



AperTO - Archivio Istituzionale Open Access dell'Università di Torino

The effects of sodium chloride on the aesthetic value of Buxus spp

| This is the author's manuscript | | | | | |
|---|----------------------------|--|--|--|--|
| Original Citation: | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| Availability: | | | | | |
| This version is available http://hdl.handle.net/2318/140618 | since 2017-05-24T14:52:00Z | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| Terms of use: | | | | | |
| Open Access | | | | | |
| Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use | | | | | |

of all other works requires consent of the right holder (author or publisher) if not exempted from copyright

(Article begins on next page)

protection by the applicable law.



UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

Caser M, Scariot V, Gaino W, Larcher F, Devecchi M. The effects of sodium chloride on the aesthetic value of Buxus spp EUROPEAN JOURNAL OF HORTICULTURAL SCIENCE (2013) 78

| 333 | The Effects of Sodium Chloride on the Aesthetic Value of Buxus spp. |
|------------|---|
| 334 | |
| 335 | M. Caser ¹⁾ , V. Scariot ¹⁾ , W. Gaino ¹⁾ , F. Larcher ¹⁾ and M. Devecchi ¹⁾ |
| 336 | |
| 337 338 | ¹⁾ Department of Agricultural, Forest and Food Sciences, University of Turin, Italy |
| 339 | |
| 340 | |
| 341 | |
| 342 | |
| 343 | |
| 344 | |
| 345 | |
| 346 | |
| 347 | |
| 348 | |
| 349 | |
| 350 | |
| 351 | |
| 352 | |
| 353 | |
| 354 | |
| 355 | |
| 356 | |
| 357 | |
| 358 | |

359 Summary

| 360 | The use of saline water is an option for the irrigation of salt tolerant ornamentals as |
|------------|--|
| 361 | competition for high quality water increases. However, despite the importance of ornamental |
| 362 | shrubs in Mediterranean areas, salt tolerance of such species has received little attention. Our |
| 363 | investigations focused on two species of Buxus L. used in urban green design: B. |
| 364 | sempervirens L. and B. microphylla Sieb. & Zucc. 'Faulkner'. Plants were subjected to |
| 365 | treatments with two NaCl solutions (125 mM and 250 mM) and distilled water (control), |
| 366 | applied by immersion and sprinkler. Injury symptoms by means of visual check were |
| 367 | regularly evaluated each two weeks, and SPAD values, chloride and sodium concentrations |
| 368 | were measured in leaves at the end of the experiment. Buxus resulted an interesting genus as |
| 369 | no severe damages were detected in all salt-treated plants. With this background, B. |
| 370 | microphylla 'Faulkner' resulted less affected by salt stress. B. sempervirens showed more |
| 371 | relevant foliar damages (bronzing, yellowing and scorching) and higher chloride and sodium |
| 372 | leaf concentration when treated by immersion. |
| 373 | |
| 374 | Key words. abiotic stress - ornamental shrub – NaCl - salinity scorching |
| 375 | |
| 376 | |
| 377 | |
| 378 | |
| 379 | |
| 380 | |
| 381 | |
| 382 383 | |
| 384 | |
| 385 | |
| 386 | |
| 387 | |
| 388 | |
| 389 | |
| 390 | |

391 Introduction

392 In the world, about one third of irrigated land are salt affected, causing a significant 393 reduction in crop productivity (FLOWERS and YEO 1995; RAVINDRAN et al. 2007; CHEN and 394 POLLE 2010). The rapid population growth and thus the competition for limited water 395 resources have forced the use of low quality water for irrigation, especially for ornamental 396 plants in urban green areas (MORALES et al. 1998; CHARTZOULAKIS et al. 2002; CARTER et al. 397 2005; NAVARRO et al. 2008). Besides, in ice affected areas, road de-icers are mostly composed 398 of sodium chloride with traces of other mineral salts (BECKERMAN and LERNER 2009). This 399 salt is the most efficient and cheap antifreeze element spread on the streets in winter, even if it 400 has a high toxic action towards different plants, especially when compared with other salts 401 such as calcium chloride and sodium sulphate (DEVECCHI et al. 2005).

402 Salinity may affect the growth of ornamental shrubs by reducing stem and leaf 403 expansion resulting by toxicity from high ionic concentration of constituents such as Na⁺ and 404 Cl⁻ (USEPA 1992). When toxic ions are present in the rhizosphere, they can disrupt the 405 uptake of nutrients by interfering with transporters in the root plasma membrane (TESTER and 406 DAVENPORT 2003). The influence of salt stress on plant growth alone is, however, not 407 sufficient to evaluate the salt tolerance of ornamentals: slight bronzing and leaf-tip yellowing 408 followed by tip death and general necrosis as consequence of ion toxicity have to be 409 considered (FRANCOIS 1982) due to their influence on decorative value (MARSCHNER 1995; 410 WU et al. 2001; FERGUSON and GRATTAN 2005; VALDEZ-AGUILAR et al. 2011). Any negative 411 effects of salts on plant growth have to be taken into consideration mainly for their influences 412 on aesthetic value which is an important component of ornamental plants (TOWNSEND 1980). 413 Salt sensitive and tolerant plants show different abilities in preventing salts to reach 414 toxic levels in transpiring leaves, by retaining these ions in the root and lower stem

415 (MAATHUIS and AMTMANN 1999; MURILLO-AMADOR et al. 2006). Another key determinant 416 of salt tolerance is likely to be the capacity of plants to maintain a high K^+/Na^+ ratio in their 417 tissues (MUNNS and JAMES 2003), which is liable to be reduced because of the competitive 418 effect of Na⁺ concentration in the rhizosphere on K⁺ uptake (DEL-AMOR et al. 2001; AKTAS et 419 al. 2006). MUNNS and TESTER (2008) highlighted that species differ in their ability to retain 420 Na⁺ in lignified roots and stems, while Cl⁻ is not retained and continues to be transported to 421 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 422 423 roots or the restricted translocation of these ions to the aerial parts.

424 Despite the potential ability of several ornamental shrubs to adapt to adverse conditions, 425 only few species belonging to *Pyracantha* L. and *Cotoneaster* Medik. genera are commonly 426 used in parks and public areas (CASSANITI et al. 2008). Poor information on salt tolerance 427 mechanisms are currently available about other species. FERRANTE et al. (2011) showed an 428 high tolerance in preventing reduction in chlorophyll content and leaf damages of Westringia 429 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 430 431 decrease in plant height, leaf area, shoot biomass, and ornamental characters (NAVARRO et al. 432 2008). CASSANITI et al. (2009) studied 12 widely cultivated ornamental shrubs, investigating 433 possible relation with different salinities (10, 40 and 70 mM NaCl). Cotoneaster lacteus, 434 Grevillea juniperina and Pyracantha 'Harlequin' were indicated as the most sensitive species. 435 In all these studies, the influence of salt stress on ornamental plant was evaluated considering 436 mainly marginal leaf burn and their influence on decorative value. Indeed, the overall 437 appearance as well as survival should be the ultimate criteria for planting landscape shrubs. 438 *Buxus* has always played an important role in historical gardens and topiary art but it is 439 also suitable in urban greening (COSTA and DEVECCHI 2006). Poor data about the response of 440 this genus to saline stress are available (WU et al. 2001; BECKERMAN and LERNER 2009; 441 VALDEZ-AGUILAR et al. 2011). Therefore, on the basis of previous investigations about 442 injuries caused by salt stress in Mediterranean plants (DEVECCHI et al. 2005), the effects of 443 two NaCl concentrations, applied by immersion and sprinkler irrigation systems, were 444 evaluated in B. sempervirens L. and B. microphylla Sieb. & Zucc. 'Faulkner' during container 445 production.

446

447 Materials and Methods

448 Experiments were conducted in a glasshouse at the Experimental Centre of the 449 Agricultural Faculty of the University of Turin (Italy; 45°03'59.73''N, 7°35'24.72''E). One-450 year-old plants of *B. sempervirens* and *B. microphylla* 'Faulkner' were cultivated in 14 cm 451 diameter pots (1.2 l volume) filled with a mixture of peat and pomice (2:1, v/v). Fertilization 452 took place with a slow-release fertilizer (Osmocote 15:11:13; Scotts Europe). Air 453 temperature, humidity, and Photosynthetically Active Radiation (PAR) were monitored 454 (Table 1). After 15 days from transplanting, 120 plants of each species were subjected to 455 treatments (5 months long from December to April 2007). Solutions containing 125 mM NaCl 456 and 250 mM NaCl, and distilled water (control) were applied every 15 days for a total of nine 457 applications for both the immersion and sprinkler systems (WU et al. 2001). Immersion

458 irrigation system was applied by placing plants with vase on 2 L of treatment solutions for 15 459 minutes. In the sprinkler system, solutions were sprayed through sprinklers for 20 min with a 460 precipitation rate of 2.5 cm/h. In order to minimize salt accumulation in the soil, vases were 461 completely covered by a nylon cloth. A randomized block was adopted as the experimental 462 design.

463 The effect of NaCl on ornamental characteristics was evaluated on ten apical leaves 464 randomly selected on one branch per plant, after two weeks from each application for a total 465 of nine surveys. Foliar damage (FD) was visually estimated in terms of bronzing and 466 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 467 468 quantity of chlorophyll present in leaf tissue was conducted on 25 leaves of each plant using 469 the Chlorophyll Meter SPAD-502 Konica Minolta Sensing Inc. (Osaka, Japan). This 470 instrument measures the transmittance of the leaf in the red and infrared regions (650 nm and 471 940nm, respectively). Leaves were then collected and stored at -18° C for subsequent analyses 472 (Na⁺ and Cl⁻ concentration). In order to measure ion concentration in leaves, the analysis of 473 chlorides and sodium was performed in double. A total of 10 g of fresh weight of leaves from 474 10 plants per treatment were dried in a ventilated oven at 90 °C for 48 hrs and grounded 475 finely. One g of the dry ground leaves was put in platinum dishes beneath an IR lamp at 200 476 °C and then in oven at 400 °C for 5 hrs. Ashes were placed inside a drying apparatus to avoid 477 the humidity absorption. Then samples were added with water to reach a volume of 25 ml and 478 the concentration of Cl⁻ and Na⁺ was determined using the chromatograph DIONEX 479 (Sunnyvale, California) with an anionic exchange column AS 14A IonPac and a cationic 480 exchange column CS 12A IonPac.

481 The effect of salt treatments on visual leaf damages (bronzing, yellowing and 482 scorching) was evaluated by a 2x3x2 factorial arrangement (species, salt concentration and 483 application method as fixed factors) with interaction. Differences were analysed with the non 484 parametric Tamhane's post-hoc test. SPAD values, chlorophyll content, and ions amount were 485 firstly subjected to the homogeneity of the variances and then post-hoc tested using Ryan-486 Einot-Gabriel-Welsch-F test (REGW-F). The critical value for statistical significance was 487 P < 0.05. All the data were computed by means of the SPSS statistical package (version 17.0; 488 SPSS Inc., Chicago, Illinois).

489

490 **Results**

491 During the experimental period, foliar damages were measured. Regarding foliar 492 bronzing, a statistical effect of the species was highlighted (Table 3). With the exception for 493 the 2^{nd} , 7^{th} , 8^{th} , and 9^{th} surveys, *B. microphylla* 'Faulkner' showed the lowest percentage of 494 leaves with damages (< 5% FD). After the 6^{th} survey no foliar bronzing damages were 495 observed in both species.

496 Concerning NaCl concentrations, differences were found only at the 1st and at the 6th 497 survey in which the highest salt concentration (250 mM NaCl) was more effective to induce 498 bronzing damages than 125 mM NaCl and 0 mM NaCl. No differences between the 499 application methods and no interaction among the fixed factors (species, salt concentrations 500 and application method) were noted. Regarding the foliar yellowing damages and scorching (data not shown), a statistical difference between species was obtained. B. sempervirens 501 502 showed the highest leaf yellowing values at the 6^{th} survey (up to 25% FD) and the highest leaf scorching at the 7th survey (up to 5% FD). No statistically visually detectable salt-stress-503 504 induced symptoms were noted among salt concentrations, administration type, and among the 505 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*.

513 The Na⁺ and Cl⁻ concentrations measured in the leaves of the species are presented in 514 Figs. 3 and 4. Species showed a slight variability in their ion concentrations. In general, less 515 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 516 517 was increased to 250 mM NaCl, leaf Na⁺ concentrations statistically increased in both species 518 and administration type in relation with control (Fig. 3). In B. sempervirens plants treated by 519 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 520 521 comparison with control, except for *B. sempervirens* treated by immersion in which the 522 highest Cl⁻ were noted (Fig. 4). A rise in NaCl (250 mM NaCl) in the irrigation water led to

523 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).

531

532 **Discussion**

533 Salinity stress is a common environmental problem which can limit crop production 534 since it affects different morphological, physiological, and biochemical processes (MUNNS 535 and TESTER 2008). The presence of salinity-induced damage is one of the principal indicators 536 of salt stress in plants (BANON et al. 2005). The main effect of salinity is growth reduction 537 (MUNNS and TERMAAT 1986) which has been used in many studies as a measure of resistance 538 to saline conditions (SANCHEZ-BLANCO et al. 1991). For ornamental shrubs, a decrease in 539 growth rate is not enough to characterize salt tolerance as other more important traits such as 540 visible symptoms of injury (leaf bronzing and scorching) contribute to their aesthetic value 541 (FRANCOIS 1982; CASSANITI et al. 2009).

542 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 543 544 experiment, resulting a tolerant cultivar. Similarly, no salt stress symptoms, growth effects, 545 and leaf chlorosis symptoms were detected also by WU et al (2006) on *B. microphylla* plants 546 treated with 8.55 mM NaCl and 25.63 mM NaCl. On the opposite, VALDEZ-AGUILAR et al. 547 (2011) rated *B. microphylla* var. *japonica* as a sensitive genotype already at low 548 concentrations (7.18 mM NaCl), due to severe leaf bronzing. Authors concluded that bronzing 549 may have been the result of high leaf concentrations of Na⁺ and Cl⁻, which caused direct 550 toxicity (MUNNS AND TESTER 2008). In the present study, although B. microphylla 'Faulkner' 551 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 552 553 damages, suggesting an efficient salt compartmentalisation (SANCHEZ-BLANCO et al. 2004; 554 RODRIGUEZ et al. 2005). The retention of either Na+ and Cl- in leaves has been proposed to 555 be a trait related to salt tolerance plants (BOURSIER and LAUCHLI 1990; PEREZ-ALFOCEA et al. 556 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.

570 HORMAETXE et al. (2004) demonstrated that the accumulation of protective red carotenoids is 571 fully reversible upon transfer to room temperature or during spring and summer, occurring in 572 parallel with recovery of photochemical efficiency. HORMAETXE et al. (2006) indicated that 573 over production of red carotenoids was recorded both in summer and winter, indicating a 574 clear pattern of induction proportional to the stress intensity. The authors individuated the 575 optimal temperature for *B. sempervirens* plants, ranging between 9°C-13°C. Both supra- and 576 suboptimal temperatures induced similar responses. In the present study, both *Buxus* species 577 showed foliar bronzing damages until the first half of March, period in which the mean 578 temperature was always lower than the optimum The salt applications in March and April 579 produced foliar yellowing and scorching only in *B. sempervirens* plants. Besides, this species 580 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.

586

587 Acknowledgments

588 We are grateful to Andrea Schubert and Dario Sacco for precious advices, and Mario589 Russo for his practical help.

591 **References**

- AKTAS, H., K. ABAK and I. CAKMAK 2006. Genotypic variation in the response of pepper to
 salinity. Sci. Hortic. 110, 260-266.
- BAÑON, S., J.A. FERNÁNDEZ, J. OCHOA and M.J. SÁNCHEZ-BLANCO 2005. Paclobutrazol as an
 aid to reduce some effects of salt stress in oleander seedlings. Europ. J. Hort. Sci. 70,
 43-49.

597 BARRACLOUGH, P.B. and J. KYTE 2001. Effect of Water Stress on Chlorophyll Meter Readings

- 598 in Winter Wheat. In: Plant Nutrition Food Security and Sustainability of Agro-
- 599 Ecosystems, Horst, W.J., M.K. Schenk, A. Burkert, N. Claassen and H. Flessa et al.
- 600 (Eds.). Kluwer Academic Publishers, Netherlands, ISBN: 978-0-7923-7105-2, pp: 722601 723.
- BECKERMAN, J and B.S. LERNER 2009. Salt damage in landscape plants. Purdue University,
 pp. 1-11.
- BOURSIER, P. and A. LÄUCHLI 1990. Growth responses and mineral nutrient relations of salt
 stressed sorghum. Crop Sci. 30, 1226-1233.
- 606 CARTER, C.T., C.M. GRIEVE, J.A. POSS and SUAREZ 2005. Production and ion uptake of
 607 *Celosia argentea* irrigated with saline wastwater. Sci. Hortic. **106**, 381-394.
- 608 CASSANITI, C., A. LI ROSI and D. ROMANO 2008. Salt tolerance of ornamental shrubs mainly
 609 used in the Mediterranean landscape. Acta Hortic 807, 675-680.
- 610 CASSANITI, C., C. LEONARDI and T.J. FLOWERS 2009. The effect of sodium chloride on
 611 ornamental shrubs, Sci. Hortic. 122, 586-593.
- 612 CASTELLI, F., R. CONTILLO and F. MICELI 1996. Non-destructive determination of leaf
 613 chlorophyll content in four crop species. 177, 275-283.
- 614 CHARTZOULAKIS, K., A. PATAKAS, G. KOFIDIS, A. BOSABALIDIS and A. NASTOU 2002. Water
- 615 stress affects leaf anatomy, gas exchange, water relations and growth of two avocado
 616 cultivars. Sci. Hortic. **95**, 39-50.
- 617 CHEN, S. and A. POLLE 2010. Salinity tolerance of *Populus*. Plant Biology **12**, 317-333.
- COSTA, E. and M. DEVECCHI 2006. Characterization of species and cultivars of genus *Buxus* for germplasm productive purposes. Italus Hortus 13, 676-678.
- 620 DE JONG, S., E.A. ADDINK, P. HOOGENBOOM and W. NIJLAND 2012. The spectral response of
- Buxus sempervirens to different types of environmental stress A laboratory
 experiment. J. Photo. rem. Sens. 74: 56-65.
- 623 DEL AMOR, F.M., V. MARTINEZ and A. CERDA 2001. Salt tolerance of tomato plants as
- affected by stage of plant development. HortScience **36**, 1260-1263.

- DEVECCHI, M., V. SCARIOT and A. SCHUBERT 2005. The use of traditional and alternative
 antifreeze salts on herbaceous and shrub species for urban decoration: experimental
 results. Adv. Hortic. Sci. 19, 86-93.
- FERGUSON, L. and S.R. GRATTAN 2005. How salinity damages citrus: osmotic effects and
 specific ion toxicities. HortTechnology 15, 95-99.
- FERRANTE, A., A. TRIVELLINI, F. MALORGIO, G. CARMASSI, P. VERNIERI and G. SERRA 2011.
 Effect of seawater aerosol on leaves of six plant species potentially useful for
- 632 ornamental purposes in coastal areas. Sci. Hortic. **128**, 332–341.
- FLOWERS, T.J. and A.R. YEO 1995. Breeding for salinity resistance in crop plants: where next?
 Austr. J. Plant Phys. 22, 875-884.
- FRANCOIS, L.E. 1982. Salt tolerance of eight ornamental tree species. J. Am. Soc. Hortic. Sci. **107**, 66-68.
- GARCIA-PLAZAOLA, J.I., A. HERNANDEZ and J.M. BECERRIL 2000. Photoprotective responses
 to winter stress in evergreen mediterranean ecosystems. Plant Biol. 2, 530-535.
- HORMAETXE, K., A. HERNANDEZ, J.M. BECERRIL and J.I. GARCIA-PLAZAOLA 2004. Role of
 red carotenoid in photoprotection during winter acclimation in *Buxus sempervirens*leaves. Plant Biol. 6, 325-332.
- 642 HORMAETXE, K., J.M. BECERRIL, A. HERNANDEZ, R. ESTEBAN and J.I. GARCIA-PLAZAOLA
- 643 2006. Plasticity of photoprotective mechanisms of *Buxus sempervirens* L. leaves in
 644 response to extreme temperatures. Plant Biol. 9: 59-68.
- IDA, K., K. MASAMOTO, T. MAOKA, Y. FUJIWARA, S. TAKEDA and E. HASEGAWA 1995. The
 leaves of the common box, *Buxus sempervirens* (Buxaceae), become red as the level of
 a red carotenoid, anhydroeschscholtzxanthin, increases. J. Plant Res. 108: 369-376.
- MAATHUIS, F.J.M. and A. AMTMANN 1999. K⁺ nutrition and Na⁺ toxicity: the basis of cellular
 K+/Na+ ratios. Ann. Bot. 84, 123-133.
- MARSCHNER, H. 1995. Mineral Nutrition of Higher Plants, 2nd ed. Academic Press, London.
 pp. 527-528.
- MORALES, M.A., E. SANCHEZ-BLANCO, E. OLMOS, A. TORRECILLAS and J. ALARCON 1998.
 Changes in the growth, leaf water relations and cell ultrastructure in *Argyranthemum coronopifolium* plants under saline conditions. J. Plant Phys. 153, 174-180.
- MUNNS, R. and A. TERMAAT 1986. Whole plant response to salinity. Austr. J. Plant Phys. 13,
 143-160.
- MUNNS, R. 2002. Comparative physiology of salt and water stress. Plant Cell. Env. 25, 239250.

- MUNNS, R. and R.A. JAMES 2003. Screening methods for salinity tolerance: a case study with
 tetraploid wheat. Plant Soil 253, 201-218.
- MUNNS, R. and M. TESTER 2008. Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. 59,
 662 651-681.
- 663 MURILLO-AMADOR, B., E. TROYO-DIÉGUEZ, J.L. GARCÍA-HERNÁNDEZ, R. LÓPEZ-AGUILAR,
- 664 N.Y. ÁVILA- SERRANO, S. ZAMORA-SALGADO, E.O. RUEDA-PUENTE and C. KAYA 2006.
- Effect of NaCl salinity in the genotypic variation of cowpea (*Vigna unguiculata*) during
 early vegetative growth. Sci. Hortic. 108, 423-441.
- 667 NAVARRO, A., S. BAÑÓN, W. CONEJERO and M.J. SÁNCHEZ-BLANCO 2008. Ornamental
- characters, ion accumulation and water status in *Arbutus unedo* seedlings irrigated with
 saline water and subsequent relief and transplanting. Env. Exp. Bot. 62, 364-370.
- 670 PÉREZ-ALFOCEA, F., M.E. BALIBREA, J.J. ALARÇON and M.C. BOLARÍN 2000. Composition of
- 671 xylem and phloem exudates in relation to the salt tolerance of domestic and wild tomato
 672 species. J. Plant Phys. 156, 367-374.
- 673 RAVINDRAN, K.C., K. VENKATESAN, V. BALAKRISHNAN, K.P. CHELLAPPAN and T.
- BALASUBRAMANIAN 2007. Restoration of saline land by halophytes for Indian soils. Soil
 Biol Biochem 39, 2661-2664.
- RODRÍGUEZ, P., A. TORRECILLAS, M.A. MORALES, M.F. ORTUÑO and M.J. SÁNCHEZ-BLANCO
 2005. Effects of NaCl salinity and water stress on growth and water relations of
- 678 *Asteriscus maritimus* plants. Env. Exp. Bot. **53**, 113-123.
- SANCHEZ-BLANCO, M.J., M.C. BOLARIN, J.J. ALARCON and A. TORRECILLAS 1991. Salinity
 effects on water relations in *Lycopersicon esculentum* and its wild salt-tolerant relative
 species *L. pennellii*. Phys. Plant. 83, 269-274.
- 682 SÁNCHEZ-BLANCO, M.J., P. RODRÍGUEZ, E. OLMOS, M. MORALES and A. TORRECILLAS 2004.
- Differences in the effects of simulated sea aerosol on water relations, salt content, and
 leaf ultrastructure of rock-rose plants. J. Env. Quality 33, 1369-1375.
- TESTER, M. and R. DAVENPORT 2003. Na⁺ tolerance and Na⁺ transport in higher plants. Ann.
 Bot. 91, 503-527.
- TOWNSEND, A.M. 1980. Response of selected tree species to sodium chloride. J. Hortic. Sci.
 105: 878-883.
- USEPA 1992. Manual: guidelines for water reuse, USEPA, Rep. 625/R-92/004. USEPA,
 Washington, DC.
- 691 VALDEZ-AGUILAR, L.A., C.M. GRIEVE, A. RAZAK-MAHAR, M. MCGIFFEN and D.J. MERHAUT
- 692 2011. Growth and ion distribution is affected by irrigation with saline water in selected

- landscape species grown in two consecutive growing-seasons: spring-summer and fallwinter. HortScience 46: 632-642.
- WATANABE, S., Y. HATANAKA and K. INADA 1980. Development of digital chlorophyll meter:
 I Structure and performance. Jap. J. Crop Sci. 49, 89-90.
- WU, L., X. GUO and A. HARIVANDI 2001. Salt tolerance and salt accumulation of landscape
 plants irrigated by sprinkler and drip irrigation systems. J. Plant Nutr. 24: 1473-1490.
- 699ZOU, W., H. FANG, Y.D. BAO and Y. HE 2011. Detection of nitrogen content changes of rape
- 700leaf using hyperspectral imaging. Adv. Mat. Res. 210, 131-134.
- 702 Received / Accepted
- 703 Addresses of authors: Matteo Caser (corresponding author), Valentina Scariot, Walter Gaino,
- 704 Federica Larcher, and M. Devecchi, Department of Agricultural, Forest and Food Sciences,
- via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy, e-mail: matteo.caser@unito.it

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





Tables

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

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

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

| 840 | 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 | |
| 841 | | | | |
| | | | | |
| 842 | | | | |
| 843 | | | | |
| 844 | | | | |
| | | | | |
| 845 | | | | |
| | | | | |

- 848 Table 3. Main effects of species, salt (NaCl) concentration, application method, and
- 849 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 | | | | | |
|------------------------------|---------------------|----------|-----------------|----------|----------|----------|
| | 1 st | 2^{nd} | 3 rd | 4^{th} | 5^{th} | 6^{th} |
| 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 |
| Р | * | 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 |
| Р | * | 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 |
| Р | 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 |

851 [§]Mean values showing the same letter are not statistically different at $P \leq 0.05$. The statistical relevance of

852 Between-Subjects Effects' Test was subjected to Tamhane Test ANOVA for non parametric data (*=P≤0.05,

853 ns=non significant).