_{PSII} indicates that quantum yield of PSII depends on efficient photosynthetic quenching of light energy which in turn depends on efficient gas exchange evaluated via E. Furthermore, the fact that Φ_{PSII} is strongly and negatively correlated with NPQ (Table 3, upper half) is also indicative of the dependence between Φ_{PSII} and low energy load in PSII. Such well coordinated parameters of the photosynthetic machinery are indicative of absence of irreversible damage, despite that salinity treatments resulted in decreased photosynthetic performance. This statement is also supported by the values of the Φ_{PSIIo} parameter which showed no accumulation of damage, i.e. photoinhibition (Table 2). Contrary to the above, on the long term, some damage had been accumulated in the photosynthetic machinery due to the salinity treatments as indicated from the respective values of the Φ_{PSIIo} parameter (Table 2). SB1 plants were affected similarly to S1 and S2 plants while SB2 plants showed the lower photosynthetic performance, indicating that the effect of boron was not beneficial any longer possibly due to its accumulation in toxic levels together with NaCl. According to the correlations, photosynthetic rate was primarily depended on the Φ_{PSII} parameter and, notably, also on the Φ_{PSIIo} parameter, i.e. the absence of photoinhibition (Table 3, lower half). Additionally, the correlation between Φ_{PSII} and Φ_{PSIIo} indicates that the effectiveness of PSII photochemistry to use light energy itself was determined to a large extent by the level of photoinhibition and not by the set of parameters determining actual photosynthetic performance such as E (note the absence of correlation between Φ_{PSII} and E; Table 3, lower half). Furthermore, it is important that Φ_{PSII} was also not correlated with NPQ under these circumstances. The effects of salt spray on photosynthesis and related parameters can be expected to be highly variable, depending predominantly on the plant species, even when species of the same type and from the same biome are examined. The effects of artificial sea spray (applied twice per week for ca. two months) on photosynthetic parameters of two mediterranean evergreen sclerophylls, *Arbutus unedo* and *Quercus ilex* where strikingly different, i.e. photosynthesis of *Q. ilex* was considerably affected while that of *A. unedo* was

Table 2. Maximum quantum yield of PSII photochemistry of *Salvia officinalis*. Other details are as in Table 1.

Date	C	W	S1	S2	SB1	SB2
11/7 (20)	0.81±0.00 ^a	0.80±0.00 ^a	0.81±0.01 ^a	0.78±0.01 ^a	0.81±0.01 ^a	0.80±0.00 ^a
5/9 (76)	0.79±0.01 ^{ab}	0.81±0.00 ^a	0.77±0.01 ^{abc}	0.73±0.01 ^c	0.76±0.03 ^{bc}	0.73±0.01 ^c

practically unaffected (Mereu et al., 2011). In general, interactions between boron and salinity are considered either on the basis of the alleviation of the negative effects of salinity when boron is present (El-Hamdaoui et al., 2003; Yermiyahu et al., 2008; Díaz et al., 2011; Del Carmen Rodríguez-Hernández et al., 2013) or on the basis of the reduction of boron uptake under saline conditions (Edelstein et al., 2005; Tripler et al., 2007). Ben-Gal and Shani (2002) and Bañón et al., (2012) have reported that excess boron decreased yield and transpiration in tomato, but the negative effect of high boron is mitigated when plants are exposed to NaCl. Proportionately, salinity may reduce high boron uptake (Alpaslan and Gunes, 2001; Ismail, 2004; Wimmer and Goldbach, 2012; Smith et al., 2013). However, in other studies, the combined effect of high external boron supply and increased salinity often results in increased uptake of boron and/or NaCl which limits plant growth (Alpaslan and Gunes, 2001; Ben-Gal and Shani, 2002; Wu and Guo, 2006; Masood et al., 2012; Shapira et al., 2013; Smith et al., 2013). According to recent results (Shapira et al., 2013; Smith et al., 2013), boron and salinity interact in a complex manner and the results of this interaction may be different depending on plant species, specific levels of salt and boron, time of application and climate. In the present study, NaCl and boron were applied directly on the aerial plant parts, therefore, the antagonistic effect between boron and salinity seems not to be determined from water uptake mechanisms (i.e. transpirational flow (Wimmer and Goldbach, 2012) or modulation of aquaporin activity (Martinez-Ballesta et al., 2008; Del Carmen Rodríguez-Hernández et al., 2013)) since root uptake and translocation were not participating in the entry and distribution of Na⁺ and Cl⁻ ions and boric acid in the aerial parts. Two reasons may explain the above effect: a) it may be due to the modulation of leaf surface penetration by one component (e.g. boron) which reduces the diffusion of the other (e.g. NaCl) or b) it may be due to biochemical interactions between the two components in the cell walls or plasma membranes. As for the first supposed mechanism, we were unable to measure ion concentrations in leaf tissues for two reasons: firstly, leaves of sage are highly pubescent which makes hair removal and leaf surface purification prior to determination of any inorganic element almost impossible and secondly, leaves of some treatments were highly affected at the end of the experiment (curved or rolled lamina, appearance of necrotic areas, etc.) making handlings even harder. As for the second supposed mechanism, we attempted to measure electrolyte leakage in intact leaves through determination of potassium by atomic absorption spectroscopy but our results were highly inconsistent, probably due to the pubescent nature of the leaf surface of sage. Bastias et al., (2005) examined the effect of high boron supply (334 µM or 668 µM in nutrient solution) under various salinity levels (0, 100 or 430 mM NaCl in nutrient solution) on several physiological parameters of *Zea mays* L. amylaceae 'Lluta' (a salinity tolerant local variety) including growth, membrane permeability and photosynthetic performance. In the 430 mM NaCl treatment, high boron supply resulted in lower membrane permeability compared to the zero boron supply treatment.

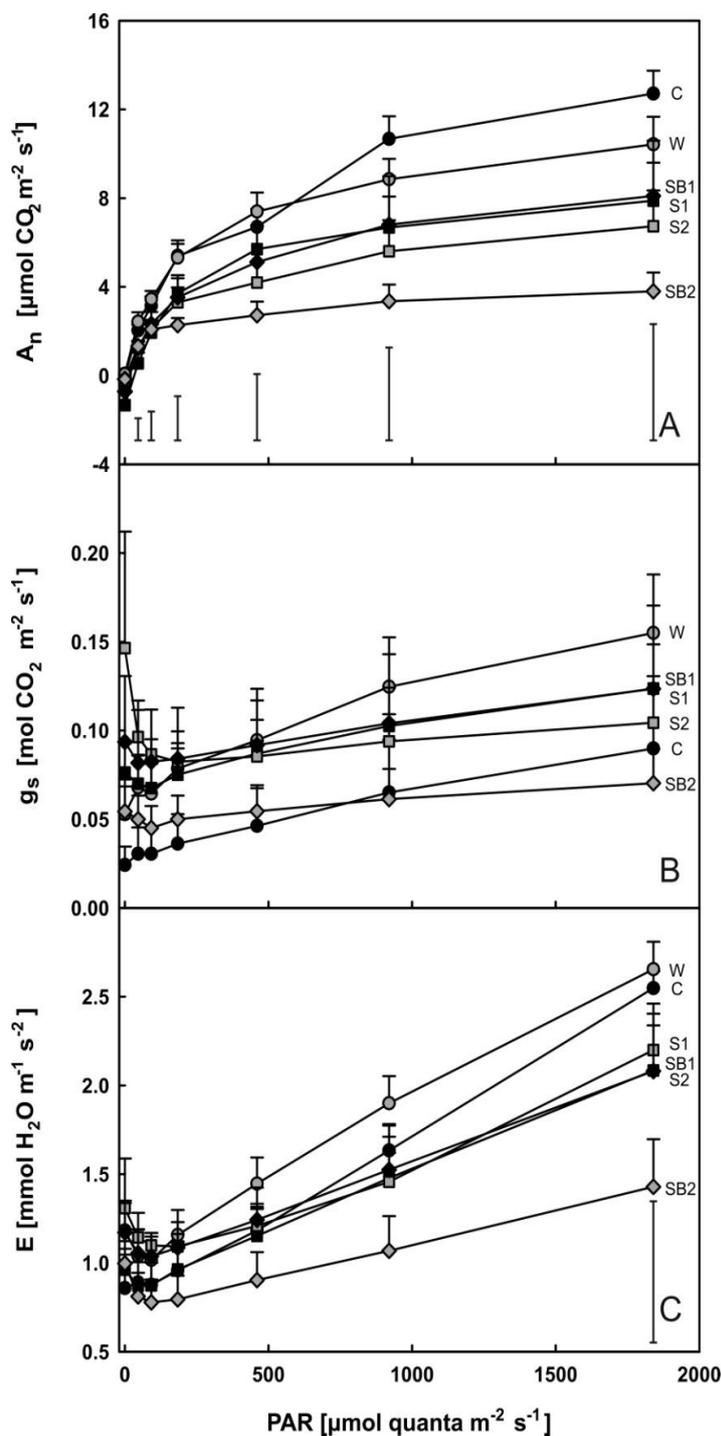


Fig 2. Light response curves of photosynthetic rate (A), stomatal conductance to CO₂ (B) and transpiration rate (C) of *Salvia officinalis* recorded ca. 76 days after the initiation of treatments.

Table 3. Values of correlation coefficients (r_s) of Spearman's bivariate correlations between photosynthetic parameters of all treatments. Values for the analyses (concerning all parameters except Φ_{PSIIo}) were taken at the maximum light intensity of each light curve. Correlations were performed separately for the two sampling periods. Asterisks denote level of correlation significance (*: $P \leq 0.05$; **: $P \leq 0.01$; blank cells: not statistically significant correlations).

ca. 20 days after the initiation of treatments					
Parameter	A	g_s	E	Φ_{PSIIo}	Φ_{PSII}
g_s					
E	0.943**				
Φ_{PSIIo}					
Φ_{PSII}	0.997**		0.943**		
NPQ	-0.886*				-0.886*
ca. 76 days after the initiation of treatments					
Parameter	A	g_s	E	Φ_{PSIIo}	Φ_{PSII}
g_s					
E					
Φ_{PSIIo}	0.986**				
Φ_{PSII}	0.943**			0.899*	
NPQ					

However, neither photosynthetic rate nor stomatal conductance were higher under high boron supply compared to zero supply although these parameters were not further reduced due to the presence of boron (Bastías et al., 2005). Del Carmen Rodríguez-Hernández et al., (2013) have shown that the addition of boron (ca. 166 or 400 μM) resulted in some membrane protection in root cells against destabilization due to NaCl (80 mM) but only in one of the two broccoli cultivars examined and only at the low boron concentration (166 μM). On the contrary, higher membrane permeability was reported for maize and sorghum during germination (Ismail, 2004) or tomato and cucumber during cultivation (Alpaslan and Gunes, 2001) although boron concentrations tested in the former were extreme (5-20 mM) and NaCl concentrations were considerably lower than those of sea water (50 mM in the former or 30-40 mM in the latter). Having taken all the above into account, it is possible that the contribution of boron to the maintenance of membrane integrity may partially explain the antagonistic effect between high boron supply and salinity irrespective of modulation of root function, such as in the case of the present study. Wu and Guo (2006) tested the response of two coast redwood (*Sequoia sempervirens*) varieties to sprinkle irrigation (which can be considered as similar to spraying) using water containing NaCl or NaCl and boron at lower concentrations in comparison to the present study (ca. 8.5 mM for NaCl vs. 410 mM in the present study; 185 μM for boron vs. 420 μM in the present study). According to their study, the addition of boron in the irrigation solution caused additional damage to the needles, i.e. the combined effect of NaCl and boron was synergistic. It seems that an antagonistic effect exists between high boron and salinity in such a way that one stress factor may reduce the effect of the other and vice-versa. Our results (growth parameters and parameters of photosynthesis) are in accordance with the above in the short-term (in this study ca. 20 days under the treatments), since the combined effect of both stress factors was clearly antagonistic (less detrimental) compared to the effect of NaCl alone. However, in the longer term (in this study ca. 76 days under the treatments), the combined effect of high-boron and saline spray applied twice per week (SB2 treatment) was more detrimental on photosynthetic parameters compared to salinity alone (S2 treatment) and it is expected that plant growth would be affected in the long-term. To our knowledge, it is reported for the first time that an antagonistic / synergistic effect between salinity stress and boron may occur independently of root uptake function because both components were directly

applied to the aerial plant parts.

Materials and Methods

Experimental site, plant material and cultivation

Experiments were conducted in the experimental site of the Laboratory of Floriculture and Landscape Architecture of Agricultural University of Athens (37° 58'57.73" N, 23°42'19.42" E) from 10/5 to 10/9/2012. Sage plants (*Salvia officinalis* L.) were obtained from a commercial nursery and transplanted in 4 L plastic pots filled with peat:perlite (1:1 v/v), pH 6.5. A commercial fertilizer (Nutrileaf 20-20-20, Miller Chemical and Fertilizer Corporation, USA) was applied in 19/6 at a concentration of 5 g L⁻¹. Average daily temperature for the duration of the study was 24.3°C whereas average daily minimum and maximum were 16.9 °C and 32.5 °C respectively. No rainfall was recorded during the experimental period (Laboratory of General and Agricultural Meteorology, Agricultural University of Athens). Irrigation was applied every 1-2 days (May-June) or everyday (June to September) at 06:00 am using an automated drip system supplied with tap water, delivering a volume of ca. 170 mL per irrigation cycle and per pot.

Treatments and samplings

Thirty plants were assigned to six treatments (five plants/replicates per treatment) and arranged in a complete randomized design. Two groups of control plants were used: plants receiving no spray (C treatment) and plants sprayed with deionized water twice per week (W treatment). Four groups of plants received four different saline solution spraying treatments: two groups were sprayed with NaCl solution (410 mM NaCl, Ferak GmbH, Berlin, Germany) either once per week (S1 treatment) or twice per week (S2 treatment) and two groups were sprayed with NaCl (410 mM)+H₃BO₃ (420 μM , Merck KGaA, Darmstadt, Germany) solution either once per week (SB1 treatment) or twice per week (SB2 treatment). Before the application of each spraying, the soil surface was covered with plastic film to avoid contact between the soil and the spraying solution. Each plant was sprayed evenly with 15 mL of the appropriate solution. All treatments began in 21/6. All leaf samples were taken from fully expanded leaves. Care was taken to measure or collect sun-lit leaves, preferably from the south-facing side of the canopy. Leaves that were measured by gas-exchange

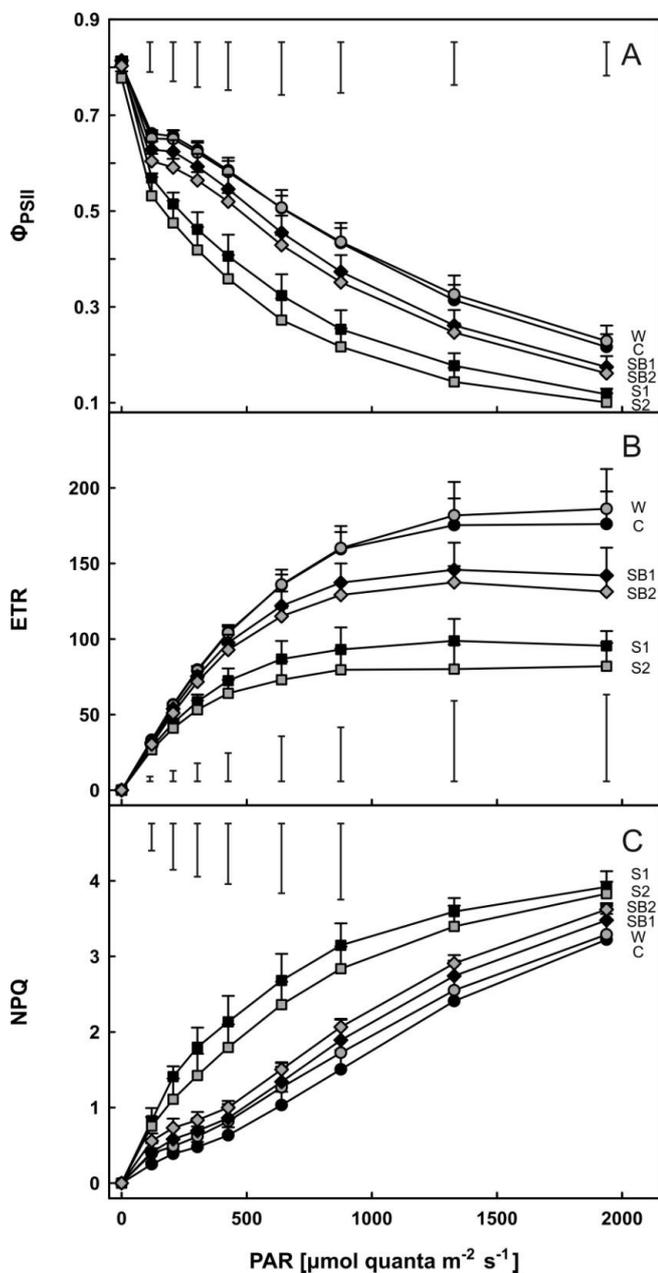


Fig 3. Light response curves of effective quantum yield of PSII photochemistry (A), electron transport rate (B) and non-photochemical quenching (C) of *Salvia officinalis* recorded ca. 20 days after the initiation of treatments. Other details are as in Fig 1.

or chlorophyll fluorescence were then collected for determination of the concentration of chlorophylls.

Growth, morphological parameters and relative water content

Plant height was measured from the bottom of the above ground plant portion in the following dates: 11/6, 13/7 and 5/9. Leaf area index (LAI) was measured with a LAI-2000 Plant Canopy Analyzer (Li-COR Inc., Lincoln, NE, USA) in the following dates: 28/6 and 25/9. Leaf thickness was measured with an IP54 digital micrometer (PREISSER Messtechnik GmbH, Gammertingen, Germany) by averaging three samplings in each leaf at the following dates: 17/7 and

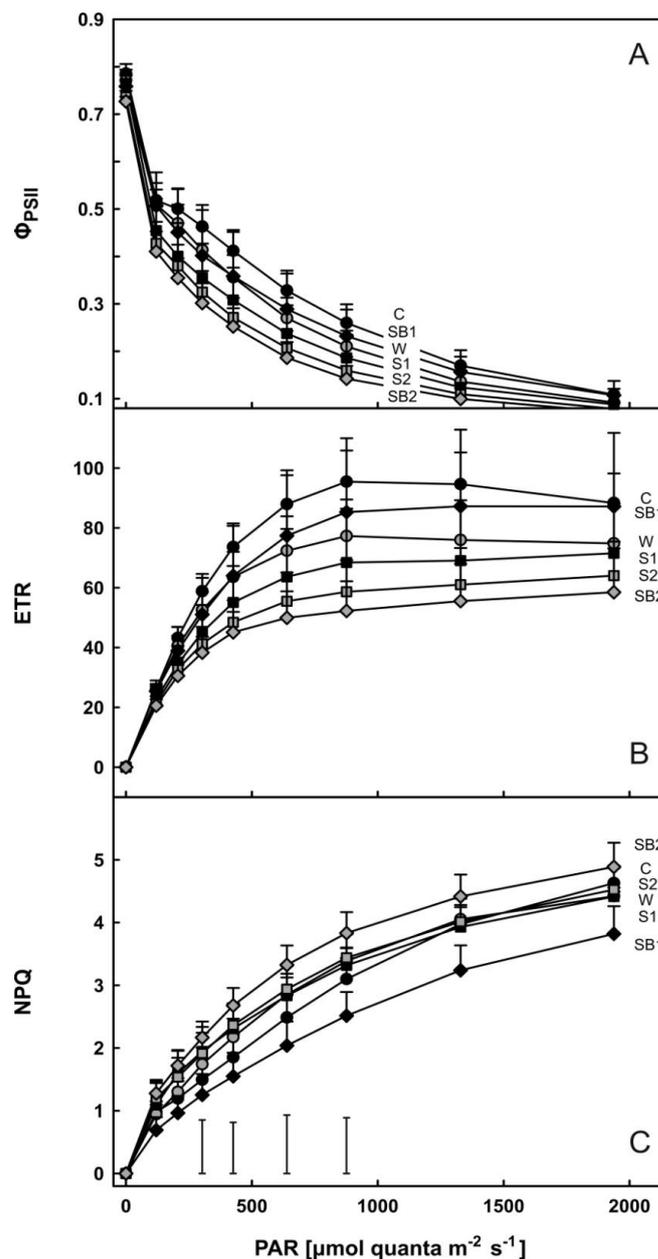


Fig 4. Light response curves of effective quantum yield of PSII photochemistry (A), electron transport rate (B) and non-photochemical quenching (C) of *Salvia officinalis* recorded ca. 76 days after the initiation of treatments. Other details are as in Fig 1.

12/9. Relative water content (RWC) was measured by weighting (fresh weight, FW) leaf disks of 10.5 mm diameter. Disks were then water-saturated for 24 h and measured again (saturated weight, SW). Finally, dry weights (DW) of the disks were measured after air drying at 60°C for 48 h. RWC was calculated as $RWC = [(FW - DW) / (SW - DW)] \times 100$. RWC was measured at the following dates: 17/7 and 12/9.

Concentration of chlorophylls, gas exchange and chlorophyll fluorescence

Concentration of chlorophylls was measured using 80% acetone extracts according to Lichtenthaler (1987). Measurements were obtained in the following dates: 12/6, 17/7 and 12/9.

Gas exchange was measured between 07:00 am and 12:30 pm on sunny days, between 10 and 13/7 and between 4 and 7/9, using a LC Pro+ portable photosynthesis system (ADC Bioscientific Ltd., Hoddesdon, UK) at various light intensities, at PAR (photosynthetically active radiation) PFD (photon flux density) between 0 and 1500 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (steady state was accomplished by applying each intensity for 3 min) to construct light response curves using the artificial red-blue LED light source and the broad leaf chamber (measuring 6.25 cm^2 of leaf area). Concentration of CO_2 was ambient (ca. 380 ppm) and air temperature inside the leaf chamber was maintained at ca. 27°C. The Walz mini-PAM portable chlorophyll fluorescence instrument (Heinz Walz GmbH, Effeltrich, Germany) was used to study the parameters of photosystem II (PSII) photochemistry. These measurements were taken on the same leaves and just before measuring gas exchange parameters. Before recording chlorophyll fluorescence kinetics, samples were darkened for 30 min using dark leaf clips. After dark adaptation, the PAM measuring light (650 nm; PFD ca. 0.15 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$; kept steady during all experiments) was turned on and the minimal fluorescence at the dark-adapted state (F_0) value was determined prior to the determination of the maximal fluorescence at the dark-adapted state (F_m). Afterwards, a range of actinic light intensities (using the instrument's halogen lamp; white light) were applied, at PAR PDF between 120 and 1938 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (steady state was accomplished by applying each intensity for 3 min) to construct light response curves. For each light level, maximum fluorescence in the light (F_m') and steady state fluorescence (F_s) were determined. Maximum (intrinsic) quantum yield of PSII photochemistry (Φ_{PSII_0}), effective quantum yield of PSII photochemistry (Φ_{PSII} , Genty et al., 1989), electron transport rate (ETR) and Stern-Volmer non-photochemical quenching (NPQ) were calculated according to Bilger and Björkman (1990).

Statistical analysis

Statistics were performed using Statgraphics Centurion XVI Version 16.1.11 (StatPoint Technologies, Inc., Warrenton, VA, USA). Significant differences between treatments were determined by one-way ANOVA and LSD tests ($P \leq 0.05$). Spearman bivariate correlations between the pairs of all parameters were performed with SPSS Statistics version 17.0 (IBM SPSS Statistics, IBM Corp., Somers, NY, USA) and correlation coefficients r_s were recorded when correlation was statistically significant at either level ($P \leq 0.05$ or $P \leq 0.01$).

Conclusion

Photosynthetic parameters showed significant decline due to simulated salt spray. The addition of boron alleviated most of the negative effects of salinity in the short-term (20 days after the beginning of treatments) while in the long-term (76 days after the beginning of treatments) the effect of boron was not beneficial, possibly due to its accumulation at toxic levels together with NaCl. We showed, in accordance with previous studies, that boron may alleviate the negative effects of salinity. Additionally, we state that this effect was observed by salinity spray, suggesting that the interaction between the two components was not due to changes in root function. Saline spray experiments may yield different results if boron is also included.

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