Productivity and Quality Responses of Salt-Stressed Roses to Supplemental Calcium[1]

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Abstract

Plants of *Rosa* × spp. L. 'Happy Hour' grafted on the rootstocks *R.* × 'Manetti' and *R.* × 'Natal Briar' were salinized with 12 mM NaCl and received supplemental calcium (Ca) applications (as $CaSO_4$) of 0, 2.5, 5.0, 7.5 and 10 mM. Additional plants were salinized with 6 mM Na_2SO_4 and supplemented with 5 mM $CaSO_4$ and compared to non-salinized, no supplemental Ca control plants. Cumulative flowers harvested, shoot length and leaf chlorophyll index were similar for both rootstocks across salt treatments, but Manetti plants had higher dry weights in flowers and most plant tissues except roots. Productivity and water relations in NaCl-salinized plants were not responsive to supplemental Ca. Conversely, calcium-supplemented plants salinized with Na_2SO_4 had better productivity and quality than those with NaCl, and were similar to non-stressed control plants. Salt injury symptoms were evident only on NaCl-treated plants, regardless of Ca supplements, and closely associated with chloride, but not sodium, accumulation, in leaf tissues. The extent of the ameliorative properties of supplemental calcium applications on salinized rose plants is influenced by the salinity level, the chemical composition of the salinizing solution (major ions and counter-ions) and the cultivar (scion) and rootstock selection.

Index words: cut flowers, chloride, salinity, sodium, sulfate, rose, rootstocks.

Species used in this study: Rose (*Rosa* × spp. L., *R.* × 'Happy Hour', *R.* × 'Manetti', *R.* × 'Natal Briar').

Significance to the Horticulture Industry

Drought and competition for irrigation-quality water resources are forcing green industry activities (nursery and greenhouse production, landscape maintenance) to seriously consider the use of alternative, poorer-quality irrigation sources. Amendment of irrigation waters and nutrient solutions with supplemental calcium applications has been used to ameliorate adverse effects of salinity on several crops. We evaluated this practice in 'Happy Hour' roses grafted on two rootstocks and exposed to salt stress with NaCl. The biomass, flower yields and water status of NaCl-salinized rose plants were not responsive to supplemental calcium applications, and salt-injury symptoms were closely associated with chloride accumulation in tissues. Conversely, when salinity stress was primarily derived from Na_2SO_4 salts, the addition of a calcium supplement allowed the rose plants to sustain productivity and quality similar to non-salinized plants. Roses grafted on the rootstock Manetti performed better under salt stress than those grafted on Natal Briar. The positive and ameliorative properties of supplemental calcium applications on salinized plants is influenced by the level of salinity stress, the chemical composition of the saline water or nutrient solution, and the selection of cultivars and rootstocks (in grafted plants).

Introduction

Modern roses (*Rosa* × spp. L.) have been classified as having poor salt tolerance, with significant reductions in biomass and quality when exposed to an electrical conductivity (EC) above 2 to 3 $dS \cdot m^{-1}$ in saturated paste extracts (Bernstein et al. 1972; Hughes and Hanan 1978). These values are equivalent to 1.3 to 2.0 $dS \cdot m^{-1}$ and 4.0 to 6.0 $dS \cdot m^{-1}$ in irrigation water/nutrient solution and soil solution, respectively (Farnham et al. 1985). These thresholds were originally developed with older garden and greenhouse cultivars and rootstocks primarily grown in mineral soils. Recent studies suggest that modern rose cultivars used for greenhouse cut flower production can tolerate higher levels of salt stress, up to 1.5 to 2.0 $dS \cdot m^{-1}$ in NaCl (15 to 20 mM) above the EC provided by the typical fertigation solution EC of 1.0 to 2.0·dS m^{-1} (Cabrera and Perdomo 2003, Wahome et al. 2000, 2001), with tolerance influenced by rootstock selection (Cabrera et al. 2009, Niu and Rodriguez 2008). The greenhouse rose industry in North America relied for several decades on the rootstock *R.* × 'Manetti' and to a lesser extent on *R. indica* 'Major' (aka *R. odorata*), but these were almost completely displaced in the last two decades by the vigorous *R.* × 'Natal Briar' (Cabrera 2002). Recent research results and anecdotal grower comments suggest that despite its significant effects on flower productivity and quality, and ease of propagation, the yield and quality of rose cultivars grafted on 'Natal Briar' is significantly diminished when exposed to salt stress (NaCl) as compared to 'Manetti' (Cabrera et al. 2009).

The major constraints for plant growth under salt stress include water deficits due to low soil water potential that hinder water uptake and transport through the soil-plant-atmosphere continuum, ion toxicity associated with excessive uptake of inorganic ions, mainly chloride (Cl^-) and sodium (Na^+), and lastly, internal ion imbalances that affect the uptake, transport and internal distribution of other ions within the plant, calcium (Ca^{2+}) in particular, that eventually lead to physiological and qualitative disorders (Cassaniti et al. 2013, Grattan and Grieve 1999, Marschner 1995). Increases in exchangeable Na^+, characteristic of salinity dominated by Na salts, are balanced by decreases in exchangeable Ca^{2+}, potassium (K^+) and magnesium (Mg^{2+}), leading to deficiencies when the concentrations of these elements in solution are low (Gorham, 2007). Sodium-dominated salinity not only reduces Ca^{2+} availability, but its transport and mobility to growing regions of the plant, affecting the growth and quality of both vegetative and reproductive organs (Cramer, 2002; Grattan and Grieve, 1999). Application of gypsum ($CaSO_4$) is a common practice under saline-sodic conditions to increase

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salt tolerance by improving soil structure and aeration, and by increasing the Ca^{2+}/Na^{+} ratio, which supports the capacity of roots to restrict Na^{+} influx (Marschner 1995).

Amendment of saline solutions with Ca has been shown to ameliorate adverse effects of salinity on several agronomic and horticultural crops (Cramer, 2002). For example, in navel orange [*Citrus sinensis* (L.) Osbeck] budded on two rootstocks and subjected to 0 and 45 mM NaCl in the nutrient solution, raising the $[Ca^{2+}]$ from 3 to 30 mM mitigated the effects of salinity on plant growth, defoliation and leaf injury (Bañuls et al. 1991). In container-grown May hawthorn (*Crataegus opaca* Hook. & Arn.) 25 mM NaCl applied to the nutrient solution was more inhibitory to growth, water use, and ion uptake selectivity in the absence of additional calcium as compared to inclusion of 2 and 5 mM Ca^{2+} as $CaCl_2$ (Picchioni and Graham 2001). The supply of 10 mM of $Ca(NO_3)_2$ had optimal effects on growth and metabolism of guava seedlings (*Psidium guajava* L.) stressed with 30 and 60 mM NaCl (Ebert et al. 2002).

The main objective of this study was to determine if application of supplemental Ca to fertigation solutions ameliorates the effects of NaCl-salinity stress in greenhouse roses and if these are differentially modulated by rootstock selection. An additional treatment was added to permit preliminary assessment of rose plant response to supplemental Ca applications under two salt types (NaCl vs Na_2SO_4).

Materials and Methods

Plant culture. On January 26, a total of 84 bare-rooted 'Happy Hour' rose plants, grafted on the rootstocks 'Manetti' and 'Natal Briar' (42 plants of each), were transplanted into #4 (15 L) black plastic containers (Nursery Supplies, Inc. Kissimmee, FL) filled with a peat moss:pine bark:sand (3:1:1 v/v) substrate. The substrate was amended with 3.0 kg·m^{-3} (5 lb·yd^{-3}) dolomitic limestone (Carl Pool Products, Gladewater, TX) and 0.6 kg·m^{-3} (1 lb·yd^{-3}) each of Micromax fertilizer (The Scotts Company, Marysville, OH) and Aqua-GroG 2000 (The Scotts Company, Marysville, OH). Plants were placed on raised benches in a greenhouse, with 25/16C (77/61F) day/night set points. Containers were arranged three abreast, spaced on 30 cm (12 in) centers on benches located in the middle of the greenhouse, and surrounded by border benches with roses growing under similar conditions. The plants were initially fertigated with 15N-2.2P-12.5K water soluble fertilizer (15-5-15 Cal-Mag, The Scotts Company, Marysville, OH) adjusted to deliver 10 mM (140 ppm) of nitrogen (N). The plants were managed by conventional pruning practices to induce synchronized growth and flowering flushes (Cabrera 2002).

Salinity-supplemental calcium treatments. Starting on April 19, a modified ½ strength Hoagland formulation was used as a base fertigation solution, containing (in mM): 8.0 NO_3–N, 1.0 NH_4–N, 0.5 P (as $H_2PO_4^-$), 3.0 K^+, 2.25 Ca^{2+}, 1.0 Mg^{2+}, 1.0 S (as SO_4^{2-}), 1.0 mg·L^{-1} Fe (as Fe-EDDHA) and half-strength Hoagland's micronutrient concentrations. The solutions were prepared in tap water (pH = 7.9; EC = 0.5 dSm^{-1}) with pH was adjusted to 6.2 ± 0.03 with HNO_3 before addition of salts. Treatments (Table 1) included a non-salinized control (solution 1), a series of NaCl-salinized (12.0 mM) treatments with supplemental Ca (0, 2.5, 5, 7.5 and 10 mM as $CaSO_4$; solutions 2–6), and a Na_2SO_4-salinized treatment (6.0 mM) with 5.0 mM $CaSO_4$ (solution 7). The control treatment allowed for evaluation of the general effect of salinity, whereas the Na_2SO_4 treatment allowed comparing the influence of the Na^+ counter-anion (chloride versus sulfate) on the response to the addition of Ca^{2+} to the salinized solutions.

Solutions were pumped from 150-L (40 gal) containers with submersible pumps (Model 2E-38N, Little Giant Pump Co., Oklahoma City, OK) feeding 1.3 cm (1/2") polyethylene irrigation lines connecting 3.2 mm (1/8") spaghetti tubing to calibrated spray-stake emitters (Spot Spitter®, Roberts Irrigation Products, San Marcos, CA), one per container. Evapotranspiration (ET) was gravimetrically determined in representative treatment plants, and used to calculate fertigation volumes to apply per treatment, set at ET plus an additional target leaching fraction of 25%. Electrical conductivity (Conductivity Meter Mod. 2052, VWR International, Inc. Irving, TX), pH (AP63 Accumet®, Fisher Scientific, Pittsburgh, PA) and chloride concentrations (Chloridometer Model 4425000, Labconco Co., Kansas City, MO) were monitored on leachate samples collected from selected treatments every two weeks.

Data collection. There were a total of five harvest events during the experiment. Flower shoots were harvested at commercial maturity, recording dry weight (DW) and number (FS), average length (SL) and leaf chlorophyll index (LCI;

Table 1. Composition of the salinity and supplemental calcium treatments added to the base fertigation solution (modified 0.5× Hoagland), including their total calculated, measured and adjusted electrical conductivities.

Nutrient solution[z]	Treatments (mM)			EC (dS m^{-1})[y]		
	NaCl	Na_2SO_4	$CaSO_4$	Calculated	Measured	Adjusted
1	0.0	0.0	0.0	1.6	1.6	1.6
2	12.0	0.0	0.0	2.8	2.7	2.7
3	12.0	0.0	2.5	3.3	3.1	3.1
4	12.0	0.0	5.0	3.8	3.3	3.3
5	12.0	0.0	7.5	4.3	3.6	3.7
6	12.0	0.0	10.0	4.8	3.8	4.0
7	0.0	6.0	5.0	3.8	3.1	3.1

[z]The base fertigation solution in all treatments consisted of a modified 0.5× Hoagland formulation prepared on tap water (see Materials and Methods).

[y]Calculated EC includes the EC of the tap water, the base 0.5× Hoagland salt formulation, the stressing salts (NaCl or Na_2SO_4) and supplemental Ca^{2+} ($CaSO_4$), using the empirical equation EC = Sum of cations or anions (in meq/L) divided by 10 (10). Measured EC was determined immediately after preparing each solution. Adjusted EC was calculated using the free ion concentrations determined by a chemical speciation program (Barak 1990).

Chlorophyll Meter SPAD-502, Minolta Co. LTD, Japan) per plant. Three whole plants per treatment were destructively harvested at the end of the experiment and analyzed for nutrient content and biomass partitioning.

Relative water content (RWC), stem water potential (SWP) and leaf osmotic potential (LOP) were determined at midday (between 12:00 PM and 1:30 PM) in three plants per treatment during each harvest. Three leaflets were sampled from one flower shoot per plant and their RWC determined according to Jiang and Huang (2001). Stem water potential was measured with a pressure chamber (Model 610, PMS Instrument Co., Corvallis, OR). For leaf osmotic potential, tissue sap was extracted from the first five-leaflet leaf of a flowering shoot per plant and analyzed with a vapor pressure osmometer (Model 5520, Wescor, Inc., Logan, UT). Osmometer readings in mmol·kg^{-1} were converted to MPa using the van't Hoff relation: $\prod_s = RT\sum c_j$, where $\prod_s$ is osmotic pressure of the sap solution, and its negative equivalent is defined as osmotic potential; R is the gas constant; T is temperature in °K; and c_j is the concentration of sap solutes.

During each harvest, the three uppermost five-leaflet leaves from each flowering shoot were collected and pooled for each plant, dried and ground. Samples from harvests II (71 DAT) and IV (133 DAT) were sent to the Louisiana State University AgCenter Soil Testing and Plant Analysis Laboratory for total nutrient analyses. Phosphorous, K, Ca, Mg, S, B, Cu, Fe and Zn were extracted in nitric acid/peroxide digests (Havlin and Soltanpour, 1989) and determined by inductively coupled plasma spectrometry. Nitrogen was quantified by dry combustion with a Leco CN 628 Analyzer (LECO Corporation, St. Joseph, MI). Chloride in all harvested tissues was quantified by silver ion titration in CH_3COOH extracts with a digital chloridometer (Model 4425000, Labconco Co., Kansas City, MO), while sodium was done in HCL extracts by flame emission (Spectrometer AA240FS, Varian, Inc., Australia).

For harvests III, IV and V (99, 133 and 184 DAT), a salt injury rating evaluation was made immediately after harvest of all flowering shoots, using a scale from 0 to 5 (0 = no visible damage, 1 = 1–20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80% and 5 = 81–100% of foliage exhibiting salt damage). This evaluation was performed on the leaves remaining on the plant after harvesting the flowering shoots.

Statistical analyses. The experimental design was a randomized complete block design with a factorial arrangement of treatments, each with six replications. For variables evaluated at one point in time only, rootstock selection (RS; 'Manetti' and 'Natal Briar') and salt treatments (control, the NaCl-series and the Na_2SO_4 treatment) were the factors. To exclude seasonal influence on productivity over time, DW, FS, and FSL data from each harvest were normalized into a relative data scale and then subjected to arc sine transformation. Data from the series of NaCl-salinized treatments with supplemental Ca (solutions 2–6) were evaluated with trend analyses (regressions), with RS and Ca amendment concentration as factors. Orthogonal contrasts were performed between control treatment (solution 1) and the NaCl-salinized treatment series with supplemental Ca. Pair-wise comparisons were performed between the control treatment (solution 1) and the Na_2SO_4-salinized treatment with 5.0 mM $CaSO_4$ (solution 7) and between the NaCl and Na_2SO_4 salinized treatments, both with 5.0 mM $CaSO_4$ (solutions 4 and 7). All analyses were performed with SAS® 9.1 for Windows (SAS Institute Inc., Cary, NC). For all statistical analyses *ns*, *, **, *** denote non-significant, and significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

Results and Discussion

Nutrient solutions and leachate EC (EC_L), Cl concentration ($[Cl_L]$) and pH (pH_L). Addition of supplemental Ca to the solutions increased the EC of the salinized treatments. Compared to the expected EC values initially calculated for the treatment formulations, however, the measured EC of the freshly prepared solutions was lower when supplemented with Ca (Table 1). The chemical composition of the initial formulations was used to calculate the actual free ion concentrations using the chemical speciation program SPECIES (Barak 1990). The resulting free ion concentrations were utilized to re-calculate the EC, and these adjusted values were most similar to measured values. According to the ion speciation results, ion pairing, particularly between calcium and sulfate, was largely responsible for differences between the calculated and measured EC of the solutions with increasing Ca supplementation. Nevertheless, increases in the EC of the salt treatments were evident, as expected, with the supplemental Ca additions (Table 1). Leaching fraction was similar between RS and among all treatments ($P < 0.05$), averaging 27% throughout the whole experimental period (data not shown).

Within the NaCl-series, EC_L from 'Manetti' plants increased as the concentration of supplemental Ca in the saline solution increased; while in 'Natal Briar', EC_L was similar across Ca levels (Fig. 1A, B). Leachate $[Cl_L]$ was similar across Ca levels for 'Manetti' plants while in 'Natal Briar' it tended to decrease linearly as the levels of Ca increased (Fig. 1C, D). In both RS, pH_L showed a quadratic response across Ca levels, decrasing as Ca level increased (Fig. 1E, F).

As expected, control plants had lower EC_L and $[Cl_L]$ compared to those from the NaCl-salinized series (Fig. 1A, B, C, D). On the other hand, pH_L was greater in leachates from the control plants than in the NaCl series, on average by 0.8 and 1.1 units for 'Manetti' and 'Natal Briar', respectively ($P < 0.0001$ for both RS). The EC_L did not differ between RS for either control or Na_2SO_4 treatments. Leachate $[Cl_L]$ was the same across RS and these two fertigation treatments. Leachates from control plants had on average lower EC_L and greater pH_L values than those from the Na_2SO_4 treatment ($P < 0.0001$ for both variables). Comparing between the 12 mM NaCl and 6 mM Na_2SO_4, both at the 5 mM supplemental Ca level, the first treatment had greater EC_L and $[Cl_L]$ values across RS ($P < 0.001$; averages of 7.3 vs. 6.4 dS·m^{-1}, and 1,289 vs. 203 mg·L^{-1}, respectively). Leachate pH was greater in the Na_2SO_4 treatment only for 'Natal Briar' plants.

Flower productivity. Total cumulative flower shoots harvested per plant, average SL, and LCI were similar for both RS across salt treatments ($P < 0.05$; data not shown), so data were pooled. 'Manetti' plants had greater harvested flower DW than 'Natal Briar' ($P = 0.007$; 137 vs. 124 g, respectively).

Flower shoot DW, FS, SL and LCI were similar among the NaCl-salinized treatments regardless of the concentration of supplemental Ca in the saline solutions ($P < 0.05$ for all variables; Fig. 2A–D). Compared to the NaCl-salinized treatments, plants from the control treatment had slightly

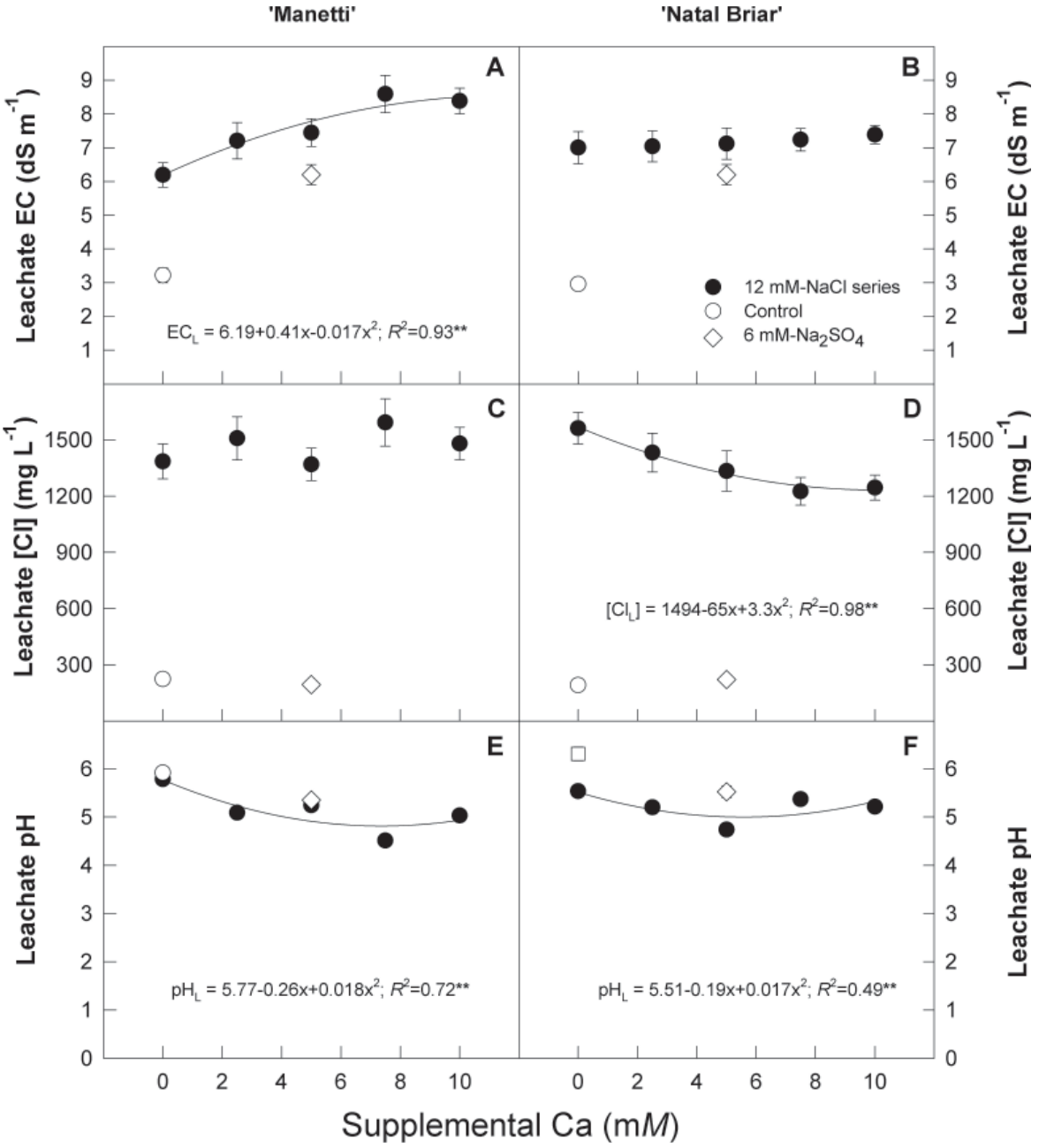

Fig. 1. Electrical conductivity (A, B), chloride concentration (C, D) and pH (E, F) in leachates collected from 'Happy Hour' roses budded on 'Manetti' or 'Natal Briar' rootstocks, and subjected to NaCl or Na_2SO_4 based salinity in a 0.5× Hoagland's solution amended with supplemental calcium. Symbols are means ± s.e. of 6 plants. No curves were drawn for data sets with non-significant regressions.

greater DW and FS ($P = 0.05$ and $P = 0.004$, respectively; Fig. 2A–B), whereas average SL and LCI were similar ($P < 0.05$; Fig. 2C–D).

Plants salinized with Na_2SO_4 had DW, FS, and LCI similar to control plants ($P < 0.05$; Fig. 2A, B and D), but longer (by 1.4 cm) flower shoots ($P = 0.006$; Fig. 2C). Sodium chloride based salinity was more detrimental to DW and FS than Na_2SO_4 ($P = 0.01$ and 0.04, respectively; Fig. 2A–B), while SL and LCI were not affected by the salt composition ($P < 0.05$; Fig. 2C–D).

Whole plant biomass partitioning. When compared with 'Natal Briar' across salt treatments, 'Manetti' plants had greater top DW (stems and leaves; $P = 0.03$, 80 vs. 69 g), lower root:shoot ratio ($P = 0.0002$; 0.5 vs. 0.6), and similar root DW (averaging 20 g). Plant organ biomass was not affected by salt source or supplemental Ca compared to the non-salinized controls ($P < 0.05$ for all organs; data not shown). Conversely, the DW of old leaves of plants subjected to NaCl salt stress was lower those with Na_2SO_4 salt stress ($P = 0.03$; 12 vs. 8 g, respectively); although the DW of their roots and stems were similar ($P < 0.05$).

Foliar salt injury. Plants exposed to NaCl showed salt injury symptoms that increased incrementally over time on the leaves of their harvested flowers, and the foliage left on

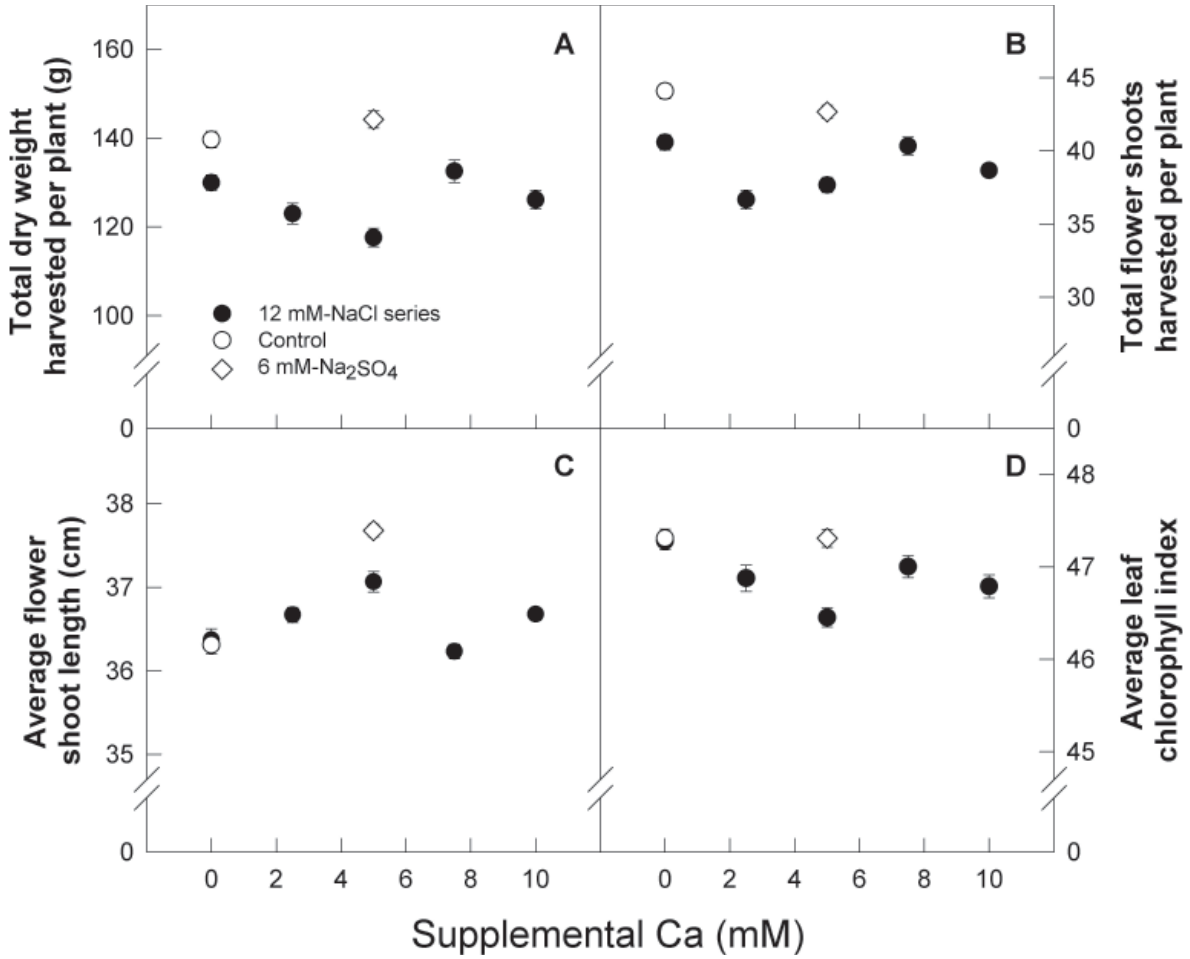

Fig. 2. Total cumulative dry weight (A) and flower shoots (B) harvested per plant; average shoot length (C) and leaf chlorophyll index (D) of 'Happy Hour' roses subjected to NaCl or Na_2SO_4 based salinity in a 0.5× Hoagland's solution amended with supplemental calcium. No significant differences were observed in rootstocks across supplemental Ca rates. Symbols are means ± s.e. of 12 plants (averaged across rootstock). No curves were drawn for data sets with non-significant regressions.

the plants, compared to plants exposed to Na_2SO_4 and the non-salinized controls (data not shown). Supplemental Ca did not influence foliar salt damage symptoms on the NaCl-salinized plants.

Water relations variables. Relative water content, SWP, and LOP were not affected by RS or by the level of supplemental Ca for the NaCl-salinized series ($P < 0.05$ for all variables; Fig. 3A–C). The non-salinized control plants had greater values for RWC, SWP, and LOP than plants from the NaCl-salinized series ($P = 0.02$, 0.005, and 0.01, respectively; Fig. 3A–C), and greater RWC and SWP than those subjected to the Na_2SO_4 ($P = 0.02$ and 0.01, respectively; Fig. 3A–B).

The three water relation variables were similar for the NaCl and the Na_2SO_4 salt treatments ($P < 0.05$; Fig. 3A–C), and SWP was affected by RS ($P = 0.009$) with 'Manetti' plants having less negative values than 'Natal Briar' (–0.73 vs. –0.84 MPa).

Tissue mineral concentrations — chloride. All the plants subjected to NaCl salt stress (across RS and Ca treatments) had similar incremental accumulations of Cl in the foliage of harvested flowers, reaching 9.4 $g \cdot kg^{-1}$ by 133 DAT; data not shown). Accumulation of Cl in leaves of harvested shoots from the control plants and those exposed to Na_2SO_4 was minimal, averaging 1.7 ± 0.1 and 2.1 ± 0.2 $g \cdot kg^{-1}$, respectively, across the experimental period. Rootstock and supplemental Ca did not affect Cl accumulation in roots, main stems, old stems and old leaves remaining on the plants salinized with NaCl, averaging 7.5, 6.4, 6.8 and 17.6 $g \cdot kg^{-1}$, respectively. These values were 24, 33, 87 and 164% greater than those observed in the control plants (Fig. 4A).

When comparing the NaCl and Na_2SO_4 salt treatments at the 5.0 mM supplemental Ca level, 'Manetti' plants had greater Cl amounts in roots (38%) and main stem (31%) than 'Natal Briar' plants ($P < 0.05$; data not shown). The Cl in roots and main stems was the same for these two salt treatments ($P < 0.05$) while in old stems and old leaves [Cl] was greater for NaCl plants by 77 and 140%, respectively (salt composition effect, $P = 0.001$ and 0.002, respectively; Fig. 4A).

Tissue mineral concentrations — sodium. Leaf [Na] in harvested flower shoots was not affected by sampling date, RS, salt source or supplemental Ca, averaging 0.08 $g \cdot kg^{-1}$ across all treatments, including control plants (Fig. 4). For the destructive harvest of whole plants, in the NaCl-series, both RS had the same [Na] in all organs evaluated except for roots,

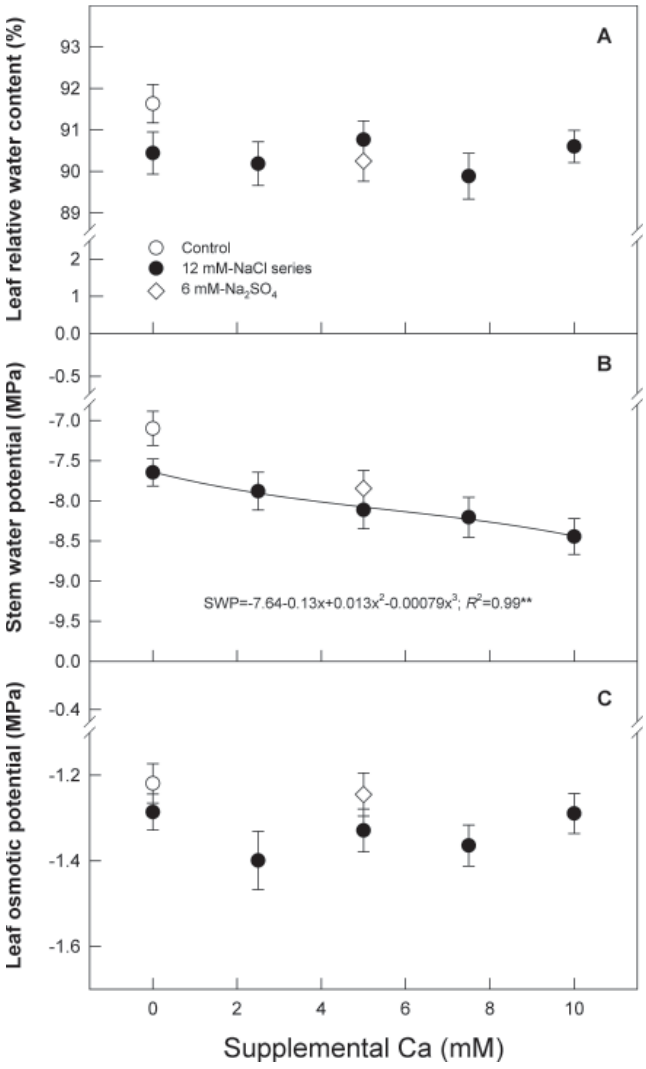

Fig. 3. Relative water content (A), stem water potential (B) and leaf osmotic potential (C) of 'Happy Hour' roses subjected to NaCl or Na_2SO_4 based salinity in a 0.5× Hoagland's solution amended with supplemental calcium. No significant differences were observed in rootstocks across supplemental Ca rates. Symbols are means ± s.e. of 12 plants (averaged across rootstock). No curves were drawn for data sets with non-significant regressions.

with 'Manetti' plants having slightly greater concentrations than in 'Natal Briar' ($P = 0.06$; 8.5 vs. 7.7 g·kg^{-1}). Sodium concentration in roots, main stems and old stems of NaCl-treated plants were 60, 29 and 117% greater, respectively, than in controls plants, but were similar in old leaves remaining on the plants (Fig. 4B). Similarly, [Na] in roots, main stems and old stems of Na_2SO_4 plants were 58, 19 and 95% greater than in the controls (Fig. 4B). The RS affected [Na] in old leaves left on the plants at the end of the experiment, with 'Natal Briar' plants having [Na] 78% higher than 'Manetti' (0.66 vs. 0.37 g·kg^{-1}, respectively).

Comparing the NaCl and Na_2SO_4 salt treatments, there were no effects due to RS selection or salt source for [Na] in main stems, old stems and old leaves ($P > 0.05$; Fig. 4 B) with overall averages of 4.6, 1.4 and 0.8 g·kg^{-1}, respectively. For roots [Na], however, there was an interaction between RS and salt treatment ($P < 0.05$). In the Na_2SO_4 treatment root [Na] was similar for both RS (8.0 g·kg^{-1}), but in the NaCl salt treatment it was greater in roots of 'Manetti' plants by 30% ($P < 0.05$; 9.1 vs. 7.0 g·kg^{-1}).

Tissue mineral concentrations — calcium. Within NaCl-plants, RS and supplemental Ca treatments did not affect the leaf [Ca] of harvested flower shoots (15.4 g·kg^{-1}), and was similar to values observed both in control and Na_2SO_4 plants (14.4 and 13.6 g·kg^{-1}, respectively) (data not shown). Of the plants with 5 mM Ca supplement, those salinized with NaCl had greater leaf [Ca] than those exposed to the Na_2SO_4 salt ($P = 0.04$; 16.0 vs. 13.6 g·kg^{-1}, respectively).

Correlations between leaf [Cl], [Na] and [Ca] and plant productivity. Simple correlations were performed between selected leaf nutrient concentrations and plant productivity variables (data not shown). Leaf [Cl], exhibited a negative association with harvested DW, FS, and SL ($P < 0.05$; $r = -0.45$, -0.47 and -0.43, respectively), whereas no relationships were found with leaf [Na]. On the other hand, leaf [Ca] were positively correlated to DW, FS, SL and LCI ($P < 0.05$; $r = 0.59$, 0.25, 0.68 and 0.42, respectively).

The measured EC of both the applied salinized nutrient solutions (2.7–4.0 dS·m^{-1}; Table 1) and the collected leachates from all treatments (Fig. 1A, B) exceeded the soil solution salinity thresholds (2–3 dS·m^{-1}) historically recommended for roses (Bernstein et al. 1972, Hughes and Hanna 1978) and other flower crops (Cassaniti et al. 2013). Recent studies, however, have shown that greenhouse roses could be more tolerant to greater levels of salinity than previously

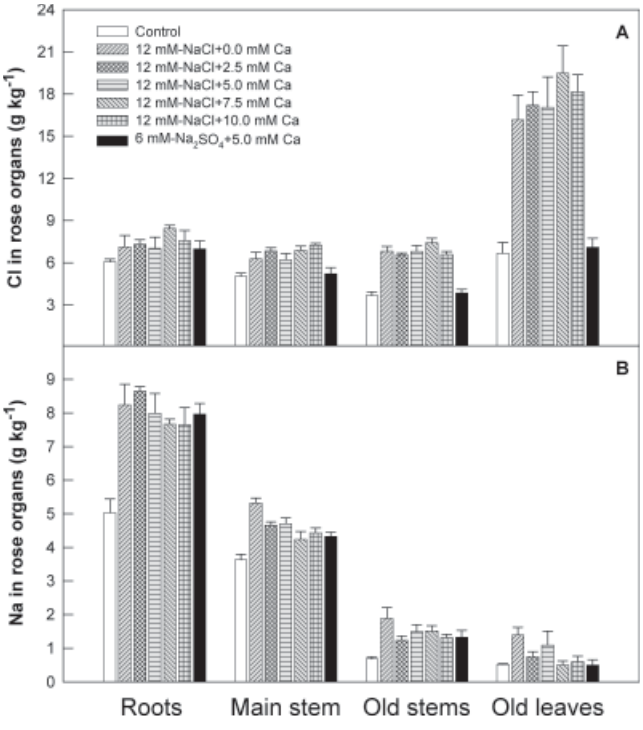

Fig. 4. Chloride (A) and sodium (B) concentrations in plant organs of 'Happy Hour' roses subjected to NaCl or Na_2SO_4 based salinity in a 0.5× Hoagland's solution amended with supplemental calcium. Data are means ± s.e. of 12 plants (averaged across rootstock).

established levels. This tolerance was attributed to substrate composition, irrigation management, cultivars, and rootstock selection (Cabrera and Perdomo 2003, Cabrera et al. 2009, Niu et al. 2008, Wahome et al. 2000). Based on results from these recent studies, we inferred that the NaCl or NaCl-$CaCl_2$ salinity tolerance limit for greenhouse roses, although influenced by rootstock selection, is between 10 and 15 mM (Cabrera and Perdomo 2003; Cabrera et al. 2009). As in previous studies, results from the present experiment also point to 'Manetti' as the rootstock selection to be used in production systems that are exposed to salt stresses resulting from use of naturally-saline irrigation waters, reclaimed water, and/or when employing recirculating or recycling drainage effluents laden with high salt contents (Cassaniti et al. 2013).

Compared to the control plants, the stress imposed by the NaCl-salt treatments caused reductions in plant productivity (Fig. 2A–B) and aesthetic quality, and negatively affected plant water relations (Fig. 3). Detrimental effects of NaCl salt treatments on dry biomass were more evident on the aerial parts of the plants (harvested shoots and foliage), while the lower plant organs (main stems and roots) were not affected to the same extent. In contrast with NaCl, Na_2SO_4 salt stress was not as detrimental to rose productivity or quality, and more comparable with the control treatment, in agreement with previous observations (Niu and Rodriguez 2008).

Supplementing saline solutions with additional Ca has been reported to alleviate the detrimental effects caused by salinity in navel orange, May hawthorn, guava, cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) (Bañuls et al. 1991, Ebert et al. 2002, Kaya et al. 2003, Picchioni and Graham 2001). Plant response to supplemental Ca, however, is modulated not only by plant genotype and salt stress level but by the chemical composition of the imposed salt stress (Cramer, 2002). In rabbiteye blueberries (*Vaccinium virgatum* Aiton) subjected to 0, 25 or 100 mM Na as NaCl or Na_2SO_4, supplemental Ca (0, 1, 3 or 10 mM as $CaSO_4$) improved shoot growth of plants exposed to Na_2SO_4, but not of those exposed to NaCl (Wright et al. 1992). In our experiment, supplementing the saline solution with Ca did not alleviate the harmful effects caused by salinization with NaCl on rose productivity, quality and water relations. Detrimental effects of Na-based salinity on plant growth can be more severe when its counterion is Cl^- rather than another anion (i.e. SO_4^{2-}, NO_3^-); there is an apparent synergistic effect between Na and Cl, with greater injury in the presence of both ions (Martin and Koebner 1995, Picchioni and Graham 2001). This contention is supported by the results from our study, where rose plants stressed with Na_2SO_4 supplemented with 5 mM Ca^{2+} had significantly better yield and quality responses compared to those exposed to an equivalent (equinormal) stress imposed by NaCl (with 5 mM Ca^{2+}), in fact, resembling the performance of the non-salinized control plants.

Some results from studies asserting the beneficial effects of supplemental Ca in salt stressed plants might have had misinterpretations. In a study with salinity and supplemental Ca in navel orange plants, the base saline solution contained 45 mM NaCl but lacked $Ca(NO_3)_2$ (Bañuls et al. 1991). Calcium sulfate and $Ca(NO_3)_2$ were added to the treatments to give final [Ca^{2+}] ranging from 3–30 mM, and NH_4^+ and NO_3^- levels were maintained constant by adding NH_4NO_3 and $(NH_4)SO_4$ to the treatments. The salinized orange plants showed pronounced increases in plant dry biomass when raising the supplemental [Ca^{2+}] from 3 and 10 mM, but were lessened at higher supplemental concentrations. As calcium was not included in their basic NaCl-salinized solutions, we contend that the dry biomass increases observed at lower supplemental [Ca^{2+}] could have been due simply to the inclusion of this essential major cation in the nutrient solution, more than to its ameliorative effects on saline stress. Higher supplemental Ca applications would have raised effectively the overall salt stress of the solutions, even with the potential for precipitation and/or ion-pair formation (mostly $CaSO_4$), as we observed in our study (Table 1). Some other salinity-supplemental calcium studies have used NO_3^- as the Ca^{2+} counter-anion (Ebert et al. 2002, Kaya et al. 2003). In these studies, adding NO_3^- resulted in reduction in Cl^- uptake and accumulation, and improved plant responses due to a NO_3^-/Cl^- antagonism (Marschner 1995). As such, the alleviating effects of supplemental calcium as $Ca(NO_3)_2$ could be attributed to either Ca^{2+} and NO_3^- separately, or to their synergistic effect(s).

Reductions in rose plant productivity and LOP were influenced by the Na^+ accompanying-anion. Exposure to NaCl-salinity resulted in more detrimental effects on flower shoot productivity, old foliage DW, and lower LOP in 'Happy Hour' roses in comparison with exposure to an equivalent Na_2SO_4 stress level (Figs. 2A, B; 3C). Also, those plants having Cl^- as the Na-accompanying ion exhibited more foliar salt injury compared to those exposed to the counter-anion SO_4^{2-}. Furthermore, as previously indicated, the plants exposed to Na_2SO_4 effectively yielded cumulative DW and harvested flower shoots similar to those in the non-salinized control plants.

The calculated, measured and adjusted EC (Table 1) for the 12 mM NaCl and 6 mM Na_2SO_4 solutions (both having 12 mM Na^+ and 5 mM supplemental Ca^{2+}), were similar between these two treatments, and therefore the total salt stress and the Na-specific effects imposed on the rose plants would have been the same. Thus, the observed differential effects between these treatments are assumed to be due to the Na-counter anions, with Cl^- being more detrimental than SO_4^{2-}, with the degree of the responses being also modulated by the rootstock selection. Similarly, Niu and Rodriguez (2008) reported differential responses of rose rootstocks to chloride- and sulfate-salinities, with *Rosa fortuniana* having greater DW reductions with Cl-dominated salinity. Conversely, DW reductions in *R.* × 'Dr. Huey', *R. multiflora*, and *R. odorata* were similarly affected by both salt sources. They observed, however, that Cl-dominated salinity led to lower visual quality of all rootstocks, especially in *R. fortuniana*. In other horticultural crops, like tomato (*Lycopersicon esculentum* Mill, Yokas et al. 2008), sweet pepper (*Capsicum annuum* L.; Navarro et al. 2002), and rabbiteye blueberries (Wright et al. 1992), SO_4-based salinity has also been reported as being less deleterious than Cl-based salinity.

In general, Cl accumulation in NaCl-treated rose plants was progressive over time and more pronounced in the foliage (old and new) than in the lower woody organs. Leaf [Na], conversely, remained low (similar to the control treatment) and stable in the harvested flower shoots, compared to its significant accumulation in lower woody organs (particularly roots and old stems). Considering that the molar ratio of applied Cl^- and Na^+ (in the nutrient solutions) was 1:1, and that by 133 DAT the average molar ratio of leaf [Cl] and [Na] in flower shoots from the NaCl-salinized plants was approximately 75:1, the greater transport to and accumulation

of chloride in the foliage is clearly evident, as observed in many other plants and crops (Marschner 1995).

Greater [Cl] in the upper parts (shoots or leaves) and/or greater [Na] in the woody organs have also been reported in NaCl-treated seedlings of red-osier dogwood (*Cornus stolonifera* Michx; Renault et al. 2001), *R.* × 'Mandelon' roses (Bass and van der Berg 1999), May hawthorn (Picchioni and Graham 2001), the rose rootstocks *R.* × 'Dr. Huey', *R. fortuniana*, *R. multiflora* and *R. odorata* (Niu and Rodriguez 2008), and *R.* × 'Bridal Pink' rose plants on the rootstock 'Manetti' (Cabrera and Perdomo 2003). Leaf Na toxicity is less widespread than Cl toxicity. Many crop species with relatively low salt tolerance are typical Na excluders and capable, at low and moderate salinity levels, of restricting Na transport into the leaves where it is highly toxic in salt sensitive species (Marschner 1995). In roses the ability to sequester Na in roots and/or restrict transport to the leaves appears to be dependent of the rootstock selection, with 'Manetti' possessing a higher degree in these abilities compared to other greenhouse rose rootstocks (Cabrera et al. 2009).

While leaf [Ca] exhibited a positive association with the response variables DW, FS, FSL and LCI, [Cl] showed a negative association with the first three, whereas [Na] did not show apparent relationships with these. Our previous experiments have also found very close negative relationships between [Cl] concentration and productivity and quality variables (Cabrera et al. 2009). This could be explained by the observation that Cl^- is highly mobile in the soil, readily absorbed via passive means in plants, and highly mobile, enabling both short- and long-distance transport (Kaya et al. 2003, Marschner 1995). Toxicity due to Cl will generally build upon the adverse effects induced by osmotic effects alone (Grattan and Grieve 1999). Based on this, the detrimental effects exhibited by NaCl-salinized rose plants are likely due to the combination of osmotic stress and specific-ion toxicity, caused chiefly by the ion Cl^- (much more than by Na^+).

Based on the results from the present study and previous reports, it is evident that the response of salt-stressed roses to supplemental Ca is influenced by several factors. These include the inherent salt tolerance of the scions (cultivars), rootstocks and their combinations, levels of [Ca^{2+}] found in the growing substrate and irrigation water, the concentration and composition of the salinizing agents, and the supplemental Ca^{2+} counter-anions (i.e. Cl^-, NO_3^-, SO_4^{2-}). Further experimentation is needed to establish sound and practical nutrient management recommendations to effectively deal with salt stress conditions in commercial greenhouse rose production.

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