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The Effects of Sodium Chloride on the Aesthetic Value of Buxus spp.

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Summary

The use of saline water is an option for the irrigation of salt tolerant ornamentals as competition for high quality water increases. However, despite the importance of ornamental shrubs in Mediterranean areas, salt tolerance of such species has received little attention. Our investigations focused on two species of *Buxus* L. used in urban green design: *B. sempervirens* L. and *B. microphylla* Sieb. & Zucc. ‘Faulkner’. Plants were subjected to treatments with two NaCl solutions (125 mM and 250 mM) and distilled water (control), applied by immersion and sprinkler. Injury symptoms by means of visual check were regularly evaluated each two weeks, and SPAD values, chloride and sodium concentrations were measured in leaves at the end of the experiment. *Buxus* resulted an interesting genus as no severe damages were detected in all salt-treated plants. With this background, *B. microphylla* 'Faulkner' resulted less affected by salt stress. *B. sempervirens* showed more relevant foliar damages (bronzing, yellowing and scorching) and higher chloride and sodium leaf concentration when treated by immersion.

Key words. abiotic stress - ornamental shrub – NaCl - salinity scorching

Introduction

In the world, about one third of irrigated land are salt affected, causing a significant reduction in crop productivity (FLOWERS and YEO 1995; RAVINDRAN et al. 2007; CHEN and POLLE 2010). The rapid population growth and thus the competition for limited water resources have forced the use of low quality water for irrigation, especially for ornamental plants in urban green areas (MORALES et al. 1998; CHARTZOULAKIS et al. 2002; CARTER et al. 2005; NAVARRO et al. 2008). Besides, in ice affected areas, road de-icers are mostly composed of sodium chloride with traces of other mineral salts (BECKERMAN and LERNER 2009). This salt is the most efficient and cheap antifreeze element spread on the streets in winter, even if it has a high toxic action towards different plants, especially when compared with other salts such as calcium chloride and sodium sulphate (DEVECCHI et al. 2005).

Salinity may affect the growth of ornamental shrubs by reducing stem and leaf expansion resulting by toxicity from high ionic concentration of constituents such as Na^+ and Cl^- (USEPA 1992). When toxic ions are present in the rhizosphere, they can disrupt the uptake of nutrients by interfering with transporters in the root plasma membrane (TESTER and DAVENPORT 2003). The influence of salt stress on plant growth alone is, however, not sufficient to evaluate the salt tolerance of ornamentals: slight bronzing and leaf-tip yellowing followed by tip death and general necrosis as consequence of ion toxicity have to be considered (FRANCOIS 1982) due to their influence on decorative value (MARSCHNER 1995; WU et al. 2001; FERGUSON and GRATTAN 2005; VALDEZ-AGUILAR et al. 2011). Any negative effects of salts on plant growth have to be taken into consideration mainly for their influences on aesthetic value which is an important component of ornamental plants (TOWNSEND 1980).

Salt sensitive and tolerant plants show different abilities in preventing salts to reach toxic levels in transpiring leaves, by retaining these ions in the root and lower stem (MAATHUIS and AMTMANN 1999; MURILLO-AMADOR et al. 2006). Another key determinant of salt tolerance is likely to be the capacity of plants to maintain a high K^+/Na^+ ratio in their tissues (MUNNS and JAMES 2003), which is liable to be reduced because of the competitive effect of Na^+ concentration in the rhizosphere on K^+ uptake (DEL-AMOR et al. 2001; AKTAS et al. 2006). MUNNS and TESTER (2008) highlighted that species differ in their ability to retain Na^+ in lignified roots and stems, while Cl^- is not retained and continues to be transported to the leaves. MUNNS (2002) highlighted some tolerance differences between close species. The same author described that this tolerance is based on the limited uptake of Na^+ and Cl^- by roots or the restricted translocation of these ions to the aerial parts.

Despite the potential ability of several ornamental shrubs to adapt to adverse conditions, only few species belonging to *Pyracantha* L. and *Cotoneaster* Medik. genera are commonly used in parks and public areas (CASSANITI et al. 2008). Poor information on salt tolerance mechanisms are currently available about other species. FERRANTE et al. (2011) showed an high tolerance in preventing reduction in chlorophyll content and leaf damages of *Westringia fruticosa* after 77 days of seawater aerosol. On the other hand, plants of *Arbutus unedo* submitted to two irrigation treatments (5.45 dSm^{-1} and 9.45 dSm^{-1}) showed significant decrease in plant height, leaf area, shoot biomass, and ornamental characters (NAVARRO et al. 2008). CASSANITI et al. (2009) studied 12 widely cultivated ornamental shrubs, investigating possible relation with different salinities (10, 40 and 70 mM NaCl). *Cotoneaster lacteus, Grevillea juniperina* and *Pyracantha* 'Harlequin' were indicated as the most sensitive species. In all these studies, the influence of salt stress on ornamental plant was evaluated considering mainly marginal leaf burn and their influence on decorative value. Indeed, the overall appearance as well as survival should be the ultimate criteria for planting landscape shrubs.

Buxus has always played an important role in historical gardens and topiary art but it is also suitable in urban greening (COSTA and DEVECCHI 2006). Poor data about the response of this genus to saline stress are available (WU et al. 2001; BECKERMAN and LERNER 2009; VALDEZ-AGUILAR et al. 2011). Therefore, on the basis of previous investigations about injuries caused by salt stress in Mediterranean plants (DEVECCHI et al. 2005), the effects of two NaCl concentrations, applied by immersion and sprinkler irrigation systems, were evaluated in *B. sempervirens* L. and *B. microphylla* Sieb. & Zucc. 'Faulkner' during container production.

Materials and Methods

Experiments were conducted in a glasshouse at the Experimental Centre of the Agricultural Faculty of the University of Turin (Italy; 45°03'59.73''N, 7°35'24.72''E). One-year-old plants of *B. sempervirens* and *B. microphylla* 'Faulkner' were cultivated in 14 cm diameter pots (1.2 l volume) filled with a mixture of peat and pomice (2:1, v/v). Fertilization took place with a slow-release fertilizer (Osmocote 15:11:13; Scotts Europe). Air temperature, humidity, and Photosynthetically Active Radiation (PAR) were monitored (Table 1). After 15 days from transplanting, 120 plants of each species were subjected to treatments (5 months long from December to April 2007). Solutions containing 125 mM NaCl and 250 mM NaCl, and distilled water (control) were applied every 15 days for a total of nine applications for both the immersion and sprinkler systems (WU et al. 2001). Immersion irrigation system was applied by placing plants with vase on 2 L of treatment solutions for 15 minutes. In the sprinkler system, solutions were sprayed through sprinklers for 20 min with a precipitation rate of 2.5 cm/h. In order to minimize salt accumulation in the soil, vases were completely covered by a nylon cloth. A randomized block was adopted as the experimental design.

The effect of NaCl on ornamental characteristics was evaluated on ten apical leaves randomly selected on one branch per plant, after two weeks from each application for a total of nine surveys. Foliar damage (FD) was visually estimated in terms of bronzing and yellowing variations based on a scale of six classes (0-5), while scorching by means of a scale of five classes (0-4; Table 2; DEVECCHI et al. 2005). At the end of the experiment the relative quantity of chlorophyll present in leaf tissue was conducted on 25 leaves of each plant using the Chlorophyll Meter SPAD-502 Konica Minolta Sensing Inc. (Osaka, Japan). This instrument measures the transmittance of the leaf in the red and infrared regions (650 nm and 940nm, respectively). Leaves were then collected and stored at -18°C for subsequent analyses (Na^+ and Cl^- concentration). In order to measure ion concentration in leaves, the analysis of chlorides and sodium was performed in double. A total of 10 g of fresh weight of leaves from 10 plants per treatment were dried in a ventilated oven at 90 °C for 48 hrs and grounded finely. One g of the dry ground leaves was put in platinum dishes beneath an IR lamp at 200 °C and then in oven at 400 °C for 5 hrs. Ashes were placed inside a drying apparatus to avoid the humidity absorption. Then samples were added with water to reach a volume of 25 ml and the concentration of Cl^- and Na^+ was determined using the chromatograph DIONEX (Sunnyvale, California) with an anionic exchange column AS 14A IonPac and a cationic exchange column CS 12A IonPac.

The effect of salt treatments on visual leaf damages (bronzing, yellowing and scorching) was evaluated by a 2x3x2 factorial arrangement (species, salt concentration and application method as fixed factors) with interaction. Differences were analysed with the non parametric Tamhane's post-hoc test. SPAD values, chlorophyll content, and ions amount were firstly subjected to the homogeneity of the variances and then post-hoc tested using Ryan-Einot-Gabriel-Welsch-F test (REGW-F). The critical value for statistical significance was $P<0.05$. All the data were computed by means of the SPSS statistical package (version 17.0; SPSS Inc., Chicago, Illinois).

Results

During the experimental period, foliar damages were measured. Regarding foliar bronzing, a statistical effect of the species was highlighted (Table 3). With the exception for the 2nd, 7th, 8th, and 9th surveys, *B. microphylla* 'Faulkner' showed the lowest percentage of leaves with damages (< 5% FD). After the 6th survey no foliar bronzing damages were observed in both species.

Concerning NaCl concentrations, differences were found only at the 1st and at the 6th survey in which the highest salt concentration (250 mM NaCl) was more effective to induce bronzing damages than 125 mM NaCl and 0 mM NaCl. No differences between the application methods and no interaction among the fixed factors (species, salt concentrations and application method) were noted. Regarding the foliar yellowing damages and scorching (data not shown), a statistical difference between species was obtained. *B. sempervirens* showed the highest leaf yellowing values at the 6th survey (up to 25% FD) and the highest leaf scorching at the 7th survey (up to 5% FD). No statistically visually detectable salt-stress-induced symptoms were noted among salt concentrations, administration type, and among the fixed factors.

With the aim to evaluate leaf nitrogen status, after the last application, SPAD values were evaluated for each species (Fig. 1). In *B. microphylla* 'Faulkner' no differences were revealed. While, in *B. sempervirens* plants treated with both NaCl concentrations (125 mM and 250 mM NaCl) and treated by immersion method showed the lowest values (27.9 and 29.9, respectively). For both species, no differences between sprinkler and control treatments were found. Generally, data obtained in *B. microphylla* 'Faulkner' plants were higher than in *B. sempervirens*.

The Na^+ and Cl^- concentrations measured in the leaves of the species are presented in Figs. 3 and 4. Species showed a slight variability in their ion concentrations. In general, less Na^+ was accumulated than Cl^- (the average Na^+/Cl^- ratio was 0.32). More in deep, this ratio was 0.30 in *B. microphylla* 'Faulkner' and 0.23 in *B. sempervirens*. When the external NaCl was increased to 250 mM NaCl, leaf Na^+ concentrations statistically increased in both species and administration type in relation with control (Fig. 3). In *B. sempervirens* plants treated by immersion, above all, Na^+ concentration reached a value 65-fold higher than control. Concerning Cl^- concentrations, at 125 mM NaCl, no significant differences were found in comparison with control, except for *B. sempervirens* treated by immersion in which the highest Cl^- were noted (Fig. 4). A rise in NaCl (250 mM NaCl) in the irrigation water led to significant increases of Cl^- in leaf concentrations in both species.

The tendency of the species to accumulate Na^+ and Cl^- in the leaves was investigated by calculating the linear regression between the increasing of the ion concentrations in the irrigation water and application method (data not shown). Compared to Na^+ accumulation, the highest slope was reached in *B. microphylla* 'Faulkner' plants (r^2 = 1.000) treated by sprinkler. While the lowest in *B. sempervirens* (r^2 = 0.7912) treated by immersion. Concerning Cl^- concentrations, *B. microphylla* 'Faulkner' showed the highest slope for both application methods (r^2 = 0.8386 and r^2 = 0.9313 for immersion and sprinkler treatments, respectively).

Discussion

Salinity stress is a common environmental problem which can limit crop production since it affects different morphological, physiological, and biochemical processes (MUNNS and TESTER 2008). The presence of salinity-induced damage is one of the principal indicators of salt stress in plants (BANON et al. 2005). The main effect of salinity is growth reduction (MUNNS and TERMAAT 1986) which has been used in many studies as a measure of resistance to saline conditions (SANCHEZ-BLANCO et al. 1991). For ornamental shrubs, a decrease in growth rate is not enough to characterize salt tolerance as other more important traits such as visible symptoms of injury (leaf bronzing and scorching) contribute to their aesthetic value (FRANCOIS 1982; CASSANITI et al. 2009).

In general, between the two studied species, *B. microphylla* 'Faulkner' showed no yellowing and scorching leaf damages and only limited bronzing symptoms during all the experiment, resulting a tolerant cultivar. Similarly, no salt stress symptoms, growth effects, and leaf chlorosis symptoms were detected also by WU et al (2006) on *B. microphylla* plants treated with 8.55 mM NaCl and 25.63 mM NaCl. On the opposite, VALDEZ-AGUILAR et al. (2011) rated *B. microphylla* var. *japonica* as a sensitive genotype already at low concentrations (7.18 mM NaCl), due to severe leaf bronzing. Authors concluded that bronzing may have been the result of high leaf concentrations of Na^+ and Cl^-, which caused direct toxicity (MUNNS AND TESTER 2008). In the present study, although *B. microphylla* 'Faulkner' plants treated by immersion with 250 mM NaCl accumulated the highest concentrations of Na^+ and Cl^-, they did not show any symptoms of yellowing and scorching and low bronzing damages, suggesting an efficient salt compartmentalisation (SANCHEZ-BLANCO et al. 2004; RODRIGUEZ et al. 2005). The retention of either Na+ and Cl- in leaves has been proposed to be a trait related to salt tolerance plants (BOURSIER and LAUCHLI 1990; PEREZ-ALFOCEA et al. 2000).

SPAD chlorophyll meter measures the ‘green degree’ which relates to chlorophyll content to reflect the value of leaf nitrogen content (ZOU et al. 2011). SPAD chlorophyll meter is frequently used as a quantitative measure of the severity of leaf damages associated with different stresses (WATANABE et al. 1980; BARRACLOUGH and KYTE 2001) and, to a limited extent of leaf photosynthetic capacity (CASTELLI et al. 1996). In the present study, *B. microphylla* 'Faulkner' presented no differences in SPAD values among treatments. All this underlines its healthy and good aesthetic value, even under salt-stressed conditions.

Limited knowledge on salt stress responses are available in *B. sempervirens*. DE JONG et al. (2012) reported that this species showed evident foliar damages when exposed to abiotic stresses such as cold, drought, and salinity. IDA et al (1995), GARCIA-PLAZAOLA et al. (2000), and HORMAETXE et al. (2004) reported that during winter acclimation *B. sempervirens* leaves vary to red as a response to photoinhibitory conditions. The leaf bronzing would reduce light intercepted by chlorophyll, contributing to the adjustment of the source-sink ratio. HORMAETXE et al. (2004) demonstrated that the accumulation of protective red carotenoids is fully reversible upon transfer to room temperature or during spring and summer, occurring in parallel with recovery of photochemical efficiency. HORMAETXE et al. (2006) indicated that over production of red carotenoids was recorded both in summer and winter, indicating a clear pattern of induction proportional to the stress intensity. The authors individuated the optimal temperature for *B. sempervirens* plants, ranging between 9°C-13°C. Both supra- and suboptimal temperatures induced similar responses. In the present study, both *Buxus* species showed foliar bronzing damages until the first half of March, period in which the mean temperature was always lower than the optimum The salt applications in March and April produced foliar yellowing and scorching only in *B. sempervirens* plants. Besides, this species showed also SPAD values lower than *B. microphylla* 'Faulkner' and higher chloride and sodium leaf concentration when treated by immersion.

We conclude that *Buxus* is an interesting genus in salt stress conditions as no severe visual damages were detected in salt-treated plants (125 mM and 250 mM NaCl). Between the two species, *B. microphylla* 'Faulkner' was the most salt-tolerant, showing no foliar damages also at high salt concentration.

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Figure captions

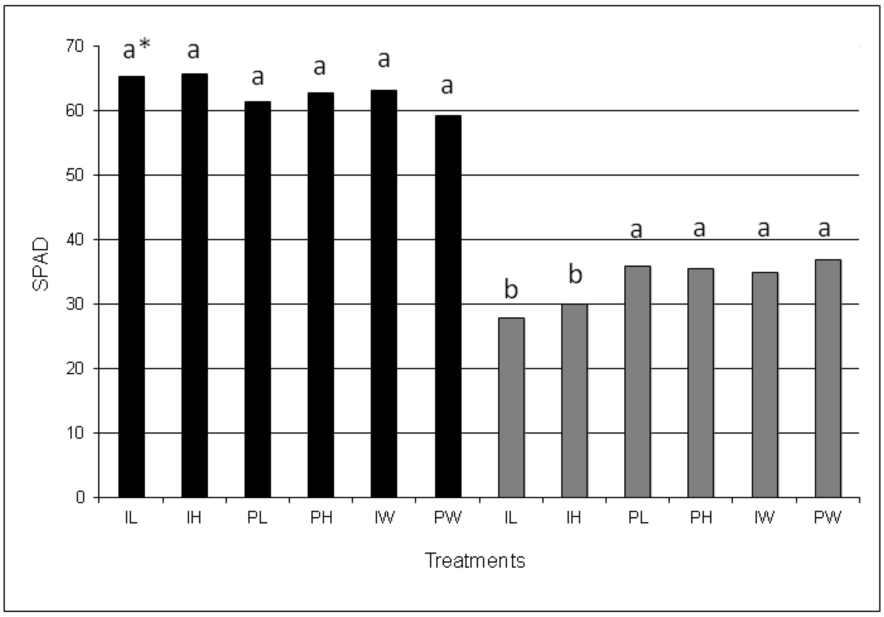

Fig. 1. Mean foliar SPAD values in *B. microphylla* 'Faulkner' (black histograms) and *B. sempervirens* (grey histograms) plants. Within each species, mean values showing the same letter are not statistically different at $P \leq 0.05$ (according to the REGW-F test). IL = immersion at 125 mM NaCl; IH = immersion at 250 mM NaCl; PL = sprinkler at 125 mM NaCl; PH = sprinkler at 250 mM NaCl; IW = immersion at 0 mM NaCl; PW = sprinkler at 0 mM NaCl

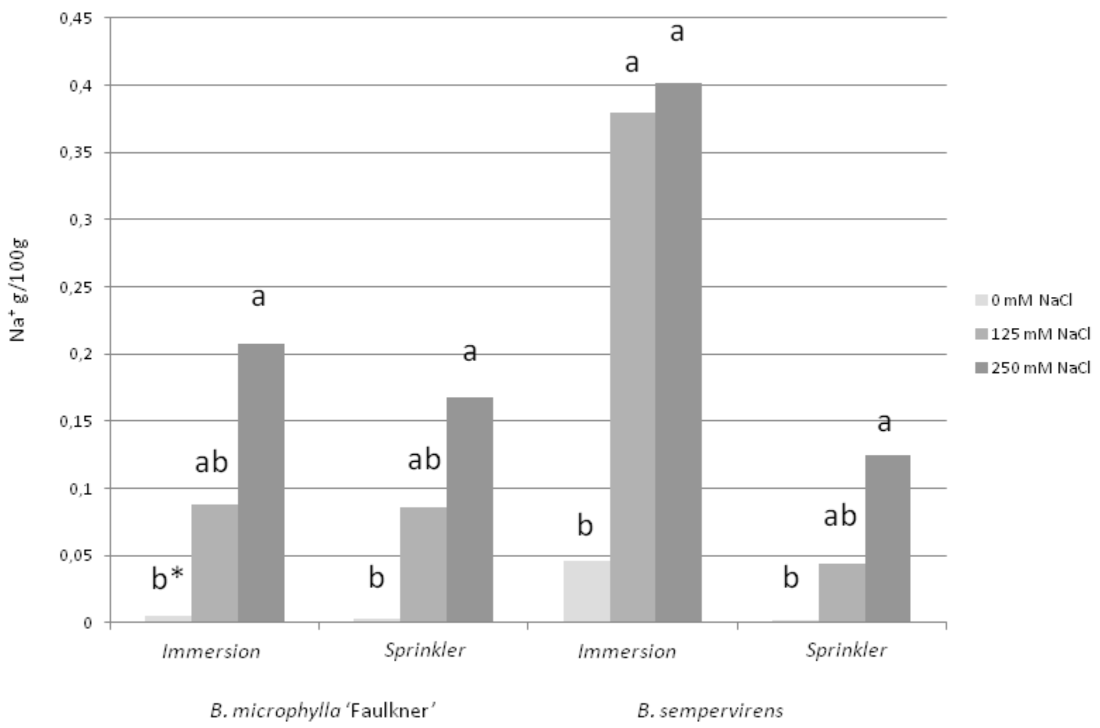

Fig. 2. Na^+ concentration (g/100g dry matter) in leaves for *B. microphylla* 'Faulkner' and *B. sempervirens* under salt treatments (0, 125, 250 mM NaCl) by immersion and spraying application. Within each species and application method, different letters indicate differences according the REGW-F test ($P \leq 0.05$).

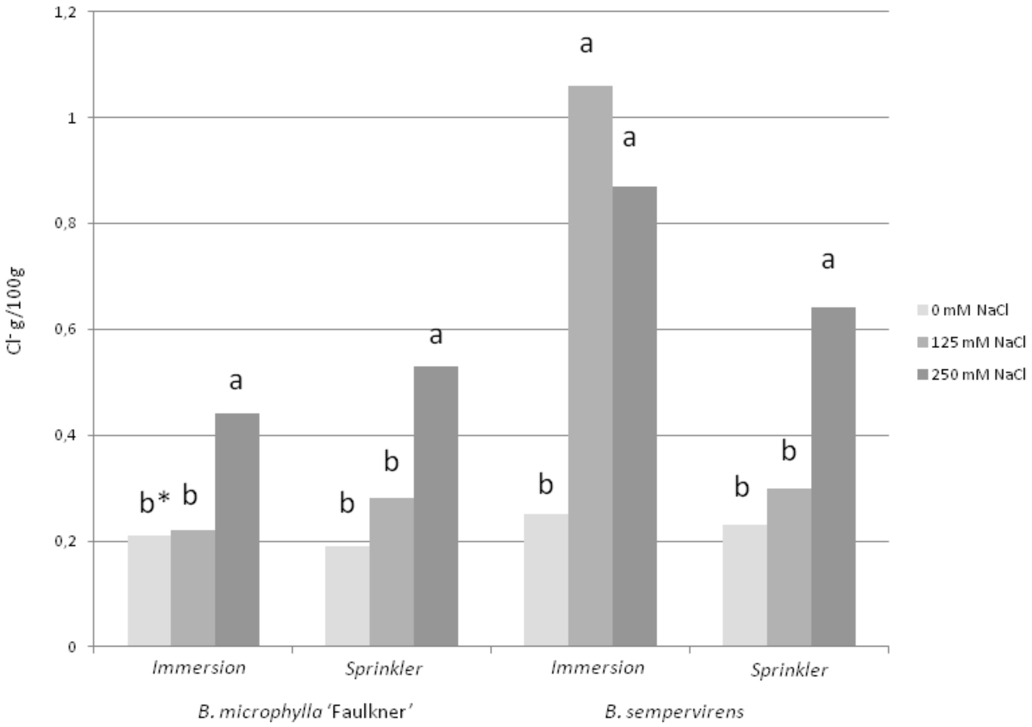

Fig. 3. Cl^- concentration (g/100g dry matter) in leaves for *B. microphylla* 'Faulkner' and *B. sempervirens* under salt treatments (0, 125, 250 mM NaCl) by immersion and spraying application. Within each species and application method, different letters indicate differences according the REGW-F test (P≤0.05).

Tables

Table 1. Temperature (mean, minimum and maximum), relative humidity (UR; %), and mean Photosynthetically Active Radiation (PAR) variation during the nine surveys in the greenhouse.

Survey	T mean (°C)	T min (°C)	T max (°C)	UR (%)	PAR (µmol/m^{2}s)
1st	1.47	-1.51	6.35	96.59	112.90
2nd	3.62	-0.29	9.63	92.99	120.25
3rd	3.62	0.08	9.21	92.18	139.40
4th	4.11	-1.01	11.39	90.43	187.25
5th	6.14	-0.29	14.12	87.13	239.65
6th	9.54	1.77	18.04	76.74	351.90
7th	8.30	2.41	14.56	73.23	349.65
8th	13.27	7.01	20.08	78.41	433.25
9th	17.31	9.56	25.33	71.25	566.20

Table 2. Classification of foliar bronzing, yellowing, and scorching damages (% FD) by means of visual evaluation.

Classes	Bronzing (% FD)	Yellowing (% FD)	Scorching (% FD)
0	0	0	0
1	< 5	< 5	< 10
2	5-25	5-25	10-25
3	26-45	26-45	26-50
4	46-65	46-65	> 50
5	> 65	> 65	

Table 3. Main effects of species, salt (NaCl) concentration, application method, and interaction among fixed factors on leaf bronzing values during the first six surveys. For the seventh, eighth, and ninth surveys no damages were observed in both species.

	Surveys					
	1st	2nd	3rd	4th	5th	6th
Species	Mean class values					
Buxus microphylla ‘Faulkner’	0.93 b§	1.43	1.15 b	0.73 b	0.00 b	0.00 b
Buxus sempervirens	1.33 a	2.07	2.03 a	1.56 a	0.27 a	0.19 a
P	*	ns	*	*	*	*
Salt (NaCl) concentration						
0 mM	1.10 b	1.51	1.40	0.98	0.40	0.02 b
125 mM	1.05 b	1.93	1.58	1.18	0.13	0.07 b
250 mM	1.25 a	1.80	1.79	1.28	0.23	0.19 a
P	*	ns	ns	ns	ns	*
Application method						
Immersion	1.12	1.71	1.45	1.12	0.14	0.11
Sprinkler	1.15	1.79	1.73	1.17	0.13	0.07
P	ns	ns	ns	ns	ns	ns
Interactions						
Species X Salt	ns	ns	ns	ns	ns	ns
Species X Application	ns	ns	ns	ns	ns	ns
Salt X Application	ns	ns	ns	ns	ns	ns

§Mean values showing the same letter are not statistically different at $P \leq 0.05$. The statistical relevance of Between-Subjects Effects’ Test was subjected to Tamhane Test ANOVA for non parametric data (*=$P \leq 0.05$, ns=non significant).