Effects of salinity and B excess on the growth, photosynthesis, water relation and mineral composition of laurustinus grown in greenhouse

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Abstract

A greenhouse study was conducted to determine the interactive effects of NaCl salinity and boron on the growth, plant water status, gas exchange, chlorophyll fluorescence and concentrations of sodium (Na), chloride (Cl) and boron (B) in laurustinus (*Viburnum tinus* L.). Potted plants were grown in a factorial combination of salinity (2 and 6 dS m^{-1}) and boron (1 and 6 mg L^{-1}). Plant dry weight (DW) decreased with salinity and B excess, particularly as a result of the former. The salinity × B interaction on the plant DW was not significant (additive effects). Salinity increased Na and Cl concentrations in leaf (20 and 35 mg g^{-1} DW, respectively) resulting in foliar injury. The application of 6 mg L^{-1} of B (B toxicity or B excess) produced injury symptoms in old leaves (leaf tip and edge burn). Salinity and B toxicity led to leaves dropping, especially the former. B toxicity led to higher B concentrations in insured leaves (1385 mg kg^{-1} DW) and salinity reduced it to 425 (B x NaCl antagonistic effect). Boron excess did no alter Na and Cl concentrations in leaf. Salinity decreased stomatal conductance (g_s) as a regulatory mechanism against osmotic stress, which resulted in a dropping photosynthesis (P_n). Leaf water parameters were only affected by salinity, which enhanced a process of osmotic adjustment and improving the plant water status. Salt-stressed plants showed an adaptive response to salinity, which decreased g_s, P_n and quantum yield of photosystem II (éPSII), and dissipated the excess radiant energy as heat (increased non-photochemical quenching [NPQ]). The combination of salinity and B excess maintained éPSII and decreased the effectiveness of stomatal regulation, NPQ and P_n. This caused the lowest plant DW and suggests disorders in electron transport (photorespiration). Our findings suggest that: (1) laurustinus is a B excess sensitive species, (2) salinity reduced the accumulation of B in leaves of the B excess stressed plants but was not enough to prevent injuries in PSII, and (3) B excess or/and salinity provide plants of poor commercial quality.

INTRODUCTION

Marginal waters contain high salt levels and, in some cases, a high boron (B) concentration. Salinity and B toxicity may stress and limit plant growth. The adverse effects of salinity on crop growth from two characteristics: (1) the increased osmotic potential of the soil solution with salinity, which reduces the availability of water for plants and (2) the specific effects of some elements (Na, Cl, B, etc.) present in excess.

Although B is an essential element for plant growth, it can be toxic to plants when its concentration in soil solution exceeds a threshold value (Gupta et al., 1985). For example, a B concentration higher than 1 mg L^{-1} can cause severe leaf burn to some sensitive landscape plants (Wu and Dodge, 2005). Irrigation with saline groundwater containing high B occurs, with notables examples found in the world (Yermiyahu et al., 2008). In recent years, B toxicity has become more a problem because of the increased demand for desalinated water, in which the B concentration may be high (Parks and Edwards, 2005). The addition of desalinated water to municipal water in the south of the Iberian Peninsula is increasing the B content in reclaimed sewage. Several studies suggest that salinity and B excess effects were inter-dependent rather that additive and some of these studies have suggested an antagonistic combined effect of both factors (Yermiyahu et al., 2008).

The objective of the current study was to determine the effects of B excess in non-saline or saline conditions on the physiological and agronomic performance of potted laurustinus.

MATERIALS AND METHODS

The experiment was carried out in Cartagena, SE Spain (Lat. 37º47´N, Long. 0º 54´W) in a greenhouse covered with polycarbonate. During the experiment temperatures were 10.3 °C (minimum), 24.7 °C (average) and 36.9 °C (maximum); minimum relative humidity was 22.4 %, and the maximum 100 %, with a mean of 65.5 %. *Viburnum tinus* L. seedling were grown in 17 cm ø pots filled with a mixture of coconut fiber-bark and perlite (4:1 v/v). The experiment began on 4 March 2009 and finished on 29 September 2009. Plants were irrigated daily by a computer-controlled drip irrigation system to reach 100 % water holding capacity (leaching 20-25%). On 6 April 2009, four irrigation treatments were applied: (1) irrigation with saline water and B low (6 dS m^{-1} and 1 mg L^{-1}); (2) irrigation with non-saline water and B toxicity (2 dS m^{-1} and 6 mg L^{-1}): (3) irrigation with saline water and B toxicity (6 dS m^{-1} and 6 mg L^{-1}); and (4) irrigation with non-saline water and B low (control irrigation, 2 dS m^{-1} and 1 mg L^{-1}). Boron and salinity were supplied as boric acid and sodium chloride.

At the end of the experiment, leaf area and DW of roots and aerial part were determined in five plants per treatment. The growth index determined was the shoot/root. Blade area was determined with a leaf are meter (LI-3100C, LI-COR Biosciences, NE, USA) in the leaves present in the plant at the end of the experiment. Leaf fall were collected and their area measured as they fell during the growth period and the cumulative area of leaves fall per plant was recorded, and was calculated as a percentage of the leaf area (blade surface of leaves non-drop and drop). Stomatal conductance (g_s) and photosynthesis (P_n) were measured at midday using a CIRAS-2 Portable Photosynthesis System. Leaf water potential (Ψ_h), leaf osmotic potential (Ψ_o), leaf pressure potential (Ψ_p) and leaf osmotic potential at full turgor (Ψ_{os}) were estimated as described by Bañón et al. (2005). B content was determined by inductively coupled plasma-mass spectrometry (Agilent 7500 ICP-MS, International Equipment Trading Ltd., Illinois, USA), and the contents of Cl and Na by ion chromatography (Hewlett Packard/HP 5972 GC/MSD System, AIR Inc., CO, USA). Chlorophyll fluorescence was determined using a FMS-2 modulated chlorophyll fluorometer from Hansatech Instruments (Maxwell and Johnson, 2000).

The two factors (B and salinity) and their interaction were analysed by two-way analysis of variance using Statgraphics Plus for Windows. Treatment means were separated by Least Significant Difference Test ($P<0.05$). Ratios and percentages was arcsine transformed before statistical analysis to ensure homogeneity of variance.

RESULTS AND DISCUSSION

Salinity and B toxicity symptoms

B toxicity symptoms appeared at 6 mg L^{-1}. These first included yellow or orange spots at the tips and margins of leaves (basal and middle part of plant), which resulted in progressive necrosis, the leaves curling inwards and sometimes dropping (about 12%). Salinity resulted in necrotic lesions, wilting starting from the tips and spreading to the blade centre, leaves curled inwards and finally some dropped (about 24%). Leaf injury due to salinity was more evident than that caused by B excess, and affected leaves in every part of the plant. In salt and B excess stressed plants salinity symptoms but no B symptoms were visible. Salinity x B interaction was significant for the leaf fall (Table 1), meaning that B excess under salinity did not increase the leaf fall (antagonistic effect, see Table 2).

Plant growth

Salinity decreased plant dry weight (DW) by 60%, which indicates that laurustinus growth was seriously affected by 6 dS m^{-1}, while 6 mg L^{-1} of B reduced plant DW by 12%. The combined effect of both B and salinity on plant DW was additive (interaction no significant, see Table 1). The shoot/root ratio increased under salinity, which suggests, contrary to most crop species, than the laurustinus root system might be more sensitive to salt stress than its aerial part. B excess increased the shoot/root too (Yermiyahu et al., 2008). Salinity and B excess irrigated plants probably decrease their capacity to absorb water and nutrients, because a high shoot/root ratio means that the roots are not as abundant, and that the seedling is more likely to suffer from water stress. B excess and salinity reduced leaf area which was caused by blade necrosis and leaf drop. The B x salt interactive effect on leaf area was similar to that found in plant DW (antagonistic effect).

Concentrations of Na, Cl and B in plant

Salinity altered the Na and Cl contents in leaf, but not in the root. Toxic concentrations of Cl and Na in the leaves of laurustinus were about 35 and 20 mg g^{-1} DW, respectively (Table 1). As a result, salinated plants become susceptible to specific-ion injury as well as to nutritional disorders, which resulted in reduced growth. The application of 6 mg L^{-1} of B did not modify the Cl or Na contents in leaf compared with the 1 mg L^{-1}.

The B content in leaves and roots were significantly increased by a high B supply. Higher B contents were found in leaf than in the root. This result is in agreement with those reported for citrus and other species, where the B content was high in leaves and low in roots. In this experiment, the highest B contents were found in insured leaves (~1380 mg kg^{-1} DW), while levels in uninjured leaves were between 54 and 71 mg kg^{-1}. These values confirm that leaf injury symptoms were due to B excess because laurustinus accumulates a lot of B in its basal leaves. The leaf B content in B excess treated plants was four times lower in saline than in non-saline conditions (B x salt antagonistic effect). Poss et al. (1999) also observed that B toxicity was mitigated under conditions of increasing salinity in *Eucalyptus camaldulensis*.

Gas exchange, chlorophyll fluorescence and water relations

Salinity decreased g_s at both concentrations of B studied, which suggests an effective stomata regulation of laurustinus to avoid salt-induced osmotic stress. A low g_s and leaf area in salt stressed plants reduces B transport, because B is transported to the leaves via the transpiration stream (Ben-Gal and Shani, 2002). This led to a lower B content in the leaves of salinity and B excess treated plants than in non-salinity and B excess treated plants. Stomata regulation of this plant in saline conditions was lower in the presence of excess B (B x NaCl antagonistic effect), but not sufficiently to prevent a substantial fall in P_n (Table 2). A drop in P_n may be related to stomatal factors or may be result of non-stomatal factors. Our data suggest that stomatal factors mainly induced decrease P_n in laurustinus as a result of salinity in 1 mg L^{-1} of B. However, when 6 mg L^{-1} of B was applied in saline conditions P_n declined sharply, which indicates that non-stomatal factors are the most relevant.

Both salinity and B excess reduced the maximum quantum yield of photosystem II (Fv/Fm) and the combined effect of the two factors was additive, which meant that this diminution was greater in the combination of salinity and B excess. This supports the conclusion that the combination of salinity and B excess harmed photosynthetic performance in laurustinus. An increase in non-photochemical quenching (NPQ) may occur as a result of processes that protect the leaf from light-induced damage or of the damage itself (Maxwell and Johnson 2000). Salinity increased NPQ in 1 mg L^{-1} of B and decreased NPQ in 6 mg L^{-1} of B (antagonistic effect, see Table 2). Moreover, 6 dS m^{-1} and 1 mg L^{-1} treated plants showed a decline in both the quantum yield of photosystem II (éPSII) and P_n (which means low photochemical activity), and an increase in NPQ (increase of radiant energy dissipated as heat). These findings indicated that plants are adapting to saline stress. Salinity and B excess treated plants showed a strong decline in P_n (which reduced plant DW) but not in éPSII, which suggests a disorder in electron transport and photorespiration (Fryer et al., 1998), and, as a result, a fall in Fv/Fm.

Salinity resulted in a decrease of Ψ_h, Ψ_o and Ψ_{os}, while B excess did not modify any water parameters (Table 1). Salt stressed and non-stressed plants presented statistically equal values of Ψ_p because the plants underwent osmotic adjustment as a result of the salt stress imposed (Ψ_{os} fell in salinated plants).

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Tables

Table 1. Level of signification from the two-way ANOVA conducted to determine the effects of boron (B), salinity (S) and interaction S x B on studied parameters [(1)].

PARAMETERS	Salinity (S)		Boron (B)		Statistic		
	2 dS m^{-1}	6 dS m^{-1}	1 mg L^{-1}	6 mg L^{-1}	S	B	SxB
Leaf fall (%)	6.25	23.93	12.04	18.15	***	**	**
Plant dry weight (g)	229.10	89.08	170.71	147.47	***	*	ns
Shoot/root	2.92	3.43	3.05	3.31	**	*	ns
Leaf area (dm^2)	69.08	35.60	56.57	48.11	***	**	**
Leaf Cl^- (mg g^{-1} DW)	4.98	36.07	20.22	20.83	***	ns	ns
Root Cl^- (mg g^{-1} DW)	8.45	5.92	8.65	5.72	**	ns	ns
Leaf Na^+ (mg g^{-1} DW)	0.68	20.14	10.38	10.44	***	ns	ns
Root Na^+ (mg g^{-1} DW)	7.33	6.25	7.96	5.61	ns	ns	ns
Leaf B^- (mg Kg^{-1} DW)	721.27	248.07	64.84	904.50	**	***	**
Root B^- (mg Kg^{-1} DW)	118.66	89.64	32.69	175.60	**	**	**
g_s (mmol m^{-2} s^{-1})	32.55	20.67	24.39	28.83	**	**	*
P_n (μmol m^{-2} s^{-1})	5.31	3.42	4.68	4.15	***	**	*
Fv/Fm	0.83	0.78	0.83	0.78	*	*	ns
NPQ	2.03	2.86	2.73	3.06	**	**	*
éPSII	0.20	0.19	0.21	0.20	ns	*	**
Ψ_h (MPa)	-1.35	-1.81	-1.61	-1.55	**	ns	ns
Ψ_p (MPa)	1.44	1.46	1.45	1.45	ns	ns	ns
Ψ_o (MPa)	-2.75	-3.26	-3.00	-3.02	**	ns	ns
Ψ_{os} (MPa)	-1.91	-2.61	-2.32	-2.19	**	ns	ns

[(1)]Values in each row which do not have any letter in common are significantly different as described by LSD test. ns: non-significant. *Significant at $P < 0.05$. **Significant at $P < 0.01$. ***Significant at $P < 0.001$

Table 2. Interactive effects of salinity and boron on parameters studied [2]

PARAMETERS	Salinity (dS m^{-1})	Boron (mg L^{-1})	
		1	6
Leaf fall (% FA)	2	*0.65a	*11.86b
	6	23.43a	24.44a
Plant dry weight (g)	2	*242.02b	*216.19a
	6	99.41b	78.76a
Shoot/root ratio	2	*2.77a	*3.08b
	6	3.33a	3.54b
Leaf area (dm^2)	2	*73.22 b	*64.95a
	6	39.92 b	31.28a
Lower leaf B^- (mg Kg^{-1} DW)	2	58.32a	*1384.23b
	6	71.36a	424.78b
Root B^- (mg Kg^{-1} DW)	2	*19.27a	*139.53b
	6	27.78a	88.75b
g_s (mmol m^{-2} s^{-1})	2	*32.08a	*33.02a
	6	16.70a	24.63b
P_n (µmol m^{-2} s^{-1})	2	*5.34a	*5.48a
	6	4.02b	2.82a
NPQ	2	*2.62a	*3.25b
	6	2.85a	2.86a
éPSII	2	*0.22b	*0.19a
	6	0.19a	0.20a

[2] Asterisks indicate significant differences by salinity, and different small letters indicate significant differences between B concentration, both according to the Least Significant Difference Test (P<0.05).