CHARACTERIZING SALINITY TOLERANCE IN GREENHOUSE ROSES

A Dissertation

by

ALMA ROSA SOLÍS PÉREZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2009

Major Subject: Horticulture

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Approved by:

Co-Chairs of Committee,	Raúl I. Cabrera
	Michael A. Arnold
Committee Members,	Leonardo Lombardini
	W. Todd Watson
Head of Department,	Tim D. Davis

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ABSTRACT

Characterizing Salinity Tolerance in Greenhouse Roses. (May 2009)

Alma Rosa Solís Pérez, B.S., Universidad Autónoma Chapingo, México;

M.S., Colegio de Postgraduados en Ciencias Agrícolas, México

Co-Chairs of Advisory Committee: Dr. Raúl I. Cabrera
Dr. Michael A. Arnold

Among ornamental plants, roses (*Rosa* L.) are considered the most economically important, being among the most popular garden shrubs, as well as the favorite cut flowers sold by florists. In the past roses have been classified as fairly salt-sensitive, however, recent nutrition studies suggest that they may actually tolerate moderate to relatively high salinities. The general objective of this research was to reassess the limits of tolerance to salinity of roses and the influence of the rootstock used, to determine the ameliorative properties of supplemental Ca^{2+} on the response to salt stress, and to establish the influence of Na^{+}- and Cl^{-}-counter ions on the detrimental effects caused by these salinizing elements.

The NaCl or NaCl-$CaCl_2$-salinity tolerance limit for greenhouse roses, although greatly influenced by the rootstock, was between 12 and 15 $mmol \cdot L^{-1}$. Plants grafted on 'Manetti' sustained their productivity/quality characteristics for longer time periods, tolerated greater salinity concentrations, and accumulated less Cl^{-} and Na^{+} in leaves of flowering shoots than those grafted on 'Natal Briar', confirming the greater ability of the former rootstock to tolerate salt stress.

Supplementing the saline solution with 0-10 $mmol \cdot L^{-1}$ Ca^{2+} (as $CaSO_4$) did not alleviate the harmful effects caused by NaCl-salt stress (12 $mmol \cdot L^{-1}$) on the productivity and quality responses of roses.

The detrimental effects caused by Na- and Cl-based salinity were greatly influenced by the composition of the salt mixtures (i.e. their counter ions).

Sodium sulfate and $CaCl_2$ were the least harmful salts; NaCl had intermediate effects, while $NaNO_3$ and KCl were the most deleterious. Among the most distinguishable effects caused by the more toxic Na^+ and Cl^- counter ions were lower osmotic potential (π_{SS}) and greater electrical conductivity (EC_{SS}) of the salinized solutions, markedly increased uptake and/or transport of either Na^+ or Cl^- to the flowering shoot leaves, and altered uptake and/or transport of other mineral nutrients.

Computations of the saline solutions' chemical speciation revealed that salts containing divalent ions had lower ionization and exhibited greater ion associations compared to monovalent ion salts, rendering a lower number in free ions/molecules in solution which caused greater π_{SS} and lower EC_{SS} in those solutions.

DEDICATION

To my son, Luis Eduardo Becerra Solís.

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CHAPTER I

INTRODUCTION

Increasing demand for high quality water has encouraged use of low quality, non-potable water for landscape and agricultural irrigation (Quist and Williams, 1999). The most common water quality problem is high total dissolved solids (TDS), mainly composed of soluble salts (Reed, 1996) such as NaCl, $CaSO_4$, $MgSO_4$, and $NaHCO_3$ (Grattan, 2002). Waters with salt levels in excess of drinking water standards (1000 $mg \cdot L^{-1}$ TDS) are among the readily available resources for irrigation purposes and include saline groundwater, agricultural drainage water, industrial wastewater, and reclaimed municipal effluent with elevated salinity (Miyamoto and White, 2002).

Dissolved salts in irrigation water form ions (cations and anions). The most common cations are calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^{+}) while the most common anions are chloride (Cl^{-}), sulfate (SO_4^{2-}) and bicarbonate (HCO_3^{-}); potassium (K^{+}), carbonate (CO_3^{2-}), and nitrate (NO_3^{-}) also exist in water supplies, but concentrations of these constituents are comparatively low (Grattan, 2002). Some irrigation waters, particularly from ground water sources, contain boron at levels that may be detrimental to certain crops (Grattan, 2002).

Excessive soil salinity can result from natural processes, from crop irrigation with saline irrigation water under poor drainage conditions and from fertilizers (Handreck and Black, 2002; Neumann, 1997). Salts accumulate in the root zone by two processes: the upward movement of a shallow saline water table and salts left in the soil due to insufficient leaching (Grattan, 2002). Irrigation can contribute a substantial amount of salt to a field over the season (Grattan, 2002). For example, a water source with an electrical conductivity

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(EC) of 1.0 $dS \cdot m^{-1}$, a quality suitable for irrigation of most crops, contains nearly 1 ton of salt in every acre-foot of water applied. Under normal irrigation management, soil salinity will typically be about 1.5 times that of the water (Petersen, 1996).

All fertilizers increase the salinity of the substrate to which they are applied. The extent of increase depends on the amount of fertilizer applied, the solubility of the fertilizer, and whether or not the fertilizer interacts with the substrate so that ions are removed from solution (Handreck and Black, 2002). Many ornamentals are produced under constant liquid fertilization; therefore, the contribution of nutrient salts to the salinity of the irrigation water must be taken into account (Petersen, 1996).

There are three major constraints for plant growth on saline substrates: 1) water deficit arising from high osmotic pressures (more negative osmotic potentials) making it more difficult for plants to establish a continuous gradient of water potential between the soil solution and the atmosphere; 2) ion toxicity associated with excessive uptake mainly of Cl^- and Na^+, in part due to osmotic adjustment in order to draw water into their tissue solutions by the accumulation of inorganic ions; and, 3) nutrient imbalance caused by alterations in nutrient availability or competitive uptake, depression in uptake and/or shoot transport and impaired internal distribution of mineral nutrients such as potassium, nitrate and calcium (Gorham, 2007, Marschner, 1995). Competitive interactions between ions, due to ion concentrations within plants and soil, alters ionic balance within plants and ultimately results in nutrient element deficiencies or toxicities (Quist and Williams, 1999).

Among the most common effects of salinity is growth inhibition by Na^+ and Cl^-. For some plants, especially woody perennials, Na^+ is retained in the roots and stems and it is the Cl^- that accumulates in the shoot and is most damaging to the plant (Flowers, 1988). Sodium is not considered an essential element for most plants, but it beneficially affects the growth of some at

concentrations below the threshold of salt tolerance. At concentrations above the threshold, Na^+ can directly and indirectly affect plants to their detriment (Maas, 1990).

Salts carried in the transpirational stream are deposited in leaves as the water evaporates, and salt gradually builds up with time. Salt concentrations in older leaves are therefore much greater than in younger leaves. In older leaves, the salt concentrations eventually become high enough to kill the cells (Munns, 2002).

Mechanisms for salt tolerance are of two main types: those minimizing the entry of salt into the plant, and those minimizing the concentration of salt in the cytoplasm. Halophytes (plants adapted to saline habitats) have both types of mechanisms, while most glycophytes (plants adapted to non-saline habitats) have poor ability to exclude salt, and it concentrates to toxic levels in the transpiring leaves (Munns, 2002).

In contrast to agronomic species and crops, when establishing permissible levels of salinity for ornamentals, aesthetic characteristics of the plant are as or more important than growth or yield. Loss or injury of leaves due to salt stress is unacceptable for ornamentals, even if their growth remains unaffected (Maas, 1990).

Although salt tolerance is relatively low in most crop species and cultured woody species, genetic variability exists not only among species but also among cultivars within a species (Marschner, 1995). To date, little is known of the impacts of low quality water on ion uptake and salt tolerance of most ornamental plants (Quist and Williams, 1999). There are some classifications of salt tolerance on ornamental shrubs, trees, and groundcovers reported by Maas (1990), including around 48 ornamental species, and some studies on irrigation water quality in ornamental trees (Quist and Williams, 1999). Considering the number of ornamental species with potential use in cultivated landscapes, in most cases their salinity tolerance remains unknown**.**

Among ornamental plants, roses (*Rosa* L.) can be considered a staple ornamental crop because of their economic importance. They are among the most popular garden shrubs, as well as the most common flowers sold by florists. Thus, roses have been the subject of intensive research on several aspects of their production including physiology of management and plant breeding. Mineral nutrition of this species has received much attention due to its high production costs and more recently salinity has become an important issue, as the scarcity of high quality water is forcing the use of low quality water for irrigation.

Bernstein et al. (1972) classified roses as having very poor tolerance to salinity with a 25-50% decrease in shoot growth at electrical conductivity values in the saturation extract (EC_e) between 2 and 3 $dS \cdot m^{-1}$, and experiencing lethal effects at EC_e of 4 $dS \cdot m^{-1}$. These reference thresholds to salinity conditions for roses are still in use today despite the fact that they were developed under crop management conditions that are being phased out and with cultivars that are now obsolete (Cabrera and Perdomo, 2003).

Recent information from nutrition studies suggest that soilless-grown roses may actually tolerate relatively greater salinities resulting from more intensive production practices such as greater fertility rates and drainage recycling, without significantly, or minimally, affecting flower yield and quality.

Baas and van den Berg (1999), for example, reported decreases in yield ($stems \cdot m^{-2}$) of only 2% per $dS \cdot m^{-1}$ of increase in electrical conductivity (EC) of the recycled solution (range in EC of 2-4.8 $dS \cdot m^{-1}$) with an accumulation of Na^+ of 6 or 12 $mmol \cdot L^{-1}$ in the recirculation tank. Wahome et al. (2000, 2001) studied the effect of salinity on two rootstocks finding differences in tolerance between them, but both exhibited salt damage at 20 and 30 $mmol \cdot L^{-1}$ NaCl in the irrigation water. Similarly, Lorenzo et al. (2000) reported a gradual reduction both in shoot length and elongation rate at 20 or 30 $mmol \cdot L^{-1}$ Na^+. Cabrera and Perdomo (2003) evaluated the effects of 0, 5, 10, 15 and 30 $mmol \cdot L^{-1}$ of NaCl, finding

visual symptoms of salt injury most severely on the oldest foliage of plants receiving concentration of 30 $mmol \cdot L^{-1}$ but not on the foliage of harvested shoots. This response was affected by the rootstock selection (Cabrera and Perdomo, 2003).

The objectives of studies herein were to reassess the limits of tolerance to salinity of roses grown in soilless substrates and the influence of the rootstock used; to determine the ameliorative properties of supplemental Ca^{2+} on the response to salt stress, and to establish the influence of Na^{+}- and Cl^{-}-counter ions on the detrimental effects caused by these salinizing elements.

CHAPTER II

DETERMINATION OF SALINITY TOLERANCE LIMITS OF ROSE (*ROSA* L. 'RED FRANCE') AND THE INFLUENCE OF THE ROOTSTOCK

INTRODUCTION

Scarcity and decreasing quality in many parts of the world are making water one of the main limiting factors in agricultural production (Raviv and Blom, 2001). Ground and surface water depletion is forcing agricultural production to use irrigation water of increasingly poor quality (Reed, 1996). Among the readily available resources for irrigation are saline ground water, agricultural drainage water, industrial wastewater, and reclaimed municipal effluent with elevated salinity (Miyamoto and White, 2002).

Soluble salts that occur in soils consist mostly of various proportions of the ions Cl^-, SO_4^{2-}, HCO_3^-, Na^+, Ca^{2+}, Mg^{2+}, and, rarely, NO_3^- or K^+ (Bernstein, 1975; Richards, 1954). These ions may be indigenous, but are more commonly brought into an area in irrigation water or in waters draining from adjacent areas (Bernstein, 1975).

Water very high in salinity has a ratio of $Na^+/(Na^+ + Ca^{2+})$ near one indicating that Na^+ is the major salinizing cation; however, the bulk of the water used to irrigate most horticultural crops with low [electrical conductivity (EC)=0.1 $dS \cdot m^{-1}$] to intermediate salinity (EC=2.0 $dS \cdot m^{-1}$) have ratios between 0.1-0.7, indicating Ca^{2+} is a major contributor to salinizing the media (Grattan and Grieve, 1999).

Surprisingly, a large percentage of salinity studies on horticultural or agronomic crops use NaCl as the sole salinizing agent, limiting the extent to which the results can be interpreted (Gattan and Grieve, 1999). Results obtained from NaCl salinization may lead to misleading and erroneous

interpretations about plant responses caused by salinity since they are ignoring the fundamental distinction between saline and sodic conditions (Maas and Grieve, 1987). By definition saline soils contain soluble salts in quantities that adversely affect plant growth but which have a sodium-adsorption-ratio (SAR) of less than 15, while sodic soils have a SAR greater than 15 and contain sufficient exchangeable sodium to interfere with plant growth. Clearly, the addition of a large quantity of NaCl to base nutrient solutions produces a highly sodic medium (Maas and Grieve, 1987).

Roses are an economically important ornamental plant that is cultivated in greenhouses and nurseries under intensive irrigation and fertilization management (Cabrera, 2003b). Bernstein et al. (1972) classified roses as having very poor tolerance to salinity, with a 25-50% decrease in shoot growth at electrical conductivity values in the saturation extract (EC_e) between 2 and 3 $dS \cdot m^{-1}$, and experiencing lethal effects at EC_e of 4 $dS \cdot m^{-1}$. Baas and van den Berg (1999), however, reported decreases in yield (flowering $shoots \cdot m^{-2}$) of only 2% per $dS \cdot m^{-1}$ of increase in EC of the recycled solution (range in EC of 2-4.8 $dS \cdot m^{-1}$) with an accumulation of Na^+ of 6 or 12 $mmol \cdot L^{-1}$ in the recirculation tank. Wahome et al. (2000, 2001) studied the effect of salinity on two rootstocks, finding differences in tolerance between them, but both exhibiting salt damage at 20 and 30 $mmol \cdot L^{-1}$ NaCl in the irrigation water. Similarly, Lorenzo et al. (2000) reported a gradual reduction both in rose shoot length and elongation rate at 20 or 30 $mmol \cdot L^{-1}$ Na. Cabrera (2001; 2003a) evaluated the effects of 0, 5, 10, 15 and 30 $mmol \cdot L^{-1}$ NaCl, finding visual symptoms of salt injury most severely on the oldest foliage of plants receiving 30 $mmol \cdot L^{-1}$, but not on the foliage of harvested flowering shoots. This response may have been affected by the rootstock selection (Cabrera, 2003a).

According to some of these recent studies, roses might be more salt tolerant than previously thought. However, most of them involved artificial salinization of nutrient solutions or sand cultures using a single salt, NaCl.

Results obtained from NaCl salinization with concurrent increases in Na^+/Ca^{2+} ratios and in Cl^- not only cause problems in interpretation but also are irrelevant in an ecological sense (Greenway and Munns, 1980).

Rosa L. 'Manetti' and *R. indica* 'Major', the oldest clonal rootstocks known, were selected in the nineteenth century and became very popular in southern Europe in a short time (De Vries, 2003a,b). Both clones are still being used for garden roses or greenhouse cut roses in the Mediterranean area and in the USA (De Vries, 2003c). In the late twentieth century several newly selected clonal rootstocks for greenhouse were introduced, but despite their favorable characteristics, they were replaced by *Rosa* L. 'Natal Briar' which has almost completely dominated the western European, North American and South American cut-rose industry from about 1990 onwards. (De Vries, 2003a,c). Originating in South Africa, 'Natal Briar' did not result from a breeding program, neither was it systematically selected from a population, but is just a genotype that became highly successful once it was accidentally tried out as a rootstock, and now it has practically displaced most other clonal rootstocks (De Vries, 2003b).

The main objective of this experiment was to identify the salinity tolerance limits of *Rosa* L. 'Red France', budded on two rootstocks differing in their salinity tolerances, to increasing salinity concentrations of mixed NaCl-$CaCl_2$ and the extent to which they would sustain flower yield and quality.

MATERIALS AND METHODS

Plant culture and management

On 16 January 2004 XX-grade bare-rooted 'Red France' rose plants (*Rosa* L. 'Red France'), budded on two rootstocks, *Rosa* L. 'Manetti' and *Rosa* L. 'Natal Briar' (42 per rootstock) were transplanted into 20 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 previously amended with 3.0 $kg \cdot m^{-3}$ of dolomitic limestone (Carl Pool Products, Gladewater, TX) and 0.6 $kg \cdot m^{-3}$ of each Micromax Micronutrients fertilizer (The Scotts Company, Marysville, OH) and Aqua-GroG 2000 (The Scotts Company, Marysville, OH). Plants were spaced at 30 cm between centers, with three plants abreast on gravel beds covered with weed-barrier fabric. The experiment was conducted at the Texas A&M University Research and Extension Center, Dallas, TX, in a 6 m x 12 m glass-covered greenhouse fitted with an evaporative wet-pad cooling system and heat provided by thermostatically-controlled gas burners. Greenhouse temperatures were set at 25°C day and 16°C night. Temperature, humidity and photosynthetically active radiation were monitored with sensors connected to a Campbell CR510 Datalogger (Campbell Scientific Inc., Logan, UT).

On 12 February 2004 the plants irrigation began with a nutrient solution containing 15-5-15 Cal-Mag (The Scotts Company, Marysville, OH) and adjusted to deliver 150 $mg \cdot L^{-1}$ of nitrogen until 16 March 2004, when a hard pinch (removal of the terminal portion of a soft shoot, including two to four leaves; Langhans, 1987a) was imposed. Plants were then grown for an entire flowering flush cycle and all flowering shoots were harvested on 15 April 2004. During the length of the experiment plants were managed following conventional pruning practices (Langhans, 1987b), inducing synchronized flushes of growth and flowering.

On 23 April 2004 the salinity treatments consisting of a base nutrient solution plus salt mixtures were implemented. A modified ½ strength Hoagland solution (Hoagland and Arnon, 1950) was used as a base solution supplemented with six NaCl-$CaCl_2$ salt mixtures (2:1 $mmol \cdot L^{-1}$ ratio; Table 2.1). The base solution contained (in $mmol \cdot L^{-1}$): 9.5 N (5.3:1 NO_3^-:NH_4^+ ratio), 0.5 P (as $H_2PO_4^-$), 3.0 K, 2.0 Ca, 1.0 Mg, 1.0 S (as SO_4^{2-}), 1.0 mg $Fe \cdot L^{-1}$ as Fe-

EDDHA and half-strength Hoagland's micronutrient concentration. Before adding any salts (base nutrient solution and salt treatments) pH of the tap water was adjusted to 6.82±0.06 using 6.0 M H_2SO_4.

Table 2.1. Saline solution treatments added to a base nutrient solution (modified half strength Hoagland solution).

Solution	[NaCl] ($mmol \cdot L^{-1}$)	[$CaCl_2$] ($mmol \cdot L^{-1}$)	[NaCl-$CaCl_2$] ($mmol \cdot L^{-1}$)	Calculated EC*
1	0.0	0.0	0.0	1.05
2	1.0	0.5	1.5	1.25
3	2.0	1.0	3.0	1.45
4	4.0	2.0	6.0	1.85
5	8.0	4.0	12.0	2.65
6	16.0	8.0	24.0	4.25

* Sum of cations or anions (in $meq \cdot L^{-1}$) divided by 10. This calculation includes the EC of the base solution, but does not include the EC provided by the tap water (average of 0.6 $dS \cdot m^{-1}$).

Solutions were pumped from 150-L containers with submersible pumps (Model 2E-38N, Little Giant Pump Co., Oklahoma City, OK) feeding 1.3 cm polyethylene irrigation lines that supported spray–stake Spot Spitter® emitters (Roberts Irrigation Products, San Marcos, CA), connected via 3.2 mm spaghetti tubing. Each plant container was fitted with one calibrated emitter. Representative plants from selected treatments were routinely weighed to gravimetrically determine the evapotranspiration rate (ET). Total base irrigation volume consisted of ET plus an additional leaching target fraction of 25%.

Electrical conductivity (Portable Conductivity Meter, Model 2052, VWR International, Inc. Irving, TX), pH (pH/mV/Ion meter AP63 Accumet® portable; Fisher Scientific, Pittsburgh, PA) and Cl concentrations (Digital Chloridometer

Model 4425000, Labconco Co., Kansas City, MO) were monitored on three leachate samples collected from selected treatments on a bi-weekly basis. Chloride concentrations were determined according to Adriano and Doner (1982).

Data collection

Plant productivity and flowering shoots quality

There were a total of six harvest events during the experiment. Flowering shoot dry weight (DW), number (FS) and length (FSL), and leaf chlorophyll index (LCI; measured as the optical density, SPAD reading; portable SPAD chlorophyll meter, SPAD-502, Minolta Co. LTD, Japan) were recorded per plant. Harvested flowering shoots were put into paper bags and oven-dried at 70^{o}C. At the end of the experiment, immediately after the sixth harvest of flowering shoots, three whole plants per treatment were destructively harvested and analyzed for biomass partitioning and nutrient content. Plants were cut into three portions: roots (all roots and the rootstock stem portion below the graft union), old stems and old leaves.

Flowering shoots DW and FS per plant from each harvest were added to get the total DW and total FS harvested per plant. For LCI and FSL the average per harvest and general average from the six harvests were used in the data analysis.

Water relations measurements

Leaf relative water content (RWC) and stem water potential (SWP) were determined on four selected plants from each treatment during each harvest event (except at 192 DAT). For RWC three leaflets from one flowering shoot per

plant were cut and weighed to determine their fresh weight (FW); soaked in deionized water in petri dishes and refrigerated for a 24 h period to determine their turgid weight (TW); and, oven-dried at 70^{o}C for 48 h or until they recorded a stable weight to determine their dry weight (DW). Relative water content was determined by the formula RWC=[(FW-DW)/(TW-DW)*100] (Jiang and Huang, 2001). Stem water potential was measured prior to the harvest events at 101, 144 and 265 DAT using a pressure chamber (Model 610, PMS Instrument Co., Corvallis, Oregon, USA). Sampling time for these water relations variables was midday (between 12:00 PM and 1:30 PM) when the evaporative demand was at its peak.

Tissue analyses

During each harvest the three uppermost five-leaflet leaves from each flowering shoot were collected and pooled for each plant, dried and ground (to pass a 40-mesh screen). Samples from harvests II (67 DAT) and V (192 DAT) were sent to the Louisiana State University AgCenter Soil Testing and Plant Analysis Laboratory to be analyzed for total nutrient concentration. Phosphorous, K, Ca, Mg, S, B, Cu, Fe, Zn, and Na were determined by ICP procedures, while N was measured with a Leco N analyzer. Analysis of Cl in leaves of flowering shoots from all harvests (except III), and Cl and Na in all the organs (roots, old stems and old leaves) after destructively harvesting the plants at the end of the experiment were done locally. Chloride in tissue was determined according to Gilliam (1971) with a digital chloridometer (Model 4425000, Labconco Co., Kansas City, MO) while Na was determined by flame emission with a Fast Sequential Atomic Absorption Spectrometer (AA240FS, Varian, Inc. Australia).

Salt burn damage

A salt burn rating evaluation was taken between harvests III and IV (101 and 144 DAT) by two different evaluators 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 burn damage). This evaluation was performed on the leaves remaining on the plant after harvesting the flowering shoots.

Experimental design and statistical analyses

The experimental design was a randomized complete block design (RCBD) with a factorial arrangement of treatments. For variables evaluated at one point in time only, rootstock selection (RS) and salt mixture concentration level (SC) were the factors, with two levels for rootstock ('Manetti' and 'Natal Briar') and six levels for the NaCl-$CaCl_2$ salt mixture (Table 2.1), yielding 12 different combinations with seven replications (one container with one plant was used as a replication) per combination for a total of 84 experimental units. For variables analyzed at different points in time (i.e. during the harvests of flowering shoots), in addition to RS and SC, harvest (as days after treatment, DAT) was included as a third factor (repeated measures over time), with six levels (31, 67, 101, 144, 192 and 265 DAT).

Physiology and productivity of roses are highly influenced by the season of the year and differences in yield may be caused by different environmental factors (Maas, 1990; Mastalerz, 1987). Due to these circumstances variance heterogeneity that is independent of treatment effects is introduced. To overcome this problem, yields can be expressed on a relative basis (Mass, 1990). Hence, for some of the variables evaluated at harvest events over time (i.e. DW, FS, and FSL) data were first converted to a comparable scale (relative data) and then subjected to an arc sine transformation (Gomez and Gomez,

1984) to allow for a distinction of true treatment effects.

Quantitative data were analyzed by GLM, regression, correlation and mixed procedures while qualitative, categorical data were analyzed by chi-square procedures. For all procedures performed the Statistical Analysis System (SAS ® 9.1 for Windows, SAS Institute Inc., Cary, NC) was used.

RESULTS

Total irrigation volume, Cl, Na and Ca applied; leachate pH (pH_L), EC (EC_L) and Cl concentration ($[Cl_L]$)

The same volume of solution was applied to both rootstocks at each irrigation event (results not shown). It was only for the different salt levels that the volume of solution applied per irrigation event was different (estimation based on ET plus a target leaching fraction of 25%). Total irrigation volumes applied per treatment (sum of partial volumes throughout the 38-week experimental period) decreased as the concentration of the NaCl-$CaCl_2$ salt mixture in the nutrient solution increased (Fig. 2.1A). Leaching fractions were similar between RS and among SC averaging 25.0±2.7% and 25.1±2.4% for 'Manetti' and 'Natal Briar' plants, respectively. Conversely, the total masses of Cl^-, Na^+ and Ca^{2+} applied throughout the whole experiment increased as the SC increased (Fig. 2.1A).

There were no differences between RS for EC_L and $[Cl_L]$ (P>0.05 for both variables), thus data were pooled accordingly. Leachate EC and $[Cl_L]$ were greatly correlated (r=0.96, P<.0001) and both were positively affected by increasing SC (Fig. 2.1B). Leachate EC and $[Cl_L]$ throughout the experiment averaged 3.0±0.29, 3.6±0.24, 6.4±0.28 and 7.2±0.48 $dS \cdot m^{-1}$ and 177±35, 463±47, 1473±65 and 1960±150 $mg \cdot L^{-1}$ for the 0, 3, 12 and 24 $mmol \cdot L^{-1}$ SC, respectively.

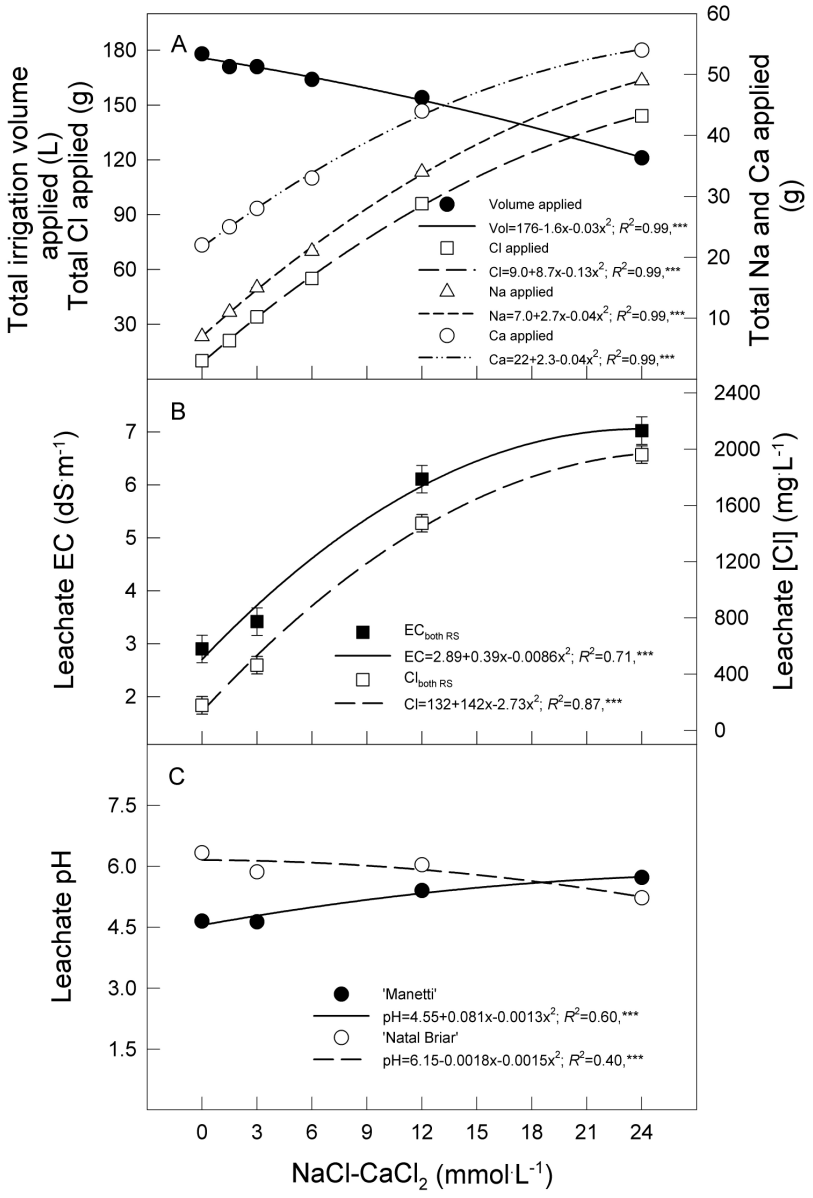

Fig. 2.1. [A] Total irrigation volume applied, total chloride (Cl), sodium (Na) and calcium (Ca) applied in the irrigation water; [B] electrical conductivity (EC) and Cl concentration ([Cl]); and, [C] pH in leachates from 'Red France' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. Symbols represent the mean ± standard error of 14 plants for plots A and C and of 28 plants for plot B. Symbols obscure the error bars that are not apparent. Significance according to general linear models (GLM) procedure: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Leachate pH decreased over time in both RS ranging from 5.6 and 6.2 at the beginning to 4.7 and 5.5 at the end of the experiment for 'Manetti' and 'Natal Briar' plants, respectively. Salt mixture concentration level and RS had interactive effects on pH_L (P<0.0001). For the low SC, pH_L was greater in leachates from 'Natal Briar' than those from 'Manetti' plants (6.3 and 5.9 in 'Natal Briar' and 4.6 and 4.6 in 'Manetti' for 0 and 3 $mmol \cdot L^{-1}$, respectively (Fig. 2.1C). However, in 'Natal Briar' pH_L tended to decrease as SC increased while for 'Manetti' plants pH_L increased as SC increased, resulting in slightly greater pH_L values for 'Manetti' plants at the 24 $mmol \cdot L^{-1}$ SC (Fig 2.1C).

Biomass and flower productivity

Similarly for both RS, both DW and FS harvested per plant at each harvest event decreased over time for all of the SC. However, their degree of reduction was different among SC (Fig. 2.2A and Fig. 2.2B), indicating interactive effects between SC and DAT were present for both variables (P=0.0003 and P<0.0001 for DW and FS, respectively). Plants subjected to 0 to 6 $mmol \cdot L^{-1}$ NaCl-$CaCl_2$ had, in general, similar reductions on DW and FS over time, while those subjected to 12 and 24 $mmol \cdot L^{-1}$ exhibited significantly greater decreases in both variables by the third harvest (101 DAT) for 24.0 $mmol \cdot L^{-1}$ and the fourth harvest (144 DAT) for 12.0 $mmol \cdot L^{-1}$ (Fig. 2.2A and Fig. 2.2B). For FSL and LCI only data from harvests I to V (31, 67, 101, 144 and 192 DAT) and from SC from 0.0 to 12.0 $mmol \cdot L^{-1}$ NaCl-$CaCl_2$ were used in the repeated measures statistical analysis since for the greatest SC (24 $mmol \cdot L^{-1}$) complete sets of data were available only for the first three harvests at 31, 67 and 101 DAT, and for the last harvest at 265 DAT several plants subjected to the two greatest SC (12 and 24 $mmol \cdot L^{-1}$) did not bear any flowering shoots. There were no interactions present between RS and DAT or between SC and DAT on both FSL and LCI (P>0.05 for all cases).

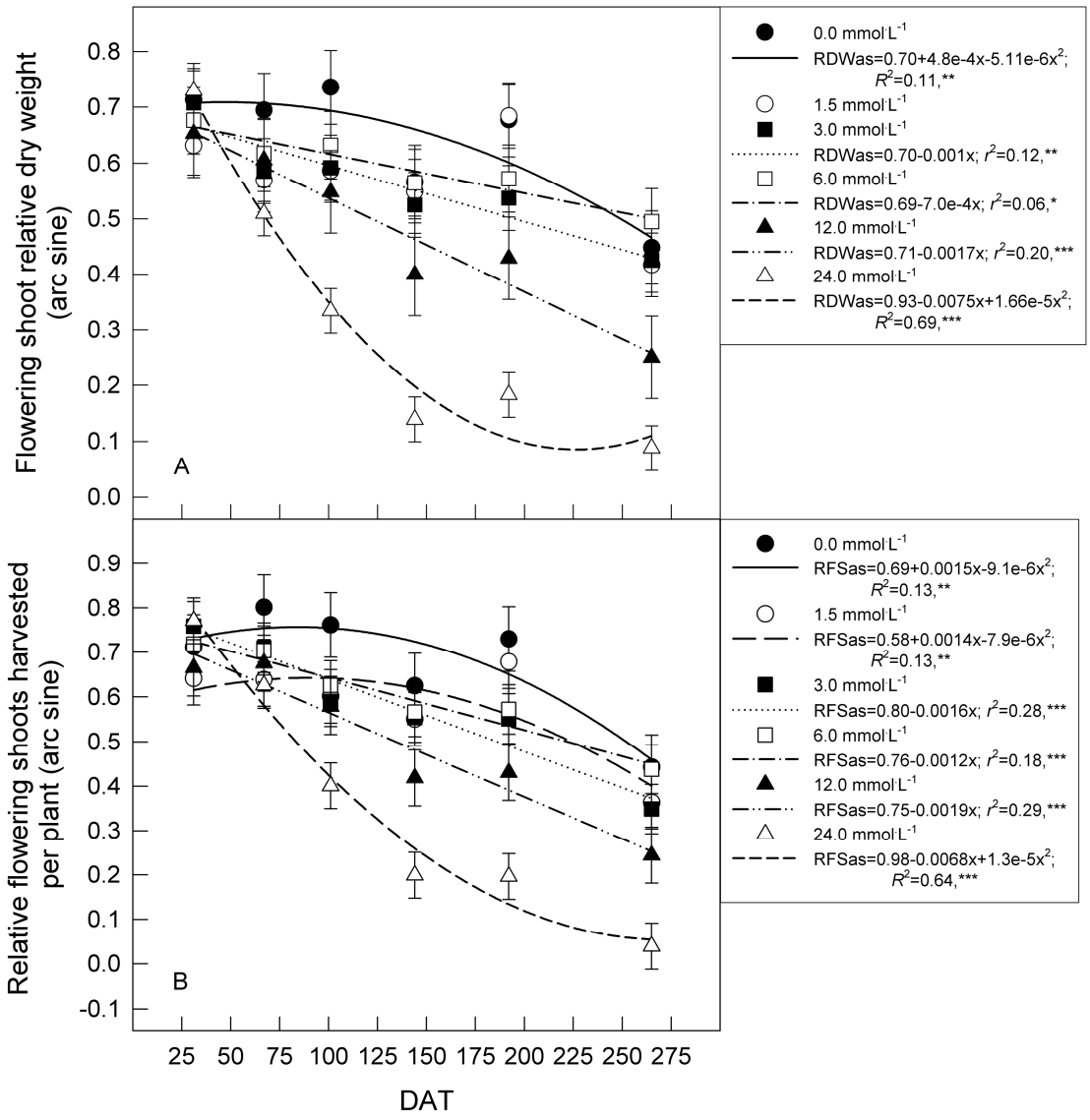

Fig. 2.2. (A) Flowering shoot relative dry weight and (B) relative number of flowering shoots harvested per plant, over six harvest events (at 31, 67, 101, 144, 192 and 265 DAT) in 'Red France' roses subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. Data and equations presented based on arc sine transformed data. Symbols represent the mean ± standard error of 14 plants. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Table 2.2. Total cumulative flowering shoot dry weight (DW) and total flowering shoots (FS) harvested per plant; total average flowering shoot length (FSL) and leaf chlorophyll index (LCI); dry weights of roots, old stems, old leaves, top part and whole plant; and root:shoot (R:S) ratio from the destructive harvest of 'Red France' roses budded on 'Manetti' and 'Natal Briar' rootstocks, and subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. For variables evaluated during regular harvests of flowering shoots, the means per rootstock selection and per salt concentration level are the average of 42 and 14 plants, respectively. For variables evaluated during the destructive harvest of whole plants means per rootstock selection and per salt concentration level are the average of 18 and 6 plants, respectively.

Rootstock		Variables evaluated during the harvest of flowering shoots throughout the experimental period				Variables evaluated during the destructive harvest of whole plants at end of experimental period					
						Dry weight (g)					
		DW (g)	FS	FSL (cm)	LCI	Roots	Old stems	Old leaves	Top part	Whole plant	R:S ratio
'Manetti'		141	39	38	51	32	46	8.81	55	87	0.61
'Natal Briar'		119	35	37	51	23	32	5.77	37	61	0.65
Difference		*22*	*4*	*1.0*	*0.0*	*9*	*14*	*3.04*	*18*	*26*	*-0.04*
Significance [z]		**	*	**	*ns*	**	**	**	***	**	*ns*
NaCl-$CaCl_2$ ($mmol \cdot L^{-1}$)	EC[y] ($dS \cdot m^{-1}$)	TDW (g)	TFS	SL (cm)	LCI	Roots	Old stems	Old leaves	Top part	Whole plant	R:S ratio
0.0	1.65	156	44	38	52	29	41	8.1	49	78	0.62
1.5	1.85	141	39	38	51	28	39	9.0	48	76	0.58
3.0	2.05	139	39	37	51	33	50	11.9	62	95	0.52
6.0	2.45	145	40	38	52	30	37	7.8	45	75	0.68
12.0	3.25	117	34	36	51	29	38	7.0	45	74	0.68
24.0	4.85	82	25	37	49	19	27	0.00	27	47	0.71
Significance		***	***	*	***	*	*	***	**	**	*
Regression[x]		L	L	Q	Q	Q	L	Q	L	L	L

[z] Significance according to GLM: ns, *, **, *** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively.
[y] EC=estimated EC (sum of cations or anions in $meq \cdot L^{-1}$ divided by 10) + EC from tap water used to make nutrient solutions (0.6 $dS \cdot m^{-1}$).
[x] Regression: L=linear, Q=quadratic.

Total flowering shoot DW and total FS harvested per plant (sum of all harvests), and total average FSL were affected by the RS, with 'Manetti' plants having greater values for all variables. Leaf chlorophyll index was the same for both RS (Table 2.2).

Increasing SC in the nutrient solution had a detrimental effect on total flowering shoot DW and total FS harvested per plant, and on the total average FSL and LCI (Table 2.2; Fig. 2.3A and Fig. 2.3B).

Dry weights of all plant tissues (roots, old stems, old leaves, top part and whole plant) were affected by RS, with greater values observed in all variables for 'Manetti' plants (Table 2.2). The root:shoot ratio was similar in both RS (Table 2.2).

Increasing concentrations of NaCl-$CaCl_2$ affected negatively the dry weights in all the organs (Table 2.2; Fig. 2.4A and 2.4B). The root:shoot ratio increased slightly as the SC increased (Table 2.2; Fig. 2.4B).

Foliar salt injury

For the salt injury five categories were defined based on the percentage of foliage exhibiting salt burn damage: 0=no visible damage, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80% and 5=81-100%. Since the ratings received by the plants were based on a categorical scale, data were analyzed by the Chi-square test. However, for the Chi-square test to be valid, at least 80% of the expected cell values should be greater than five and all should be greater than 1 (Daya, 2001; Elliot and Woodward, 2007). With our data analyzed as a 2 x 6 factorial arrangement of treatments we had a total of seven repetitions per treatment, a sample size too small to be divided into 5 categories. We analyzed the data with rootstocks as the only factor (salt levels pooled), but still our data did not comply entirely with the expected cell number rule, therefore we proceeded to combine adjacent columns (0 and 1, 4 and 5) with low expected numbers to increase the

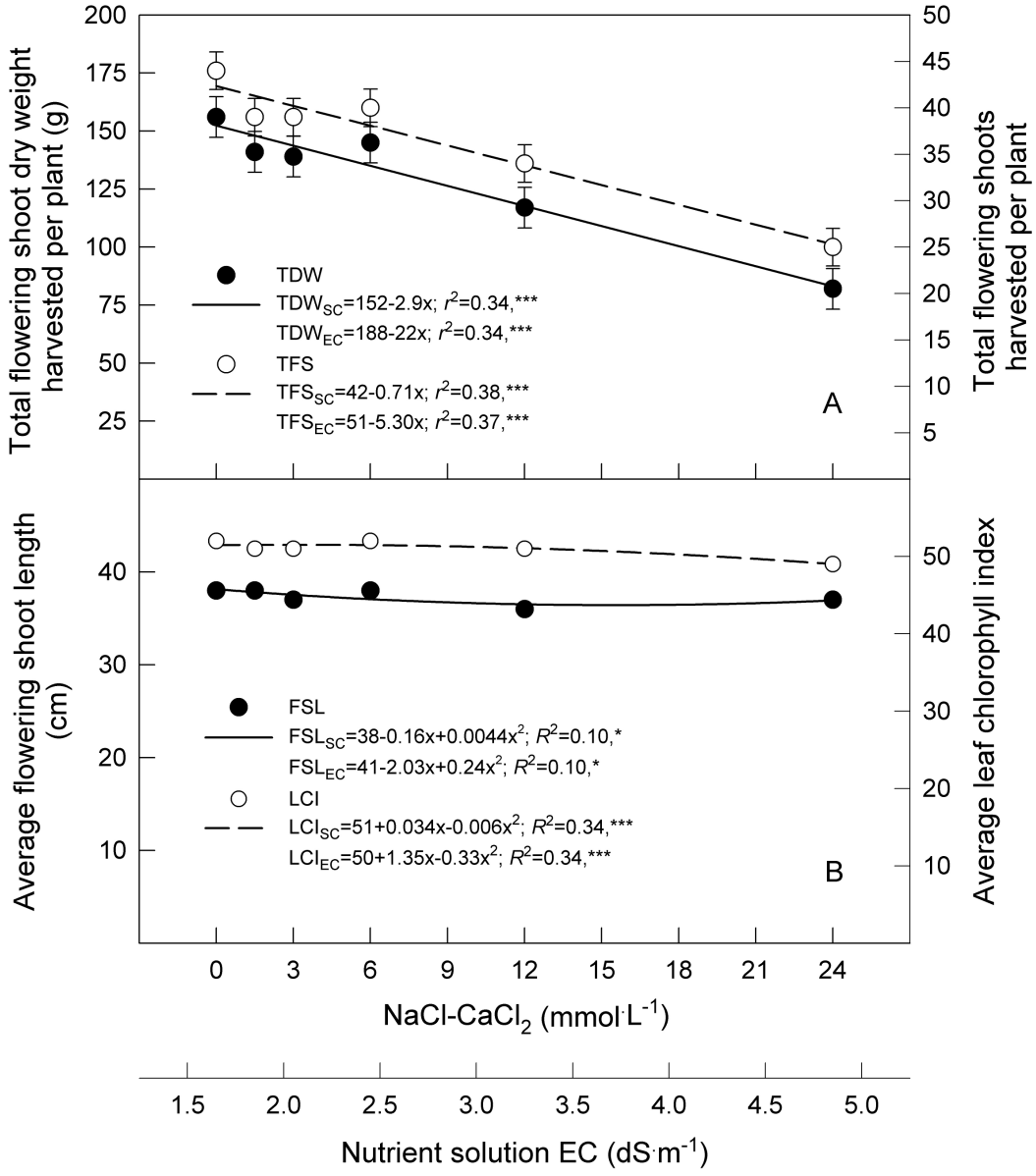

Fig. 2.3. (A) Total cumulative flowering shoot dry weight and flowering shoots harvested per plant; (B) total average flowering shoot length and average leaf chlorophyll index of 'Red France' roses subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. Symbols represent the mean ± standard error of 14 plants. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Data are averages and cumulative sums for the entire experimental period (38 weeks). SC=salt concentration; EC=electrical conductivity.

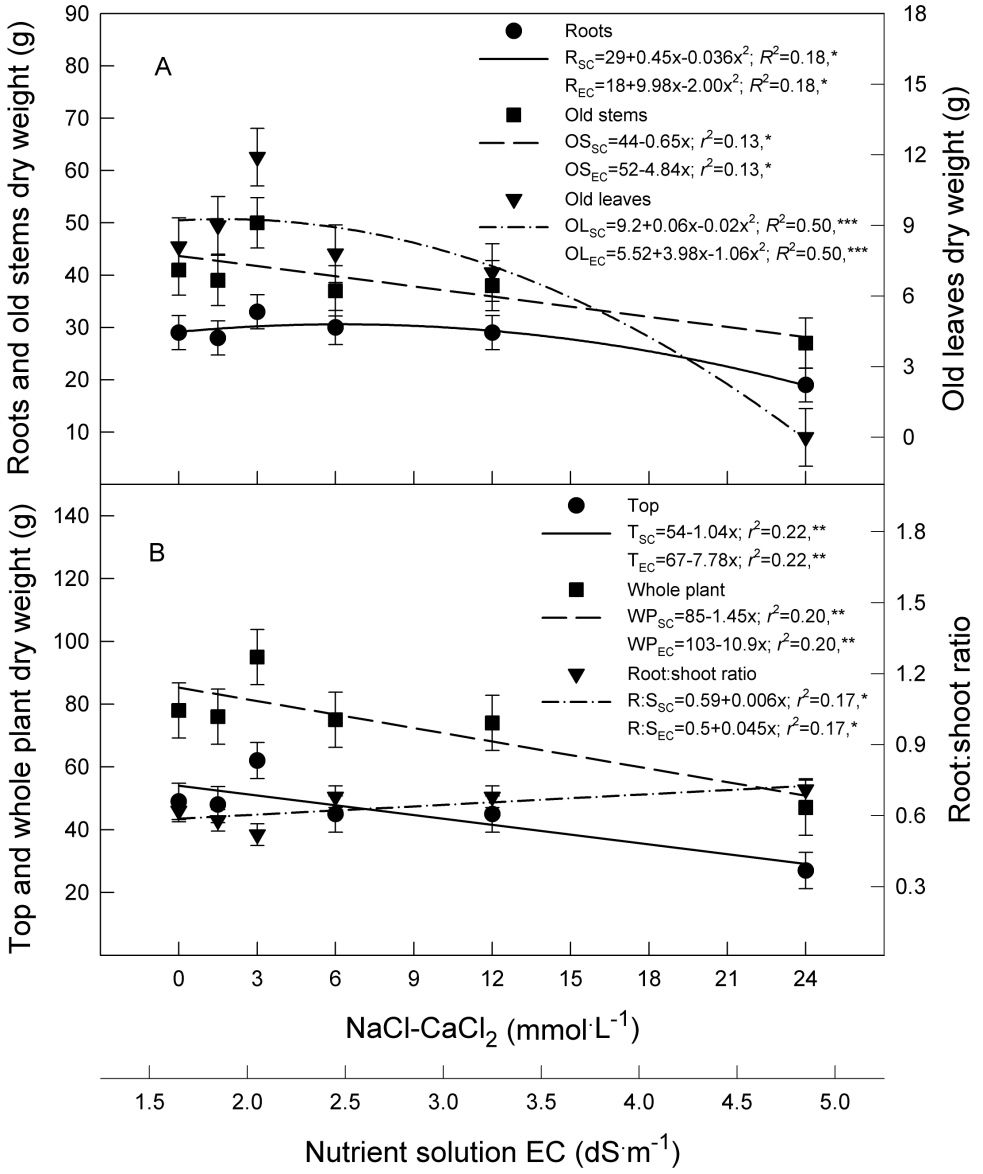

Fig. 2.4. (A) Roots, old stems and old leaves dry weights; (B) top part and whole plant dry weights, and root:shoot ratio of 'Red France' roses subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. Symbols represent the mean ± standard error of 6 plants. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. Plants were destructively harvested at the end of the experimental period (38 weeks), immediately after removing the flowering shoots. SC=salt concentration; EC=electrical conductivity.

numbers in each cell, as recommended by Daya (2001) and Elliot and Woodward (2007).

The extent of salt injury on the lower foliage of the plants (older leaves), after removing flowering shoots, was different between RS across SC (X^2=35.21; P<0.0001). Foliage of 'Manetti' plants was less affected by salinity since approximately 83.3% of the plants presented salt injury on only 0-20% of the foliage, 14.2% had salt damage on 21-60%, and only 2.5% of the plants had salt injury on 61-100% of the foliage (Fig. 2.5). On the other hand, in 'Natal Briar' 19% of the plants had damage on 1-20% of the foliage, 33.3% on 21-40%, and 47.7% had salt burn damage on 41-100% of the foliage (Fig. 2.5). It is important to mention that in 'Manetti' all of the plants subjected to 0-6 $mmol \cdot L^{-1}$ of NaCl-$CaCl_2$ received ratings between 0 and 1 and only those subjected to 12 and 24 $mmol \cdot L^{-1}$ presented salt burn damage corresponding to Categories 3 and 4 (no plants were rated as Category 5 for this RS). As for 'Natal Briar' mostly plants receiving 0-1.5 $mmol \cdot L^{-1}$ were rated as Category 1 while ratings of 2 and 3 started at SC as low as 3.0 $mmol \cdot L^{-1}$. All of the plants subjected to 12 $mmol \cdot L^{-1}$ were rated between Categories 2 to 4 and all of those under 24 $mmol \cdot L^{-1}$ fell into Categories 4 and 5 (data not shown).

Salt injury (as bronzing and scorching of leaf edges) on the lower leaves of flowering shoots of both rootstocks started to appear by the harvest event IV (at 144 DAT), in 'Manetti' plants only for the greatest SC (24.0 $mmol \cdot L^{-1}$) while in 'Natal Briar' plants salt damage was present on plants from the 3.0 to the 24.0 $mmol \cdot L^{-1}$ SC. By harvest V (at 192 DAT) flowering shoots of 'Manetti' plants subjected to the two greatest salt concentrations had salt damage on the lower leaves. In 'Natal Briar' plants, those subjected to 12.0 $mmol \cdot L^{-1}$ had salt damage at 192 DAT as well, while those subjected to 24 $mmol \cdot L^{-1}$, 43% did not produce flowering shoots at all and the rest had very small and slow developing leaves.

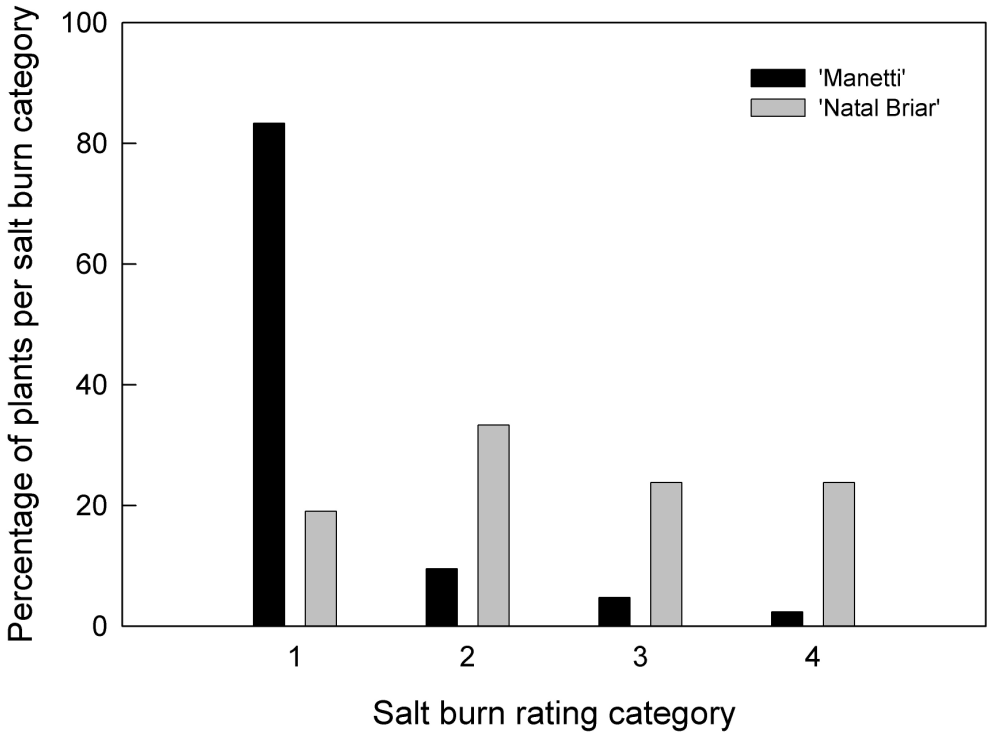

Fig. 2.5. Salt damage ratings of foliage of 'Red France' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. Salt damage rating is based on a 0 to 5 scale in which 0=no visible damage, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80%, and 5=81-100% of the foliage exhibiting salt burn damage. (n=42).

Water relations variables

The patterns of RWC and SWP were similar over time, thus data from all sampling dates were pooled accordingly. Leaf relative water content was similar between both RS (average of 92.07%; $P>0.05$). Stem water potential was affected by RS with 'Manetti' plants having lower values than 'Natal Briar' plants (-0.69 MPa versus -0.61 MPa, respectively; $P=0.0331$). Both variables were negatively affected by SC (Fig. 2.6).

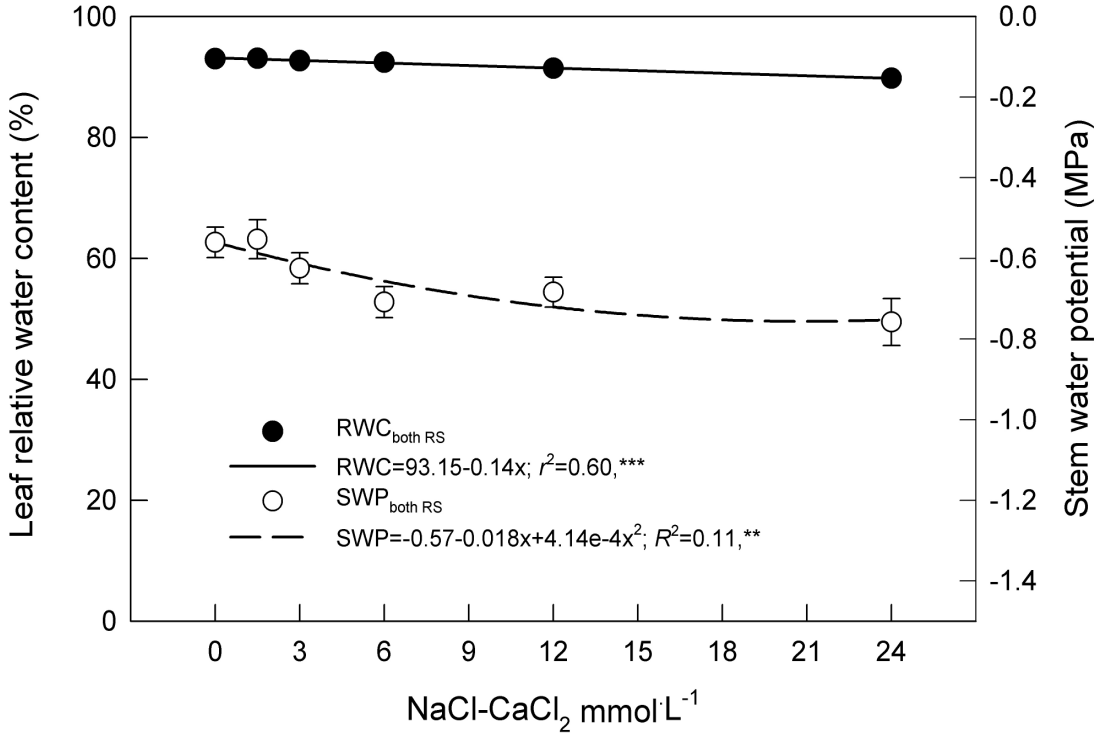

Fig. 2.6. Leaf relative water content and stem water potential of 'Red France' roses subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. Symbols represent the mean ± standard error of 30 observations. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Tissue mineral nutrient content

Chloride [Cl], sodium [Na], and calcium [Ca] concentrations

For leaf [Cl] data from five harvest events (not for harvest III, at 101 DAT) were available, which made it possible to consider time as a continuous variable. Leaf [Na] and [Ca] data sets were available only for two harvests (67 and 192 DAT), therefore both harvests were included in the data analysis as the levels of a discrete factor (harvest).

Chloride [Cl]. In the two greatest SC (12.0 and 24 mmol·L^{-1}) some plants did not yield any flowering shoots for the last harvest events. In repeated measures missing data from an experimental unit during a sampling date causes its elimination for the entire experimental period. Due to this restriction data from some of the harvest events were not included for the 12.0 and 24.0 mmol·L^{-1} salt levels (harvest VI at 265 DAT for 12.0, and harvests III to VI, 101-265 DAT for 24.0 mmol·L^{-1}). In the SC ranging from 0.0 to 6.0 mmol·L^{-1} all plants yielded flowering shoots at all the harvest events, therefore their regression lines include data from the entire experimental period (31-265 DAT).

There were interactions between RS and DAT (P<0.0001), SC and DAT (P<0.0001), and between RS and SC (P=0.0368).

Even though flowering shoots were removed from the plants during each harvest event, leaf [Cl] increased from one harvest to the next in both RS and in all SC (Fig. 2.7), although their rate of change was not the same. Plants budded on 'Manetti' had greater leaf [Cl] increase rates over time for all SC, except for the 24 mmol·L^{-1} level (Fig. 2.7). Across SC leaf [Cl] in 'Manetti' plants averaged 2.32±0.18, 4.28±0.42, 7.42±1.04, 7.62±0.89, and 8.82±0.84 g·kg^{-1}, at 31, 67, 144, 192 and 265 DAT, respectively, with an average increase from 31 to 265 DAT of approximately 280%. For 'Natal Briar' leaf [Cl] values were 8.02±0.30, 9.25±0.62, 9.96±0.77, 11.67±1.28 and 12.27±0.87 g·kg^{-1}, at 31, 67, 144, 192 and 265 DAT, respectively, with an average increase from 31 to 265 DAT of approximately 53%. Nevertheless, across salt treatments and harvest events flowering shoots of plants budded on 'Natal Briar' had on average 73% greater leaf [Cl] than those budded on 'Manetti' with overall averages of 10.2±0.38 g·kg^{-1} versus 5.9±0.37 g·kg^{-1}, for 'Natal Briar' and 'Manetti' plants, respectively.

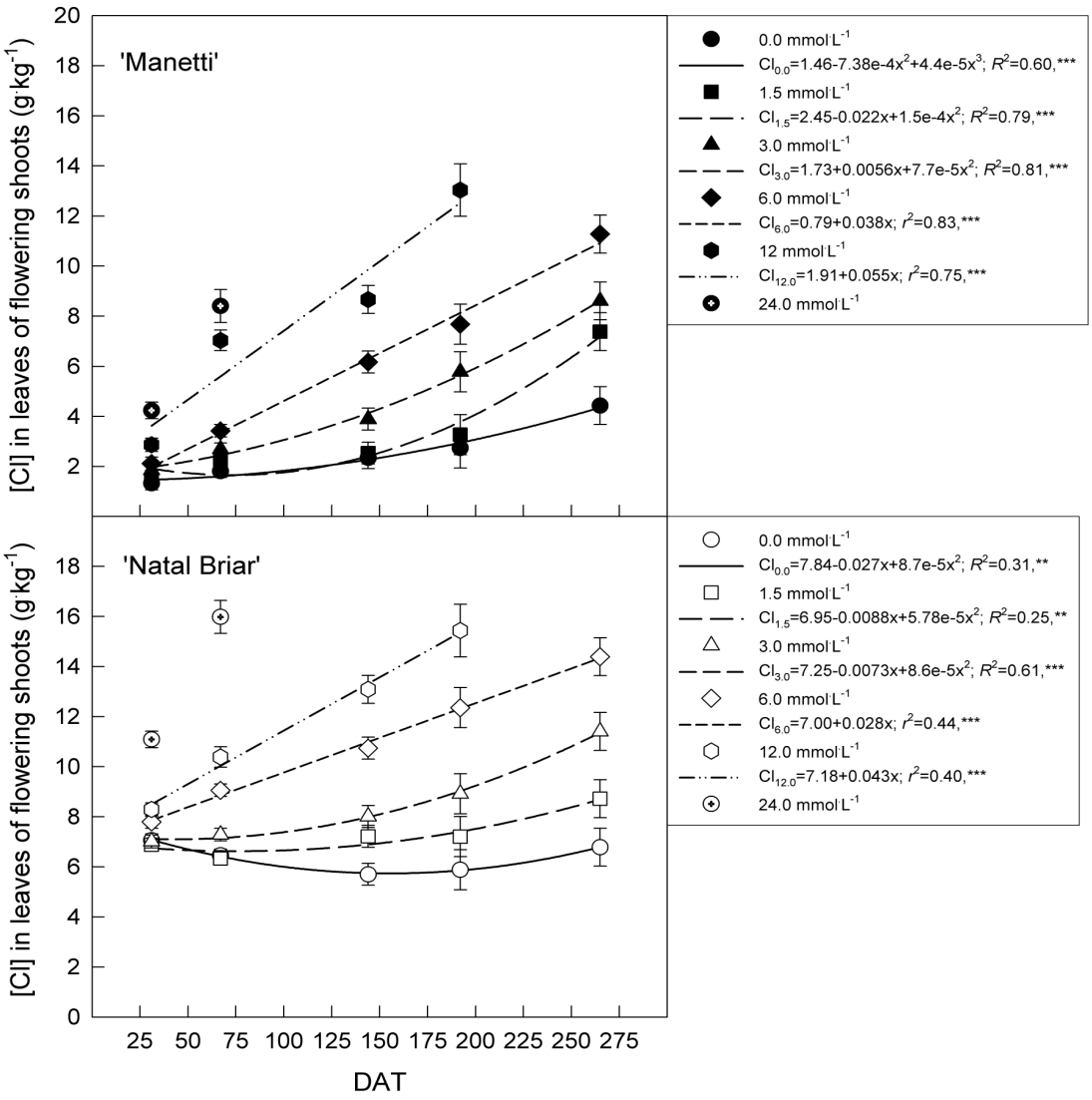

Fig. 2.7. Increase of leaf chloride concentration ([Cl]) over time in flowering shoots of 'Red France' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. Symbols represent the mean ± standard error of 7 plants. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Due to the missing data for the two greatest SC (as explained at the beginning of this section), in Fig. 2.8A the regression lines including all SC were fitted with data from harvests I and II (31 and 67 DAT), since harvest III (101 DAT) was not included in the leaf Cl analysis and at harvests IV, V and VI (144, 192 and 265 DAT) several plants from the 24.0 $mmol \cdot L^{-1}$ did not yield any flowering shoots. For the same reasons the regression lines including 0.0 to 12.0 $mmol \cdot L^{-1}$ SC contain data from harvests I to V (31 to 192 DAT), while the regression lines for the 0.0 to 6.0 $mmol \cdot L^{-1}$ salt levels include data from all six harvests (31 to 265 DAT), since in this lower salinity range all plants yielded flowering shoots for all harvest events. Leaf [Cl] was positively affected by the salinity level (P<0.0001) similarly for both RS (Fig. 2.8A). However, the rate of change over salt concentration levels increased as [Cl] data from subsequent harvests were included in the data analysis (Fig 2.8A).

In both RS leaf [Cl] had a negative correlation to flowering shoot DW and FS harvested per plant (P<0.0001 for both RS and variables; r=-0.65 and r=-0.55 in 'Manetti', respectively; and, r=-0.36, and r=-0.36 in 'Natal Briar, respectively). Leaf chlorophyll index was negatively associated with leaf [Cl] in 'Natal Briar' plants only (P=0.0016; r=-0.23).

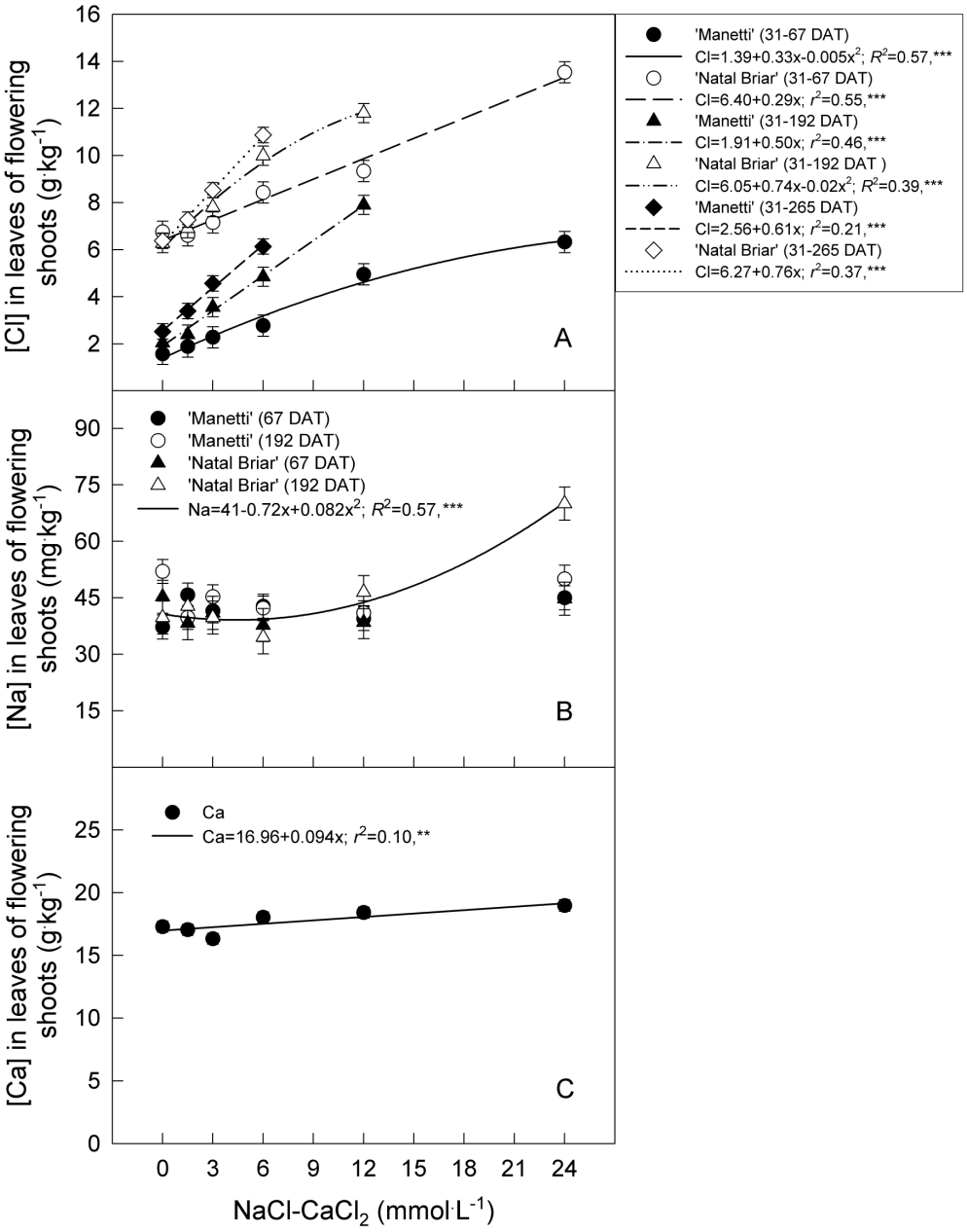

Fig. 2.8. (A) Leaf chloride, (B) sodium, and (C) calcium concentrations in flowering shoots of 'Red France' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to increasing NaCl-$CaCl_2$ in a half strength Hoagland's nutrient solution. Symbols represent the mean ± standard error of 7, 4 and 8 plants for plots A, B and C, respectively. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

For the whole plants destructively harvested at the end of the experiment, [Cl]'s increased in roots, old stems and old leaves as the salinity in the nutrient solution increased with greater increasing rates for the upper organs (Fig 2.9A). In roots and old leaves the rate of increase was similar for both rootstocks (no interaction present) but 'Manetti' plants had higher [Cl] in roots by 39%; leaf [Cl] was similar for both rootstocks (no leaves were available for the greatest salt treatment). In old stems [Cl] increased for 'Manetti' plants at greater rates as the salinity levels increased (interaction between RS and SC, P=0.0359).

Sodium [Na]. For leaf [Na] there was an interaction among RS, SC and harvest events (P=0.0103). In 'Manetti' plants [Na] was similar between harvest events and among SC, with an average concentration of 43.4±1.0 $mg \cdot kg^{-1}$ (Table 2.3; Fig. 2.8B). In 'Natal Briar', on the other hand, harvest event and SC had interactive effects (P=0.0148). In harvest II (67 DAT) all salinity levels had similar [Na], averaging 41.0±1.2 $mg \cdot kg^{-1}$ (Fig. 2.8B), while in harvest V (192 DAT) [Na] was positively affected by SC (Fig. 2.8B), reaching concentrations as great as 70 $mg \cdot kg^{-1}$ for the 24.0 $mmol \cdot L^{-1}$ salt level (72% greater than the average of the 0.0 to 12.0 $mmol \cdot L^{-1}$ salt levels).

In 'Natal Briar' plants [Na] showed a negative correlation to flowering shoots DW, FS, FSL and LCI (P=0.0004, 0.0013, 0.0649, and <0.0001; and, r=-0.49, -0.45, -0.27,-0.54, respectively). No association between [Na] and productivity and/or quality variables was found in 'Manetti' plants (P>0.05).

From the destructive harvest of plants [Na] was affected by SC in roots, old stems and old leaves (Fig. 2.9B). Sodium concentration in roots was the same in both RS. In stems [Na] was greater in 'Natal Briar' plants for the first three SC (0.0 to 3.0 $mmol \cdot L^{-1}$), less in the 6.0 $mmol \cdot L^{-1}$ and similar to 'Manetti' plants for the last two SC (12 and 24 $mmol \cdot L^{-1}$; interaction between RS and SC, P=0.045). Sodium concentration in old leaves was greater in 'Natal Briar' plants by 71% (Fig. 2.9B).

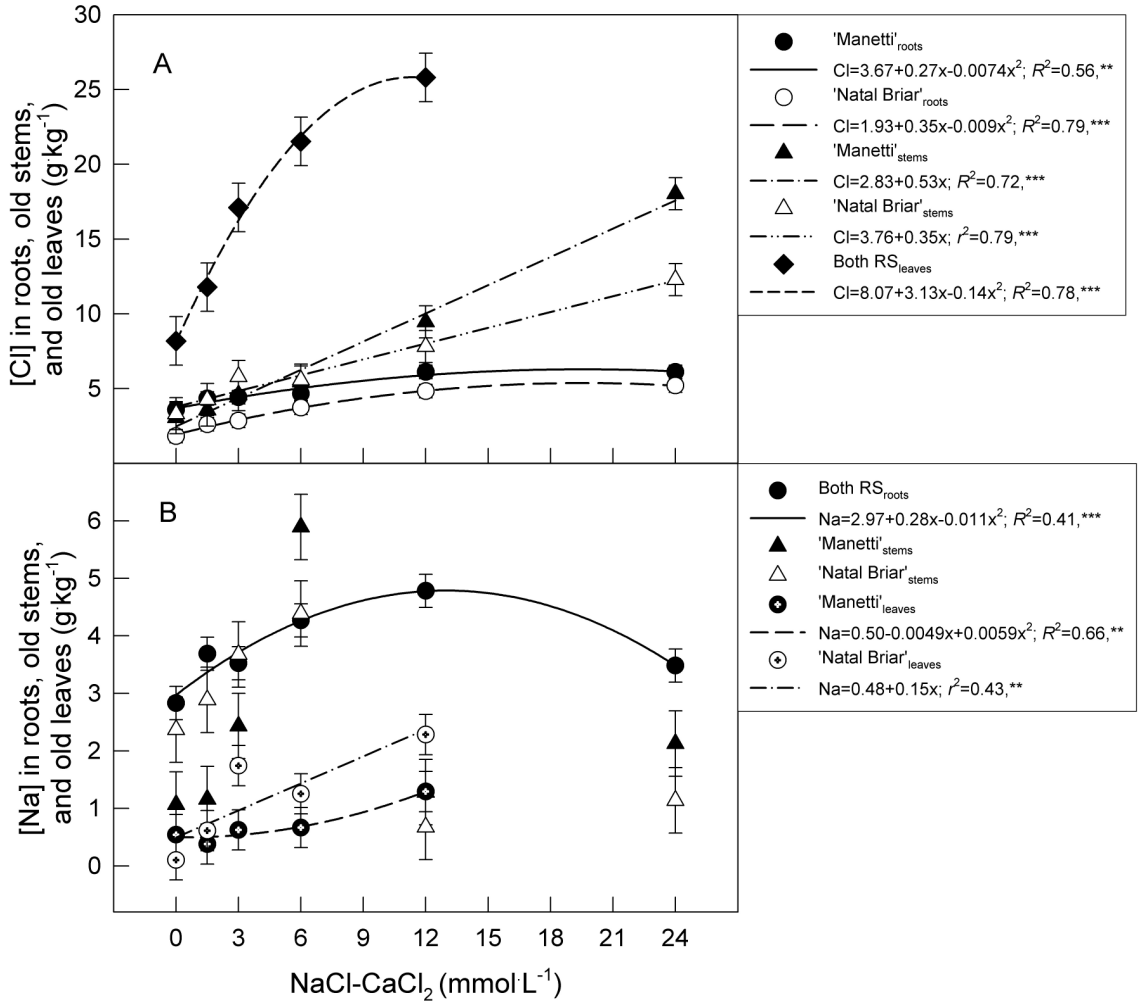

Fig. 2.9. (A) Chloride and (B) sodium concentrations in roots, old stems and old leaves of 'Red France' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. Symbols represent the mean ± standard errors of 3 plants for plots of single rootstocks and 6 plants for plots with both rootstocks pooled. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Table 2.3. Mineral nutrient concentration in leaves of flowering shoots of 'Red France' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to increasing NaCl-$CaCl_2$ salinity in a half strength Hoagland's nutrient solution. Leaf samples correspond to harvests events II and V (at 67 and 192 DAT). Means are the average of 4 plants.

Macronutrients ($g \cdot kg^{-1}$)																		
	N			P			K			Ca			Mg			S		
	67 DAT	192 DAT	*Diff.*	67 DAT	192 DAT	*Diff.*	67 DAT	192 DAT	*Diff.*	67 DAT	192 DAT	*Diff.*	67 DAT	192 DAT	*Diff.*	67 DAT	192 DAT	*Diff.*
'Manetti'	31.9	29.6	*2.3* *	2.40	2.42	*-.02 ns*	23.2	23.9	*-0.7 ns*	17.6	16.7	*0.9 ns*	2.36	1.81	*0.55* ***	2.38	3.48	*-1.1* ***
'Natal Briar'	31.2	29.4	*1.8 ns*	2.58	2.36	*0.22* ***	20.6	21.6	*-1.0* *	19.9	16.6	*3.3* ***	2.88	2.34	*0.54* ***	2.29	3.05	*-0.76* ***
Diff. Signif.[z]	*0.7 ns*	*0.2 ns*		*-0.18* **	*0.06 ns*		*2.6* ***	*2.3* ***		*-2.3* ***	*0.1 ns*		*-0.52* ***	*-0.53* ***		*0.09 ns*	*0.43* ***	
Micronutrients ($mg \cdot kg^{-1}$)																		
	Mn			Fe			B			Zn			Na					
	67 DAT	192 DAT	*Diff.*	67 DAT	192 DAT	*Diff.*	67 DAT	192 DAT	*Diff.*	67 DAT	192 DAT	*Diff.*	67 DAT	192 DAT	*Diff.*			
'Manetti'	187	228	*-41 ns*	68.2	52.9	*15.3* ***	37.4	37.4	*0.0 ns*	34.9	32.8	*2.1 ns*	42.0	45.0	*-3.0 ns*			
'Natal Briar'	165	195	*-30 ns*	59.4	48.2	*11.2* ***	97.8	82.8	*15.0* **	43.2	32.5	*10.7* ***	40.9	45.5	*-4.6* *			
Diff.	*22 ns*	*33 ns*		*8.8* ***	*4.7* **		*-60.4* ***	*-45.4* ***		*-8.3* ***	*0.3 ns*		*1.1 ns*	*-0.5 ns*				

[z] Significance according to GLM: ns, *, **, *** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively.

Calcium [Ca]. For leaf [Ca] RS and DAT had interactive effects (*P*=0.0010). In 'Manetti' plants [Ca] was similar between harvest II and V (67 and 192 DAT; average of 17.2 $g \cdot kg^{-1}$) while in 'Natal Briar' plants leaf [Ca] decreased by 17% from harvest II to harvest V (Table 2.3). At 67 DAT, 'Natal Briar' plants had greater leaf [Ca] than 'Manetti' plants, but by 192 DAT both RS had similar leaf [Ca] (Table 2.3).

Salt concentration level had a linear positive effect on leaf [Ca] (Fig. 2.8C).

In 'Natal Briar' plants leaf [Ca] was correlated with FSL (*P*=0.0323; *r*=0.31) and LCI (*P*=0.0277; *r*=-0.32). In 'Manetti' plants no association between [Ca] and productivity and/or quality variables was found (results not shown).

Leaf concentration of other mineral nutrients

The concentration of several mineral nutrients exhibited a significant decrease from harvest II (67 DAT) to harvest V (192 DAT) in both RS (Table 2.3). In 'Manetti' plants leaf [N], [Mg] and [Fe] decreased by 7, 23 and 22%, respectively. In 'Natal Briar' leaf [P], [K], [Ca], [Mg], [Fe], [B] and [Zn] decreased by 8.5%, 5%, 17%, 19%, 19%, 15%, and 25%, respectively. Only leaf [S] increased 46% in 'Manetti' and 33% in 'Natal Briar' from 67 to 192 DAT (Table 2.3). 'Natal Briar' plants had higher leaf concentrations of P, Ca and Zn at 67 DAT, but by 192 DAT their concentration became similar in both RS. At 67 DAT both RS had similar leaf [S], but by 192 DAT 'Manetti' plants surpassed 'Natal Briar' by 14% (Table 2.3).

Leaf concentrations of Mg, Fe, and B were affected by RS. 'Manetti' plants had greater leaf [Fe] (13%) and 'Natal Briar' plants had greater leaf [Mg] (26%), and [B] (142%; data not shown).

Leaf N, K, Mg, S, Mn and Fe concentrations were affected by SC in the nutrient solution (Table 2.4). Leaf [N], [Mg], and [Fe] decreased and [Mn] and

[K] increased with increasing SC ([K] only for 'Natal Briar' plants). Leaf [S] decreased only for the two greatest SC (12 and 24 $mmol \cdot L^{-1}$), but no linear or quadratic models were significant (Table 2.4).

Leaf [Cl] was positively correlated to leaf [Ca], [Mn], [B], and [Na], and negatively correlated to [N] and [Fe] (r=0.28, 0.33, 0.30, 0.56, -0.68 and -0.54, respectively). Leaf [Na] was positively correlated to [K], and negatively correlated to [N], [Mg] and [Fe] (r=0.26, -0.68, -0.29 and -0.25, respectively). Leaf [Ca] was positively correlated to [P], [Mg], [Mn], [B], [Zn] and [Cl], and negatively correlated to [S] (r=0.24, 0.54, 0.25, 0.38, 0.47, 0.28 and -0.36, respectively).

Table 2.4. Effect of increasing salinity levels of a NaCl-$CaCl_2$ (2:1 molar ratio) salt mixture in a half strength Hoagland's nutrient solution on leaf mineral nutrient concentration of flowering shoots of greenhouse 'Red France' roses. Leaf samples correspond to harvests II (67 DAT) and V (192 DAT). Means for the element K are the average of four plants, for the rest of the elements means are the average of eight plants.

NaCl-$CaCl_2$ ($mmol \cdot L^{-1}$)	N ($g \cdot kg^{-1}$)	K ($g \cdot kg^{-1}$)		Mg ($g \cdot kg^{-1}$)	S ($g \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)
Rootstock		'Manetti'	'Natal Briar'				
0.0	31.55[z]	22.99	21.13	2.55	2.89	132	60.3
1.5	31.37	24.43	19.88	2.39	2.85	147	59.6
3.0	30.75	23.66	20.68	2.36	2.82	177	57.3
6.0	31.91	23.23	21.37	2.39	2.90	221	58.5
12.0	30.67	23.23	20.79	2.31	2.73	232	55.4
24.0	26.96	23.57	22.76	2.09	2.61	255	51.8
	L***	ns	L**	L*	ns	Q*	L**
R^2	0.15		0.15	0.06		0.18	0.08
Parameter							
B_0	31.95		20.44	2.46		135.00	59.72
B_1	-0.182		0.085	-0.014		13.68	-0.328
B_2						-0.373	

[z] significance according to GLM: ns, *, **, *** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively.

DISCUSSION

Overall salinity stress response

The first three SC (0.0, 1.5 and 3.0 $mmol \cdot L^{-1}$ NaCl-$CaCl_2$) had calculated EC values of 1.65, 1.85 and 2.05 $dS \cdot m^{-1}$ (including the tap water's contribution ~0.6 $dS \cdot m^{-1}$), which corresponds to EC values of 2.48 $dS \cdot m^{-1}$, 2.78 $dS \cdot m^{-1}$ and 3.08 $dS \cdot m^{-1}$ in the saturation extract, respectively (Farnham et al., 1985). For the rest of the SC (6.0, 12.0 and 24.0 $mml \cdot L^{-1}$) the calculated EC values were 2.45 $dS \cdot m^{-1}$, 3.25 $dS \cdot m^{-1}$ and 4.85 $dS \cdot m^{-1}$, equivalent to EC values in the saturation extract of 3.68 $dS \cdot m^{-1}$, 4.88 $dS \cdot m^{-1}$ and 7.28 $dS \cdot m^{-1}$, respectively (Farnham et al., 1985), which are above the salinity thresholds recommended for roses in the past (EC 2-3 $dS \cdot m^{-1}$ in the saturation extract; Bernstein et al.,1972; Davidson and Boodley, 1987; Hughes and Hanan, 1978). However, EC in leachates collected from plants in all treatments (Fig. 2.1B), (including the non-salinized control), surpassed the maximum leachate salinity thresholds (EC 1.4-1.8 $dS \cdot m^{-1}$) previously recommended for roses (Brun and Settembrino, 1996), (Fig. 2.1B).

Flower productivity and quality parameters, and plant water relations were negatively affected by increasing SC in the nutrient solution (Table 2.2; Fig. 2.3-2.6). However, in general terms, there were no significant differences in the periodic and cumulative data collected in the salinity range of 0.0-6.0 $mmol \cdot L^{-1}$ NaCl-$CaCl_2$ (nutrient solution total EC of 1.65-2.45 $dS \cdot m^{-1}$; and EC_L of 2.9-4.9 $dS \cdot m^{-1}$). It was only for the two greatest SC, 12.0 and 24.0 $mmol \cdot L^{-1}$ (nutrient solution EC of 3.25 and 4.85 $dS \cdot m^{-1}$; and EC_L of 6.3 and 7.3 $dS \cdot m^{-1}$, respectively) that substantial reductions in all evaluated variables were found during regular flowering shoot harvests and at the destructive harvest of whole plants (Table 2.2). Plants subjected to these greatest SC showed significant decreases in their productivity (flowering shoot DW and FS harvested per plant) by the fourth

and third harvest events (144 and 101 DAT), respectively, compared to those plants under the 0.0 to 6.0 $mmol \cdot L^{-1}$ salinity range (Fig. 2.2A-B).

Cabrera and Perdomo (2003) found no effects of salinity concentrations ranging from 0 to 10 $mmol \cdot L^{-1}$ NaCl on flowering shoot number and dry weight of 'Bridal Pink' roses budded on 'Manetti' during four harvest events (approximately 145 DAT). It was only after increasing the salinity concentrations by 3X (0, 15 and 30 $mmol \cdot L^{-1}$) during a 175-day second experimental phase (total experimental period of approximately 11 months) that foliar salt injury on the lower, older leaves started to appear (79 days after starting the second phase). They reported no reductions in DW or FS due to the salt treatments for the entire 11-month trial. Leachate EC and $[Cl_L]$ from our study are in agreement with those reported by Cabrera and Perdomo (2003), but the leaching fraction (LF) they reported for their study was greater than in the present study (38% versus 25%, respectively). This LF of 25% corresponds to the overall average for the entire experimental period (38 weeks). However, it is possible that plants experienced some degree of water stress (LF< of the 25% targeted) through the study period. In a 25-month salinity trial Wahome et al. (2000) reported that 'Kardinal' roses budded on *R. rubiginosa* could tolerate up to 10 $mmol \cdot L^{-1}$ NaCl in irrigation water without a significant reduction in the total and root dry weights. However, the proportion of marketable cut-flowers was significantly reduced compared to the non-salinized controls.

Conversely, Hughes and Hanan (1978) reported increasing problems due to Na and Cl at concentrations above 4.0 $mmol \cdot L^{-1}$ on 'Forever Yours' roses budded on 'Manetti' rootstock growing on gravel and soil. However, the two salinized solutions that they reported to reduce rose shoot yield, length and weight (salt concentrations > 4.0 $mmol \cdot L^{-1}$ NaCl) had 4.0 $mmol \cdot L^{-1}$ of HCO_3, while a third solution yielding the greatest mean stem length and weight had the same salt concentration but no HCO_3. Bernstein et al. (1972) also reported severe damage and plant death of 'Grenoble' roses budded on 'Dr. Huey' (*Rosa*

L. 'Shafter') growing at 4 $dS \cdot m^{-1}$ salinities over a one-year experimental period and the tap water used in their experiment contained 2.4 $mmol \cdot L^{-1}$ of HCO_3 Fernández-Falcón et al., (1986) evaluated the effects of Cl and HCO_3 in irrigation water on 'Mercedes' roses on *R. canina* L. 'Inermis'. The water treatments used had [Cl] ranging from 2.6 to 13.6 $mmol \cdot L^{-1}$, [HCO_3] from 3.5 to 9.6 $mmol \cdot L^{-1}$ and pH values ranging from 8.4 to 8.9. Bailey (1996) reported that the suggested maximum alkalinity (as $CaCO_3$ or HCO_3) for long term greenhouse crops is 2.6 $mmol \cdot L^{-1}$. Hughes and Hanan (1978) found HCO_3 to be highly toxic to rose production, resulting in chlorosis whenever concentrations exceeded 2.0 $meq \cdot L^{-1}$. As bicarbonate has been reported to exacerbate the salinity detrimental effects caused by NaCl alone on tomato (*Lycopersicon esculentum* Mill.; Navarro et. al., 2000) and pinto bean (*Phaseolus vulgaris* L. 'Poncho') plants (Valdez-Aguilar et al., 2008), thus, it could be that the increasing problems attributed to Na and/or Cl in some of the rose salinity studies cited above were at least partially compounded or exacerbated by high alkalinity. In addition to the possible masking effects of alkalinity in some of these studies, the duration of the experiments was different, an important factor to consider when establishing or determining salinity tolerance (Bernstein, 1975; Munns, 2002; Yaron et al., 1969). According to a biphasic model of the plant growth responses to salinity proposed by Munns (1993), during phase 1 (time scale of weeks) plant growth is reduced by the decrease in soil water potential (water-stress effect). In this short term phase closely related genotypes differing in salt tolerance respond identically to salt stress. Later, during phase 2 (after a long-term exposure to salinity), the salt-specific effects appear as salt injury in the old leaves which die because of a rapid rise in salt concentrations in cell walls or cytoplasm when the vacuoles can no longer sequester incoming salts. If the rate of leaf death approaches the rate of new leaf production, then eventually there is a substantial drop in the supply of assimilates to the growing leaves, or a change in the supply of growth regulators, and growth is further

reduced. It is here, during phase 2 that differences in varieties differing in salt tolerance become apparent.

Rootstock effects on the response to salinity

Plants budded on the 'Manetti' rootstock were more vigorous, produced more and longer flowering shoots per plant, and their foliar salt burn injury was considerably less compared those plants budded on 'Natal Briar' (Table 2.2, Fig. 2.5). These greater DW produced by 'Manetti' plants with similar volumes of irrigation water applied confers them a greater water-use efficiency (WUE; carbon gain per water used) in spite of being under slightly greater water stress (as indicated by their lower stem water potentials). Obiol and Cardús (1974) reported *Rosa* L. 'Manetti' as more productive than *R. indica* 'Major' and *R. canina* L. while Cabrera (2002), on the other hand, found no differences among four rose rootstocks [*Rosa* L. 'Manetti', *R. indica* 'Major', *Rosa* L. 'Natal Briar' and Dr. Huey (*Rosa* L. 'Shafter')] in flower and dry yield biomass over four flowering flushes. However in both cases plants were grown with non-salinized solutions.

Response of garden rose rootstocks to salinity stress varies among rose genotypes (Niu and Rodriguez, 2008a; Wahome et al., 2000, Wahome et al., 2001). 'Manetti' has been previously reported as able to tolerate moderate salinity (up to 15 $mmol \cdot L^{-1}$ NaCl) in the irrigation water without significant effects on flowering shoot dry weight and number (Cabrera and Perdomo, 2003). 'Natal Briar', on the other hand, growing under 8.0 $dS \cdot m^{-1}$ salinity performed poorly yielding the lowest budtake (grafting success) percentage compared to *R. indica* 'Major', *R. damascene* Mill., *R. banksiae* R. Br., *R. bourboniana* L. 'Edouard', *R. rugosa* Thunb. and the Hybrid Tea 'Crimson Glory' (Singh and Chitkara, 1984). Differences in the responses of plant growth to salt stress are expected to appear only after a long-term exposure to salinity (weeks to months; Neumann,

1997). Greater rates of salt accumulation in the mature leaves of more salt-sensitive varieties then lead to toxic effects, i.e. accelerated leaf senescence and/or necrosis (Neumann, 1997). Tolerant varieties and rootstocks resist the uptake and accumulation of toxic ions in the stem and leaf tissue (Grattan, 2002).

Chloride, Na and Ca accumulation in flowering shoot leaves

Even though flowering shoots were removed from the plants at each harvest event, their leaf [Cl] increased progressively from one harvest to the next (Fig. 2.7), results consistent with those reported by Cabrera and Perdomo (2003). This cumulative increase implies that over time greater amounts of Cl were absorbed from the growing substrate and/or retranslocated from the lower portions of the plant to the newly developing flowering shoots. By 31 DAT 'Natal Briar' plants already had leaf [Cl] that was 246% greater than in 'Manetti' plants (8.02 $g \cdot kg^{-1}$ versus 2.32 $g \cdot kg^{-1}$, respectively). 'Manetti' plants registered their greatest average leaf [Cl] at 265 DAT (8.82 $g \cdot kg^{-1}$), but it barely surpassed the average concentration registered by 'Natal Briar' plants during the first harvest event at 31 DAT (8.02 $g \cdot kg^{-1}$).

Chloride concentrations were very low in old, woody tissues (roots and old stems) compared with young, non-woody tissues, i.e. leaves (Fig. 2.8A and Fig. 2.9A). Considering that Cl moves readily with the soil (substrate) water and is taken up by the roots, then transported to the stems and leaves (Grattan, 2002), the greater [Cl] in leaves could be related to a certain degree to their greater transpiration rates compared to old stems and roots.

In woody tissues (roots and old stems; unfortunately no Cl analysis of flowering stems was made) [Cl] was greater in 'Manetti' plants compared to roots and old stems from 'Natal Briar' plants (Fig. 2.9A). Since there were no differences in $[Cl_L]$ between both rootstocks (Fig. 2.1B), the amount of Cl that

entered the plant should be similar in both RS. Hence, the remarkable difference in leaf [Cl] of flowering shoots is apparently due to the ability of the 'Manetti' rootstock to sequester some of the Cl in the lower woody organs and to restrict to a greater extent its transport to the flowering shoot leaves over time. Leaf Cl accumulation in old leaves occurred to a maximum concentration of 26.74 $g \cdot kg^{-1}$ (2.67%) at the 12 $mmol \cdot L^{-1}$ salt level (no leaves were available at the 24 $mmol \cdot L^{-1}$ salt level).

Contrary to leaf [Cl], leaf [Na] remained steady in both RS, averaging 43 $mg \cdot kg^{-1}$ and consistent with the results reported by Cabrera and Perdomo (2003). It was only at the 24 $mmol \cdot L^{-1}$ SC and for harvest V (192 DAT) that leaf [Na] increased considerably in leaves of 'Natal Briar' plants (Fig. 2.8B). Probably over time and after the 12.0 $mmol \cdot L^{-1}$ SC this rootstock could not keep up the restriction in Na uptake and/or transport to the upper leaves.

Leaves die sooner in a more salt-sensitive variety because salts arrive sooner, or because cells are unable to compartmentalize the salt in vacuoles to the same high concentration as the tolerant variety (Munns, 1993). 'Manetti' seems to restrict the transport of Na and Cl to the flowering shoots to a greater extent than 'Natal Briar', producing higher yields and quality of cut-flowers. However, 'Natal Briar' is the rootstock being predominantly used in the greenhouse cut rose industry having displaced 'Manetti' and other clonal rootstocks since the 1990's (De Vries, 2003a, b and c).

The ratio of Na:Cl supplied in the irrigation water was approximately 1:2.6 (taking into account their respective concentrations in the tap water), and in leaves of flowering shoots this ratio was 1:137 for 'Manetti' and 1:237 in 'Natal Briar' plants. Sodium concentrations were much higher in roots, old stems and old leaves than in the flowering shoots leaves for both rootstocks (Fig. 2.8B and 2.9B). This confirms the previously reported greater restriction of Na transport to the upper leaves compared to Cl (Bernstein et al., 2006; Cabrera, 2003a; Cabrera and Perdomo, 2003; Niu and Rodriguez, 2008a; Sadasivaiah and

Holley, 1973), although this Na exclusion is not general to all rose rootstocks (Fernández-Falcón et al., 1986; Baas and van den Berg, 1999, Cabrera, 2003a). Given the closer, and negative correlations between tissue [Cl] with the productivity and quality variables evaluated, it seems that the reductions in flower yield and quality were due to a greater extent to Cl rather than to Na toxicity as reported by Baas and van den Berg (1999) and Bernstein et al. (1972).

Leaf [Ca], similar to leaf [Cl], increased linearly with increasing SC (Fig. 2.8A and Fig. 2.8C) and both elements were positively correlated, which is not surprising since both were constituents of the salt treatments applied. The lack of difference between RS in leaf [Ca] could be explained by the fact that both received similar volumes of irrigation water and, given the close relationship between transpiration and Ca transport within the plant (Baas et al., 2003) it is assumed that the amount of Ca entering the plant was the same in both RS. The amount of water absorbed by the plant was negatively affected by increasing salinity levels (Fig. 2.1A), possibly due to reductions in the transpiration rates as reported by Baas and van den Berg (1999). But, even though the total amount of water absorbed by plants decreased as the SC increased, leaf Ca accumulation increased with increasing concentrations of NaCl-$CaCl_2$ in the irrigation water (Fig. 2.8C). This was due to the fact that even with lower total water irrigation volumes applied, the total mass (in g) of the applied salinizing ions was greater as the SC increased (Fig. 2.1A).

Lorenzo et al. (2000) did not find significant effects of $NaSO_4$-based salinity on leaf [K] of roses. Similarly, our results show that K concentration in leaves of flowering shoots was not affected by increasing salinity, contrary to what has been reported in snapdragon (*Antirrhinum majus* L.; Carter and Grieve, 2008). Actually, it showed a slightly positive relationship to increasing salinity levels in 'Natal Briar' (Table 2.4). Interestingly, also in 'Natal Briar' (RS with greater foliar [Cl] and [Na], and greater leaf salt injury) the levels of foliar [B]

where 142% greater than in ‘Manetti’ (Table 2.3). Leaf [Cl] and [B] were positively correlated and both present in considerably greater concentrations in ‘Natal Briar’ plants. Higher Cl, Na and B accumulations in ‘Natal Briar’ were also reported by Cabrera (2002). It could be possible that their uptake and/or transport within the plant are related and that the more negative effect of salinity on the ‘Natal Briar’ RS was due to additive or synergetic effects of these two elements.

Leaf concentrations of N, Mg, S and Fe decreased as the salinity in the irrigation water increased (S only in plants subjected to the highest levels of salinity; Table 2.4). Most of these elements are associated with the chlorophyll molecule (Handreck and Black, 2002), which could explain the negative effect of increasing SC on LCI.

Contrary to what previous reports state about seasonal changes in mineral nutrient absorption in roses, lower in summer and greater in winter (Takeda and Takahashi, 1998; Terada et al., 1997), our results showed decreases in most of the leaf mineral nutrient concentrations from harvest II (67 DAT-June 29) to harvest V (192 DAT-November 1; Table 2.3). As the time of exposition of the plants to the saline stress increased, their production of flowering shoots not only decreased in number and dry weight, but their development rate slowed down, flower heads were smaller and the leaves did not reach the same degree of maturation by the time the flower bud appeared compared to those plants not exposed to salt stress. It is contented that the reduced plant growth, and impairment of its physiology, caused by the salinity stress caused the decrease in mineral nutrient concentrations in the younger, tender flowering shoot leaves as they approached winter.

CHAPTER III

EFFECTS OF SUPPLEMENTAL CALCIUM ON THE RESPONSE OF ROSE (*ROSA* L. 'HAPPY HOUR') TO SALINITY STRESS

INTRODUCTION

There are three major constraints for plant growth on saline substrates (Gorham, 2007; Lambers et al., 1998; Marschner, 1995). First, water deficit arising from low soil water potential (high osmotic pressure) associated with high salinity, making it more difficult for plants to establish a continuous gradient of water potential between the soil solution and the atmosphere. Second, ion toxicity associated with excessive uptake of inorganic ions, mainly Cl^- and Na^+. And third, nutrient imbalance by depression in uptake and/or shoot transport and impaired internal distribution of other nutrients (calcium in particular) causing ion imbalances and leading to deficiency symptoms.

Increases in exchangeable Na^+ (characteristic of salinity dominated by Na^+ salts) are balanced by decreases in exchangeable K^+, Ca^{2+} and Mg^{2+}, leading to deficiencies when the concentrations of these elements in solution become deficient (Bernstein, 1975; Gorham, 2007). But salinity dominated by Na^+ salts not only reduces Ca^{2+} availability, it also reduces Ca^{2+} transport and mobility to growing regions of the plant, which affects the quality of both vegetative and reproductive organs (Grattan and Grieve, 1999).

Due to its stabilizing effect on the plasma membrane, Ca^{2+} plays an important role in selectivity of ion uptake, the K^+/Na^+ selectivity of roots in particular (Marschner, 1995). In plants grown in the absence of calcium or, in the presence of high salinity, this ion selectivity is lost due to increased membrane permeability (Epstein, 1972; Ehret et al., 1990; Kaya et al., 2002). Sodium reduces binding of Ca^{2+} to the plasma membrane, inhibits its influx while

increasing its efflux, and depletes its internal stores from endomembranes (Rengel, 1992). Salt would almost instantly reduce the amount of Ca^{2+} being transferred to the leaf cells, with Ca^{2+} activity dropping and Na^{+} activity rising in the apoplasm of leaf cells (Rengel, 1992).

Application of gypsum ($CaSO_4$) is a common practice in reclamation of saline-sodic and sodic soils to increase salt tolerance by improving soil structure and thus soil 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 calcium has been shown to ameliorate adverse effects of salinity on several crops. For example, on wheat (*Triticum aestivum* L. 'Neepawa') and barley (*Hordeum vulgare* L. 'Abee') subjected to moderate to severe Na_2SO_4, $MgSO_4$, $CaSO_4$ and $CaCl_2$ salinities, increasing the concentration of Ca ([Ca]) in the solution, 10 to 18 $mmol \cdot L^{-1}$, ameliorated the detrimental effects of salt on wheat with increases of 138% in leaf area and 42% in plant dry weight compared to the non-amended saline solution (Ehret et al., 1990). In barley, leaf area increased by 25% but plant dry weight was not affected by the increasing [Ca] (Ehret et al., 1990). In navel orange [*Citrus sinensis* (L.) Osbeck] budded on two rootstocks and subjected to 0 and 45 $mmol \cdot L^{-1}$ NaCl in the nutrient solution, raising the [Ca] from 3 to 30 $mmol \cdot L^{-1}$ mitigated the effects of salinity on plant growth, defoliation and leaf injury (Bañuls et al., 1991). In container-grown *Crataegus opaca* Hook. & Arn. 25 $mmol \cdot L^{-1}$ NaCl applied to the nutrient solution was more inhibitory to growth, water use, and ion uptake selectivity in the absence of additional Ca^{2+} as compared to inclusion of 2.0 and 5.0 $mmol \cdot L^{-1}$ Ca^{2+} as $CaCl_2$ (Picchioni and Graham, 2001). In strawberry plants (*Fragaria x ananassa* Duch.) grown at high NaCl-salinity (35 $mmol \cdot L^{-1}$) supplementing the saline nutrient solution with 5 $mmol \cdot L^{-1}$ Ca^{2+} [as $CaCl_2$ or $Ca(NO_3)_2$], ameliorated the effects of salinity on plant growth and fruit yield (Kaya et al., 2002; 2003a). The supply of 10 $mmol \cdot L^{-1}$ of $Ca(NO_3)_2$ had optimal effects on growth and metabolism of NaCl-stressed (30

and 60 mmol·L^{-1} NaCl) guava seedlings (*Psidium guava* L.; Ebert et al., 2002). Inclusion of 20 mmol·L^{-1} Ca^{2+}, as $CaCl_2$, prevented reductions in leaf photosynthesis, and chlorophyll content caused by 70.4 mmol·L^{-1} NaCl on tomato (*Solanum lycopersicum* L.) leaves, but did not prevent reductions in leaf length and elongation rate (Montesano and van Iersel, 2007).

In the past, roses (*Rosa* L.) have been classified as having poor salinity tolerance, with a decrease in shoot growth between 25% and 50% at electrical conductivity (EC) in the saturation extract (EC_e) between 2 dS·m^{-1} and 3 dS·m^{-1}, and experiencing lethal effects at EC_e of 4 dS·m^{-1} (Bernstein et al., 1972). However, more recent studies indicate that roses can tolerate greater levels of salinity, exhibiting salt damage only after 15 or 20 mmol·L^{-1} NaCl, and this response was influenced by rootstock selection (Cabrera, 2001, 2003a; Lorenzo et al., 2000; Wahome et al., 2000, 2001).

Compared to other ornamental crops, roses grown for cut-flower production are known for their high water and mineral nutrient (or fertilizer) requirements (Cabrera, 2003b). Nutrient solutions used to irrigate roses growing in mineral soil beds and organic-based substrates usually contain 250-350 mg·L^{-1} Ca^{2+} (6.2-8.7 mmol·L^{-1}) while in hydroponic systems formulations are in general less concentrated, approximately 90 mg·L^{-1} Ca (2.2 mmol·L^{-1}) in a ½ strength Hoagland solution.

The main objective of this experiment was to determine if supplementing the saline nutrient solution with additional Ca^{2+} would increase the salinity tolerance of a greenhouse rose cultivar, ‘Happy Hour’, budded on two rootstocks.

MATERIALS AND METHODS

Plant culture and management

On January 26, 2005, 90 bare-rooted 'Happy Hour' rose plants budded on two rootstocks, *Rosa* L. 'Manetti' and *Rosa* L. 'Natal Briar', were transplanted into 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 previously amended with 3.0 $kg \cdot m^{-3}$ dolomitic limestone (Carl Pool Products, Gladewater, TX) and 0.6 $kg \cdot m^{-3}$ of each Micromax Micronutrients fertilizer (The Scotts Company, Marysville, OH) and Aqua-GroG 2000 (The Scotts Company, Marysville, OH). Plants were placed on 5.5 x 1.5 x 0.4 m raised benches, with three plants abreast in each bed and spaced at 30 cm between centers. Plants were irrigated with a nutrient solution made with 15-5-15 Cal-Mag (The Scotts Company, Marysville, OH) and adjusted to deliver 140 $mg \cdot L^{-1}$ of nitrogen until they underwent a first hard pinch (removal of the terminal portion of a soft shoot, including two to four leaves; Langhans, 1987a). Plants were grown for an entire flowering flush cycle and all flower shoots were harvested on April 16, 2005. During the length of the proceeding experiment, plants were managed following conventional pruning practices (Langhans, 1987b), inducing synchronized flushes of growth and flowering.

The experiment was conducted at the Texas A&M University Research and Extension Center, Dallas, TX, in a 6 m x 12 m, glass-covered greenhouse (331200N latitude and 963400W longitude) fitted with an evaporative wet-pad cooling system and heat provided by thermostatically-controlled gas burners. Greenhouse temperatures were set at 25°C day and 16°C night. Temperature, humidity and photosynthetically active radiation were monitored with sensors connected to a Campbell CR510 Datalogger (Campbell Scientific Inc., Logan, UT).

On April 19, 2005 the treatments were implemented. A modified ½ strength Hoagland formulation (Hoagland and Arnon, 1950) was used as a base solution containing (in $mmol \cdot L^{-1}$): 9.0 N (8:1 NO_3^-:NH_4^+ ratio), 0.5 P (as $H_2PO_4^-$), 3.0 K, 2.25 Ca, 1.0 Mg, 1.0 S (as SO_4^{2-}), 1.0 $mg \cdot L^{-1}$ Fe as Fe-EDDHA and half-strength Hoagland's micronutrient concentration. Before adding any salts pH of the tap water was adjusted to 6.16±0.03 using concentrated HNO_3. Treatments (Table 3.1) included a non-salinized control (base solution) (solution 1), a series of NaCl-salinized (12.0 $mmol \cdot L^{-1}$) treatments with increasing levels of supplemental Ca (as $CaSO_4$) (solutions 2-6), and a Na_2SO_4-salinized treatment (6.0 $mmol \cdot L^{-1}$) plus 5.0 $mmol \cdot L^{-1}$ $CaSO_4$ (solution 7). The purpose of adding the first (control) and Na_2SO_4 treatments was to evaluate the effect of saline stress with additional Ca with respect to a non-salinized control, and to compare the influence of the Na^+ counter-anion (Cl^- versus SO_4^{2-}) on the response to the addition of Ca to the salinized nutrient solution.

Table 3.1. Saline solution treatments added to a base nutrient solution (modified half strength Hoagland solution), calculated electrical conductivity (EC) and sodium adsorption ratio (SAR).

Solution	NaCl ($mmol \cdot L^{-1}$)	Na_2SO_4 ($mmol \cdot L^{-1}$)	$CaSO_4$ ($mmol \cdot L^{-1}$)	Calculated EC[z]	SAR[y]
1	0.0	0.0	0.0	1.05	-
2	12.0	0.0	0.0	2.25	6.66
3	12.0	0.0	2.5	2.75	5.00
4	12.0	0.0	5.0	3.25	4.18
5	12.0	0.0	7.5	3.75	3.66
6	12.0	0.0	10.0	4.25	3.30
7	0.0	6.0	5.0	3.25	4.18

[z] Sum of cations or anions (in $meq \cdot L^{-1}$) divided by 10. This calculation includes the EC of the base solution, but does not include the EC provided by the tap water (average of 0.53 $dS \cdot m^{-1}$).
[y] $SAR=Na^+/[(Ca^{2+}+Mg^{2+})/2]^{1/2}$; cation concentrations expressed in $meq \cdot L^{-1}$.

Solutions were pumped from 150-L containers with submersible pumps (Model 2E-38N, Little Giant Pump Co., Oklahoma City, OK) feeding 1.3 cm polyethylene irrigation lines that supported black high flow spray–stake Spot Spitter® emitters (Roberts Irrigation Products, San Marcos, CA), connected via 3.2 mm spaghetti tubing. Each plant container was fitted with one calibrated emitter. Representative plants from selected treatments were routinely weighed to gravimetrically determine the evapotranspiration rate (ET). Total base irrigation volume consisted of ET plus an additional leaching target fraction of 25%.

Electrical conductivity (Portable Conductivity Meter, Model 2052, VWR International, Inc. Irving, TX), pH (pH/mV/Ion meter AP63 Accumet® portable; Fisher Scientific, Pittsburgh, PA) and Cl concentrations (Digital Chloridometer Model 4425000, Labconco Co., Kansas City, MO) were monitored on three leachate samples collected from selected treatments on a bi-weekly basis. Chloride concentrations were determined according to Adriano and Doner (1982).

Data collection

Plant productivity and flowering shoots quality

There were a total of five harvest events during this experiment. Flower shoots were harvested at commercial maturity, recording their dry weight (DW), number (FS), length (FSL) and leaf chlorophyll index (LCI; Chlorophyll meter, SPAD-502, Minolta Co. LTD, Japan) per plant. Harvested flower shoots were put into paper bags and oven-dried at 70^{o}C. At the end of the experiment, immediately after the fifth harvest of flowering shoots, three whole plants per treatment were destructively harvested and analyzed for nutrient content and biomass partitioning. Plants were cut into four portions: roots, main stem

(rootstock stem portion below the graft union), and scion old stems and old leaves.

Flowering shoots DW and FS per plant from each harvest were added to get the total flowering shoot DW and total FS harvested per plant. For LCI and FSL the average per harvest and total average from the five harvests were used in the data analysis.

Water relations measurements

Relative water content (RWC), stem water potential (SWP) and leaf osmotic potential (LOP) were determined for three selected plants from each treatment during each harvest. For RWC three leaflets from one flower shoot per plant were cut and weighed to determine their fresh weight (FW); soaked in deionized water in petri dishes and refrigerated for a 24 hour period to determine their turgid weight (TW); and oven-dried at 70^{o}C for 48 hours or until they recorded a stable weight to determine their dry weight (DW). Relative water content was calculated by the formula RWC=[(FW-DW)/(TW-DW)*100] (Jiang and Huang, 2001). Stem water potential was measured with a pressure chamber (Model 610, PMS Instrument, CO., Corvallis, Oregon, USA). Leaves of flower stems were covered with an aluminized Mylar envelope to synchronize their water potential with that from the stems at least two hours before measuring (Kim et al., 2004). Sampling time for all three variables was midday (between 12:00 PM and 1:30 PM) when the evaporative demand was at its peak. For leaf osmotic potential, the first five-leaflet leaf of a flowering shoot per plant was excised, covered with aluminum foil, placed in a plastic bag and transported to the laboratory in an ice cooler. After a 4-hour rehydration period with deionized water, petioles, rachis and petiolules were discarded and the leaf blades were frozen at -20^{o}C until analyzed. Before analyzing, samples were thawed for 15-18 min in the plastic bags. Tissue sap was then extracted with a

leaf press and collected on a filter disc which was immediately placed in a vapor pressure osmometer (Model 5520, Wescor, Inc., Logan UT). Osmometer readings in $mmol \cdot kg^{-1}$ were converted to MPa based on the van't Hoff equation (Nobel, 1983).

Tissue analyses

During each harvest the three uppermost five-leaflet leaves from each flowering shoot were collected and pooled for each plant, dried and ground (to pass a 40-mesh screen). Samples from harvests II (71 DAT) and IV (133 DAT) were sent to the Louisiana State University AgCenter Soil Testing and Plant Analysis Laboratory to be analyzed for total nutrient concentration. Phosphorous, K, Ca, Mg, S, B, Cu, Fe, Zn, and Na, were determinate by ICP procedures while N was determinate with a Leco N analyzer. Analysis of Cl in leaves of flowering shoots from all regular harvests, and Cl and Na in all the organs (roots, main stems, old stems and old leaves) after destructively harvesting the plants at the end of the experiment were made locally. Chloride in tissue was determined according to Gilliam (1971) with a digital chloridometer (Model 4425000, Labconco Co., Kansas City, MO) while Na was determined by flame emission with a Fast Sequential Atomic Absorption Spectrometer (AA240FS, Varian, Inc. Australia).

Salt burn damage

A salt burn rating evaluation was taken immediately after harvesting all the flowering shoots for harvests III, IV and V (99, 133 and 184 DAT) by two different evaluators 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 burn damage). This evaluation was performed on the leaves remaining on the plant

after harvesting the flowering shoots.

Experimental design and statistical analyses

The experimental design was a randomized complete block design (RCBD) with a factorial arrangement of treatments. 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. This yielded 14 different combinations with six replications (one pot with one plant was used as a replication) per combination for a total of 84 experimental units. For variables analyzed at different points in time (i.e. during the harvests of flowering shoots and leachate collection), additionally to RS and salt treatment, time (as days after treatment, DAT) was included as a third factor (repeated measures over time).

For some of the variables evaluated at harvests events over time (i.e. DW, FS, and FSL) data were first converted to a comparable scale (relative data) and then subjected to an arc sine transformation (Gomez and Gomez, 1984; detailed explanation included in Chapter II).

Data were analyzed by GLM, regression, correlation, mixed, and chi-square procedures using SAS ® 9.1 for Windows (SAS Institute Inc., Cary, NC). For all variables analyzed, there were four different statistical analysis performed. First, data from the NaCl-salinized treatment series with supplemental Ca (solutions 2-6) were analyzed as a factorial experiment, with RS and level of additional Ca as factors. Second, orthogonal contrasts between the control treatment (solution 1) and the NaCl-salinized treatment series with supplemental Ca were performed. Third, a pair-comparison between the control treatment (solution 1) and the Na_2SO_4-salinized treatment with 5.0 $mmol \cdot L^{-1}$ $CaSO_4$ (solution 7) was performed. Fourth, a pair-comparison between the NaCl and Na_2SO_4 salinized treatments, both with 5.0 $mmol \cdot L^{-1}$ $CaSO_4$ (solutions

4 and 7) was performed.

RESULTS

Leachate EC (EC_L), Cl concentration ($[Cl_L]$) and pH (pH_L)

Leaching fraction was similar between RS and among all treatments ($P>0.05$), averaging, throughout the whole experimental period, 25% and 28% for 'Manetti' and 'Natal Briar' plants, respectively.

There were interactive effects between salinity treatments and DAT for EC_L and $[Cl_L]$ ($P=0.0014$ and $P=0.0004$, respectively). In general, EC_L showed a significant increase during the first 66 DAT, remaining more or less stable for the rest of the experimental period (Fig. 3.1A). Chloride concentration in leachates followed basically the same pattern for the NaCl-salinized series (Fig. 3.1B). In the control and the Na_2SO_4-salinized treatments (no additional Cl supplied in these treatments) $[Cl_L]$ remained very low. However, in leachates from the Na_2SO_4 treatment $[Cl_L]$ exhibited a quadratic pattern, slightly increasing by the final stages of the experimental period (Fig. 3.1B).

Leachate pH was affected by DAT ($P<0.0001$). Opposite to EC_L and $[Cl_L]$, pH_L decreased considerably during the first 66 DAT, showing smaller rate changes for the rest of the experimental period (Fig. 3.1C).

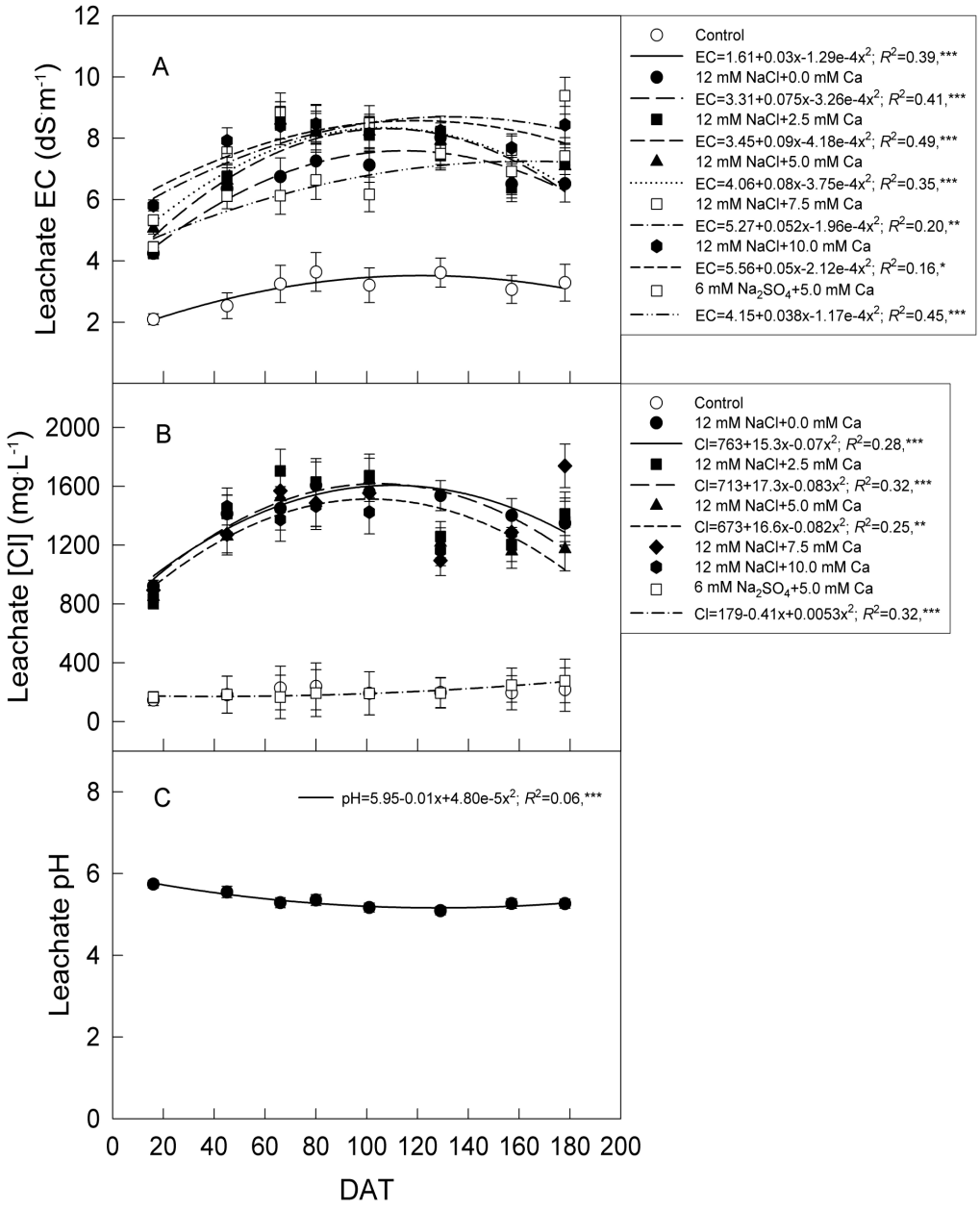

Fig. 3.1. (A) Electrical conductivity (EC); (B) Cl concentration ([Cl]); and, (C) pH in leachates from 'Happy Hour' roses subjected to a moderately high NaCl or Na_2SO_4 based salinity in a half strength Hoagland's solution amended with increasing levels of supplemental calcium. Symbols represent the mean ± standard error of 12 plants for plots A and B and of 84 plants for plot C. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

For the NaCl-salinized series (treatments 2-6), RS and level of supplemental Ca had interactive effects on EC_L, $[Cl_L]$ and pH_L (P=0.0045, P=0.0018 and P<0.0001, respectively). Electrical conductivities of leachates from 'Manetti' plants tended to increase as the concentration of supplemental Ca in the saline solution increased while in 'Natal Briar' EC_L remained the same across Ca levels (Fig. 3.2). Chloride concentration was similar across Ca levels for 'Manetti' plants while in 'Natal Briar' it tended to decrease linearly as the levels of Ca increased (Fig. 3.2). In both RS pH_L showed a quadratic response, decreasing as the concentration of supplemental Ca increased, although the decrease was slightly steeper for 'Manetti' plants (Fig. 3.2).

Leachate electrical conductivity and $[Cl_L]$ were lower in leachates from control plants compared to those from the NaCl-salized series, as expected (Fig. 3.2). 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).

Between the control and the Na_2SO_4 treatments, there were no differences between RS in EC_L and $[Cl_L]$ (P>0.05; Fig 3.2). On the other hand, RS affected pH_L (P=0.0185) with 'Natal Briar' plants having significantly greater pH_L averages (5.92 versus 5.61, respectively; Fig. 3.2). 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; Fig. 3.2). Chloride concentration was the same between RS and between treatments (Fig. 3.2).

Comparing between the 12 $mmol \cdot L^{-1}$ NaCl and 6 $mmol \cdot L^{-1}$ Na_2SO_4 both at the 5 $mmol \cdot L^{-1}$ supplemental Ca level, there were no differences between RS for EC_L and $[Cl_L]$ (P>0.05 for both; Fig. 3.2) while an interaction was present between RS and treatments for pH_L (P<0.0001). The NaCl-salinized treatment had greater EC_L and $[Cl_L]$ values (averages of 7.28 versus 6.42 $dS \cdot m^{-1}$ and 1289 versus 203 $mg \cdot L^{-1}$, respectively) (Fig. 3.2). Leachate pH was greater in the NaCl treatment only for 'Natal Briar' plants (Fig. 3.2).

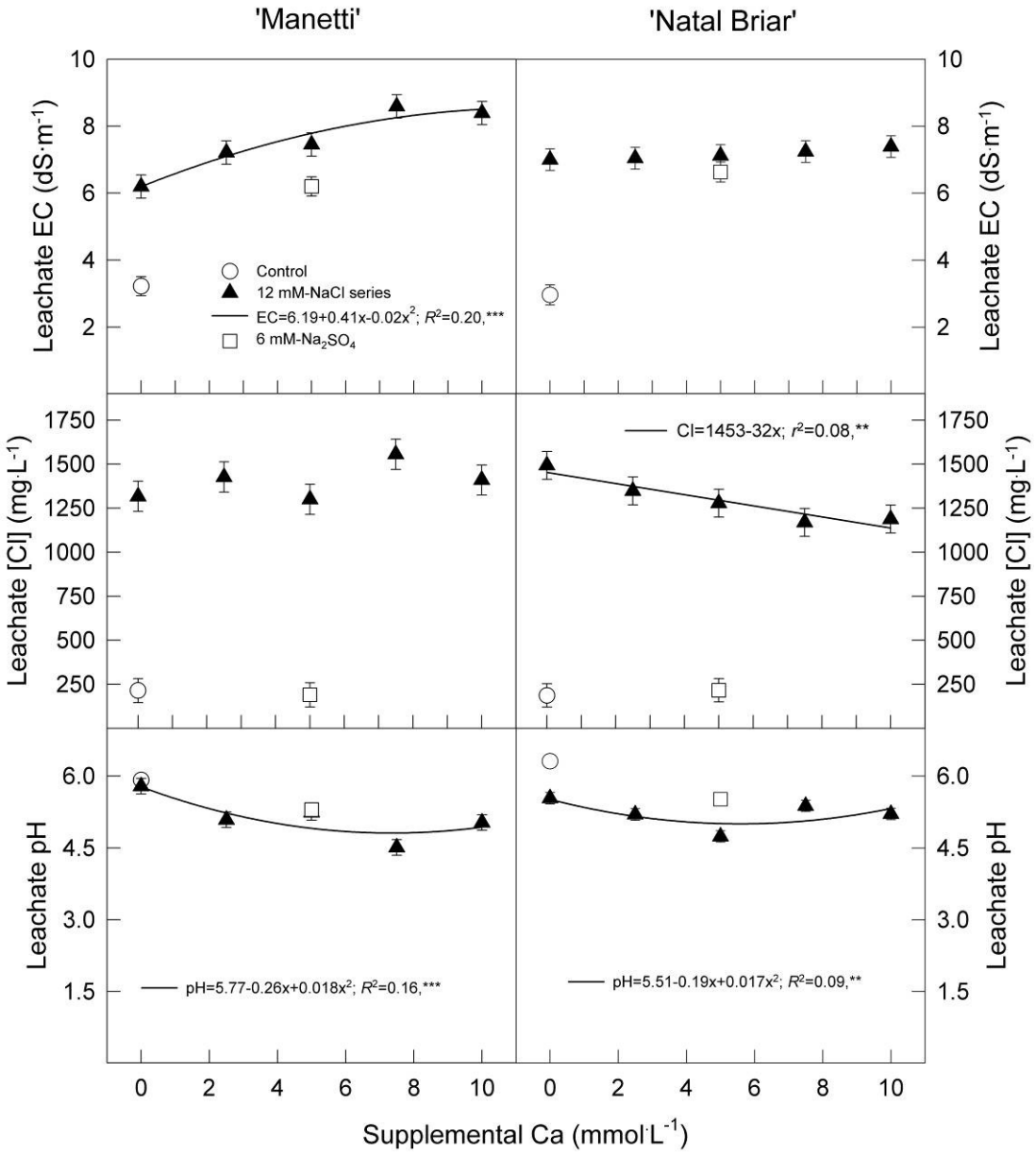

Fig. 3.2. Electrical conductivity (EC), Cl concentration [Cl] and pH in leachates from 'Happy Hour' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high NaCl or Na_2SO_4 based salinity in a half strength Hoagland's solution amended with increasing levels of supplemental calcium. Symbols represent the mean ± standard error of 12 plants for plots A and B and of 84 plants for plot C. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Biomass and flower productivity

Flowering shoot DW and FS harvested per plant, FSL and LCI had similar patterns over time in both RS and all salt treatments (P>0.05; results not shown).

Across salt treatments 'Manetti' plants had greater total flowering shoot DW harvested per plant than 'Natal Briar' plants (P=0.0065; 137 g versus 124 g, respectively). Total FS harvested per plant, and total average FSL and LCI were similar in both RS (P>0.05; results not shown).

Total flowering shoot DW and total FS harvested per plant, and total average FSL 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. 3.3A-D).

Compared to the NaCl-salinized treatments, plants from the control treatment had slightly greater total flowering shoot DW and total FS (P=0.0533 and P=0.004, respectively) (Fig. 3.3A and Fig. 3.3B). Total average FSL and LCI were similar (P>0.05; Fig. 3.3C and Fig. 3.3D).

When comparing control plants and those salinized with Na_2SO_4, total flowering shoot DW and total FS harvested per plant, and total average LCI were similar (P>0.05; Fig. 3.3A, Fig. 3.3B, and Fig. 3.3D). Flowering shoots from plants under Na_2SO_4 were on average 1.42 cm longer than those from the control treatment (P=0.0059; Fig. 3.3C).

On the other hand, when comparing the NaCl and Na_2SO_4 salt treatments at the 5.0 $mmol \cdot L^{-1}$ Ca level, total flowering shoot DW and total FS were lower when exposed to NaCl than under Na_2SO_4 (P=0.0106 and 0.0404, respectively; Fig. 3.3A and Fig. 3.3B). Flowering shoot length and LCI were not affected by the salt composition (P>0.05; Fig. 3.3C and Fig. 3.3D).

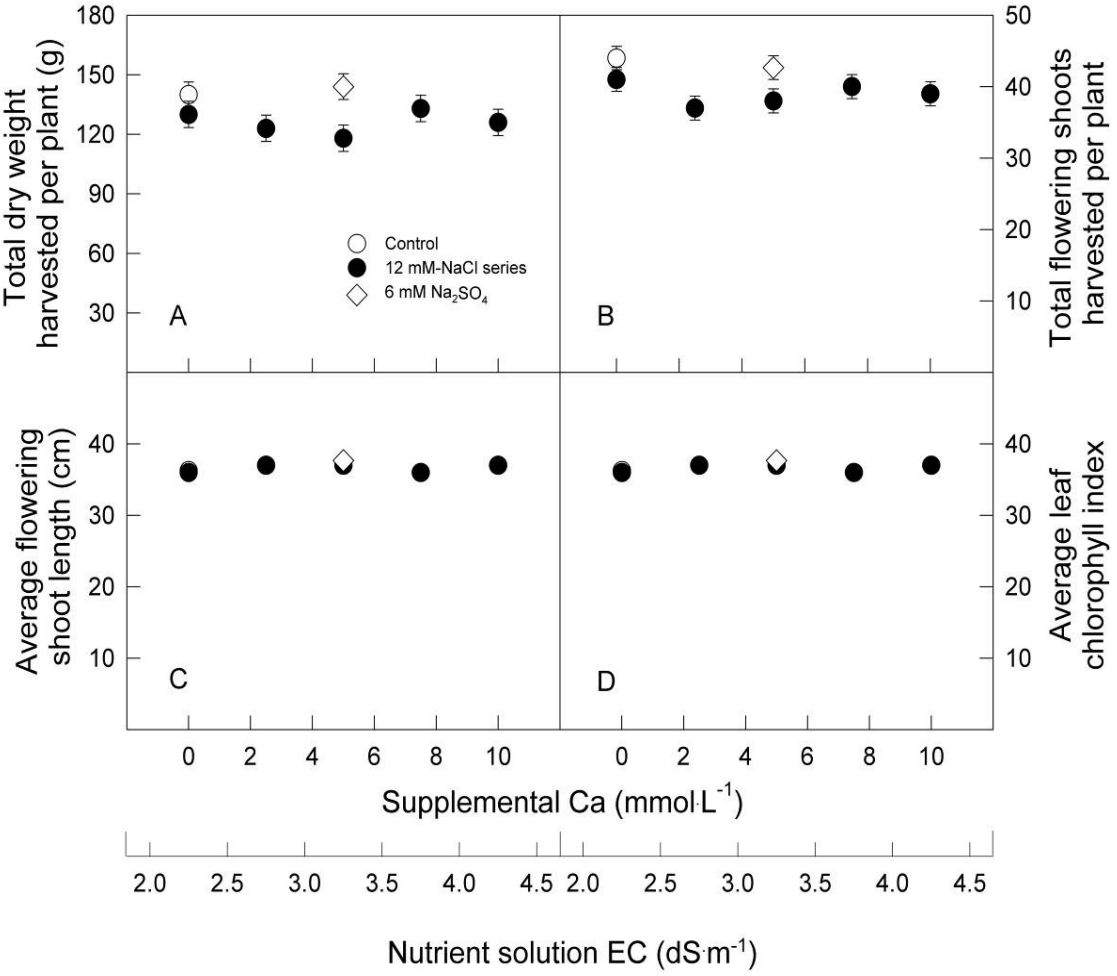

Fig. 3.3. (A) Total dry weight and (B) flowering shoots harvested per plant; (C) total average flowering shoot length and (D) leaf chlorophyll index of 'Happy Hour' roses subjected to a moderately high NaCl or Na_2SO_4 based salinity in a half strength Hoagland's solution amended with increasing levels of supplemental calcium. Nutrient solution EC represents that due to the addition of supplemental salts (NaCl, Na_2SO_4 and $CaSO_4$); EC for the control would be approximately 1.05 $dS \cdot m^{-1}$ (tap water EC~0.6 $dS \cdot m^{-1}$ not included). Symbols represent the mean ± standard error of 12 plants. Symbols obscure the error bars that are not apparent.

For the destructive harvest of the whole plants at the end of the experiment, across salt treatments 'Manetti' plants had greater old stems and

old leaves DW and lower root:shoot ratio (P=0.0289, 0.0296 and 0.0002; 69 g versus 60 g, 10.8 g versus 9.0 g, and 0.50 versus 0.58, for stems and leaves dry weights and root:shoot ratio, respectively). Roots and main stem DW were similar between RS (P>0.05), averaging 18 g and 22 g, respectively.

Level of supplemental Ca did not affect DW of any of the organs evaluated (P>0.05 for all organs; results not shown). Similarly, there were no differences in all variables evaluated when comparing the control with both the series of NaCl-salinized treatments and with the Na_2SO_4 treatments (P>0.05 for all variables in both cases; results not shown).

When comparing 12.0 $mmol \cdot L^{-1}$ NaCl versus 6.0 $mmol \cdot L^{-1}$ Na_2SO_4, DW of roots, main stem and old stems were the same between treatments (P>0.05 for all three variables), while old leaves DW was greater for those plants subjected to Na_2SO_4 compared to those under NaCl-salinity (P=0.032; 11.9 g versus 8.2 g, respectively).

Foliar salt injury

Data for salt damage were available from three different evaluation dates (99, 133 and 184 DAT). Three different Chi-square tests were performed for the salt injury data. First, to determine if the salt damage was influenced by the rootstock selection data from all salt treatments were pooled within RS for each evaluation date. In this particular case, categories four and five were pooled due to several cells yielding counts less than five (detailed explanation of procedure in Chapter II). Second, to determine the influence of the supplemental Ca on foliage salt burn, data from both RS were pooled within each Ca-level of the NaCl-salinized treatments and analyzed for the last evaluation date at 184 DAT (when salt damage was more evident). For this test, categories 0 to 4 were combined since most of the plants fell into the two highest salt burn categories (4 and 5). Third, to determine the effect of the Na-accompanying anion on the

foliage salt burn, the 12 mmol·L^{-1} NaCl and the 6 mmol·L^{-1} Na_2SO_4 treatments (both supplemented with 5 mmol·L^{-1} Ca) were compared for the last evaluation date (184 DAT). In this case categories 0, 1 and 2 were combined together in one single category and the same done was for 3, 4 and 5.

The extent of salt damage on the plants' basal foliage (old leaves left after harvesting cut-flower shoots) was similar between RS for all three evaluation dates (X>0.05 for all dates; Fig. 3.4). Plants from the control treatment in both RS showed, in general, little foliar salt injury (data corresponding to the first bar from each date in Fig. 3.4). As the exposure time increased, salt damage ratings for the rest of the salt treatments (other than the control) continued to increase with notably greater percentages of plants (74% for 'Manetti' and 60% for 'Natal Briar') falling into the greatest salt burn category for the last evaluation date at 184 DAT (Fig. 3.4). As for the foliage of flowering shoots (harvested at the end of each flowering cycle) salt burn injury as bronzing edges on the shoot basal leaves was evident on 27% of the NaCl-salinized plants by the harvest event III at 99 DAT. The percentage of plants exhibiting salt damage in the NaCl-series increased to 43% by the last harvest event at 184 DAT. Plants from the non-salinized control and those subjected to Na_2SO_4 did not show substantial salt burn injury symptoms on the foliage of flowering shoots.

Increasing the levels of supplemental Ca in the saline solution did not affect (positively or negatively) the foliage salt damage caused in the NaCl-series (X=0.2936).

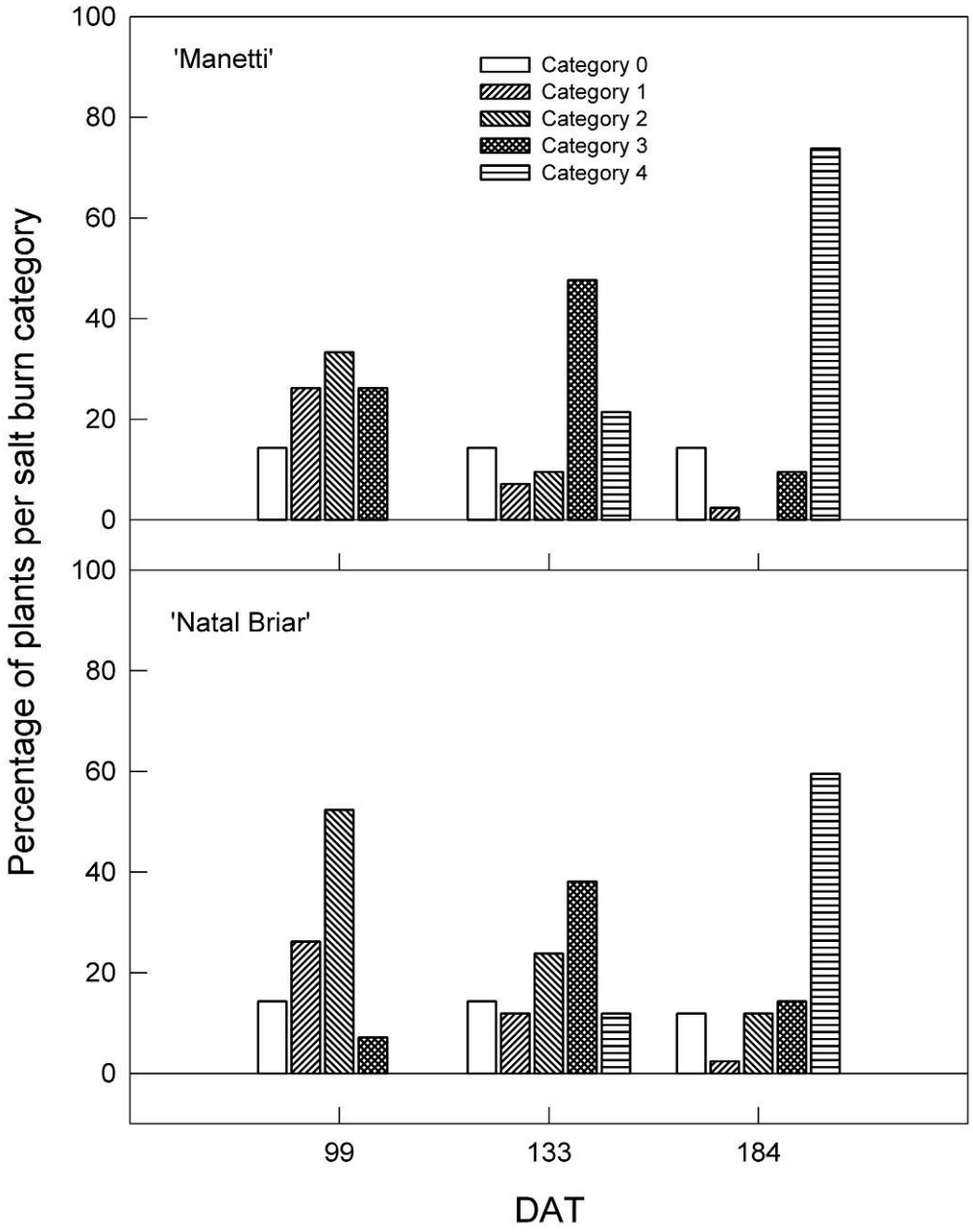

Fig. 3.4. Salt damage ratings of foliage of 'Happy Hour' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately-high salinity in a half strength Hoagland's nutrient solution amended with increasing levels of supplemental Ca. Salt damage rating is based on a 0 to 5 scale in which 0=no visible damage, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80%, and 5=81-100% of the foliage exhibiting salt burn damage. (n=42).

The Na-accompanying anion had a strong influence on the extent of salt damage on the plants' basal foliage (X=0.0047). All of the plants from the NaCl treatment exhibited salt damage on 41% of the foliage or more (salt burn categories 3-5) while in the Na_2SO_4 salt treatment 50% of the plants had salt damage on less than 40% of foliage (categories 0-2) and 50% had salt burn on 41% or more (categories 3-5; Fig. 3.5).

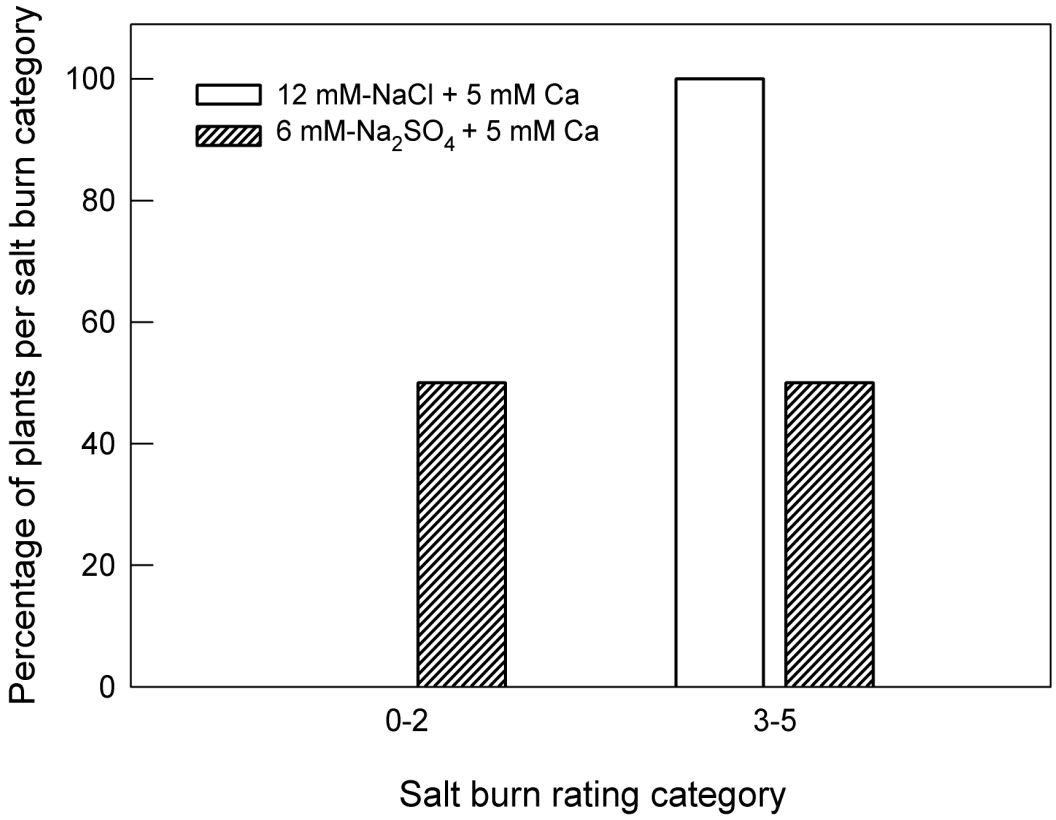

Fig. 3.5. Salt damage ratings of foliage of 'Happy Hour' roses subjected to a NaCl- or Na_2SO_4-based salinity in a half strength Hoagland's nutrient solution amended with 5 $mmol \cdot L^{-1}$ of supplemental Ca at 184 DAT. Salt damage rating is based on a 0 to 5 scale in which 0=no visible damage, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80%, and 5=81-100% of the foliage exhibiting salt burn damage. (n=12).

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. 3.6A-C).

The non-salinized control plants had greater values for RWC, SWP and LOP than plants from the NaCl-salinized series (P=0.0175, 0.0049, and 0.0118, respectively) (Fig. 3.6A-C). Similarly, RWC and SWP were greater in the non-salinized control plants than in those subjected to the Na_2SO_4 salt treatment (P=0.0226 and 0.0147, respectively) (Fig. 3.6A-B). In this last comparison (control versus the Na_2SO_4 salt treatment), the RS selection affected RWC and SWP (P=0.0450 and 0.0111, respectively) with 'Manetti' plants exhibiting greater values for both variables (91.5% versus 90.4% for RWC and -0.71 MPa versus -0.79 MPa for SWP, respectively).

On the other hand, when comparing the NaCl and the Na_2SO_4 salt treatments RWC and SWP were statistically the same between salts (P>0.05 for both), while LOP was slightly less negative for plants under Na_2SO_4 than in those under NaCl (P=0.058; -1.12 MPa versus -1.41 MPa, respectively) (Fig. 3.6A-C). Stem water potential was affected by RS (P=0.0089) with 'Manetti' plants having less negative values than 'Natal Briar' plants (-0.73 MPa versus -0.84 MPa, respectively).

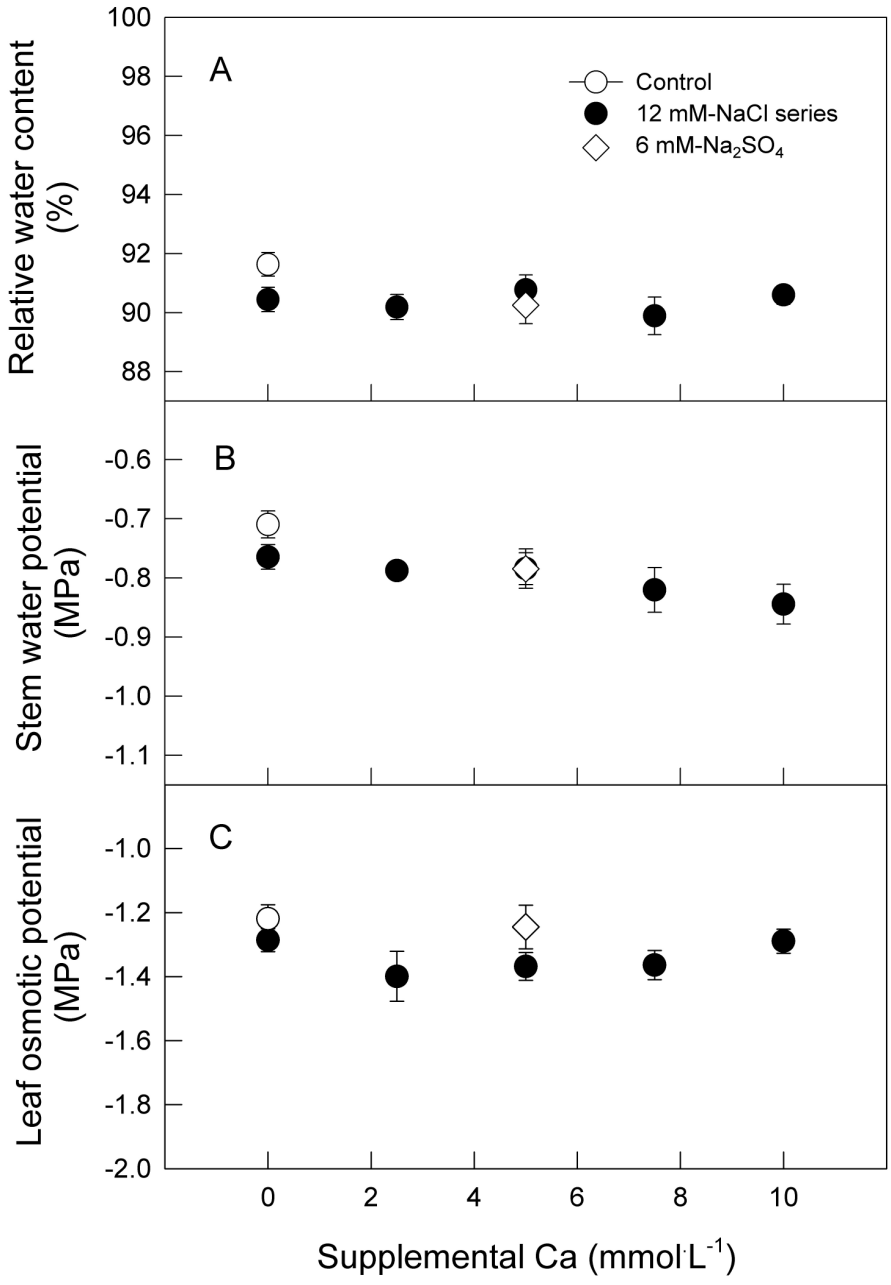

Fig. 3.6. (A) Relative water content, (B) stem water potential, and (C) leaf osmotic potential of 'Happy Hour' roses subjected to a moderately high NaCl or Na_2SO_4 based salinity in a half strength Hoagland's solution amended with increasing levels of supplemental calcium. Symbols represent the mean ± standard error of 12 plants. Symbols obscure the error bars that are not apparent.

Tissue mineral nutrient content

Chloride [Cl], sodium [Na], and calcium [Ca] concentrations

Chloride [Cl]. Even though leaf [Cl] was measured for all five harvest events, in the last harvest (V, at 184 DAT) many flowering shoots did not have enough leaves to run Cl analysis and some plants did not bear any flowering shoots. In the Mixed Procedure when one or more data are missing for an experimental unit, this experimental unit is eliminated for the entire experimental period. To avoid this, only data from harvests I-IV were included in the statistical analysis for repeated measures to determine patterns of leaf Cl accumulation over time.

There were no interactions between RS and DAT ($P>0.05$). Thus, data for both RS within every salt treatment were pooled accordingly leaving only DAT and salt treatments as factors. Similarly, data from all six NaCl-salinized treatments showed similar leaf [Cl] and it increased at similar rates over time (no effects due to Ca level and no interactions between Ca level and DAT were present; $P>0.05$) allowing for the data to be pooled and analyzed as one single NaCl treatment over time.

For the NaCl-series of treatments increases of leaf [Cl] were small for the first two months followed by a greater increase between 60 and 120 DAT, with a decreasing accumulation again for the last phase of the experimental period (Fig. 3.7). Plants for the control treatment showed a low, constant [Cl] accumulation over time (Fig. 3.7). On the other hand, plants from the Na_2SO_4 salt treatment, even though they did not receive any additional Cl in the irrigation water (same as the control treatment), exhibited a quadratic pattern with significant increases in leaf [Cl] in the last phase of the experimental period (Fig. 3.7).

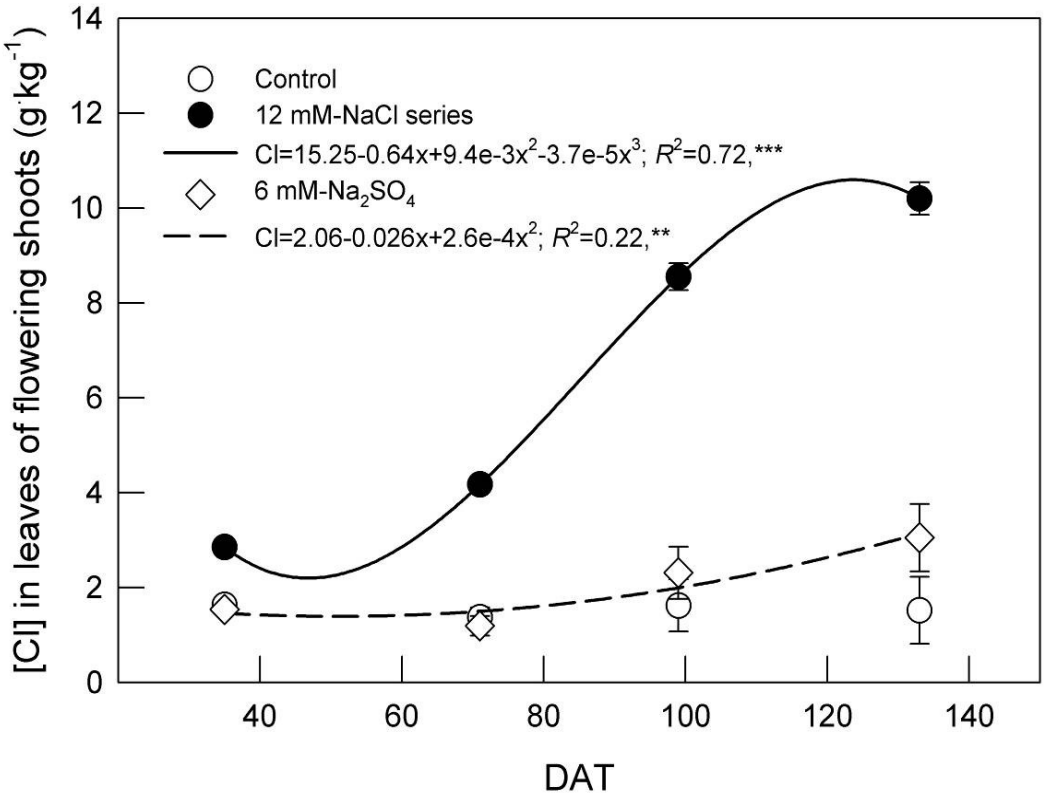

Fig. 3.7. Leaf chloride concentration over time in flowering shoots of 'Happy Hour' roses subjected to a moderately high NaCl or Na_2SO_4 based salinity in a half strength Hoagland's solution amended with increasing levels of supplemental calcium. Symbols represent the mean ± standard error of 12 plants for the control and Na_2SO_4 treatments and of 72 plants for the NaCl-series of treatments. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Overall leaf [Cl] averages (five harvests averaged) were 1.68±0.09, 7.15±0.02 and 2.07±0.17 $g \cdot kg^{-1}$ for the control, NaCl-series and the Na_2SO_4 salt treatments, respectively. Leaf [Cl] of plants in the NaCl-series were greater than those from the control by 326% ($P<0.0001$) and than those from the Na_2SO_4 salt treatment by 246% ($P<0.0001$).

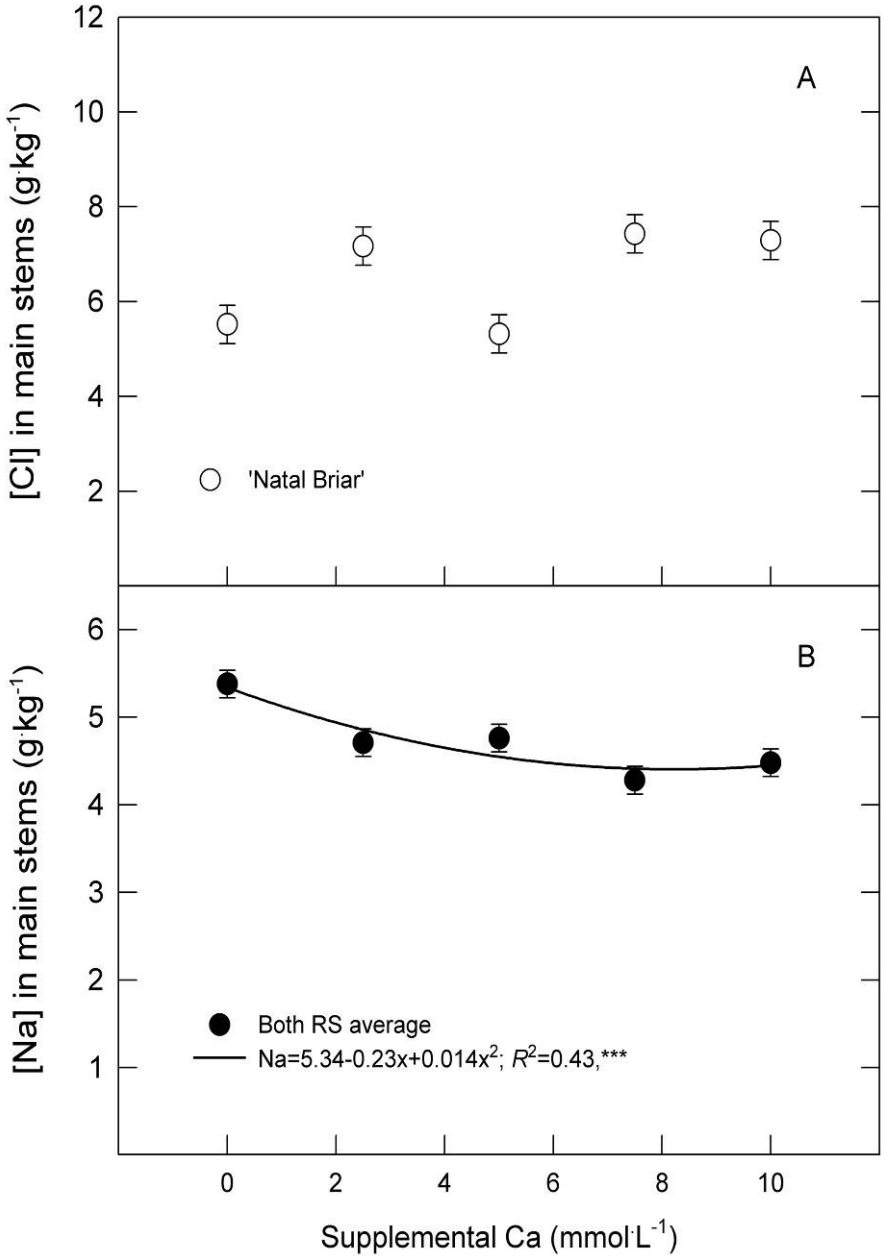

Fig. 3.8. Effect of supplemental Ca on chloride (A) and sodium (B) concentrations of main stems of 'Happy Hour' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high NaCl or Na_2SO_4 based salinity in a half strength Hoagland's solution. Symbols represent the mean ± standard error of 3 plants for plot A and 6 plants for plot B. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

When comparing leaf [Cl] total averages between plants from the control and Na_2SO_4 treatments an interaction between RS and salt composition treatment was present (*P*=0.0401). 'Natal Briar' plants showed similar leaf [Cl] averages in both salt treatments (average of 1.77 $g \cdot kg^{-1}$) while in 'Manetti' plants, those subjected to Na_2SO_4 had greater average leaf [Cl] than those from the control plants (2.39 g·kg-1 versus 1.58 $g \cdot kg^{-1}$, respectively).

For the destructive harvest of whole plants, in the NaCl-salinized series there were no effects due to RS or Ca level on [Cl] in roots, old stems or old leaves (*P*>0.05), with general averages of 7.50 $g \cdot kg^{-1}$, 6.84 $g \cdot kg^{-1}$, and 17.6 $g \cdot kg^{-1}$, respectively. As for [Cl] in main stems there was an interaction between RS and level of supplemental Ca (*P*=0.0007). In 'Manetti' plants there were no differences in main stems [Cl] among Ca levels (*P*>0.05; results not shown). In 'Natal Briar' plants there were statistical differences in main stems [Cl] among levels of Ca (*P*=0.0075; Fig. 3.8A), however the regression models were not significant (*P*>0.05).

Chloride concentrations were greater by 24%, 33%, 87% and 164% in roots, main stems, old stems and old leaves, respectively, in plants from the NaCl-salinized series compared to those from the control treatment (*P*=0.0304 for roots and *P*<0.0001 for the rest of the organs) (Fig. 3.9A).

When comparing the control and Na_2SO_4 treatments (both treatments without additional Cl in the irrigation water), [Cl] was similar between RS and salt treatments for main stem, old stems and old leaves (Fig. 3.9A). As for [Cl] in roots an interaction between RS and salt treatment was present (*P*=0.0145). Root [Cl] was similar for both salt treatments in 'Natal Briar' plants (average of 6.0 $g \cdot kg^{-1}$), while in 'Manetti' [Cl] was greater in roots from plants subjected to Na_2SO_4 than those from the control treatment (8.1 versus 6.0 $g \cdot kg^{-1}$, respectively).

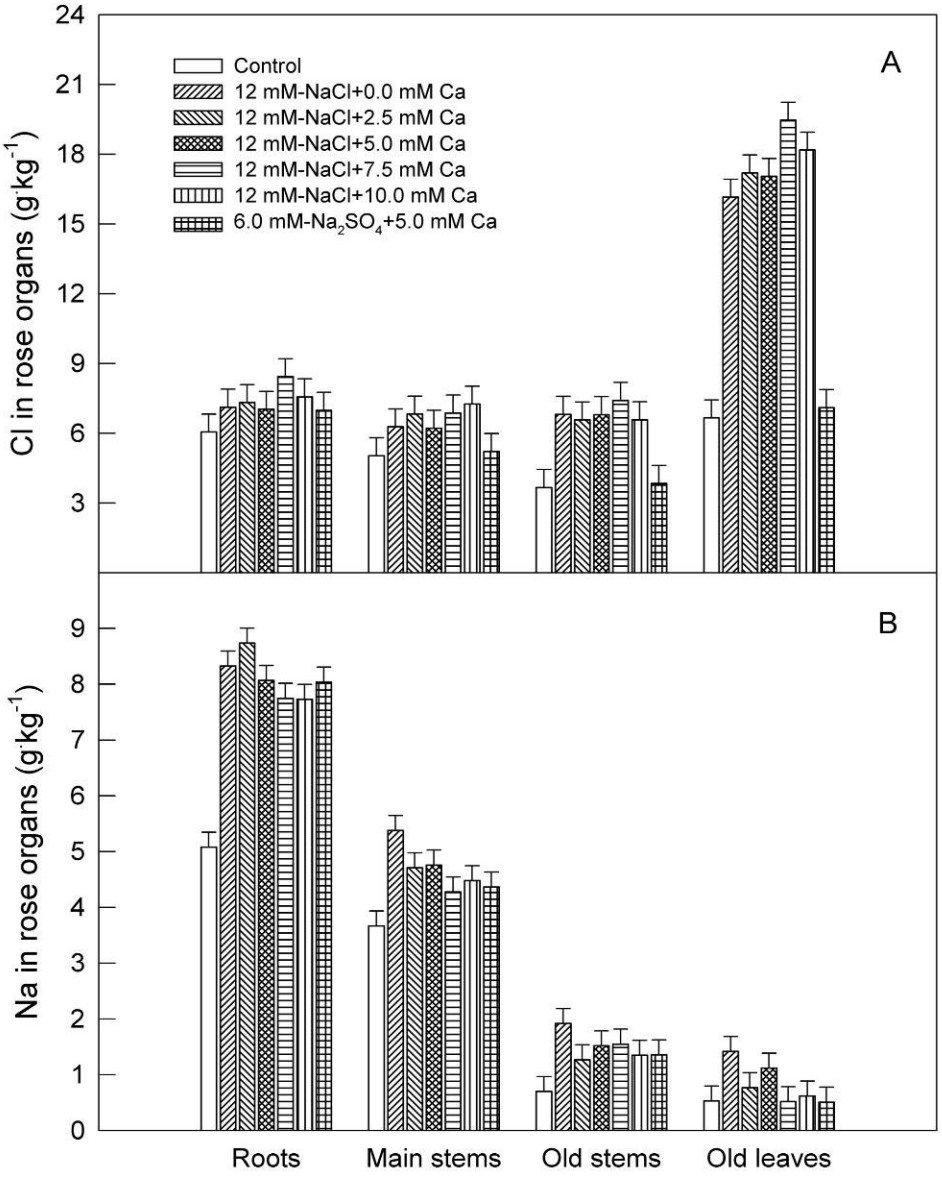

Fig. 3.9. (A) Chloride and (B) sodium concentrations in plant organs of 'Happy Hour' roses subjected to a moderately high NaCl or Na_2SO_4 based salinity in a half strength Hoagland's solution amended with increasing levels of supplemental calcium. Bars represent the mean ± standard error of 12 plants.

When comparing NaCl and Na_2SO_4 salt treatments at the 5.0 $mmol \cdot L^{-1}$ supplemental Ca level, 'Manetti' plants had greater [Cl] in roots (38%) and main stem (31%) than 'Natal Briar' plants (RS effect, *P*=0.0143 for roots and *P*=0.0095 for main stem). Root [Cl] was the same for both salt treatments (*P*>0.05) while in the main stems, old stems and old leaves [Cl] was greater for plants subjected to the NaCl salt by 19, 77 and 140%, respectively (salt composition effect, *P*=0.0542, 0.0007 and 0.0019, respectively) (Fig. 3.9A).

Sodium [Na]. For all salt treatments sodium concentrations [Na] of flowering shoots leaves were similar between harvests II and IV at 71 and 133 DAT (*P*>0.05; Table 3.2), ranging from 63-103 $mg \cdot kg^{-1}$. Within the NaCl-series, RS and level of supplemental Ca did not affect leaf [Na] of flowering shoots (*P*>0.05; Table 3.3). Control plants had the same leaf [Na] than those receiving NaCl and than those receiving Na_2SO_4 (*P*>0.05 for both comparisons). Similarly, plants from the Na_2SO_4 salt treatment had the same leaf [Na] than those from the NaCl at the 5.0 $mmol \cdot L^{-1}$ Ca level (*P*>0.05). The general leaf [Na] average of flowering shoot leaves was 79 $mg \cdot kg^{-1}$.

For the destructive harvest of whole plants, in the NaCl-series, both RS had the same [Na] in all organs evaluated except for roots, with 'Manetti' plants having slightly greater [Na] (8.5 $g \cdot kg^{-1}$ versus 7.7 $g \cdot kg^{-1}$ in 'Natal Briar'; *P*=0.0565). Level of supplemental Ca caused a decrease in the [Na] of main stems from both RS (*P*=0.0012; Fig. 3.8B).

Between the control and the NaCl-series, [Na] concentrations were greater on average for the NaCl-series in roots (60%), main stems (29%), and old stems (117%) (Fig. 3.9B). Sodium concentration in old leaves was statistically the same between the control and the NaCl series of treatments (*P*>0.05; Fig. 3.9B).

Table 3.2 Mineral nutrient concentration in leaves of flower shoots of 'Happy Hour' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to moderately high NaCl or Na_2SO_4 based salinity in a half strength Hoagland's nutrient solution amended with increasing levels of supplemental calcium. Leaf samples correspond to harvests events II and IV (at 71 and 133 DAT). Means are the average of 3 plants.

		($g \cdot kg^{-1}$)						($mg \cdot kg^{-1}$)				
'Manetti'	DAT	N	P	K	Ca	Mg	S	Mn	Fe	B	Zn	Na
Control	*71*	30.6	2.62	20.6	17.77	2.33	2.16	219	66.7	39.3	33.3	84.0
	133	28.3	2.70	21.3	11.54	1.89	2.00	179	56.0	37.3	18.7	69.3
	Difference	2.3	-0.08	-0.7	6.23	0.44	0.16	40	10.7	2.0	14.6	14.7
	Significance	ns	ns	ns	*	ns	*	ns	ns	ns	*	ns
12 mM NaCl series	*71*	28.5	2.44	20.1	17.4	2.13	2.07	290	58.3	37.5	35.9	73.1
	133	28.4	2.66	23.0	13.9	1.82	2.01	192	58.3	42.7	20.1	70.8
	Difference	0.1	-0.22	-2.9	3.5	0.31	0.06	98	0.0	-5.2	15.8	2.3
	Significance	ns	*	***	***	***	ns	***	ns	***	***	ns
6 mM Na_2SO_4	*71*	28.8	2.57	19.2	15.1	2.08	2.17	162	56.3	43.0	32.2	83.3
	133	27.6	2.67	22.8	11.9	1.85	1.86	177	53.3	39.7	19.7	64.0
	Difference	1.2	-0.1	-3.6	3.2	0.23	0.31	-15.0	3.0	3.3	12.5	19.3
	Significance	ns	ns	*	*	*	ns	ns	ns	ns	*	ns
'Natal Briar'	DAT	N	P	K	Ca	Mg	S	Mn	Fe	B	Zn	Na
Control	*71*	29.4	2.41	20.1	16.0	2.29	2.19	173	60.7	37.7	31.7	103
	133	29.5	2.73	20.7	12.4	2.12	2.03	217	59.0	40.7	18.0	63
	Difference	-0.1	-0.32	-0.6	3.6	0.17	0.16	-44	1.7	-3.0	13.7	40.0
	Significance	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns
12 mM NaCl series	*71*	28.6	2.56	20.6	16.5	2.15	2.11	238	60.6	37.9	34.1	89.5
	133	28.5	2.65	22.9	13.8	1.92	2.06	175	56.1	38.2	19.3	86.3
	Difference	0.1	-0.09	-2.3	2.7	0.23	0.05	63	4.5	-0.3	14.8	3.2
	Significance	ns	ns	***	**	**	ns	**	ns	ns	***	ns
6 mM Na_2SO_4	*71*	30.0	2.51	19.5	15.1	2.13	2.10	150	59.3	38.3	37.0	81.0
	133	29.0	2.77	21.8	12.4	1.95	2.01	200	53.0	41.0	18.0	65.3
	Difference	1.0	-0.26	-2.3	2.7	0.18	0.09	-50	6.3	-2.7	19.0	15.7
	Significance	ns	ns	ns	*	ns	ns	ns	ns	ns	*	ns

Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Table 3.3. Effect of increasing levels of supplemental Ca in a half strength Hoagland's nutrient solution salinized with NaCl or Na_2SO_4 on leaf mineral nutrient concentration of flower shoots of greenhouse 'Happy Hour' roses budded on 'Manetti' and 'Natal Briar' rootstocks. Leaf samples correspond to harvests II (71 DAT) and V (133 DAT). Means are the average of 4 plants.

'Manetti'	Ca level ($mmol \cdot L^{-1}$)	N	P	K	Ca	Mg	S	Mn	Fe	B	Zn	Na
	0.0	28.0	2.57	21.6	13.7	2.06	2.04	168	62.3	40.5	24.3	88.2
	2.5	26.8	2.50	20.3	15.8	1.99	1.98	228	52.0	42.7	27.2	67.8
	5.0	28.3	2.51	21.7	14.9	1.96	2.01	236	57.5	39.2	28.0	66.5
	7.5	29.1	2.67	22.2	16.7	1.97	2.11	280	60.8	39.7	31.8	66.2
	10.0	29.9	2.50	22.7	16.6	1.92	2.07	293	58.8	38.5	28.5	71.0
	Sig.,Model/R^2 Parameters	*L/0.17 B_o=22 B_1=0.4	ns	ns	ns	ns	ns	***L/.32 B_o=180 B_1=12	ns	ns	ns	ns
'Natal Briar'	Ca level ($mmol \cdot L^{-1}$)	N	P	K	Ca	Mg	S	Mn	Fe	B	Zn	Na
	0.0	31.0	2.89	21.6	14.7	2.15	2.26	190	63.0	36.8	27.2	90.8
	2.5	28.3	2.61	20.7	13.7	1.96	2.03	167	58.7	37.2	25.2	62.5
	5.0	28.1	2.57	22.3	17.0	2.07	2.07	238	60.3	36.8	28.8	122.2
	7.5	28.1	2.55	23.1	16.3	2.05	2.10	212	56.8	40.0	28.0	89.1
	10.0	27.2	2.42	21.2	14.1	1.95	1.96	227	53.0	39.3	24.2	74.8
	Sig.,Model/R^2 Parameters	**C/.34 B_o=31 B_1=-1.7 B_2=0.3 B_3=-0.02	**C/.37 B_o=2.9 B_1=-0.2 B_2=.03 B_3=-.002	ns	ns	ns	*C/.36 B_o=2.3 B_1=-0.17 B_2=0.04 B_3=-0.002	ns	*L/.19 B_o=63 B_1=-0.87	ns	ns	ns

Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

As for the comparison between the control and the Na_2SO_4 salt treatments there was an effect due to RS for old leaves [Na] (P=0.0469). Old leaves from 'Natal Briar' had 78% greater [Na] than those from 'Manetti' plants (0.66 versus 0.37 $g \cdot kg^{-1}$, respectively). Sodium concentration in roots, main stems and old stems were similar between RS (P>0.05). On the other hand, there were differences between these two salt treatments for [Na] in roots, main stems and old stems (P>0.0007, 0.0153 and 0.0242, respectively). Sodium concentrations were greater by 58% in roots, 19% in main stems and 95% in old stems in plants subjected to the Na_2SO_4 salt treatment than those from the control (Fig. 3.9B). In old leaves [Na] was the same between both treatments (P>0.05; Fig. 3.9B).

Comparing the NaCl and Na_2SO_4 salt treatments there were no effects due to RS selection or salt treatments for [Na] in main stems, old stems and old leaves (P>0.05; Fig. 3.9 B) with overall averages of 4.57 $g \cdot kg^{-1}$, 1.44 $g \cdot kg^{-1}$, and 0.81 $g \cdot kg^{-1}$, respectively. There was an interaction between RS and salt treatment (P=0.0527) for root [Na]. In the Na_2SO_4 salt treatment there was no difference between RS in root [Na], average of 8.04 $g \cdot kg^{-1}$. However, in the NaCl salt treatment [Na] was greater in roots of 'Manetti' plants by 30% (9.13 versus 7.02 $g \cdot kg^{-1}$, respectively).

Calcium [Ca]. Leaf [Ca] decreased from 71 to 133 DAT in both RS for the NaCl-series and the Na_2SO_4 salt treatment, and in 'Manetti' plants from the control treatment (Table 3.2). In control plants from 'Natal Briar' leaf [Ca] decreased from 71 to 133 DAT as well, however, the decrease was not significant (Table 3.2).

Within the NaCl-series there was an interaction between RS and Ca level for leaf [Ca] (P=0.0275), however none of the regression models were significant (Table 3.3).

Plants from the control treatment had similar leaf [Ca] compared to those from the NaCl-series and to those from the Na_2SO_4 salt treatment (P>0.05 in

both comparisons). Alternatively, plants from NaCl had greater leaf [Ca] than those from the Na_2SO_4 salt treatment at the 5.0 $mmol \cdot L^{-1}$ Ca level (16.0 $g \cdot kg^{-1}$ versus 13.6 $g \cdot kg^{-1}$, respectively; P=0.0399).

Leaf concentration of other mineral nutrients

Leaf sulfur [S] and zinc [Zn] concentrations decreased from harvest II (71 DAT) to harvest IV (133 DAT) in control plants from both RS (Table 3.2). For the NaCl-series leaf magnesium [Mg], manganese [Mn] and [Zn] decreased for both RS, potassium [K] increased also in both RS, while phosphorous [P] and boron [B] increased only in 'Manetti' plants (Table 3.2). As for the Na_2SO_4 salt treatment, [Mg] and [Zn] decreased and [K] increased in 'Manetti' plants, while [Zn] decreased in those from 'Natal Briar' (Table 3.2).

Plants from the control treatment had similar leaf mineral concentrations compared to those from the NaCl-series and to those from the Na_2SO_4 salinized treatment (P>0.05), except for iron [Fe] (P=0.0251), which was greater in leaves from control plants than in those subjected to Na_2SO_4 (averages of 61 $mg \cdot kg^{-1}$ versus 56 $mg \cdot kg^{-1}$, respectively). When comparing the NaCl and Na_2SO_4 treatments, the concentrations of all mineral elements were the same between both salts, except for [Ca] and [Mn] (P=0.0399 and 0.0073, respectively). Both mineral elements were greater for the NaCl salt treatment with general averages of 16 $g \cdot kg^{-1}$ versus 14 $g \cdot kg^{-1}$ for Ca and 237 $mg \cdot kg^{-1}$ and 172 $mg \cdot kg^{-1}$ for Mn, for the NaCl and Na_2SO_4 treatments, respectively.

Within the NaCl-salinized series the level of supplemental Ca had a linear positive effect on leaf nitrogen [N] and [Mn] in 'Manetti plants (Table 3.3). In 'Natal Briar' plants [Fe] decreased linearly as the Ca level increased while [N], [P], and [S] showed cubic responses (Table 3.3).

Chloride [Cl] in leaves of flowering shoots was positively correlated to K and Na, and negatively correlated to Mg and Zn (r=0.59, 0.27, -0.30 and -0.45, respectively). Sodium was positively correlated to Ca, Mg, S, Fe and Cl (r=0.24,

0.38, 0.25, 0.38 and 0.27, respectively). Calcium was positively correlated to N, Mg, S, Mn, Fe, Zn and Na (r^2=0.10, 0.31, 0.51, 0.66, 0.40, 0.69 and 0.24, respectively).

Within the NaCl-series the N:Cl, K:Na and Ca:Cl ratios were not affected by the RS selection or the level of supplemental Ca (P>0.05). The Ca:Na ratio was lower for the 12 $mmol \cdot L^{-1}$ NaCl treatment without supplemental Ca (0.0 $mmol \cdot L^{-1}$) than for the average of the NaCl-salinized treatments with 2.5-10.0 $mmol \cdot L^{-1}$ of supplemental Ca (165 versus average of 228, respectively).

Comparing the control with the NaCl-series, the Na:Cl and Ca:Cl ratios were different (P<0.0001 for both) with flowering shoots leaves of control plants having greater Na:Cl (21.5 versus average of 5.1) and Ca:Cl (10.4 versus average of 2.8) ratios, for the control and NaCl-series, respectively.

Leaf [Ca] and [S] were positively correlated to DW and FS harvested per plant, FSL and LCI (r=0.59, 0.25, 0.68 and 0.42, respectively for Ca; and r=0.37, 0.31, 0.47, and 0.36, respectively for S). Chloride, on the other hand, was negatively correlated to DW, FS, and FSL (r=-0.45, -0.47 and -0.43, respectively). None of these variables was correlated to leaf [Na] (P>0.05 for all).

DISCUSSION

Overall salinity stress response

Calculated EC values of the applied 12 $mmol \cdot L^{-1}$ NaCl- and 6.0 $mmol \cdot L^{-1}$ Na_2SO_4-saline solutions with increasing levels of supplemental Ca ranged from 2.78-4.78 $dS \cdot m^{-1}$ (including the 0.53 $dS \cdot m^{-1}$ contribution from the tap water), which corresponds approximately to EC values of 4.17-7.17 $dS \cdot m^{-1}$ in the saturation extract from a soil/substrate (Farnham et al., 1985). Except for the non-salinized control treatment (solution 1; Table 3.1), all NaCl- or Na_2SO_4-salt

treatments exceeded the soil solution salinity thresholds (EC's~2-3 $dS \cdot m^{-1}$ of the saturation extract) that have been recommended for roses in the past (Bernstein et al., 1972; Davidson and Boodley, 1987; Hughes and Hanan, 1978). However, EC in leachates collected from plants in all treatments, including the non-salinized control (Fig. 3.2), surpassed the maximum leachate salinity thresholds (EC 1.4-1.8 $dS \cdot m^{-1}$) previously recommended for roses (Brun and Settembrino, 1996).

More recent studies, however, have shown that greenhouse roses could be more tolerant to greater levels of salinity than those previously established. Irrigation solutions salinized with up to 10 $mmol \cdot L^{-1}$ NaCl did not have a significant effect on the total and root dry weights of *Rosa hybrida* L. 'Kardinal' budded on *R. rubiginosa* (Wahome et al., 2000). It was only after raising the NaCl concentration to 20 and 30 $mmol \cdot L^{-1}$ that both dry weights were significantly reduced compared to the control treatment (Wahome et al., 2000). Cabrera and Perdomo (2003) studied the effects of NaCl at concentrations ranging from 0-30 $mmol \cdot L^{-1}$ NaCl (EC of the irrigation solutions ranging from 1.6 to 4.6 $dS \cdot m^{-1}$) on *Rosa hybrida* L. 'Bridal Pink' budded on 'Manetti'. According to their results, no significant effects on cut-flower yield and quality were observed among salt treatments. In both studies, the effects of salinity stress on the rose scion cultivars were influenced by the rootstock selection used. In our first experiment (Chapter II), whose main objective was to determine the salinity tolerance limits of 'Red France' roses, reductions in DW and other productive variables were found only for NaCl-$CaCl_2$ concentrations of 12 and 24 $mmol \cdot L^{-1}$ (total EC of the saline solutions ~3.25-4.85 $dS \cdot m^{-1}$). Based on the results from these studies, we infer that the NaCl or NaCl-$CaCl_2$ salinity tolerance limit for greenhouse roses, although highly influenced by the rootstock, is between 12 and 15 $mmol \cdot L^{-1}$.

With exception of the non-salinized control, the stress imposed by the salinity treatments in the present experiment (Table 3.1) caused reductions in

plant productivity (total flowering shoot DW and total FS harvested per plant; Fig. 3.3A-B) and affected the plants' water status (lower RWC and more negative SWP and LOP; Fig. 3.6A-C). These results are in agreement with findings from our previous experiment and those from Cabrera and Perdomo (2003) and Wahome et al. (2000).

The detrimental effects caused by salinity were more evident on the aerial parts of the plants causing reductions not only on total flowering shoot DW and FS harvested per plant, but also on the old leaves DW from the plants destructively harvested at the end of the experiment. The lower plant organs DW (main stem and roots) were not affected to the same degree by the salinity stress (osmotic and/or ion-specific) as the leaves.

After approximately 3.5 months of exposure to the salinity treatments, particularly to NaCl, the plants' visual appearance was also affected by the salinity stress, exhibiting salt burn injury mostly on the plants' basal foliage (old leaves) and to a lesser degree on the basal leaves of cut-flower shoots. The extent of the damage increased as the time of exposure to the salt stress increased. Salt burn injury on old leaves was less pronounced and no substantial injury was exhibited on flowering shoot leaves of plants subjected to Na_2SO_4.

There are three major constraints for plant growth on saline substrates: (1) water deficit arising form the low water potential of the root medium; (2) ion toxicity associated with the excessive uptake mainly of Cl^- and Na^+; (3) nutrient imbalance by depression in uptake and/or shoot transport and impaired internal distribution of mineral nutrients (Marschner, 1995). Salts carried in the transpiration stream are deposited in leaves as the water evaporates, and salt gradually builds up with time as a result of a continuous, cumulative process (Munns, 2002; Neuman 1997). The long-term exposure of a plant to salinity may result mainly in ion toxicity in the older leaves because of a rapid increase in salt concentrations in cell walls or cytoplasm when the vacuoles can no longer

sequester incoming salts (Munns, 1993) and water deficit and a shortage of carbohydrates in the younger leaves (Marschner, 1995). Thus, the duration of exposure to the accumulated toxic ions may be a factor in the development of injury (Bernstein, 1975).

Effects of supplemental Ca on the response to salinity

Supplementing saline solutions with additional Ca has been reported to alleviate the detrimental effects caused by salinity in wheat and to a lesser degree in barley (Ehret et al., 1990), navel orange (Bañuls et al., 1991), *Crataegus opaca* Hook. & Arn (Picchioni and Graham, 2001), strawberry (Kaya et al., 2002; 2003a), guava seedlings (Ebert et al. 2002), cucumber (*Cucumis sativus* 'Orlando') and melon (*Cucumis melo* 'Ananas') (Kaya et al., 2003b). In rabbiteye blueberries (*Vaccinium ashei Reade* 'Tifblue' and 'Brightwell') subjected to 0, 25 or 100 mmol·L^{-1} Na as NaCl or Na_2SO_4, supplemental Ca (0, 1, 3 or 10 mmol·L^{-1}, 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 calcium (as $CaSO_4$) did not alleviate the harmful effects caused by the salinization with NaCl on both plant productivity and quality and water relations.

In the Ehret et al. (1990) study two species differing in their salinity tolerances, wheat and barley, were subjected to Na_2SO_4 plus $MgSO_4$-salinity added to the base nutrient solution and supplemented with 3.5 or 10 mmol·L^{-1} Ca (as $CaSO_4$). Plant response to Ca was determined and related to the salinity tolerance of each crop. In the $NaSO_4$-$MgSO_4$-salinized treatment supplemented with 3.5 mmol·L^{-1} Ca the growth of both species was reduced by salinity, with greater reductions in leaf area and plant DW exhibited in wheat. In the amended saline treatment containing 10 mmol·L^{-1} Ca leaf area increased 138% in wheat and 25% in barley. Plant DW increased 42% in wheat but was unaffected in

barley. In their study, Ehret et al. (1990) used SO_4^{-2} salts, where Na^+ and Mg^{2+} were the accompanying cations. The detrimental effects of Na-based salinity on plant growth can be more severe when it is accompanied by Cl^- than when these two ions are combined with other ions (i.e. SO_4, Mg or Ca) since there is an apparent synergistic effect between them, causing greater injury when both ions are present (Martin and Koebner, 1995; Picchioni and Graham, 2001). Our results showed that Cl uptake from non-NaCl salinized solution (Na_2SO_4 salt treatment) in 'Manetti' plants seemed to be enhanced by the Na-cation, exhibiting greater [Cl] in flowering shoot leaves by 51%, and in roots by 35% than those plants from the control treatment (irrigated with non-salinized nutrient solution having a [Cl] ~ 69 $mg \cdot L^{-1}$ from the tap water). Furthermore, high sulfate levels may decrease available Ca through precipitation reactions, which may, in part, be responsible for reduced Ca levels in cereals on the Canadian prairies (Janzen and Chang, 1987). According to Ehret et al. (1990), the difference in the response between wheat and barley seemed to be related to differences in Ca utilization or requirements by the salt-sensitive species (wheat), being it more dependent on Ca availability than the salt tolerant species (barley).

In Bañuls et al. (1991) study with citrus plants, the basic saline solutions containing 45 $mmol \cdot L^{-1}$ NaCl lacked $Ca(NO_3)_2$. Calcium sulfate and $Ca(NO_3)_2$ were added to the treatments to give final [Ca] ranging from 3-30 mM Ca. Ammonium (NH_4^+) and NO_3^- levels were maintained constant by adding NH_4NO_3 and $(NH_4)SO_4$ to the treatments. The addition of more sulfates to the saline solutions [as $(NH_4)_2SO_4$] could have caused precipitation of some of the supplemental Ca. In their study the increase in DW was much more pronounced between the first two external [Ca] (3 and 10 $mmol \cdot L^{-1}$) with smaller increases in DW above 10 $mmol \cdot L^{-1}$ Ca. Calcium was not included in their basic NaCl-salinized half Hoagland's solutions, therefore these increases in DW, particularly greater in the two lowest [Ca], could have been due simply to the inclusion of

this essential major cation in the mineral solution, more than to its ameliorative effects on saline stress.

Some other studies have used NO_3^- as the Ca-counter anion (Ebert et al. 2002; Kaya, et al., 2003a,b). Adding NO_3^- results in a reduction in Cl^- uptake and accumulation due to NO_3^-/Cl^- antagonism (Bernstein et al., 1974; Kafkafi et al., 1982; Martinez and Cerdá, 1989). The alleviating effects of supplemental $Ca(NO_3)_2$ could therefore be due to either Ca^{2+}, NO_3^- or to their synergistic effects.

Based on these reports it should be considered that several factors influence the degree and nature of salinized plants' responses to supplemental Ca applications such as the inherent species' salt tolerance, levels of [Ca] found in the growing substrate and irrigation water, the composition of the salinizing agents and the supplemental Ca counter-anion (i.e. Cl^-, NO_3^-, SO_4^{2-}).

Effect of the salt composition on the response to salinity

Reductions in plant productivity and LOP seemed to be highly influenced by the Na^+ accompanying-anion. Plants exhibited more detrimental effects on their flowering shoot productivity, old leaves DW and more negative LOP when exposed to NaCl-based salinity than when exposed to Na_2SO_4-based salinity (both at the 5 $mmol \cdot L^{-1}$ additional Ca level; Fig. 3.3A-B and Fig. 3.6A-C). Contrary to NaCl, the Na_2SO_4-salinized treatment did not have any negative effects on plant productivity, yielding similar total flowering shoot DW and total FS harvested per plant than the non-salinized control plants (Fig. 3.3A and Fig 3.3B).

Similar to plant productivity, the composition of the salt treatment had a substantial influence on the foliage damage caused by salinity. Those plants subjected to Cl^- as the Na-accompanying ion exhibited salt damage to a greater extent on their foliage than those exposed to the counter-anion SO_4^{2-} (Fig. 3.5).

Both saline solutions (12 mmol·L^{-1} NaCl and 6 mmol·L^{-1} Na_2SO_4, both with 5 mmol·L^{-1} of supplemental Ca) had initially equal calculated electrical conductivities (3.78 dS·m^{-1}) and equal [Na] (12 meq·L^{-1}), therefore the osmotic stress and the Na^+-specific effects imposed on the plants would had been the same in both treatments. Thus, the differential effects are assumed to be due to the Na-counter anions, Cl^- and SO_4^{2-}. Niu and Rodriguez (2008b) evaluated the response of four rose (*Rosa* L.) rootstocks to chloride- or sulfate-dominated salinities. According to their results there were interactive effects between rootstock selection and salt composition on plant DW response to salinity. At moderate salinity (EC ~ 3.9 dS·m^{-1}) Cl-dominated salinity caused greater dry weight reductions only in *R. x fortuniana* Lindl. In *Rosa* L. 'Dr. Huey', *R. multiflora* Thunb., and *R.* x *odorata* (Andrews) Sweet dry weight reductions were similar between moderate Cl- and SO_4-based salinities. Niu and Rodriguez (2008b) observed, however, that Cl-dominated salinity led to lower visual quality of all rootstocks, especially in *R. x fortuniana*. On tomato (*Lycopersicon esculentum* Mill.; Yokas et al. 2008), sweet pepper (*Capsicum annuum* L.; Navarro et al., 2002), snapbean (*Phaseolus vulgaris* L. 'Contender'; Awada et al., 1995), and rabbiteye blueberries 'Tifblue' and Brightwell' (Wright et al., 1992), SO_4-based salinities have been reported as being less deleterious than Cl-based salinities. Many crops are very sensitive to high internal chloride levels, and species are generally more tolerant to sulfate-salinity than chloride-salinity (Grattan and Grieve, 1999).

Leaf ion concentration

In general, chloride transport and deposition was progressive over time (Fig. 3.7) and more pronounced in leaves (both old leaves and flowering shoot leaves) than in roots, main stems and old stems (no Cl analysis were performed in stems from cut-flower shoots) (Fig. 3.9A). Within the NaCl-series [Cl] in old

leaves were 135%, 163% and 157% greater than in roots, main stems and old stems, respectively. In leaves from flowering shoots (grown over a single flowering flush) collected at 184 DAT, [Cl] reached levels of almost 11 $g \cdot kg^{-1}$, being greater by 43%, 60% and 56% than in roots, main stems and old stems, respectively.

Contrary to leaf [Cl], leaf [Na] of flowering shoots did not change significantly over time being similar between the control and the Na-salinized (with either Cl^- or SO_4^{2-} as counter-anions) treatments and its concentration in flowering shoot leaves was much lower compared to [Cl]. Opposite to Cl, Na transport and accumulation was greater in the lower organs, particularly roots and main stems (Fig. 3.9B). By harvest IV (133 DAT) the average ratio of Cl:Na (in mg) in leaves of flowering shoots from the NaCl-salinized plants was 149:1 for plants budded on 'Manetti' and 51:1 in 'Natal Briar' plants. Considering that their application ratio (in mg) was 1.54:1, Cl uptake and/or transport to the upper plant parts was considerably greater than for Na.

Greater [Cl] in the upper parts (shoots or leaves) and/or greater [Na] in the lower parts (roots) of the plant were found in our previous study (Chapter II) and have also been reported in NaCl-treated seedlings of red-osier dogwood (*Cornus stolonifera* Michx; Renault et al., 2001), 'Mandelon' roses (Baas and van den Berg, 1999); *Crataegus opaca* Hook. & Arn (Picchioni and Graham, 2001), cucumber and melon (Kaya et al. 2003b), snapdragon (*Antirrhinun majus* L. 'Monaco Rose'; Carter and Grieve, 2008), rose rootstocks *Rosa.* L. 'Dr. Huey', *R. x fortuniana*, *R. multiflora* and *R. x odorata* (Niu and Rodriguez, 2008b), and 'Bridal Pink' roses budded on the rootstock 'Manetti' (Cabrera and Perdomo, 2003). Leaf Na toxicity seems not to be as widespread as Cl toxicity (Marschner, 1995). Many crop species with relatively low salt tolerance are typical Na excluders and capable, at low and moderate salinity levels, of restricting the transport of Na into the leaves where it is highly toxic in salt sensitive species (Marschner, 1995). In roses the ability to restrict Na transport

to the leaves appears to be dependent of the rootstock selection (Cabrera and Perdomo, 2003). The 'Manetti' rootstock has been reported to have a greater ability to sequester Na in roots and restrict its transport to the leaves than other rose rootstocks (Cabrera and Perdomo, 2003; Sadasivaiah and Holley, 1973).

While leaf [Ca] and [S] exhibited a positive association with plant productive variables (DW, FS, FSL and LCI), Cl showed a negative association with the first three. Sodium did not appear to be related positively or negatively to any of the productive variables mentioned above. Similarly, in our first experiment (Chapter II) we found very close negative relationships between Cl and the productive and quality variables evaluated as well. Ashraf and Ahmad (2000) also reported highly negative associations between leaf [Cl] and shoot DW or cotton seed yield.

Results from the present and the previous experiment (Chapter II) indicate that Cl might be the major culprit in the reduction in plant productivity and quality caused by NaCl-salinity. This is consistent with results reported by Cabrera and Perdomo (2003). Chloride has been reported to affect plant growth to a greater extent than Na (Baas and van den Berg, 1999; Bernstein et al., 1972). Chloride is highly mobile in the soil, is readily taken up by plants and its mobility in short- and long-distance transport is high (Marschner, 1995). Chloride ions are normally taken up by plants faster than Na^+ ions and hence their concentration in plants is always greater (Greenway and Munns, 1980). For moderate-to-high external Na concentrations, a low net influx of Na into the cytoplasm could be achieved either by a low Na permeability or by a rapid active efflux balanced against passive influx (Tyerman and Skerrett, 1999). At high external Cl concentrations it is possible for the membrane potential to be less negative than the Cl equilibrium potential allowing for a passive influx (Tyerman and Skerrett, 1999). Toxicity due to chloride will generally build upon the adverse effects induced by osmotic effects alone (Ferguson and Grattan, 2005). Based on this, it is concluded that the detrimental effects exhibited by NaCl-

salinized rose plants is likely due to the combination of osmotic stress and specific-ion toxicity caused chiefly by the ion Cl (much more than with Na).

Effects of the salt treatments on the flowering shoot leaves' mineral composition was influenced by the RS (Table 3.2 and Table 3.3), but in general NaCl-based salinity seemed to affect this variable to a greater extent than the Na_2SO_4-based salinity. Plants receiving the Na_2SO_4 salt treatment (plus 5.0 $mmol \cdot L^{-1}$ $CaSO_4$) had, in general terms, a leaf mineral profile similar to the control plants (Table 3.2). At 71 DAT leaf [K] was more or less similar in all treatments and rootstocks (Table 3.2). By 133 DAT, however, leaf [K] tended to increase in the salinized treatments (Table 3.2). In our previous experiment (Chapter II), in plants budded on 'Natal Briar' (rootstock with lower salt stress tolerance and greater leaf [Cl]), leaf [K] increased as the salinity concentrations in the irrigation solutions increased. Similarly, in both experiments the uptake and transport of Cl to the leaves was considerably greater than for Na. The uptake of Na is balanced with the uptake of Cl (a negatively charged ion) and efflux of K (Tyerman and Skerrett, 1999). Roses, like several other plant species, as stated in our present and previous experiment, and other studies cited throughout this and the previous document, have developed mechanisms to exclude and/or restrict the uptake and transport of Na to the upper parts. In contrast, being that K is rarely found in saline substrates, plants have developed a highly selective uptake system of K over Na to absorb this cation (K^+) against electrochemical potential differences (Marschner, 1995). At low external concentrations K uptake is coupled to metabolic activity, where the high affinity uptake system operates against the prevailing electrochemical potential difference (Marschner, 1995). Probably, the increases in leaf [K] were due to balance of charge processes during the uptake and transport of Cl.

Conversely, leaf [Mg] and [Mn] tended to decrease in the NaCl-salinized treatments as well (Table 3.2). Chloride was a negatively associated with leaf [Mg] and [Zn]. Sodium and Ca did not show any negative associations with

other mineral elements. Contrarily, both were positively associated with leaf [Mg], [S] and [Fe]. Similarly, in our previous experiment (Chapter II) leaf [Mg] decreased as the salinity levels in the nutrient solution increased. Reductions in leaf [Mg] caused by salinity have been reported in wheat (Hu and Schmidhalter, 1997) and in three citrus rootstocks (Ruiz et al., 1997). Reports on the influence of salinity on the foliar concentrations of Mn and Zn in plants are inconsistent (Grattan and Grieve, 1999).

CHAPTER IV

EVALUATING THE INFLUENCE OF THE COUNTER ANION ON THE DETRIMENTAL EFFECTS IMPOSED BY SODIUM-BASED SALINITY ON ROSE (*ROSA* L. 'BULL'S EYE')

INTRODUCTION

There are three major constraints for plant growth on saline substrates (Gorham, 2007; Lambers et al., 1998; Marschner, 1995). First, a water deficit arising from low soil water potential (high osmotic pressure) associated with high salinity, making it more difficult for plants to establish a continuous gradient of water potential between the soil solution and the atmosphere. The second is an ion toxicity associated with excessive uptake of inorganic ions, mainly Cl^- and Na^+. And the third, a nutrient imbalance caused by depression in uptake and/or shoot transport and impaired internal distribution of other nutrients leading to ion imbalances and eventually deficiency symptoms.

By definition, saline soils have electrical conductivities of the saturation extract (EC_{SE}) greater than 4 $dS \cdot m^{-1}$ and exchangeable-sodium-percentages (ESP) less than 15% (Richards, 1954). In these kinds of soils salinity is usually caused by mixtures of salts rather than a single salt (Bernstein, 1975) and the amount of soluble salts present controls the osmotic strength of the soil solution (Richards, 1954). Ions frequently found in excess in saline soils include Cl^-, SO_4^{2-}, HCO_3^-, Na^+, Ca^{2+}, Mg^{2+}, and less frequently K^+ and NO_3^- (Martin and Koebner, 1995). Sodium seldom comprises more than half of the soluble cations and the relative amounts of Ca^{2+} and Mg^{2+} present in the soil solution may vary considerably, while soluble and exchangeable K^+ are ordinarily minor constituents (Richards, 1954). Saline-alkali soils (or saline-sodic soils), on the

other hand, have EC_{SE} greater than 4 $dS \cdot m^{-1}$ and ESP greater than 15% (Richards, 1954).

Investigations on the effects of salinity on plants have increased during the past few years (Maas and Grieve, 1987). However, a large percentage of salinity studies on horticultural or agronomic crops use NaCl as the sole salinizing agent (Grattan and Grieve, 1999). Clearly, the addition of a large quantity of NaCl to base nutrient solutions also produces a highly sodic substrate (Maas and Grieve, 1987). These results are usually purported to describe plant responses to saline conditions ignoring the fundamental distinction between saline and sodic soils (Maas and Grieve, 1987), thus limiting the extent to which the results can be interpreted (Grattan and Grieve, 1999). Under these circumstances, it is difficult to differentiate osmotic from specific ion effects, considering that both Na^+ and Cl^- may be directly toxic (Bernstein, 1975) and that a synergistic effect between them has been reported, with greater injury when both ions are present (Martin and Koebner, 1995). In saline substrates where Na^+ and Cl^- are the dominant ions, their concentrations exceed by far the demand, leading to toxicity in non salt-tolerant plants (Marschner, 1995).

Nutrient solutions for plant growth are made up of dissociated salts; however, plants need and absorb specific ions. This fact imposes the major constraint upon nutrient solutions, namely the balance of charge: the sum of the cation equivalents must be equal to the sum of the anion equivalents (Schrevens and Cornell, 1993). This constraint is the major reason for the impossibility of using classical experimental designs (factorial-type designs) with nutrient solutions (Schrevens and Cornell, 1993). The problem of experimentation with nutrient solutions in plant nutrition can be dealt with by using the theory of mixture designs and model forms (Schrevens and Cornell, 1993). The distinguishing feature of mixture experiments is that the independent variables represent proportionate amounts of the mixture rather than unrestrained

amounts. These proportions must be nonnegative, and if expressed as fractions they must sum to unity (Schrevens and Cornell, 1993).

Mixture experiments help to make predictions of the response to any mixture and to measure the influence of each component on the response (Cornell and Harrison, 1997). In a mixture experiment the components (each ingredient present in the mixture) are mixed or blended in varying proportions to form a treatment, and all the treatments have the same volume or concentration (Cornell, 2002). If each component in the mixture is expressed as a fraction, then the sum of all the components must be equal to one (Cornell, 2002). In the general mixture problem, the measured response is assumed to depend only on the proportions of the ingredients present in the mixture and not on the amount of the mixture (Cornell, 2002).

The general purpose of mixture experimentation is to make possible estimates, through a response surface exploration, of the properties of an entire multicomponent system from only a limited number of observations (Cornell, 1973). These observations are taken at preselected combinations of the components (mixtures) in an attempt to determine which of the combinations in some sense maximizes the response (Cornell, 1973). The dimension of the response surface is determined by the number of components in the mixture. Single-component mixtures, also called *pure* mixtures, are used mainly as a standard against which multicomponent blends are compared. If there are two components, then the response surface is one-dimensional and the simplex factor is a straight line, represented by a horizontal axis. With three components the response surface is bi-dimensional and the simplex space can be represented as an equilateral triangle (Fig. 4.1; Cornell and Linda, 1991; Cornell, 2002). The vertices of the simplex or triangle represent the single-component (pure) mixtures (P). The points along the edges of the triangle represent the binary blends (B) and contain two components in each mixture (the two components at both ends of that edge). The interior points of the triangle

represent mixtures in which none of the three components is absent, known as tertiary blends (T). The centroid of the triangle corresponds to the tertiary blend with equal proportions from each of the components (Cornell, 2002; Cornell and Linda, 1991). The line that departs from the middle point of the binary blends edge and ends at the opposite vertex is known as the coordinate line for the mixture component at that vertex and shows the effect of increasing its proportion in the mixture (Cornell and Linda, 1991).

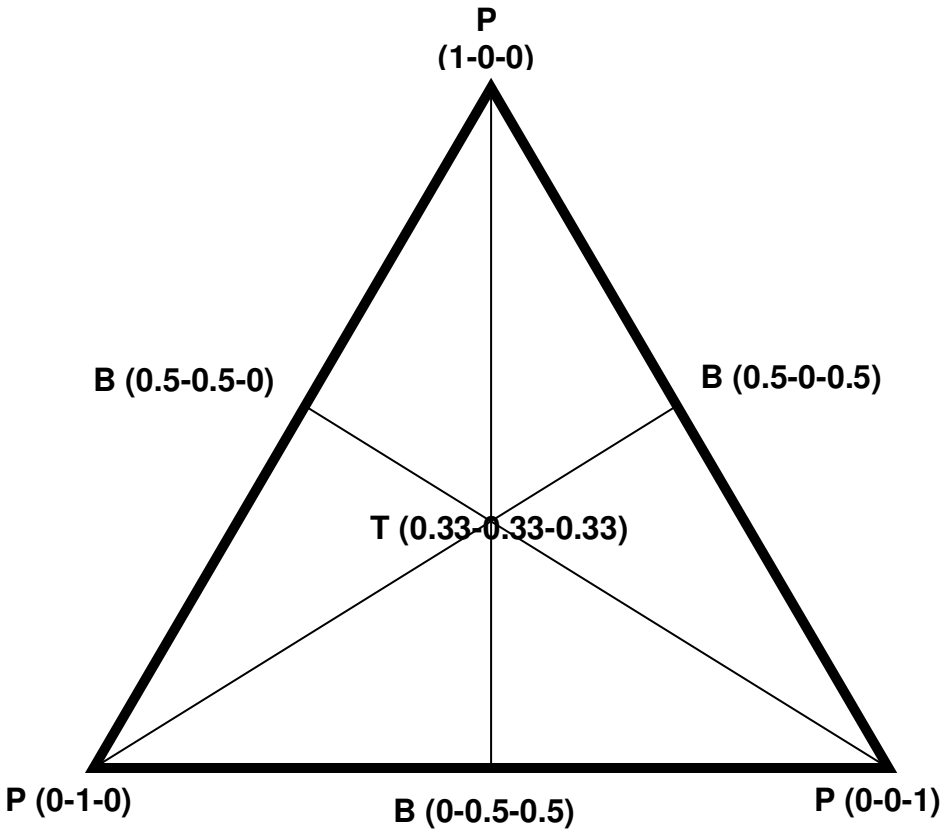

Fig. 4.1. Three component simplex region. All experimental points must lie on or inside the triangle (Cornell, 2002). Vertices represent the pure blends where only one component is present in the mixture. Along the sides of the triangle underlie the binary blends, which contain two components in each mixture. In the interior of the triangle underlie the tertiary blends where all three components are present in the mixture.

The present study was conducted to determine the effect of Na^+-based salinity (i.e. sodicity) and the effect of the Na^+-counter anions on rose plants, a species historically categorized as salt-sensitive (Cabrera, 2003a).

MATERIALS AND METHODS

Plant culture and management

On January 25, 2004, 84 bare-rooted 'Bull's Eye' rose plants budded on two rootstocks, *Rosa* L. 'Manetti' and *Rosa* L. 'Natal Briar', were transplanted into 15 L black plastic containers (Nursery Supplies, Inc. Kissimmee, FL) filled with a peat: moss, pine bark and sand (3:1:1 v/v) substrate. The substrate was previously amended with 3.0 $kg \cdot m^{-3}$ of dolomitic limestone (Carl Pool Products, Gladewater, TX) and 0.8 $kg \cdot m^{-3}$ of Micromax Micronutrients fertilizer (The Scotts Company, Marysville, OH). Plants were placed on 5.5 x 1.5 x 0.4 m raised benches, with three plants abreast in each bed and spaced at 30 cm between centers. Plants were irrigated with a nutrient solution made with 15-5-15 Cal-Mag (The Scotts Company, Marysville, OH) and adjusted to deliver 150 $mg \cdot L^{-1}$ of nitrogen until the salinized treatments were implemented. On March 10, 2004 and April 04, 2004 plants underwent a hard pinch (removal of the terminal portion of a soft shoot, including two to four leaves; Langhans, 1987a). Prior to the experiment, plants were grown for three entire flowering flush cycles. Throughout the proceeding experiment plants were managed following conventional pruning practices (Langhans, 1987b), inducing synchronized flushes of growth and flowering.

The experiment was conducted at the Texas A&M University Research and Extension Center, Dallas, TX, in a 6 m x 12 m glass-covered greenhouse fitted with exhaust fans, an evaporative wet-pad cooling system and heat provided by thermostatically-controlled gas burners. Greenhouse temperatures

were set at 25°C day and 16°C night. Temperature, humidity and photosynthetically active radiation were monitored with sensors connected to a Campbell CR510 Datalogger (Campbell Scientific Inc., Logan, UT).

On August 10, 2004, the salinity treatments, consisting of a base nutrient solution supplemented with the salt mixtures, were implemented. A modified ½ strength Hoagland formulation (Hoagland and Arnon, 1950) was used as the base solution containing (in $mmol \cdot L^{-1}$): 8.5 N (as NO_3^-), 0.5 P (as $H_2PO_4^-$), 3.5 K, 2.75 Ca, 1.0 Mg, 1.0 S (as SO_4^{2-}), 1.0 $mg \cdot L^{-1}$ Fe as Fe-EDDHA and half-strength Hoagland's micronutrient concentration. Seven saline solutions, in which the concentration of sodium [Na] was held constant at 12 $mmol \cdot L^{-1}$, were used for this experiment using NaCl, Na_2SO_4 and $NaNO_3$ salts (Table 4.1). In pure blends 100% of the Na came from a single salt source (NaCl, Na_2SO_4 or $NaNO_3$), in binary blends 50% of the Na came from each of two different salts, and in the tertiary blend 33.33% of the Na derived from each of the three different salt sources.

The expected electrical conductivity (EC_E; sum of cations or anions, in $meq \cdot L^{-1}$, divided by 10; Richards, 1954) for all the saline solutions (base nutrient solution supplemented with salt mixtures) was 2.3 $dS \cdot m^{-1}$ plus an additional 0.49±0.02 $dS \cdot m^{-1}$ contribution from the tap water used to prepare the solutions. Tap water initial pH was on average 7.9, and was adjusted to a target pH of 6.5 before adding any salts (base nutrient solution and salt treatments) using 6.0 M H_2SO_4. This acidification provided an additional contribution of approximately 0.45 $mmol \cdot L^{-1}$ sulfate (SO_4^{2-}) to all saline blends.

Table 4.1. Salt blend composition, concentration and proportion of the anions in the salt blends added to a base nutrient solution (modified half-strength Hoagland solution). Sodium was held constant at a concentration of 12 $mmol \cdot L^{-1}$ (i.e. 12 $meq \cdot L^{-1}$) in all blends.

Salt blend	Salt blend composition	Anion concentration ($meq \cdot L^{-1}$)				Anion proportion in the salt blend			Salt blend key*
		Cl^-	SO_4^{2-}	NO_3^-	Sum of anions	Cl^-	SO_4^{2-}	NO_3^-	
Pure	NaCl	12	0	0	12	1	0	0	1-0-0
Pure	Na_2SO_4	0	12	0	12	0	1	0	0-1-0
Pure	$NaNO_3$	0	0	12	12	0	0	1	0-0-1
Binary	$NaCl$-Na_2SO_4	6	6	0	12	0.5	0.5	0	0.5-0.5-0
Binary	$NaCl$-$NaNO_3$	6	0	6	12	0.5	0	0.5	0.5-0-0.5
Binary	Na_2SO_4-$NaNO_3$	0	6	6	12	0	0.5	0.5	0-0.5-0.5
Tertiary	$NaCl$-Na_2SO_4-$NaNO_3$	4	4	4	12	0.33	0.33	0.33	0.33-0.33-0.33

*Order of the anions is Cl^--SO_4^{2-}-NO_3^-.

Solutions were pumped from 150-L containers with submersible pumps (Model 2E-38N, Little Giant Pump Co., Oklahoma City, OK) feeding 1.3 cm (diameter) polyethylene irrigation lines that supported spray–stake Spot Spitter® emitters (Roberts Irrigation Products, San Marcos, CA), connected via 3.2 mm (diameter) spaghetti tubing. Each plant container was fitted with one calibrated emitter. Representative plants from selected treatments were routinely weighed to gravimetrically determine the evapotranspiration rate (ET). Total base irrigation volume consisted of ET plus an additional leaching target fraction of 25%.

Electrical conductivity (EC meter model 2052, VWR Scientific), pH (pH/mV/Ion meter AP63 accumet ® portable; Fisher Scientific) and Cl concentrations (Digital Chloridometer Model 4425000, Labconco Co., Kansas City, MO) were monitored on three leachate samples collected from all treatments on a bi-weekly basis. Chloride concentrations were determined according to Adriano and Doner (1982).

Data collection

Plant productivity and flowering shoots quality

There were a total of five harvest events during this experiment. Flowering shoots were harvested at commercial maturity, recording their dry weight (DW), number (FS), length (FSL) and leaf chlorophyll index (LCI; Chlorophyll meter, SPAD-502, Minolta Co. LTD, Japan) per plant. Harvested flowering shoots were put into paper bags and oven-dried at 70^{o}C. At the end of the experiment, immediately after the fifth harvest of flowering shoots, three whole plants per treatment were destructively harvested and analyzed for nutrient content and biomass partitioning. Plants were cut into four portions: roots, main stem (rootstock stem portion below the graft union), and scion old

stems and old leaves.

Flowering shoot DW and FS per plant from each harvest were added to obtain the total flowering shoot DW and total FS harvested per plant. For FSL and LCI the average per harvest and total average from the five harvests were used in the data analysis.

Water relations measurements

Relative water content (RWC) and stem water potential (SWP) were determined for three selected plants from each treatment during each harvest. For RWC three leaflets from one flower shoot per plant were cut and weighed to determine their fresh weight (FW); soaked in deionized water in petri dishes and refrigerated for a 24 hour period to determine their turgid weight (TW); and oven-dried at 70^{o}C for 48 hours, or until they recorded a stable weight, to determine their dry weight (DW). Relative water content was calculated by the formula RWC=[(HW-DW)/(TW-DW)*100] (Jiang and Huang, 2001). Stem water potential was measured with a pressure chamber (Model 610, PMS Instrument, CO., Corvallis, Oregon, USA). Leaves of flower stems were covered with an aluminized Mylar envelope to synchronize their water potential with that from the stems at least two hours before measuring (Kim et al., 2004). Sampling time for both variables was midday (between 12:00 PM and 1:30 PM) when the evaporative demand was at its peak.

Tissue analyses

During each harvest the three uppermost five-leaflet leaves from each flowering shoot were collected and pooled for each plant, dried and ground (to pass a 40-mesh screen). Samples from harvests II (101 DAT) and IV (225 DAT) were sent to the Louisiana State University AgCenter Soil Testing and Plant

Analysis Laboratory to be analyzed for total nutrient concentration. Phosphorous, K, Ca, Mg, S, B, Cu, Fe, and Zn, were determinate by ICP procedures while N was determinate with a Leco N analyzer. Analyses of chloride [Cl] in leaves of flowering shoots from all regular harvests were made using a digital chloridometer (Model 4425000, Labconco Co., Kansas City, MO). Sodium concentration in leaves of flowering shoots from harvests II, III and IV (101, 169 and 225 DAT) was determined by flame emission on a Fast Sequential Atomic Absorption Spectrometer (AA240FS, Varian, Inc. Australia). Similarly, [Cl] and [Na] were determined in roots, main stems, old stems and old leaves from the destructive harvest of whole plants at the end of the experiment.

Salt burn damage

On April 27, 2005, [260 DAT, between harvest IV (225 DAT) and harvest V (279 DAT) a foliage salt injury rating evaluation was taken 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 burn damage). This evaluation was performed on the leaves remaining on the plant after harvesting the flowering shoots.

Experimental design and statistical analyses

The experimental design was a randomized complete block design (RCBD) with a factorial arrangement of treatments having as factors rootstock selection (RS; 'Manetti' and 'Natal Briar') and composition of the salt blend (SB; Table 4.1). This yielded 14 different combinations with six replications (one pot with one plant was used as a replication) per combination for a total of 84 experimental units. Most of the variables were evaluated at several points in time throughout the entire experimental period (with exception of those variables

measured during the destructive harvest of plants at the end of the experiment). For all those variables evaluated over time a repeated measures procedure was performed including time, as days after treatment (DAT), as a third factor (additionally to RS and SB) to determine the pattern of the responses over time. For the total sums of flowering shoots DW and FS harvested per plant (sums of all five harvests), and all variables measured during the destructive harvest data were analyzed as a 2x7 factorial arrangement of treatments.

For some of the variables evaluated at harvest events over time (i.e. DW, FS, and FSL) data were first converted to a comparable scale (relative data) and then subjected to an arc sine transformation (Gomez and Gomez, 1984; detailed explanation included in chapter II).

Data from the factorial arrangement of treatments were analyzed by GLM, regression, correlation, mixed, and chi-square procedures using SAS ® 9.1 for Windows (SAS Institute Inc., Cary, NC). When effects due to the composition of the salt blend were present data were analyzed with Design-Expert V. 6.0.11 (Stat-Ease, Inc. Minneapolis, MN.) as a simplex-centroid design in a mixture experiment (Fig. 4.1) with a total of seven salt mixtures or blends: three pure blends, three binary blends, and one tertiary (centroid) blend. For every response this program provides an analysis of variance (ANOVA), the regression equation (best fitted model), diagnostics for the set of data and the model graphs (contour and 3-D surface). To estimate the value of a response at a given mixture or blend, the component coefficients provided by the model must be multiplied by the proportion of the components in the blend (Cornell, 2002). The number and type of coefficients provided by the model will depend on the type of the response (linear, quadratic or special cubic).

RESULTS

Leachate electrical conductivity (EC_L), Cl concentration ($[Cl_L]$) and pH (pH_L)

Volumes of irrigation solution applied were the same for all treatments throughout the entire experimental period (results not shown). Leaching fractions were similar between RS and among all SB ($P<0.05$ for both factors). Leaching fraction values ranged between 24% and 35%, and the overall averages for RS were 28% for 'Manetti' and 29% for 'Natal Briar' plants. Variations in leaching fractions over time were similar for both RS and all SB (data not shown).

Leachate electrical conductivity in 'Manetti' plants increased over time until approximately 156 DAT (Fig. 4.2A), while in 'Natal Briar' EC_L values reached a stable level at approximately 90 DAT (Fig. 4.3A). In both RS $[Cl_L]$ of SB containing NaCl (pure, binary and tertiary blends) followed patterns over time very similar to those of EC_L (Fig. 4.2B and Fig. 4.3B). Leachate pH showed a cubic pattern of change over time in both RS. It slightly decreased for the first 132 DAT, increased afterwards until around 227 DAT and decreased again for the last phase of the experimental period (Fig. 4.2C and 4.3C).

Supplemental salts were supplied at equinormal rates (i.e. $meq \cdot L^{-1}$), therefore the EC_E of the resulting saline solutions were assumed to be similar (~2.785 $dS \cdot m^{-1}$). However, numerical differences were evident in the measured electrical conductivities. Considering that all other factors and components were the same in all solutions, those differences raised the question if the type of supplemental salts used in the experiment affected electrical conductivity (EC) and pH of the applied saline solutions and the produced leachates. Thus, an analysis of variance was performed for these two chemical properties.

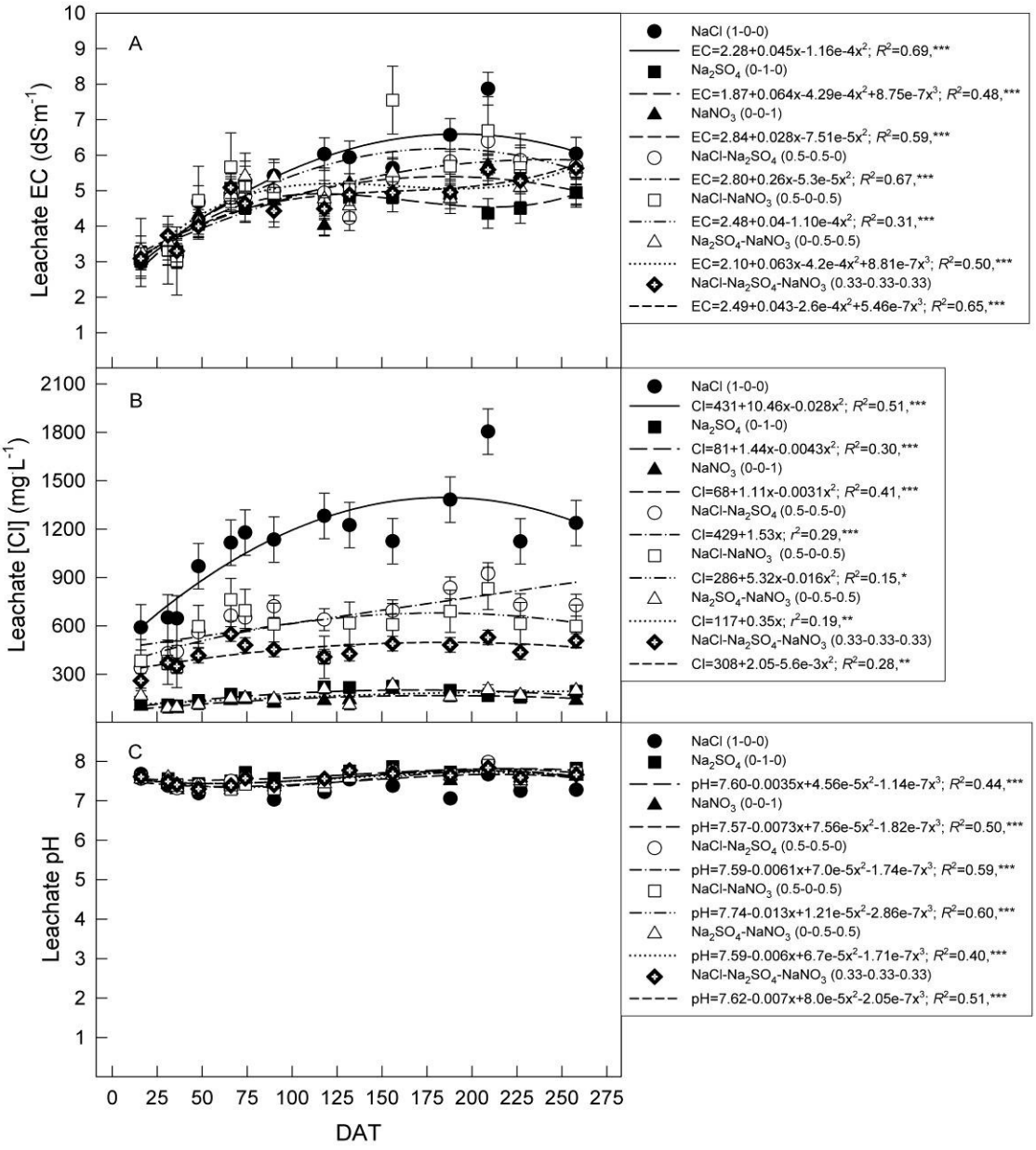

Fig. 4.2. (A) Electrical conductivity (EC), (B) Cl^- concentration [Cl], and (C) pH over time in leachates from 'Bull's Eye' roses budded on 'Manetti' and subjected to a moderately high (12 mmol·L^{-1}) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. Symbols represent the mean ± standard error of three plants. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,***=non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

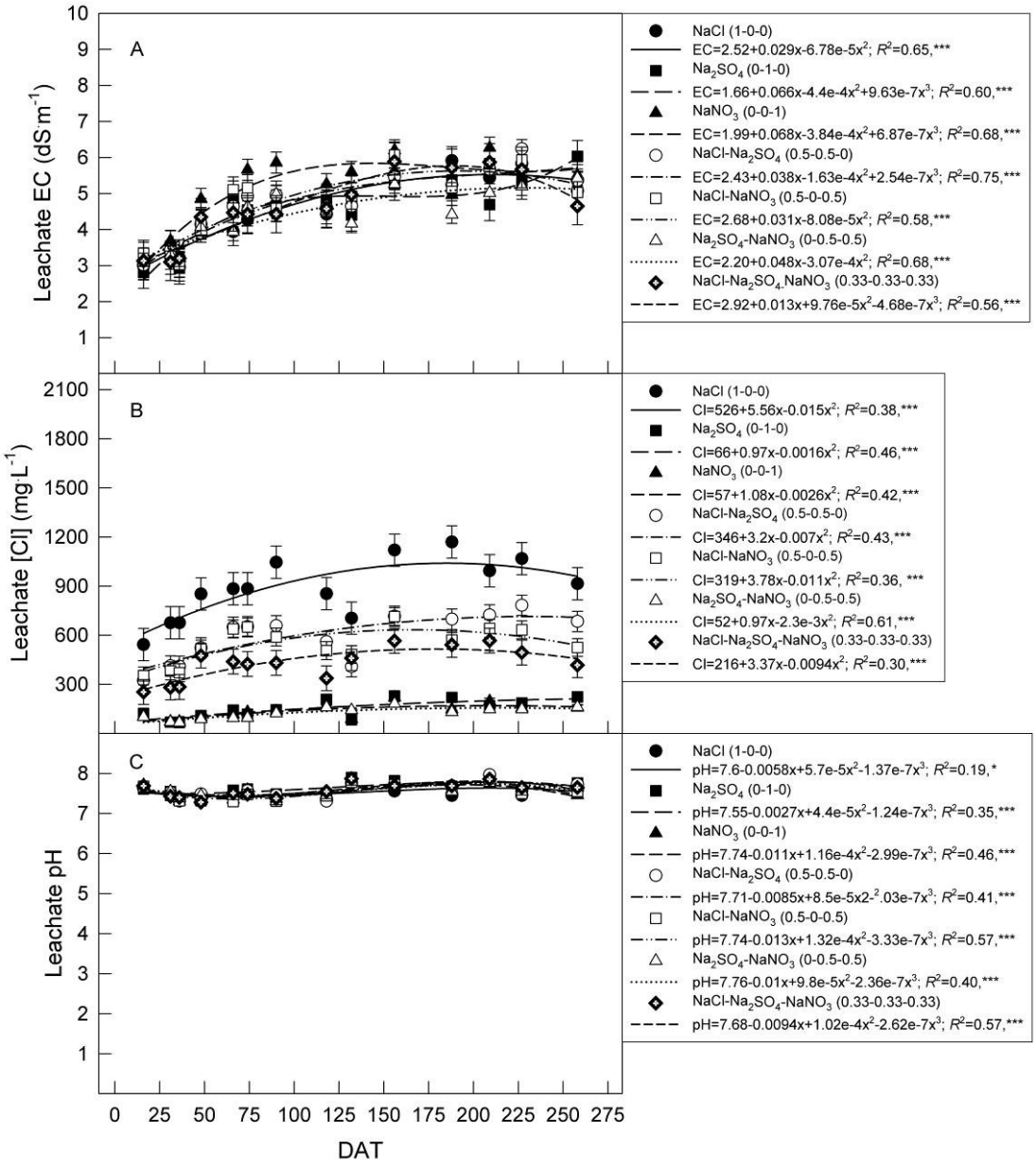

Fig. 4.3. (A) Electrical conductivity (EC), (B) Cl^- concentration [Cl], and (C) pH over time in leachates from 'Bull's Eye' roses budded on 'Natal Briar' and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. Symbols represent the mean ± standard error of three plants. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,***=non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

For all applied saline solutions pH (pH_{SS}) was the same ($P>0.05$), i.e. the salt blend composition did not affect this variable, averaging 6.59±0.03 (Fig. 4.4A; Table 4.2). In leachates, however, pH_L fitted a special cubic model ($P<0.0001$; Table 4.2). When all three anions were present in the salt blend, and when the SO_4^{2-} proportion was closer to zero, pH_L was lower compared to their pure blends' averages (depicted by the downward curvature in the central region of the response surface; Fig. 4.4B). Chloride and NO_3^- combined had a negative, synergistic effect on pH_L (Fig. 4.4B). In general terms, increasing proportions of SO_4^{2-} in the salt blend caused pH_L to increase, with its pure blend yielding the greatest average (Fig. 4.4B). Pure blends of NaCl and $NaNO_3$ yielded similar pH_L averages (Fig. 4.4B).

Electrical conductivity measured in both, saline solutions (EC_{SS}) and leachates, was affected by the anion proportion ($P<0.0001$; Table 4.2). In both cases EC values decreased linearly as the proportion of SO_4^{2-} in the salt blend increased, with its pure blend registering the lowest EC_{SS} and EC_L (Fig. 4.4C and 4.4D). Increasing proportions of either Cl^- or NO_3^- produced greater EC values in both saline solutions and leachates (Fig. 4.4C and 4.4D).

Given the clear influence of the different anions and their proportions on EC_{SS}, it was considered pertinent to determine the concentrations of free ions and ion pairs in all the saline solutions using the program SPECIES (Barak, 1990). In aqueous solutions water molecules and solutes interact with themselves and with each other, i.e. solvent and solute molecules and ions are never free from the influence of other nearby molecules and ions (Bohn et al., 1985). When ions of opposite sign are close together the energy of their mutual electrical attraction may be considerably greater than their thermal energy, so that they form a virtually new entity in the solution, of sufficient stability to persist through a number of collisions with solvent molecules (Robinson and Stokes, 1970). Ion association (i.e. ion pairs) reduces the activity of the solute as compared with a fully dissociated electrolyte (Robinson and Stokes, 1970).

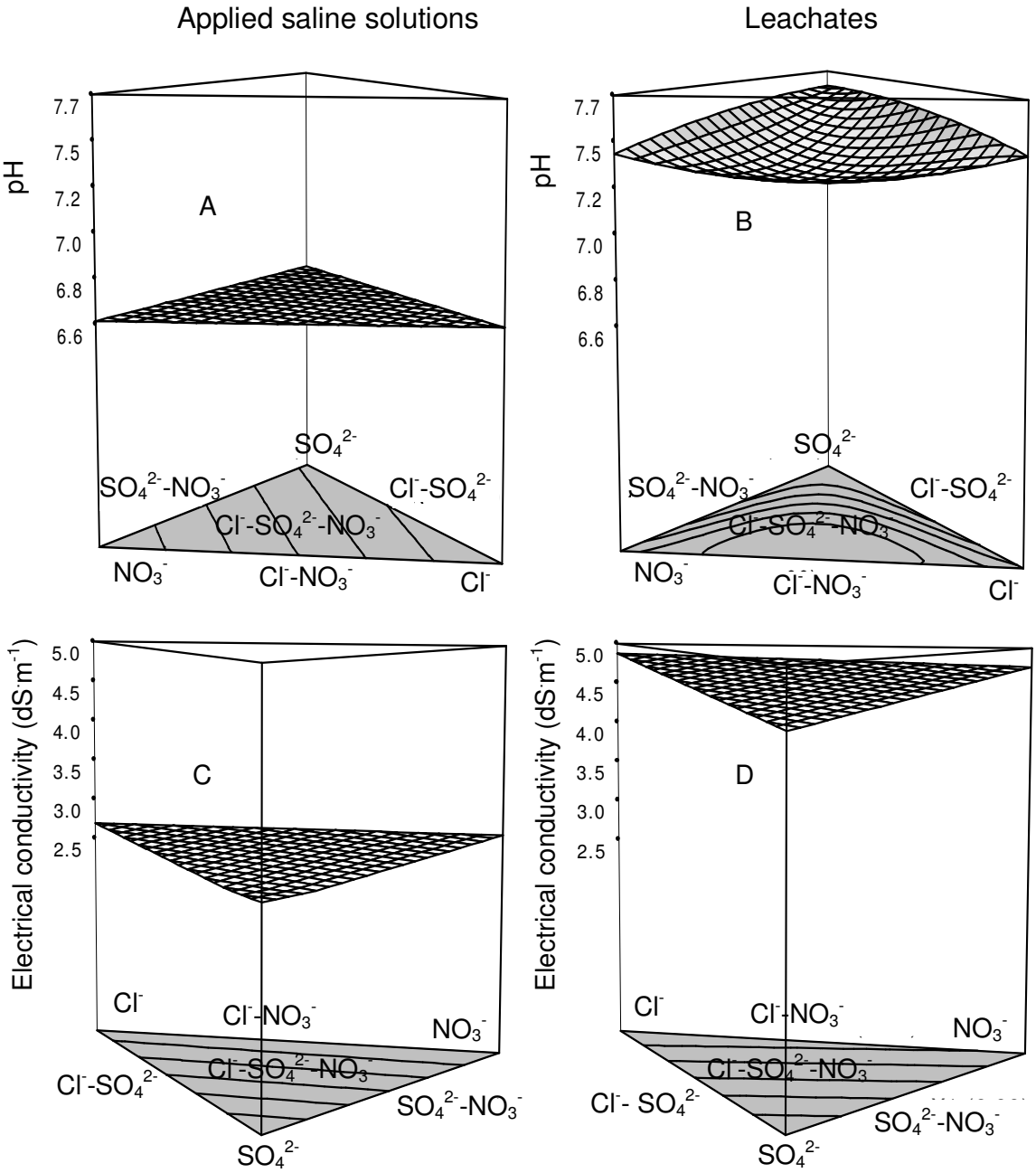

Fig. 4.4. pH (A and B) and electrical conductivity (C and D) of applied saline solutions and leachates from 'Bull's Eye' roses subjected to a moderately high (12 mmol·L^{-1}) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the counter anions Cl^-, SO_4^{2-} and NO_3^- in the salt mixture. Figures are 3-dimensional response surface. For fitted models see Table 4.2.

Table 4.2. Fitted models for pH and electrical conductivity (EC) of the saline solutions applied and of leachates from 'Bull's Eye' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

	pH	Electrical conductivity ($dS \cdot m^{-1}$)
Saline solution	pH_{ss}=6.60*(NaCl)+ 6.59*(Na_2SO_4)+6.57*($NaNO_3$); r^2=0.0604, P=0.3465	EC_{ss}=2.69*(NaCl)+ 2.45*(Na_2SO_4)+2.71*($NaNO_3$); r^2=0.75, P<0.0001
Leachates	pH_l=7.43*(NaCl)+7.62*(Na_2SO_4)+ 7.41*($NaNO_3$)+ 0.21*(NaCl*Na_2SO_4)- 0.52*(NaCl*$NaNO_3$)+0.15*(Na_2SO_4*$NaNO_3$)- 3.87*(NaCl*Na_2SO_4*$NaNO_3$); R^2=0.94, P<0.0001	EC_l=4.88*(NaCl)+4.31*(Na_2SO_4)+ 4.77*($NaNO_3$); r^2=0.33, P=0.0406

Free ion concentration data were used to estimate the osmotic potentials of the saline solutions (π_{ss}) with the van't Hoff's equation according to Nobel (1983) (Table 4.3). Additionally, the EC_E for all saline solutions were recalculated by taking into account the free concentrations of SO_4^{2-} ions in solution (Table 4.3). According to the SPECIES program SO_4^{2-} was the ion that had the lowest free ion concentrations (an average of 79% of the total applied SO_4^{-2} across all solutions) and the greatest ion associations (mainly with Ca^{2+} and Mg^{2+}; results not shown). Because of its relatively high total applied concentration, but lowest concentration as a free ion in solution, SO_4^{2-} was

considered to be the ion with the greatest impact on the effective EC of the final solutions.

Table 4.3. Applied salinity, electrical conductivities (EC), and osmotic potential (π_{ss}) of the saline solutions. Originally, the expected electrical conductivity was 2.785 $dS \cdot m^{-1}$, which included salinities provided by the base nutrient solution (1.1 $dS \cdot m^{-1}$), supplemental salts (1.2 $dS \cdot m^{-1}$), and tap water (0.485 $dS \cdot m^{-1}$).

Salt source and salt blend key	Applied salinity ($mmol \cdot L^{-1}$)	Expected EC (adjusted)** ($dS \cdot m^{-1}$)	Measured EC ($dS \cdot m^{-1}$)	Osmotic potential (MPa)
NaCl (1-0-0)*	12	2.70	2.70 ± 0.035	-0.1095
Na_2SO_4 (0-1-0)	6	2.49	2.46 ± 0.037	-0.0906
$NaNO_3$ (0-0-1)	12	2.70	2.69 ± 0.023	-0.1104
NaCl-Na_2SO_4 (0.5-0.5-0)	9	2.59	2.55 ± 0.022	-0.0999
NaCl-$NaNO_3$ (0.5-0-0.5)	12	2.70	2.72 ± 0.017	-0.1100
Na_2SO_4-$NaNO_3$ (0-0.5-0.5)	9	2.59	2.60 ± 0.033	-0.1003
NaCl-Na_2SO_4-$NaNO_3$ 0.33-0.33-0.33	10	2.62	2.61 ± 0.024	-0.1033

*Proportion of the anion (Cl^--SO_4^{2-}-NO_3^-) in the salt blend from a total of 12 $meq \cdot L^{-1}$.
**After adjusting the concentration of free SO_4^{2-} ions in solution.

While the differential electrical charge of the salinizing anions (Cl^-, SO_4^{2-} and NO_3^-) was equalized, i.e. applied on an equivalent basis, their expression in a total molar basis (in $mmol \cdot L^{-1}$) differed among saline solutions, depending on the proportion and type of anion used in each salt blend (Table 4.3).

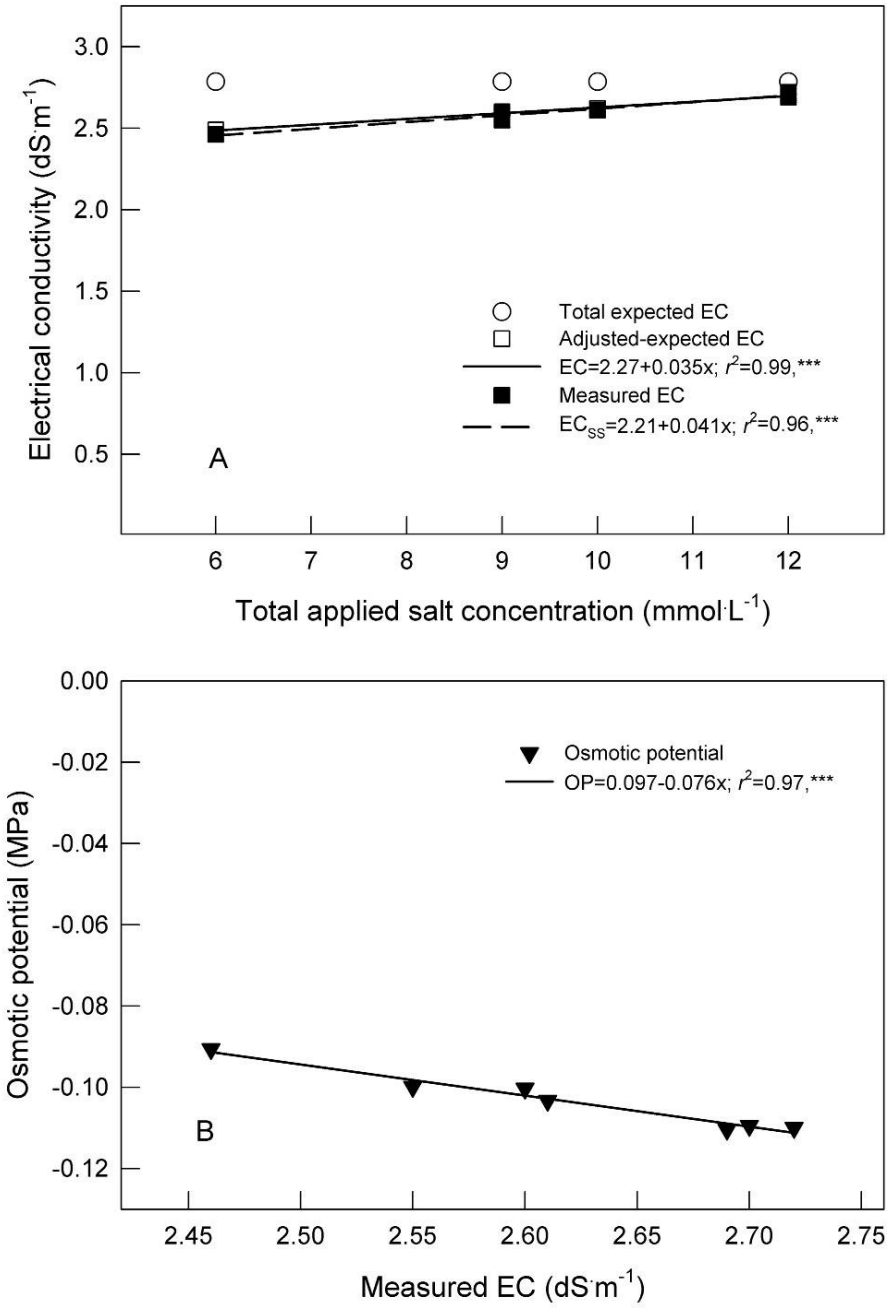

Fig. 4.5. (A) Electrical conductivities and (B) osmotic potential of the saline solutions applied to 'Bull's Eye' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. Significance according to GLM: ns, *,**,***=non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

After adjusting for the actual free SO_4^{2-} in solution, the EC_E of the saline solutions was very similar to the EC_{SS} measured throughout the experiment (r=0.98; Fig. 4.5A), and both EC values (adjusted-expected and measured) exhibited a positive, linear association with the total applied salinity expressed in a molar basis ($mmol \cdot L^{-1}$) (Fig. 4.5A). On the other hand, π_{ss} decreased linearly as EC_{SS} increased (Fig. 4.5B).

Variation in $[Cl_L]$ among SB was according to the [Cl] in the irrigation solution (Fig. 4.6). It was greater in leachates from NaCl-pure blends, followed by those from binary and tertiary blends and the lowest [Cl] were found in leachates from blends without additional Cl in the irrigation solution (only that from the tap water used to prepare the saline solutions, ~60 $mg \cdot L^{-1}$). 'Manetii' plants had greater $[Cl_L]$ in the pure blend of NaCl and the binary blend of Na_2SO_4-$NaNO_3$ than 'Natal Briar' plants (interaction between RS*SB, P=0.0055; Fig. 4.6). For the rest of the salt blends, $[Cl_L]$ was the same in both RS (Fig. 4.6).

Biomass and flower productivity

By the last harvest event at 279 DAT (harvest V) several plants did not bear flowering shoots which rendered many missing data for the analysis of LCI, and FSL since no leaves or stems were available for measurement. Therefore, for these two variables, only data from four harvests were included in the statistical analysis. For flowering shoots DW and FS harvested per plant, data from all five harvests were included in the statistical analysis since those plants not bearing flowering shoots would receive a value of zero for both variables instead of a missing value.

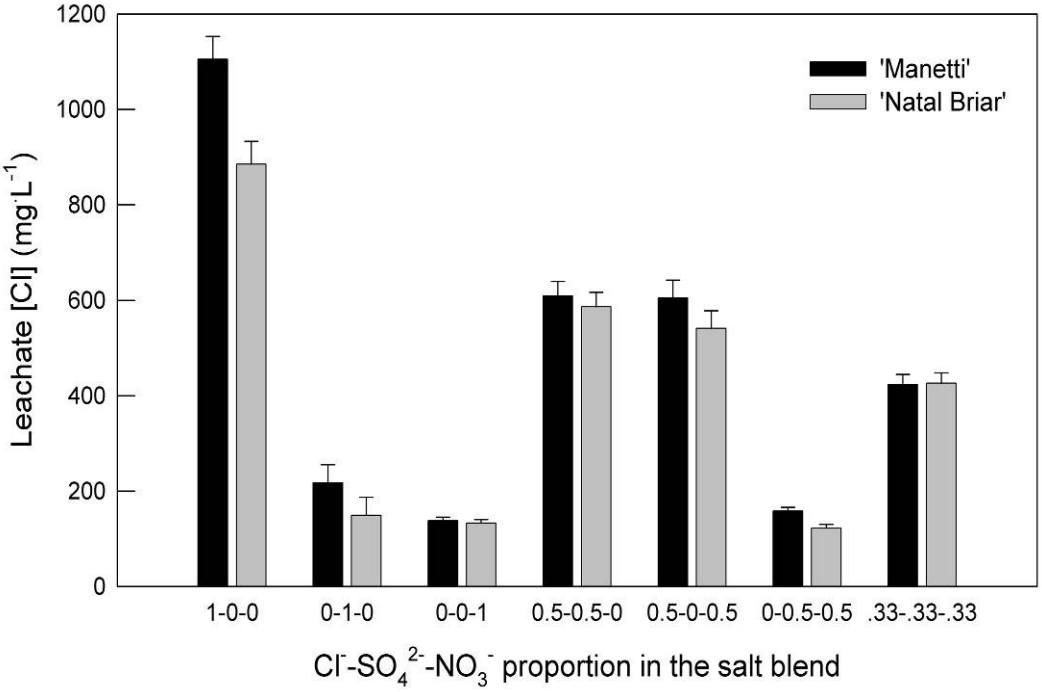

Fig. 4.6. Chloride concentration in leachates from 'Bull's Eye' roses budded 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. Bars represent the mean ± standard error of 42 observations.

Flowering shoot DW varied similarly over time for both RS and all SB (P>0.05 for both factors, data not shown). Relative FS harvested per plant and LCI varied similarly over time for all SB as well (P>0.05), but differently for both RS (RS*DAT interactions present; P=0.0388 for FS and P<0.0001 for LCI).

By the first harvest (42 DAT) plants budded on 'Natal Briar' produced slightly more FS than those on budded on 'Manetti' (Fig. 4.7A). However, by the second harvest (101 DAT) both RS produced a similar amount of FS, and afterwards 'Natal Briar' exhibited a more pronounced drop in FS produced per plant (Fig. 4.7A). As for LCI by the first harvest event (42 DAT) both RS had

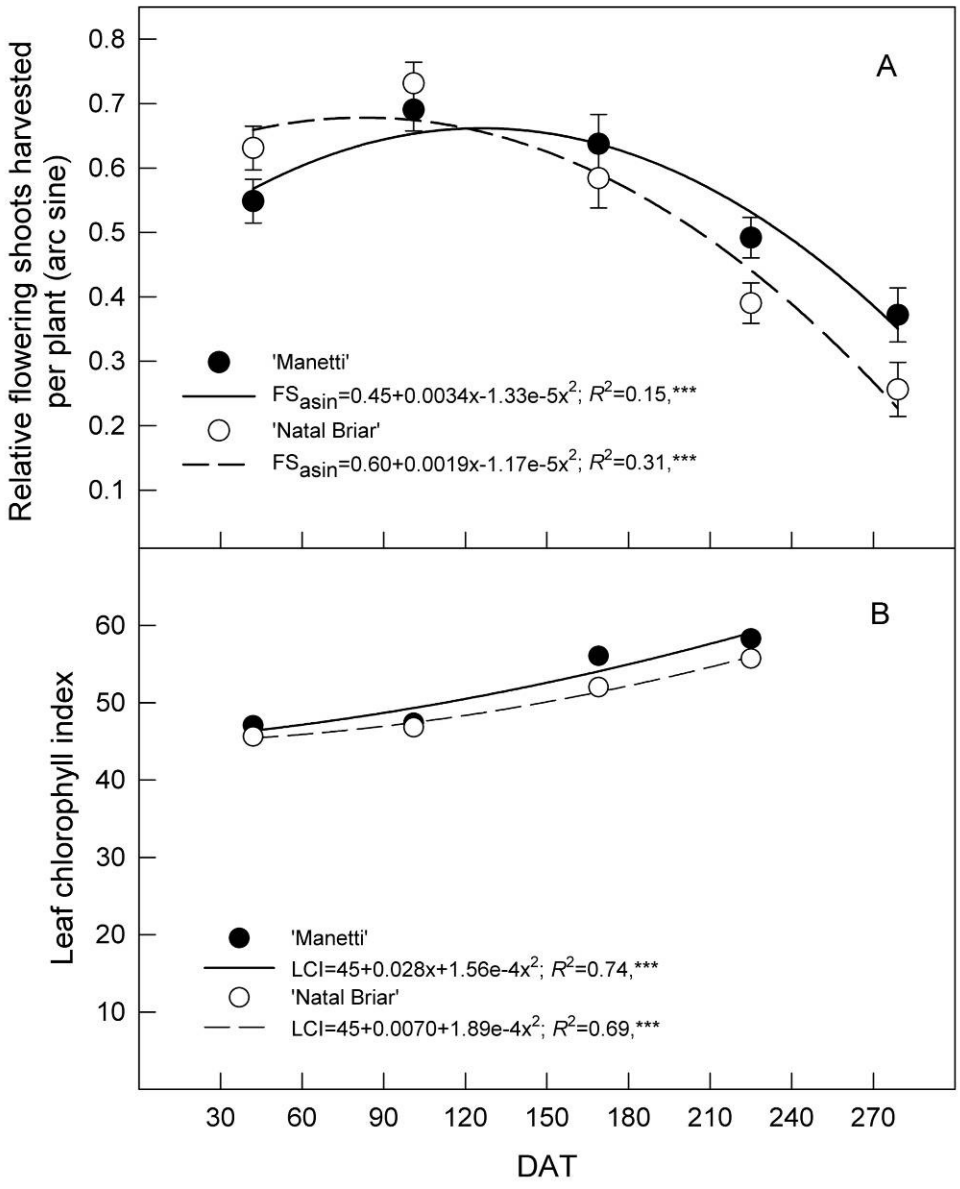

Fig. 4.7. (A) Relative flowering shoots harvested per plant and (B) leaf chlorophyll index of 'Bull's Eye' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high (12 mmol·L^{-1}) Na^+-based salinity in a half strength Hoagland's solution. Symbols represent the mean ± standard error of 12 plants. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,***=non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

similar indexes, but over time the rate of increase in LCI was greater for plants budded on ‘Manetti’ (Fig. 4.7B).

There were interactive effects between RS and SB for total flowering shoot DW and total FS harvested per plant (P=0.0306 and P=0.0141, respectively), and for the total average LCI (P=0.0611). Total average FSL was different only between RS (P=0.0017), with plants budded on ‘Manetti’ having longer stems (averages of 43.7 cm in ‘Manetti and 41.8 cm in ‘Natal Briar’).

In plants budded on ‘Manetti’ total flowering shoot DW response to SB fitted a special cubic model (P=0.0169; Fig. 4.8A; Table 4.4). Total flowering shoot DW was similar among all three pure blends (Fig. 4.8A). Chloride and SO_4^{2-} blended synergistically since the average DW from their binary blend was significantly greater (P=0.0351) than the average of their pure blends, as depicted by the upward curvature of the response surface above the edge of the triangle (Fig. 4.8A). There was some degree of upward curvature for the binary blends of Na_2SO_4-$NaNO_3$ and NaCl-$NaNO_3$, however, the increases in respect to their pure blends average was not significantly different from zero (P>0.05; Fig. 4.8A). When all three anions were present in the same proportion in the salt mixture (tertiary, centroid blend) the response of total flowering shoot DW was negative, as depicted by the depressed curvature of the central region of the response surface, with significantly lower DW average for the tertiary blend compared to the averaged DW from all three pure blends (P=0.0169; Fig. 4.8A).

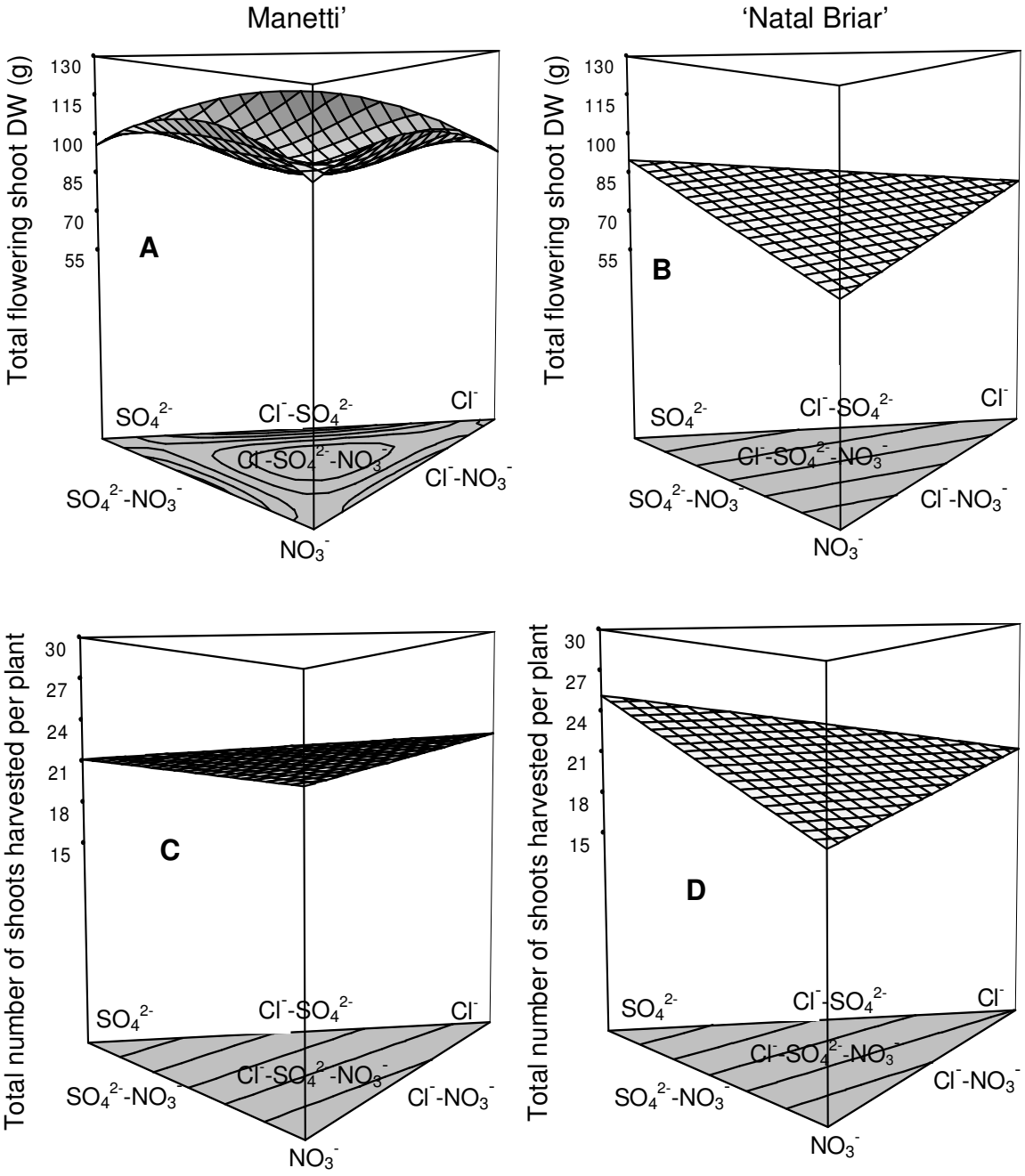

Fig. 4.8. Effect of varying the proportions of Cl^-, SO_4^{2-} and NO_3^- as the counter-anions of Na^+ in the salt mixture on total flowering shoot dry weight (A and B) and total number of shoots harvested per plant of 'Bull's Eye' roses budded on 'Manetti' (A and C) and 'Natal Briar' (B and D) rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surface. For fitted models see Table 4.4.

Table 4.4. Fitted models for productivity variables (total flowering shoots dry weight, DW; total number of shoots harvested per plant, FS; and leaf chlorophyll index, LCI) of 'Bull's Eye' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

Variable	'Manetti'	'Natal Briar'
Total flowering shoots dry weight	DW=89.83*(NaCl)+95.83*(Na_2SO_4)+98.00*($NaNO_3$)+ 90.00*(NaCl*Na_2SO_4)+47.67*(NaCl*$NaNO_3$) +60.33*(Na_2SO_4*$NaNO_3$)- 726.00*(NaCl*Na_2SO_4*$NaNO_3$); R^2=0.24, P=0.0169	DW =77.94*(NaCl)+90.21*(Na_2SO_4)+ 59.34*($NaNO_3$); r^2=0.27, P=0.0048
Total flowering shoots harvested per plant	FS=22.34*(NaCl)+21.21*(Na_2SO_4)+22.74*($NaNO_3$); r^2=0.016, P=0.7645	FS =20.49*(NaCl)+25.22*(Na_2SO_4)+18.15*($NaNO_3$); r^2=0.30, P=0.0026
Leaf chlorophyll index	LCI=51.44*(NaCl)+52.27*(Na_2SO_4)+53.27*($NaNO_3$)+ 3.60*(NaCl*Na_2SO_4)-3.07*(NaCl*$NaNO_3$)- 4.07*(Na_2SO_4*$NaNO_3$); R^2=0.19, P=0.1744	LCI =50.42*(NaCl)+50.62*(Na_2SO_4)+ 48.82*($NaNO_3$); r^2=0.16, P=0.0478

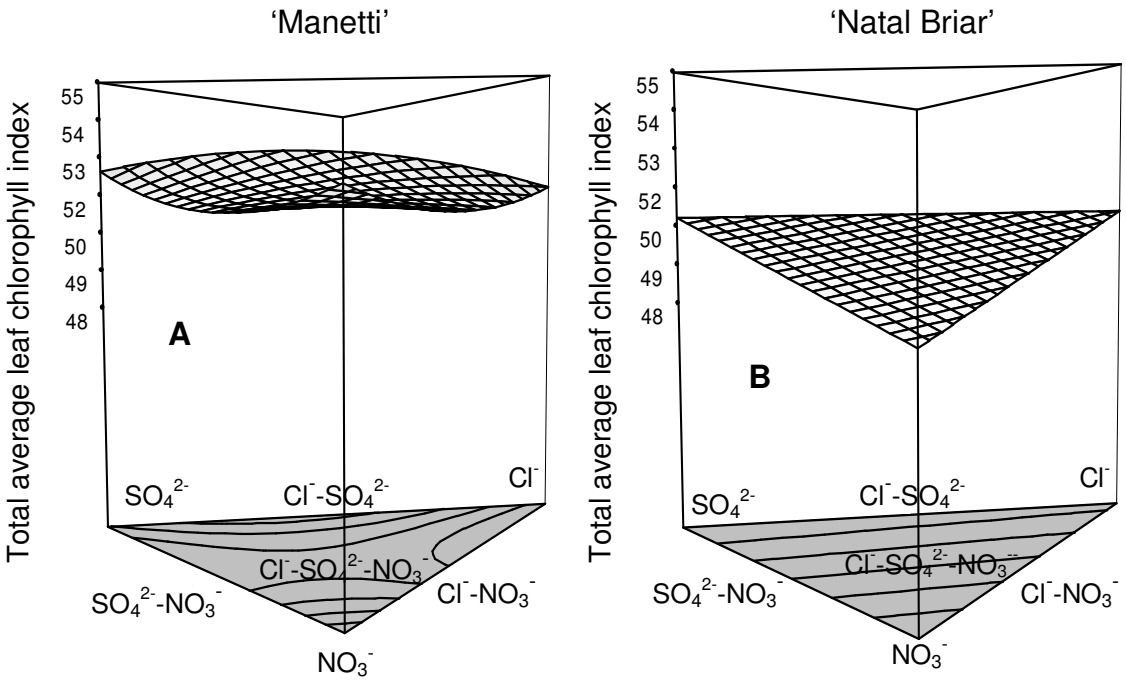

Fig. 4.9. Effect of varying the proportions of Cl^-, SO_4^{2-} and NO_3^- as the counter-anions of Na^+ in the salt mixture on total average leaf chlorophyll index of 'Bull's Eye' roses budded on 'Manetti' (A) and 'Natal Briar' (B) rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surface. For fitted models see Table 4.4.

In 'Manetti' total FS harvested per plant and total average LCI were not affected by SB (Fig. 4.8C and Fig. 4.9A; $P>0.05$ for both variables) with overall means of 22.1 and 52, respectively. The plots corresponding to these two variables are included only for visual comparative purposes between both RS. All three variables (total flowering shoot DW and total FS harvested per plant, and total average LCI) were statistically the same when comparing among pure blends in 'Manetti' plants. ($P>0.05$ for all).

In ‘Natal Briar’ plants total flowering shoots DW, total FS harvested per plant and total average LCI fitted linear models (*P*=0.0048, *P*=0.0026 and *P*=0.0478, respectively; Table 4.4).

In total flowering shoots DW and total FS responses to the mixture components there were significant effects due to Na_2SO_4 and $NaNO_3$. These effects were positive for Na_2SO_4, with both total flowering shoots DW and total FS harvested per plant increasing as the proportion of Na_2SO_4 in the salt blend increased, and negative for $NaNO_3$ with total flowering shoots DW and total FS decreasing as the proportion of $NaNO_3$ in the salt blend increased (Fig. 4.8B and 4.8D). Neither of these two variables (total flowering shoot DW and total FS) was affected by varying the proportion of NaCl in the salt mixture (Fig. 4.8B and 4.8D). Total average LCI was not affected by varying proportions of NaCl or Na_2SO_4 in the salt blend (Fig. 4.9B). Contrastingly, $NaNO_3$, had a negative effect on this response as total average LCI values tended to decrease as the proportion of the NO_3^- anion increased in the salt blend (Fig. 4.9B).

Given the differences observed in the experimental EC_{SS} and π_{ss} of the saline solutions (Table 4.3; Fig. 4.5A and 4.5B), it was considered pertinent to plot them against total flowering shoots DW and total FS harvested per plant to determine if the plants’ productivity had been affected by these two chemical properties of the saline solutions. For the evaluation of these relationships data from yield were converted to relative data to remove the inherent differences in vigor between both RS, and then transformed accordingly (Gomez and Gomez, 1984).

Neither EC_{SS} nor π_{ss} exhibited an apparent association with the productivity of plants budded on the ‘Manetti’ rootstock (Fig. 4.10A and 4.10B). Conversely, in plants budded on ‘Natal Briar’ both productivity variables (total flowering shoot DW and total FS harvested per plant) were significantly affected by EC_{SS} and π_{ss} (Fig. 4.10C and 4.10D) even though the intervals between the lowest and the greatest EC_{SS} and π_{ss} values were very narrow (0.26 $dS \cdot m^{-1}$ and

0.019 MPa, respectively). Total flowering shoot DW and total FS harvested per plant decreased with increasing values of EC_{SS} and with decreasing values (more negative) of π_{ss} (Fig. 4.10C and 4.10D).

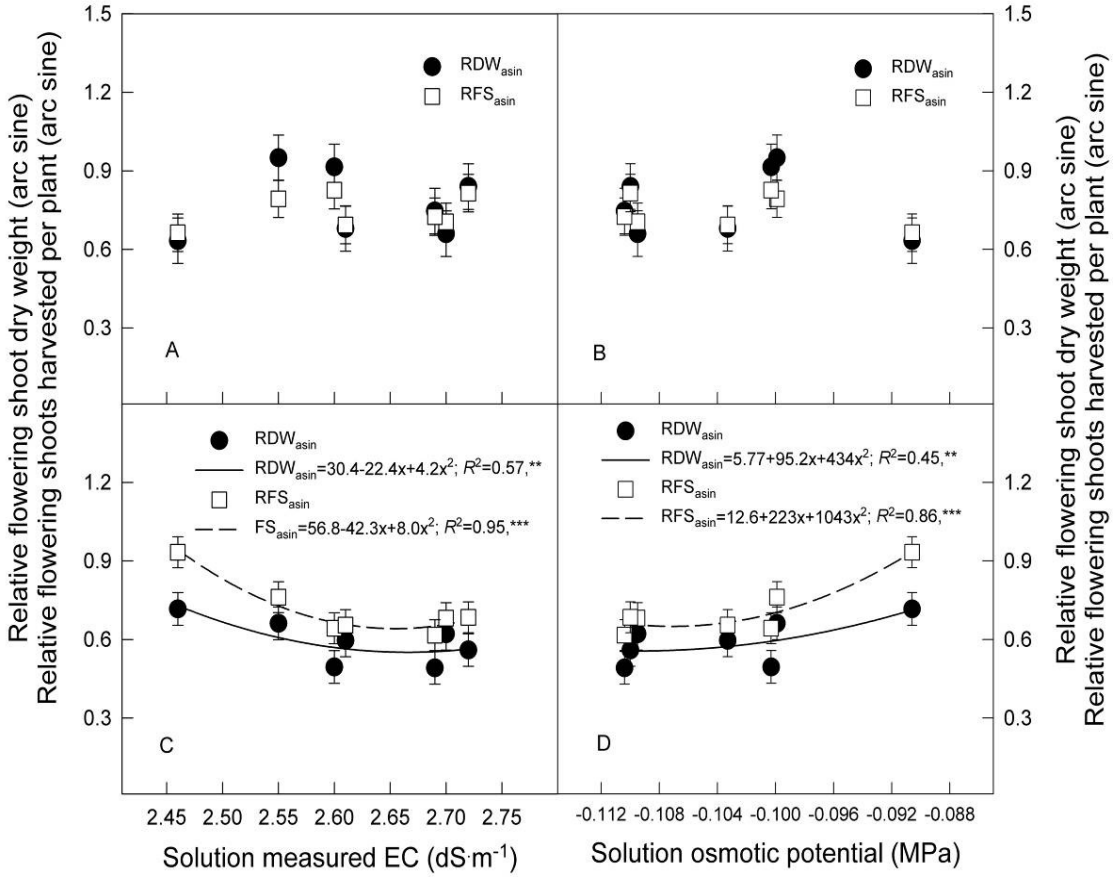

Fig. 4.10. Relative flowering shoot dry weight and relative flowering shoots harvested per plant of 'Bull's Eye' roses budded on 'Manetti' (A and B) and 'Natal Briar' (C and D) rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution. Symbols represent the mean ± standard error of 6 plants. Significance according to GLM: ns, *,**,***=non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Foliar salt injury

In order to determine if both RS were affected similarly by leaf salt injury, a Chi-square test was performed. However, due to the cell size restrictions for the Chi-square test to be valid (see details in Chapter II) each two adjacent salt injury categories were combined (0 and 1, 2 and 3, and 4 and 5), yielding three final categories: 1) up to 20% of the foliage affected, 2) salt injury affecting between 21-60% of the foliage, and 3) salt injury on 61-100% of the foliage. Additionally, 'Natal Briar' plants, which had been subjected to salt blends containing $NaNO_3$ (at 33%, 50% or 100%) exhibited substantial defoliation (mainly of those leaves grown in previous growth flushes) which interfered with the salt injury evaluation. Because of the defoliation problem treatments containing that specific salt were not included in the salt burn injury data analysis (for both RS) to avoid the problem of too many missing values. It is noteworthy here that plants budded on 'Manetti' or plants from both RS subjected to the other two mixture components (NaCl and Na_2SO_4) did not exhibit defoliation like 'Natal Briar' plants that had $NaNO_3$ in their salt mixture.

The degree of leaf injury across the salt blends evaluated (blends containing NaCl and/or Na_2SO_4) was similar in both RS (X=0.3571).

There was an interest in determining the influence of the salt mixture composition on the extent of foliar salt damage. However, due to the Chi-square restrictions (mentioned above) and because of the distribution of the salt injury ratings for the blends of NaCl and Na_2SO_4 (opposite ends of the category range), the combination of adjacent categories was not useful to perform the Chi-square test on these data. Nevertheless, considering the distinct pattern of distribution between salts a descriptive graphic is included. From those plants subjected to NaCl, 67% exhibited foliar salt injury corresponding to the greatest salt burn category while no plants fell into the first three categories (Fig. 4.11). Conversely, from those subjected to Na_2SO_4, 58% fell into the first category (0,

no visible salt damage) and no plants fell into the last three categories (greater damage ratings; Fig. 4.11). Thirty-three percent of the plants receiving the binary blends of NaCl-Na_2SO_4 fell into the first three categories and 67% of them fell into the last three (Fig. 4.11).

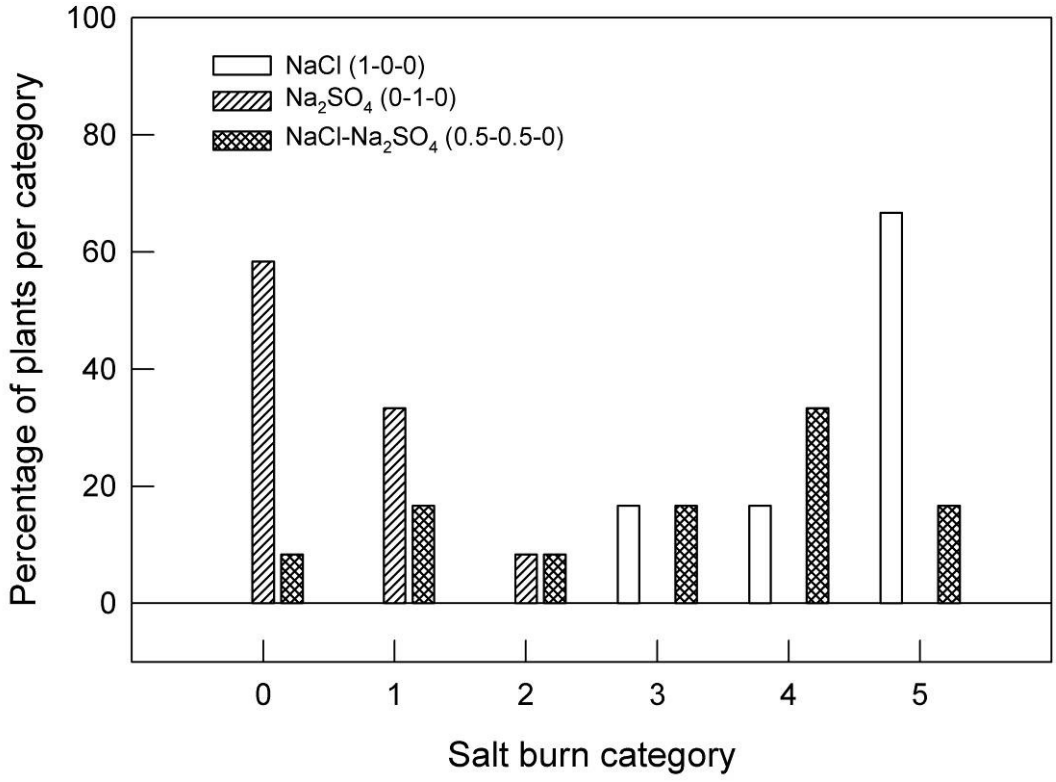

Fig. 4.11. Salt damage ratings of foliage of 'Bull's Eye' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's nutrient solution. Salt damage rating is based on a 0 to 5 scale in which 0=no foliar salt injury; 1=1%-20%; 2=21%-40%; 3=41%-60%; 4=61%-80%, and 5=81%-100% of the foliage presenting salt injury. (n=12).

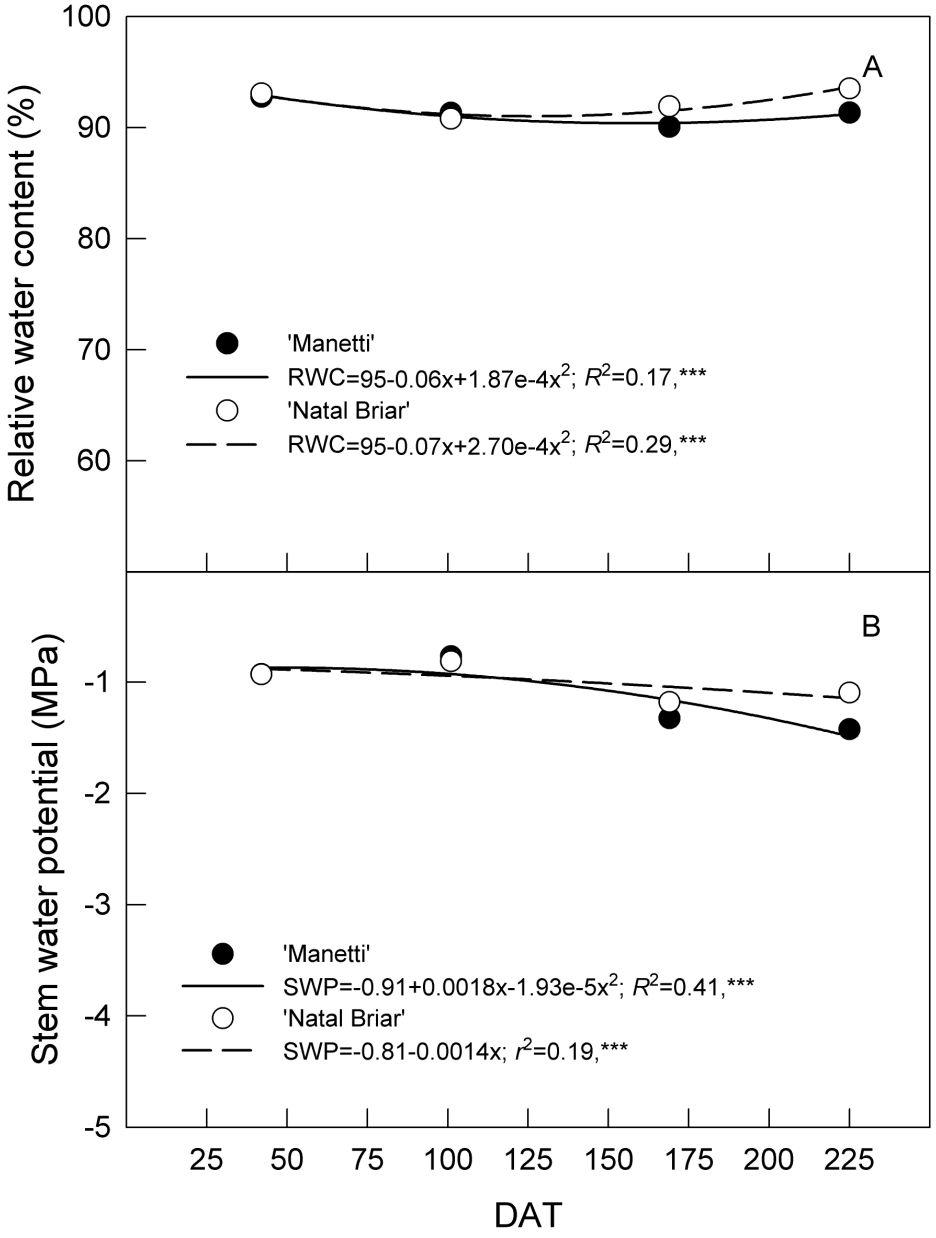

Fig. 4.12. (A) Relative water content and (B) stem water potential of 'Bull's Eye' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. Symbols represent the mean ± standard error of 84 observations. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,***=non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Water relations variables

There were interactive effects between RS and DAT for both RWC and SWP *(P*=0.0037 and *P*<0.0001, respectively). No interaction was present between RS and SB for both variables (*P*>0.05), therefore data were pooled within RS or SB, accordingly, for the graphs of RWC and SWP over time.

Both RS exhibited similar RWC and SWP patterns over time for the first half of the experimental period (until approximately 125 DAT; Fig. 4.12A and 4.12B). During the second half 'Manetti' plants experienced lower values for both variables with the greatest differences between RS recorded at the last measuring date (harvest IV, at 225 DAT; Fig. 4.12A and Fig. 4.12B).

Similarly, there were interactive effects between SB and DAT for both RWC (*P*=0.0365) and SWP (*P*=0.0261), however, the regression lines were not significant for RWC (*P*>0.05; Fig. 4.13A). As for SWP, in general, all salt blends showed decreasing linear patterns over time (Fig. 4.13B). Only the pure blend of Na_2SO_4 showed a quadratic pattern for SWP (Fig. 4.13B).

Tissue mineral nutrient content

Sodium [Na], chloride [Cl], sulfur [S] and nitrogen [N] concentrations

Sodium [Na]. Determination of leaf sodium concentrations [Na] were made at three dates (harvests II, III and IV; at 101, 169 and 225 DAT). For the statistical analysis of [Na] data, DAT was included as a discrete factor with three levels. There was a three-way interaction among RS, SB and DAT for leaf [Na] (*P*=0.0409).

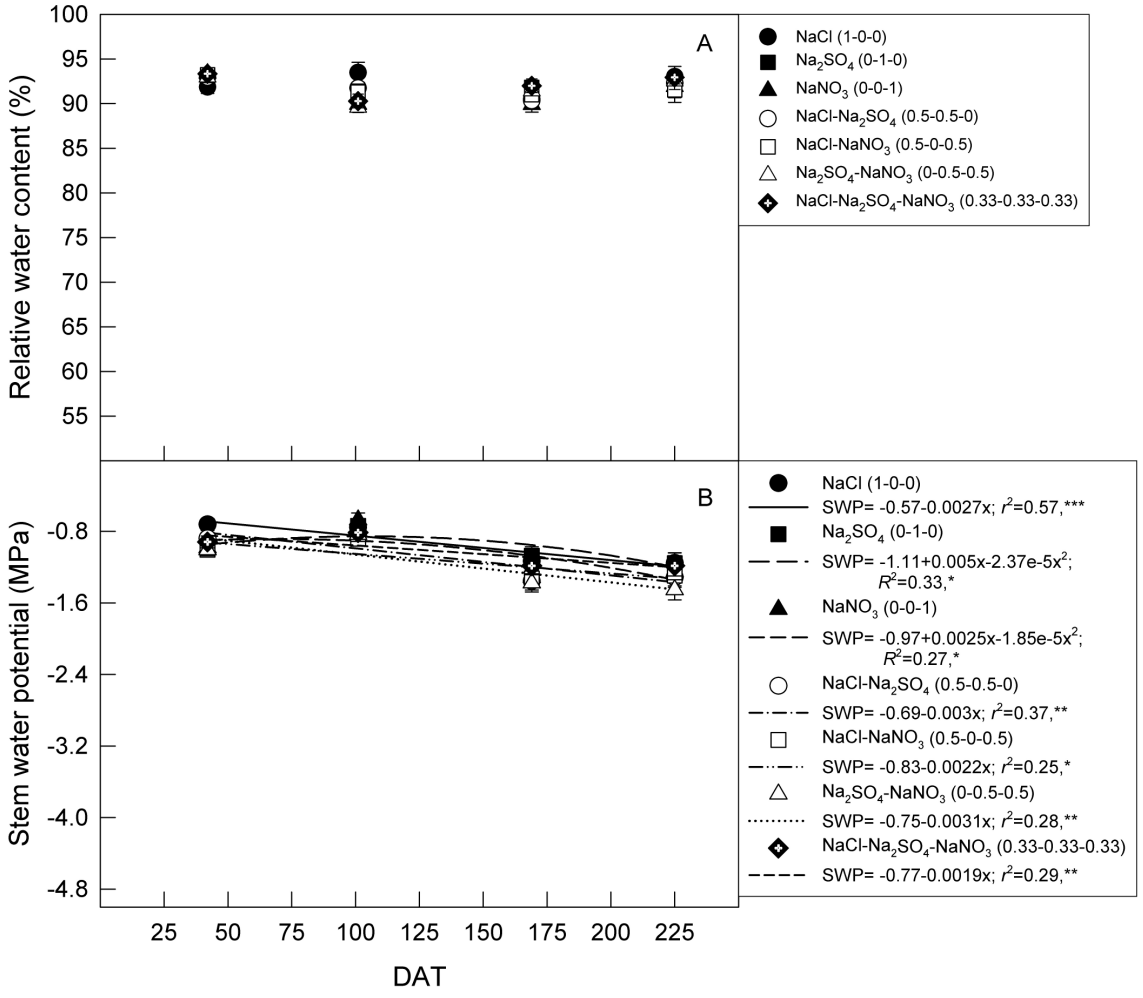

Fig. 4.13. (A) Relative water content and (B) stem water potential of 'Bull's Eye' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. Symbols represent the mean ± standard error of 24 observations. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,***=non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

By harvest II (101 DAT) [Na] was not affected by SB (model $P>0.05$). The rootstock selection, on the other hand, had a significant effect on [Na] ($P=0.0021$) with plants budded on 'Natal Briar' having greater leaf [Na] than those on 'Manetti' (290 $mg \cdot kg^{-1}$ versus 259 $mg \cdot kg^{-1}$, respectively).

By the third harvest event (169 DAT) leaf [Na] was affected by both RS ($P<0.0001$) and SB ($P=0.0141$), but no interaction between both factors was present, therefore data were pooled within RS for the statistical analysis. At 169 DAT plants budded on 'Natal Briar' had leaf [Na] 70% greater than those on 'Manetti' (620 $mg \cdot kg^{-1}$ versus 364 $mg \cdot kg^{-1}$, respectively). At 169 DAT the response of leaf [Na] fitted a linear model ($P<0.0001$; Fig. 4.14A; Table 4.5) with all three salt components having significant effects, negative for Cl^- and SO_4^{2-} and positive for NO_3^-. Increasing proportions of Cl or SO_4^{2-} caused a decrease in leaf [Na] while increases in NO_3^- caused increases in leaf [Na] (Fig. 4.14A). Comparing among the three pure blends, [Na] was similar between the Cl^- and SO_4^{2-} blends, and both had lower leaf [Na] than the NO_3^- blend (Fig. 4.14A).

By 225 DAT there were interactive effects between RS and SB ($P=0.0018$). In 'Manetti' plants leaf [Na] response to SB fitted a linear model ($P<0.0001$; Fig. 4.14B; Table 4.5). Chloride$^-$ and NO_3^- had positive effects (leaf [Na] increased as their proportions in the SB increased) while SO_4^{2-} had a negative effect as leaf [Na] tended to decrease with increasing concentrations of this anion in the salt mixture (Fig. 4.14B). Comparing among the three pure blends, plants subjected to the Cl^- blend had the greatest leaf [Na] followed by those subjected to NO_3^- and the lowest leaf [Na] were found on plants receiving SO_4^{2-} as the Na^+ counter anion (Fig. 4.14B).

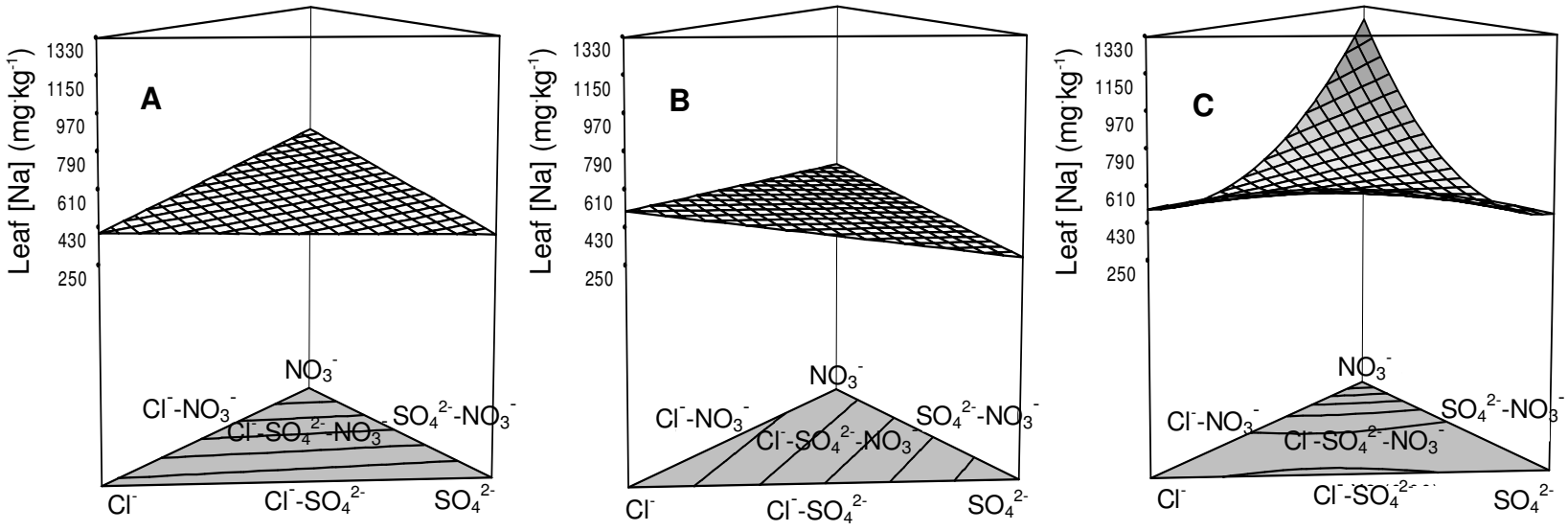

Fig. 4.14. Effect of varying the proportions of Cl^-, SO_4^{2-} and NO_3^- as the counter-anions of Na^+ in the salt mixture on sodium concentration [Na] in leaves of flowering shoots of 'Bull's Eye' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surface. (A) At 169 DAT, data from both RS averaged; (B) at 225 DAT, data from 'Manetti' and (C) at 225 DAT, data from 'Natal Briar'. For fitted models see Table 4.5.

Table 4.5. Fitted models for sodium concentration [Na] in leaves of flowering shoots (LFS) and main stems (MS) of 'Bull's Eye' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

Organ	'Manetti'	'Natal Briar'
LFS (169 DAT)	$[Na]_{\text{both RS averaged}}=407*(NaCl)+378*(Na_2SO_4)+653*(NaNO_3)$; $r^2=0.52$, $P<0.0001$	
LFS (225 DAT)	$[Na]=512*(NaCl)+267*(Na_2SO_4)+456*(NaNO_3)$; $r^2=0.61$, $P<0.0001$	$[Na]=502*(NaCl)+459*(Na_2SO_4)+1258*(NaNO_3)+436*(NaCl*Na_2SO_4)-1107(NaCl*NaNO_3)-1247(Na_2SO_4*NaNO_3)$; $r^2=0.59$, $P=0.0002$
MS	$[Na]_{\text{both RS averaged}}=4.94*(NaCl)+4.04*(Na_2SO_4)+4.48*(NaNO_3)+2.04*(NaCl*Na_2SO_4)-3.58*(NaCl*NaNO_3)+1.39*(Na_2SO_4*NaNO_3)-45.2(NaCl*Na_2SO_4*NaNO_3)$; $R^2=0.34$, $P=0.0177$	

At 225 DAT in 'Natal Briar' plants leaf [Na] response fitted a quadratic model (*P*=0.0052; Fig. 4.14C; Table 4.4). All salt blends containing NaCl and Na_2SO_4 (pure, binaries and tertiary) had leaf [Na] within the range of those in 'Manetti' plants and of the previous harvest (Fig.4.14A-B). Plants subjected to the pure blend of $NaNO_3^-$, on the other hand, exhibited [Na] considerably greater than the rest of the salt blends by approximately 100% (Fig. 4.14C).

As for [Na] in organs from the destructive harvest, there were interactive effects between organ and RS, and between organ and SB (*P*<0.0001 and *P*=0.0072, respectively). 'Natal Briar' plants had greater [Na] in main stems, old stems and old leaves than those from 'Manetti' (4.85 $g{\cdot}kg^{-1}$ versus 3.62 $g{\cdot}kg^{-1}$ for main stems, 3.05 $g{\cdot}kg^{-1}$ versus 1.11 $g{\cdot}kg^{-1}$ for old stems, and 2.35 $g{\cdot}kg^{-1}$ versus 1.36 $g{\cdot}kg^{-1}$ for old leaves, of 'Natal Briar' and 'Manetti' plants, respectively) while 'Manetti' plants had greater [Na] in roots compared to those from 'Natal Briar' (4.25 $g{\cdot}kg^{-1}$ versus 4.95 $g{\cdot}kg^{-1}$).

It was only for main stems (similarly in both RS) that the composition of the salt blend affected the response of [Na] which fitted a special cubic model (*P*=0.0177; Table 4.5). Linear and quadratic terms in the model (corresponding to the pure and binary blends, respectively) were not significant. This means that all three pure blends had similar [Na] with averages ranging between 4.0 $g{\cdot}kg^{-1}$ and 4.9 $g{\cdot}kg^{-1}$, and that the average [Na] from the binary blends between two of the three anions (Cl^-, SO_4^{2-} and NO_3^-) was not significantly different from their pure blends averaged. Only when mixed together at equal proportions in the tertiary (centroid) blend the salt components affected negatively the response of [Na] (*P*=0.0056) as depicted by the negative coefficient for the tertiary blend in the response model (Table 4.5).

Chloride [Cl]. There was a three-way interaction among RS, SB and DAT (*P*=0.0004). In both RS leaf [Cl] differed among SB according to the [Cl] in the saline solution, greater concentrations for the pure NaCl blends, followed by binary and tertiary NaCl blends, and last the blends without NaCl in the salt

mixture (Fig. 4.15A and Fig. 4.15B). Even though leaf [Cl] patterns among SB were in general terms similar between RS, plants budded on 'Natal Briar' had on average (across all SB, except the NaCl pure blends) leaf [Cl] greater by 202%, 149%, 59% and 63% by 42, 101, 169 and 225 DAT, respectively. 'Manetti' plants subjected to the NaCl pure blend had a steeper increase in leaf [Cl] than any other salt blend within the same rootstock, and than those from 'Natal Briar' (Fig. 4.15A and Fig. 4.15B). However, despite of this steep increase, 'Manetti' plants receiving NaCl at 100% still exhibited lower leaf [Cl] than those from 'Natal Briar' for the first two harvest events (at 42 and 101 DAT; Fig. 4.15A and Fig. 4.15B). By the third harvest (at 169 DAT) plants on 'Manetti' slightly surpassed those from 'Natal Briar' and by the fourth harvest (at 225 DAT) [Cl] was 22% greater in 'Manetti' plants.

Leaf [Cl] data from flowering shoots harvested at 225 DAT were analyzed separately in a factorial arrangement of treatments (with RS and SB as factors only) to determine the maximum concentrations reached in the leaves by the last stages of the experiment and the influence of RS and SB exerted on them (data from the last harvest event at 279 DAT were not used due to many plants not bearing flowering shoots).

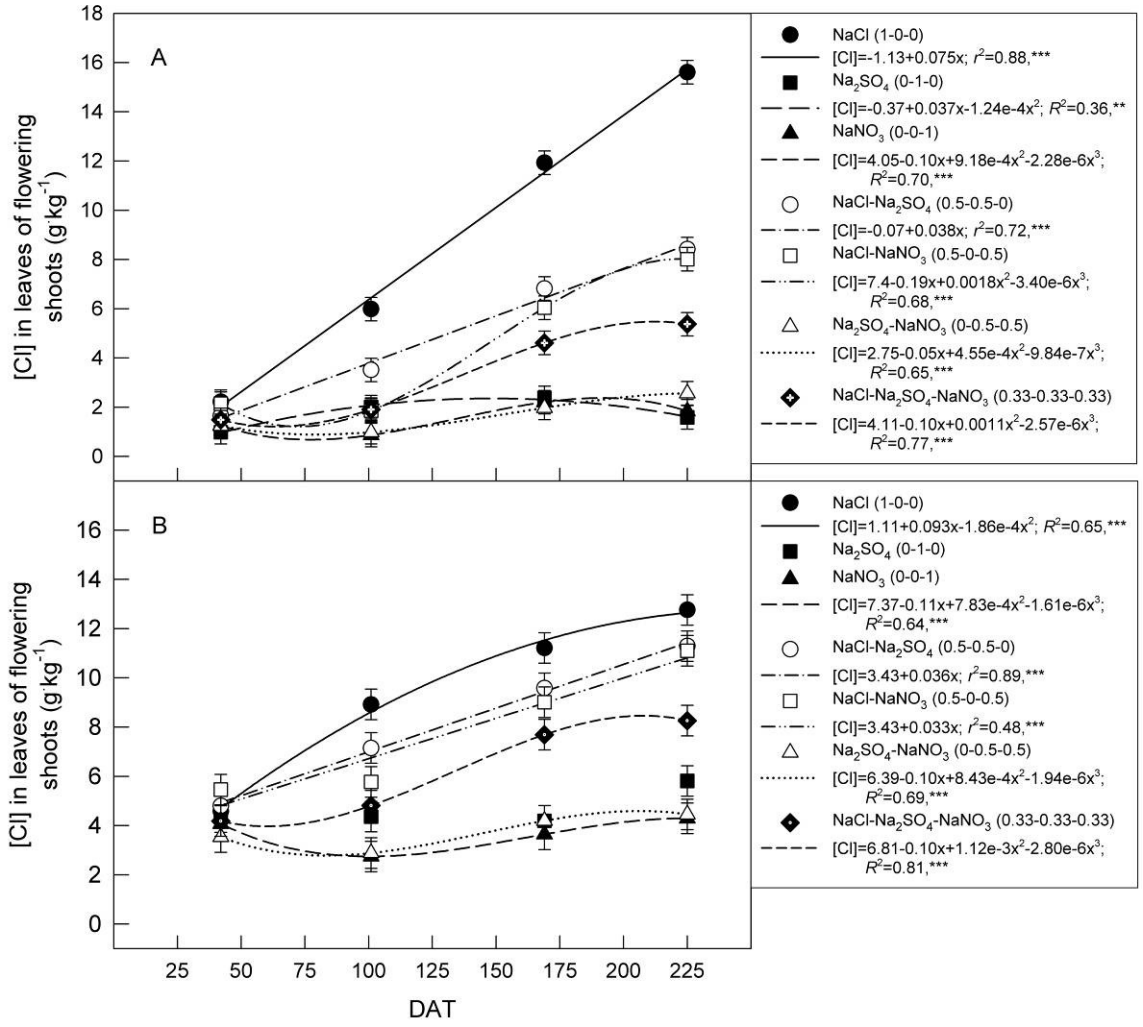

Fig. 4.15. Leaf chloride concentration over time in flower shoots of 'Bull's Eye' roses budded on 'Manetti' (A) and 'Natal Briar' (B) rootstocks and subjected to a moderately high Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. Symbols represent the mean ± standard error of six observations. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,***=non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

There was an interaction between RS and SB (P=0.0009) on leaf [Cl]. At the NaCl pure blend, leaf [Cl] was the same for both RS (P=0.1967; Fig. 4.16A). However, for the rest of the SB leaf [Cl] were considerably greater in flowering shoots of plants budded on 'Natal Briar' (P≤0.05 for all salt blends; Fig. 4.16A).

As for the organs from plants destructively harvested at the end of the experimental period, there were interactive effects between RS and SB (P=0.0480) for [Cl]. Old leaves from plants subjected the NaCl blends had considerably greater [Cl] compared to the rest of the plant organs (roots, main stems and old stems) in both RS (Fig. 4.16B and 4.16C). In plants subjected to the NaCl (1-0-0) and the NaCl-Na_2SO_4 (0.5-0.5-0) blends old leaves exhibited similar [Cl] in both RS (P>0.05; Fig. 4.16B-C). Between the NaCl binary blends (NaCl-Na_2SO_4 and NaCl-$NaNO_3$), however, [Cl] were greater when NaCl was combined with Na_2SO_4 than when combined with $NaNO_3$ (P<0.05; Fig. 4.16B-C).

Differences among the lower plant organs (roots, main stems and old stems) were more evident in plants budded on 'Natal Briar'. In general, the higher position of the organ the greater its [Cl] (Fig. 4.16C). 'Manetti' plants had, for most of the salt blends, greater [Cl] in roots and main stems than those from 'Natal Briar' (Fig. 4.16B and 4.16C).

Sulfur [S]. There were interactive effects between RS and DAT for leaf [S] (P<0.0001). Plants budded on 'Manetti' had leaf [S] greater than those in 'Natal Briar' at both sampling dates (101 and 225 DAT), however, the difference in [S] was less pronounced by 225 DAT (5.83 $g \cdot kg^{-1}$ versus 4.12 $g \cdot kg^{-1}$ for 101 DAT and 2.64 $g \cdot kg^{-1}$ versus 2.30 $g \cdot kg^{-1}$ for 225 DAT, for 'Manetti' and 'Natal Briar' plants, respectively).

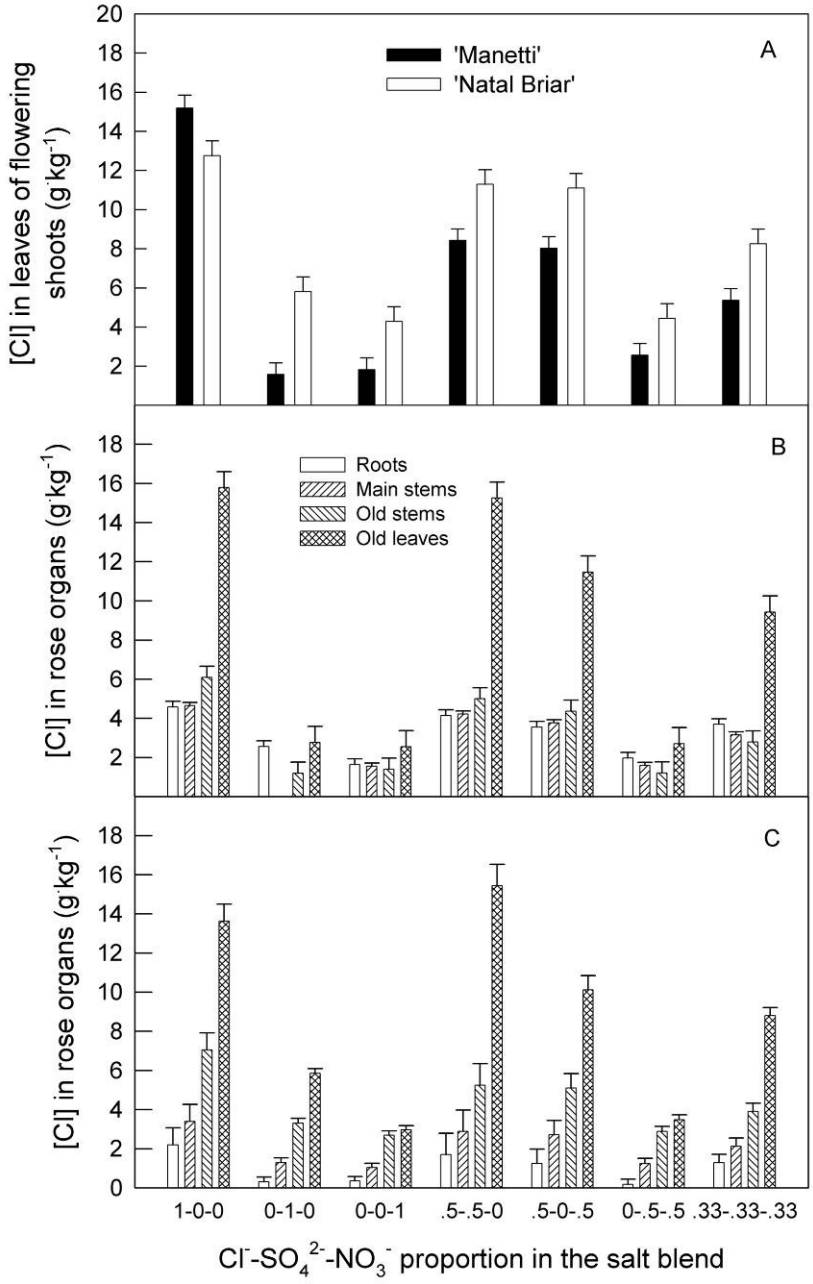

Fig. 4.16. Chloride concentration [Cl] in leaves of flowering shoots harvested at 225 DAT (A) and in plant organs of 'Bull's Eye' roses budded on 'Manetti' (B) and 'Natal Briar' (C) rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. Bars represent the mean ± standard error of 6 plants for plot A and 3 plants for plots B and C.

There were effects due to the salt blends on [S] (P<0.0001). On average (both sampling dates pooled) [S] ranged between 3.14 $g \cdot kg^{-1}$ and 4.44 $g \cdot kg^{-1}$ and were slightly greater in leaves of plants receiving Na_2SO_4 (at 33, 50 or 100%) than in plants without supplemental SO_4, except for plants subjected to the NaCl pure blend. These plants had the greatest [S] (4.44 $g \cdot kg^{-1}$) which was similar to those from plants subjected to Na_2SO_4 in the salt blend.

Nitrogen [N]. Nitrogen concentration was similar between RS (P>0.05). There were interactive effects between SB and DAT (P=0.0382). At 101 DAT there were differences in [N] only among the pure blends. Plants subjected to the pure blend of Na_2SO_4 had greater leaf [N] than plants subjected to the pure blends of NaCl or $NaNO_3$ (37.20 $g \cdot kg^{-1}$ versus 35.73 and 35.60 $g \cdot kg^{-1}$, respectively). By 225 DAT all salt blends had similar leaf [N] which ranged between 34.82 and 37.01 $g \cdot kg^{-1}$.

Leaf concentration of other mineral nutrients

Phosphorous [P]. There were interactive effects among RS, SB and DAT (P=0.0077) for leaf phosphorous concentration [P].

At 101 DAT there were effects due to RS and SB (P<0.0001 and P=0.0077, respectively), but no interaction between both factors was present (P>0.05). Thus data were pooled accordingly for the statistical tests. Plants budded on 'Manetti' had greater leaf [P] than those on 'Natal Briar' (3.26 $g \cdot kg^{-1}$ versus 2.86 $g \cdot kg^{-1}$, respectively). Leaf [P] response to SB fitted a linear model (P=0.0408; Fig. 4.17A; Table 4.6) with significant effects for SO_4^{2-} and NO_3^-. Leaf [P] increased as the proportion of SO_4^{2-} in the SB increased and decreased as the proportion of NO_3^- increased (Fig. 4.17A). There were no significant changes in leaf [P] as the proportion of Cl^- varied in the salt blend (Fig. 4.17A).

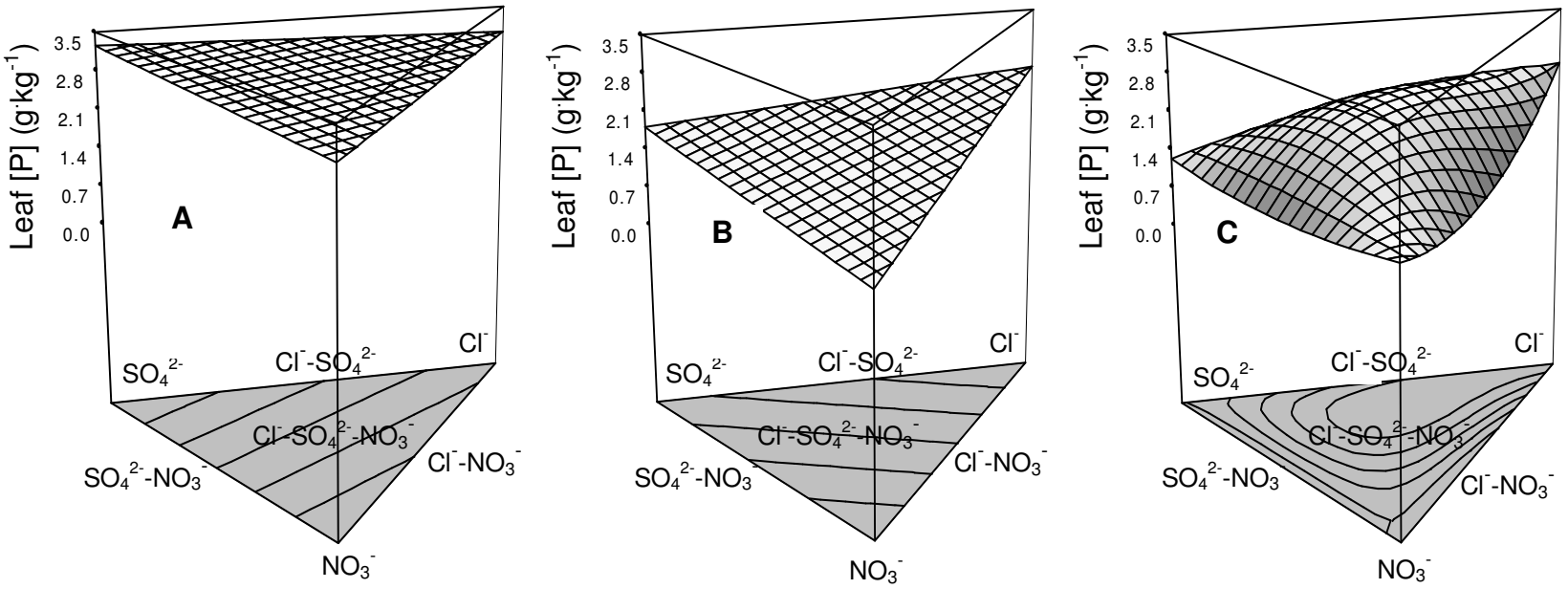

Fig. 4.17. Effect of varying the proportions of Cl^-, SO_4^{2-} and NO_3^- as the counter-anions of Na^+ in the salt mixture on phosphorous concentration [P] in leaves of flowering shoots of 'Bull's Eye' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surface. (A) At 101 DAT, data from both RS averaged; (B) at 225 DAT, data from 'Manetti' and (C) at 225 DAT, data from 'Natal Briar'. For fitted models see Table 4.6.

Table 4.6. Fitted models for leaf phosphorus concentration [P] in leaves on flowering shoots of 'Bull's Eye' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

Mineral Element/Date	'Manetti'	'Natal Briar'
P (101 DAT)	[P]=3.03*(NaCl)+3.25*(Na_2SO_4)+2.89*($NaNO_3$); r^2=0.11, P=0.0408	
P (225 DAT)	[P]=2.43*(NaCl)+1.82*(Na_2SO_4) +0.86*($NaNO_3$); r^2=0.53, P<0.0001	[P]=2.49*(NaCl)+1.23*(Na_2SO_4)+1.31*($NaNO_3$)+1.14*(NaCl*Na_2SO_4)-3.12*(NaCl*$NaNO_3$)-0.91*(Na_2SO_4*$NaNO_3$)+26.44*(NaCl*Na_2SO_4*$NaNO_3$); R^2=0.93, P<0.000

At 225 DAT there was an interaction between RS and SB (*P*=0.0093). In 'Manetti' plants the response of leaf [P] fitted a linear model (*P*<0.0001). In this case Cl- and NO_3^- had significant effects. Leaf [P] increased as the proportion of Cl- in the SB increased and decreased as the proportion of NO_3^- increased (Fig.4.17B). Varying proportions of SO_4^{2-} had no effect on leaf [P] in this rootstock at 225 DAT. In 'Natal Briar' the response of leaf [P] fitted a special cubic model (*P*<0.0001; Fig. 4.17C; Table 4.6). In this rootstock Cl^- and SO_4^{2-} had synergistic positive effects on [P] (upward curvature above the Cl-SO_4 edge of the triangle, blocked by the crest of the plot; Fig. 4.17C) while Cl^- and NO_3^- had had a negative effect when both were present in the salt mixture (downward curvature of the surface response on their edge of the triangle) (Fig. 4.17C). Sulfate and NO_3^- had no effects on [P] when combined together in the salt mixture as the average leaf [P] from their binary blend was statistically the same as their pure blends averaged (Fig. 4.17C).

Potassium [K]. There was a three-way interaction for leaf [K] among RS, SB and DAT (*P*=0.0037). At 101 DAT there were no effects due to SB (*P*=0.6373), however, RS did have significant effects (*P*<0.0001). Plants budded on 'Manetti' had greater leaf [K] than those budded on 'Natal Briar' at 101 DAT (27.1 $g \cdot kg^{-1}$ versus 25.4 $g \cdot kg^{-1}$, respectively). By 225 DAT leaf [K] was affected by SB differently in each RS (interaction between RS and SB was present, *P*=0.0028). In 'Manetti' plants the response of leaf [K] to SB fitted a linear model (*P*<0.0001; Fig. 4.18A; Table 4.7), with significant effects for Cl^- and NO_3^-. The response was positive for Cl^-, as leaf [K] increased with increasing proportions of Cl^- in the SB, and negative for NO_3^-, as leaf [K] decreased with increasing proportions of NO_3^- in the SB (Fig. 4.18A). Sulfate had no significant effects on leaf [K], i.e. there were no significant changes in leaf [K] as the proportion of SO_4^{2-} in the SB varied (Fig. 4.18A). In 'Natal Briar' plants, on the other hand, the response of leaf [K] to SB fitted a special cubic model (*P*<0.0001; Fig. 4.18B; Table 4.7). In this RS the tertiary blend (all three salt

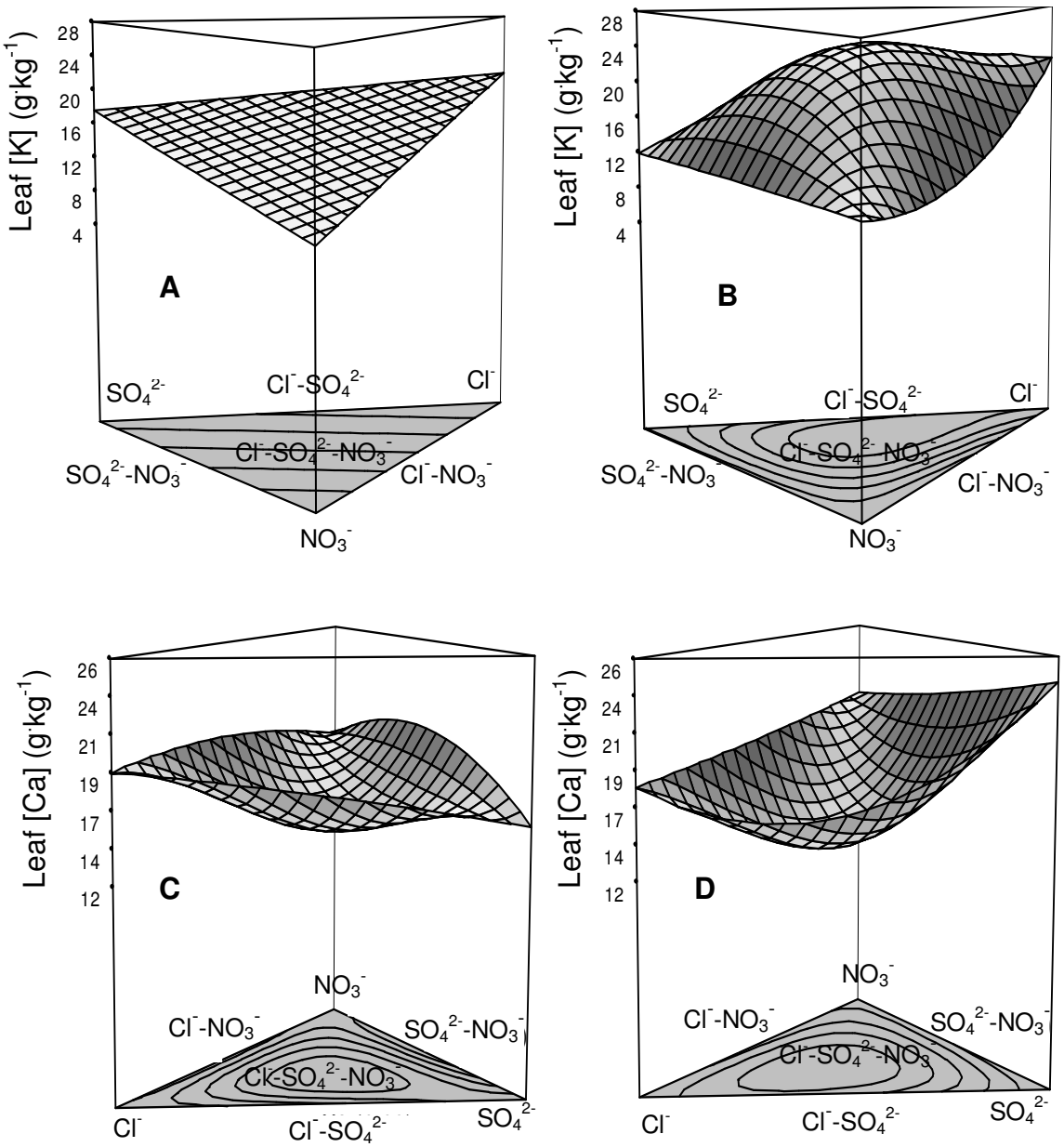

Fig. 4.18. Effect of varying the proportions of Cl^-, SO_4^{2-} and NO_3^- as the counter-anions of Na^+ in the salt mixture on potassium ([K], A and B) and calcium ([Ca], C and D) concentrations in leaves of flowering shoots of 'Bull's Eye' roses budded on 'Manetti' (A and C) and 'Natal Briar' (B and D) rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surface. Data correspond to leaves of flowering shoot harvested at 225 DAT. For fitted models see Table 4.7.

Table 4.7. Fitted models for leaf potassium [K] and calcium [Ca] concentrations in leaves on flowering shoots of 'Bull's Eye' roses budded on 'Manetti' and 'Natal Briar' rootstocks and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution with varying proportions of the accompanying anions (Cl^-, SO_4^{2-} and NO_3^-) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend. Data correspond to leaves of flowering shoot harvested at 225 DAT.

Mineral Element/Date	'Manetti'	'Natal Briar'
K	[K]=21.16*(NaCl)+17.60*(Na_2SO_4)+7.95*($NaNO_3$); r^2=0.63, P<0.0001	[K]=22.00*(NaCl)+11.97*(Na_2SO_4)+10.26*($NaNO_3$)+16.41*(NaCl*Na_2SO_4)-19.20*(NaCl*$NaNO_3$) +0.73*(Na_2SO_4*$NaNO_3$)+ 245*(NaCl*Na_2SO_4*$NaNO_3$); R^2=0.90, P<0.0001
Ca	[Ca]=19.04*(NaCl)+15.42*(Na_2SO_4)+ 18.32*($NaNO_3$)+1.05*(NaCl*Na_2SO_4)+ 4.37*(NaCl*$NaNO_3$)+13.13*(Na_2SO_4* $NaNO_3$)-141*(NaCl*Na_2SO_4*$NaNO_3$); R^2=0.46, P=0.0302	[Ca]=17.95*(NaCl)+24.39*(Na_2SO_4)+21.13* ($NaNO_3$)-20.52*(NaCl* Na_2SO_4)- 0.87*(NaCl*$NaNO_3$)-1.49*(Na_2SO_4*$NaNO_3$)- 138*(NaCl*Na_2SO_4*$NaNO_3$); R^2=0.58, P=0.0044

components present in the mixture) had a significant positive effect yielding the greatest [K] of the surface response (Fig. 4.18B). Compared to the pure blends of SO_4^{2-} and NO_3^- leaf [K] were greater for that of Cl^- (as shown by their respective coefficients in the final model equations; Table 4.7), which concentrations were almost as great as those from the tertiary blends (Fig. 4.18B). The binary blends of $NaCl$-Na_2SO_4 and $NaCl$-$NaNO_3$ exhibited quadratic effects, positive for the first, negative for the second. Chloride and SO_4^{2-} had a synergistic effect since leaf [K] were greater when both anions were present in the salt blend than their pure blends averaged (the upward curvature corresponding to the tertiary blends is blocking the Cl^--SO_4^{2-} edge; Fig. 4.18B). Contrarily, when Cl^- and NO_3^- were both present in the salt blend, leaf [K] was lower than the average of their pure blends (downward curvature on the Cl-NO_3 edge; Fig. 4.18B). Leaf [K] showed no change when both SO_4^{2-} and NO_3^- were present in the SB respect to their pure blends (Fig. 4.18B).

Calcium [Ca]. At 101 DAT leaf [Ca] was not affected by SB (P<0.05), but it was by RS (P=0.007). Plants budded on 'Natal Briar' had greater leaf [Ca] than those from 'Manetti' (17.36 $g \cdot kg^{-1}$ versus 16.01 $g \cdot kg^{-1}$, respectively). At 225 DAT an interaction between RS and SB was present (P=0.0195). In both RS the response of leaf [Ca] to the composition of the SB fitted special cubic models (P=0.0302 for 'Manetti' and P=0.0044 for 'Natal Briar'; Fig. 4.18C-D; Table 4.7).

When all three salt components were present in the salt blend the effect on leaf [Ca] was negative for both RS as depicted by the downward curvature in the central region of the surface response (Fig. 4.18C-D).

In 'Manetti' plants SO_4^{2-} and NO_3^- had a synergistic effect with their binary blend yielding slightly greater leaf [Ca] than their pure blends averaged (P=0.0163; Fig. 4.18C). In this RS leaf [Ca] was the same among all three pure blends (P>0.05; Fig. 4.18C).

In 'Natal Briar' plants, on the other hand, when mixed together Cl^- and SO_4^{2-} [Ca] exhibited a negative quadratic response (downward curvature on the

Cl^--SO_4^{2-} edge of the triangle; *P*=0.0249). In this rootstock plants subjected to the pure blend of NaCl had significantly lower leaf [Ca] compared to the pure blends of Na_2SO_4 and $NaNO_3$ (Fig. 4.18D).

Magnesium [Mg]. There were not effects due to RS (*P*>0.05) and an interaction between SB and DAT was present (*P*=0.0108), thus data from both RS were pooled. At 101 DAT there were no differences in leaf [Mg] among all salt blends (*P*>0.05; results not shown). At 225 DAT composition of the SB affected leaf [Mg] (*P*=0.0499). The response fitted a linear model (*P*=0.0037; Fig. 4.19A) and there were significant effects for SO_4^{2-} and NO_3^-. Leaf [Mg] increased as the proportion of NO_3^- in the SB increased and decreased as the proportion of SO_4^{2-} in the SB increased (Fig. 4.19A). Varying proportions of Cl^- in the salt mixture did not affect leaf [Mg] (Fig. 4.19A).

Boron [B]. There were interactions present between RS and DAT and between SB and DAT (*P*=0.0089 and *P*<0.0001, respectively). At 101 DAT there were differences between RS (*P*<0.0001) but not among SB (*P*=0.8171). 'Manetti' plants had an average leaf [B] of 39 $mg \cdot kg^{-1}$ while in 'Natal Briar' plants leaf [B] averaged 73 $mg \cdot kg^{-1}$ (87% greater). By 225 DAT there was an interaction between RS and SB (*P*=0.0298). In 'Natal Briar' there were no differences in [B] for all salt blends (*P*>0.05), averaging 118 $mg \cdot kg^{-1}$. In 'Manetti' plants on the other hand, composition of the salt mixture affected leaf [B] (*P*=0.0009). In this RS response of leaf [B] fitted a linear model (*P*<0.0001; Fig. 4.19B) with significant effects for Cl^- and NO_3^-. As Cl^- proportion in the SB increased, leaf [B] decreased while the opposite happened with NO_3^-, as its proportion in the SB increased so did leaf [B] (Fig. 4.19B).

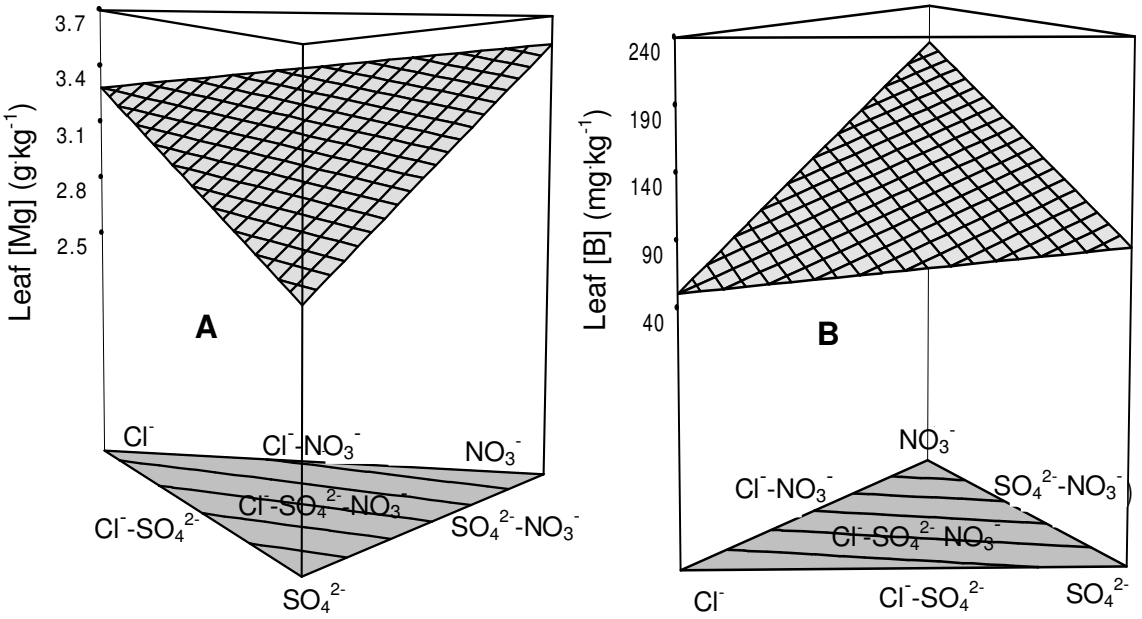

Fig. 4.19. Effect of varying the proportions of Cl^-, SO_4^{2-} and NO_3^- as the counter-anions of Na^+ in the salt mixture on magnesium ([Mg], A) and boron ([B], B) concentrations in leaves of flowering shoots of 'Bull's Eye' roses budded on 'Manetti' and 'Natal Briar' and subjected to a moderately high (12 $mmol \cdot L^{-1}$) Na^+-based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surface. Data correspond to leaves of flowering shoots harvested at 225 DAT. Plot A, both RS averaged; plot B, plants budded on 'Manetti'. Plot models: A) $[Mg]=3.29*(NaCl)+2.54*(Na_2SO_4)+3.56*(NaNO_3)$; $r^2=0.19$, $P=0.0037$;. B) $[B]=51*(NaCl)+84*(Na_2SO_4)+209*(NaNO_3)$; $r^2=0.60$, $P<0.0001$.

Iron [Fe]. There were differences between RS only ($P<0.0001$). Plants budded on 'Manetti' had greater leaf [Fe] than those on 'Natal Briar' (55.21 $mg \cdot kg^{-1}$ versus 50.15 $mg \cdot kg^{-1}$, respectively).

DISCUSSION

Overall salinity stress response

The electrical conductivity of all the applied saline solutions was expected to be around 2.785 $dS \cdot m^{-1}$, with 1.1 $dS \cdot m^{-1}$ provided from the base nutrient solution, 1.2 $dS \cdot m^{-1}$ from the supplemental salts, and 0.485 $dS \cdot m^{-1}$ from the tap water. The actual measured EC_{SS} ranged though from 2.46 to 2.72 $dS \cdot m^{-1}$ (Table 4.3, Fig. 4.5A). This corresponds to calculated EC values between 7.38 and 8.16 $dS \cdot m^{-1}$ in the soil solution and between 3.69 $dS \cdot m^{-1}$ and 4.08 $dS \cdot m^{-1}$ in the saturation extract from a soil/substrate (Farnham et al., 1985). The EC_{SS} (Fig. 4.4C) and EC_L values (Fig. 4.2A and Fig. 4.3A) surpassed the maximum soil solution salinity (EC 2-3 $dS \cdot m^{-1}$ in the saturation extract; Bernstein et al.,1972; Davidson and Boodley, 1987; Hughes and Hanan, 1978) and leachate (EC 1.4-1.8 $dS \cdot m^{-1}$; Brun and Settembrino, 1996) thresholds that have been recommended for roses in the past. More recent studies, however, have shown that greenhouse roses could be tolerant to greater levels of salinity than those previously established. 'Kardinal' roses (*Rosa hybrida* L. 'Kardinal') budded on *R. rubiginosa* could tolerate up to 10 $mmol \cdot L^{-1}$ NaCl in the irrigation water without a significant reduction in the total and root dry weights (Wahome et al., 2000). Cabrera and Perdomo (2003) subjected 'Bridal Pink' roses (*R. hybrida* L. 'Bridal Pink') budded on 'Manetti' to 0, 5 and 10 $mmol \cdot L^{-1}$ NaCl (EC of the irrigation solution ~1.6, 2.1, and 2.6 $dS \cdot m^{-1}$, respectively) without any significant effects on flower yield and quality over four growth and flowering flushes. Thereafter, the applied NaCl concentrations were increased 3-fold to 0, 15 and 30 $mmol \cdot L^{-1}$ (EC of the irrigation solution ~1.6, 3.1, and 4.6 $dS \cdot m^{-1}$, respectively) and the plants continued to be evaluated for another four flowering flushes. No significant differences in cut-flower yield and quality were observed among salt treatments despite further increases in leachate EC and Na and Cl concentrations (Cabrera

and Perdomo, 2003). In both studies, the effects of salinity stress on the rose scion cultivars were influenced by the rootstock selection used. In previous experiments (Chapters II and III), whose main objectives were to determine the salinity tolerance limits of 'Red France' roses and the ameliorative effects of supplemental calcium on the response of 'Happy Hour' roses to saline stress, respectively, it was found that the stress imposed by saline solutions ≥ 12 $mmol \cdot L^{-1}$ NaCl-$CaCl_2$ (2:1 molar ratio) caused reductions in plant productivity (flowering shoots DW and FS harvested per plant) and affected the water status of the plants (lower RWC and more negative SWP and LOP). Based on the results from our previous experiments and those from Cabrera and Perdomo (2003) and Wahome et al. (2000), it was inferred that the Na^+-based salinity tolerance limit for greenhouse roses, although highly influenced by the rootstock, was between 12 and 15 $mmol \cdot L^{-1}$.

There were marked differences in EC_{SS} and in EC_L among the different SB, which were due apparently to the type and proportion of the anion present in the salt mixture. Sulfate had a noticeable effect on lowering EC of both saline solutions and, therefore, of leachates (Fig. 4.4C-D; Table 4.3). Calculating the concentrations of free ions ([free ion]) and ion pairs ([ion pairs]) (SPECIES program; Barak, 1990), it was determined that in all solutions, independently of the type of supplemental salts used, only between 78% and 81% of the applied SO_4^{2-} was present as a free ion in solution, and 19 to 22% was associated (ion pairs), primarily with Ca^{2+} (13.30%), Mg^{2+} (3.71%), Na^+ (2.85%) and K^+ (1%). Conversely, 98.5% and 100% of the applied Cl^- and NO_3^-, respectively, were present as free ions in solution. Relative to all other ions in all solutions, SO_4^{2-} had the lowest [free ion], followed by Ca^{2+} (74-91%) and Mg^{2+} (77-91%). The greater the concentration of SO_4^{2-} applied, the lower the percentage of Ca^{2+} and Mg^{2+} as free ions in solution and vice versa, with their lowest [free ion] percentages observed at the pure blend of Na_2SO_4, (SO_4^{2-} supplied at 100%).

On an equivalent basis, the total salinity applied to all seven solutions was the same, varying only the type and proportion of the salt blend components, specifically the Na-accompanying anions. All solutions had a total of 12 molecular units of the monovalent cation Na^+, but for every applied unit of the monovalent anions Cl^- and/or NO_3^-, only half a unit of the divalent anion SO_4^{2-} was applied. Thus, the total concentration of molecular particles (or units) was lower in the SO_4^{2-}- than in the Cl^-- and/or NO_3^--based SB. In addition, according to the data obtained with the SPECIES program (Barak, 1990), 19-22% of the total SO_4^{2-} applied to all solutions formed ion pairs. Therefore, ion pair formation was greater in Na_2SO_4-based salt blends (whose initial number of SO_4^{2-} molecules was greater) compared to NaCl- and/or $NaNO_3$-based blends. Sulfate-ion associations with Ca^{2+} and Mg^{2+} not only meant a decrease in the SO_4^{2-} [free ion], but also a decrease in Ca^{2+} and Mg^{2+} [free ion]'s. In solutions with ions of symmetrical valency (i.e. the absolute values of the signed units of charge are the same) of double or higher charge, an appreciable fraction of the ions are present as closely associated pairs (Robinson and Stokes, 1970). Such pairs will have no net charge. They will therefore make no contribution to the electrical conductivity of the solution, while their thermodynamics effects will be those of removing a certain number of ions from the solution and replacing them by half the number of dipolar 'molecules' (Robinson and Stokes, 1970). Ion-associations between SO_4^{2-} and Ca^{2+} and Mg^{2+} had a dual effect on the electrochemical properties of the solutions. First, by forming molecules with no net charge, they caused a decrease in the EC_{SS} (Table 4.3). Indeed, after adjusting the actual concentration of SO_4^{2-} free in solution, the calculated EC (sum of anions in $meq \cdot L^{-1}$ divided by 10; Richards, 1954) of each saline solution was very close to their measured EC_{SS} (Fig. 4.5A). Second, due to the ion pair formation, two ions (SO_4^{2-} and either Ca^{2+} or Mg^{2+}) were replaced by one dipolar molecule, reducing even more the initially lower number of ions present in the SO_4^{2-}-salt blends (due to its divalent electrical charge) increasing, consequently,

their π_{SS} (Table 4.3) (Ben-Gal et al., 2009). This suggests a lower osmotic stress imposed on the plants by these particular SB. When the total salinity of the solutions was expressed in $mmol \cdot L^{-1}$, instead of $meq \cdot L^{-1}$, a close linear relationship was observed between total applied salinity and EC_{SS} (Fig. 4.5A). Similarly, EC_{SS} and π_{SS} were closely associated (Fig. 4.5B). These observations point to a rarely considered and/or studied situation, namely the significance of both the specific and differential contribution of each ion in solution to the actual (resultant or measured) EC and its effective osmotic strength (Ben-Gal et al., 2009). Therefore, it is contended that the common reporting of salinity on the equivalent or equinormal basis of EC alone might be masking or hiding effects that are effectively influencing soil solution chemistry and plant/crop responses. This also poses the challenging task of (re)interpreting results among both similar and dissimilar salinity studies by comparing them on a more level or integrative salt stress index or basis.

The drop in FS harvested per plant in both RS coincided approximately with the time when EC_L reached a stable level (Fig. 4.2A, Fig. 4.3A and Fig. 4.7A). By the first harvest event (42 DAT) plants budded on 'Natal Briar' were producing more FS, however, by the third harvest (169 DAT) those on 'Manetti' produced more FS, with a greater difference registered at 279 DAT (Fig. 4.7A). Similarly, approximately at the same time when 'Manetti' plants started to show greater productivity rates, they started to exhibit lower RWC and SWP (Fig. 4.12A-B). Their lower water status could be due to their greater DW production, compared to 'Natal Briar' plants, being attained with the same amounts of irrigation solution applied. The greater plant productivity (FS) exhibited by 'Manetti' plants during the last half of the experimental period (after approximately four months of salt stress exposure) is an indicator of its greater salinity tolerance ability. In a previous experiment (Chapter II), similar results were observed in 'Red France' roses grafted on 'Manetti', yielding more and longer flowering shoots under $NaCl\text{-}CaCl_2$ (0-24 $mmol \cdot L^{-1}$) saline stress

compared to ‘Natal Briar’. Obiol and Cardús (1974) have also reported *Rosa* ‘Manetti’ as more productive than *R. indica* L. ‘Major’ and *R. canina* L. Cabrera (2002), on the other hand, found no differences among *Rosa* ‘Manetti’, *R. x odorata* (Andrews) Sweet, *Rosa* ‘Natal Briar’ and *Rosa* ‘Dr. Huey’ in flower and dry biomass yield data collected over four flushes of growth. In both studies (Obiol and Cardús, 1974, and Cabrera, 2002) rose plants were grown under non-saline conditions.

In the present experiment LCI tended to increase throughout the experimental period in both RS and was in general greater in foliage of plants budded on the ‘Manetti’ rootstock (Fig. 4.7B). On one hand, rose scions have been shown to have darker foliage when budded on ‘Manetti’ than when budded on ‘Natal Briar’ or ‘Dr. Huey’ under non-saline conditions Cabrera (2002). On the other hand, leaves of salt-affected plants often have a darker green color than those of normal plants (Bernstein, 1975).

Rose leaves present a long-term acclimation to light, by adapting their photosynthetic capacity seasonally (González-Real and Baille, 2000). More photosynthetic nitrogen is allocated to leaves in autumn and spring than in summer (González-Real and Baille, 2000). Cabrera (2000) observed a close linear relationship of N concentrations with LCI and color attributes in ‘Royalty’ rose plants and also observed greater average leaf N concentrations in the winter months compared to those observed during the summer.

During the winter months, light energy is the major limiting factor for rose production in the northern parts of the United States and Western Europe (Mastalerz, 1987). In our experiment four successive harvest events took place on the months of September, November, January and March. The tendency of LCI values to increase over time could be explained by both increasing time of exposure to salt stress and leaf acclimation to reduced light conditions over the winter months. Rose plants experience several successive harvests throughout the year, and at the end of each growth and flowering flush, the flowering stems

are removed and a new growth cycle starts from the axillary buds located on the parent shoots (Cockshull and Horridge, 1977; Marcelis-van Acker, 1994). Consequently, the photosynthetic capacity of the leaves of flower stems plays a key role in achieving sustained rose flower production (González-Real and Baille, 2000). Leaf chlorophyll index or SPAD, an index of the relative chlorophyll density in a leaf, is a parameter that represents the acquisition of light at the leaf surface (Hiyama et al., 2005). Consequently greater LCI values could involve a greater capacity to intercept light and therefore achieve greater photosynthetic rates (Kozlowski and Pallardy, 1997). If plants budded on 'Manetti' have greater LCI this could confer them with a greater photosynthetic capacity over other rootstocks, especially in those months when light intensity represents a limiting factor for flower production.

Influence of the Na^+-accompanying anion on the response to salinity

Total average flowering shoot length, total FS harvested per plant and total average LCI in 'Manetti' plants responded similarly to all salt blends (Fig. 4.8C and 4.9A). On total flowering shoot DW there were effects due to the binary blend of NaCl-Na_2SO_4 and to the tertiary blend (all salts present in the blend) (Fig. 4.8A). However, total flowering shoot DW was the same among all three pure salt mixtures, i.e. no linear effects for the three anions were found, and the rest of the binary blends had similar total flowering shoot DW to their averaged pure blends (Fig. 4.8A).

In 'Natal Briar' plants, on the contrary, the counter-anion NO_3^- had a negative effect on total flowering shoot DW, total FS and total average LCI, whereas Cl^- had no effects on any these variables' responses and SO_4^{2-} had a positive effect (Fig 4.8B, Fig. 4.8D and Fig. 4.9B). In a previous experiment (Chapter III) it was found that Cl^- affected negatively plant productivity and caused foliar salt injury to a greater extent than SO_4^{2-}. In fact, plants subjected

to Na_2SO_4 yielded similar total flowering shoot DW and total FS than non-salinized control plants. Interestingly, in the present experiment it was observed that those plants subjected to the pure blends of SO_4^{2-} exhibited less foliar injury (in both RS), whereas those from 'Natal Briar' subjected to NO_3^- as the Na^+-accompanying anion experienced defoliation to a great extent.

Based on results from the present and the two previous experiments (Chapters II and III) 'Manetti' has shown to have a greater tolerance to salinity stress than 'Natal Briar'. The greater sensitivity to salinity exhibited by plants grafted on this last RS thus allowed for a clearer distinction of the detrimental effects caused by the salt treatments imposed in this experiment.

Niu and Rodriguez (2008b) evaluated the response of four rose (*Rosa* L.) rootstocks to chloride- or sulfate-dominated salinities. According to their results there were interactive effects between rootstock selection and salt composition on plant DW response to salinity. At moderate salt stress (EC ~ 3.9 $dS \cdot m^{-1}$) Cl-dominated salinity caused greater dry weight reductions only in *R. x fortuniana* Lindl., whereas in *Rosa* L. 'Dr. Huey', *R. multiflora* Thunb. and *R.* x *odorata* (Andrews) Sweet dry weight reductions were similar between moderate Cl^- and SO_4-based salinities. Niu and Rodriguez (2008b) observed, however, that Cl-dominated salinity led to lower visual quality of all rootstocks, especially in *R. x fortuniana*. Sulfate-based salinities have been reported as being less deleterious than Cl-based salinities on tomato (*Lycopersicon esculentum* Mill.; Yokas et al. 2008), sweet pepper (*Capsicum annuum* L.; Navarro et al., 2002), snapbean (*Phaseolus vulgaris* L. 'Contender'; Awada et al., 1995), and rabbiteye blueberries 'Tifblue' and 'Brightwell' (Wright et al., 1992). According to Grattan and Grieve (1999), many crops are very sensitive to high internal chloride levels and species are generally more tolerant to sulfate-salinity than chloride-salinity. Bañuls et al. (1997) studied the effects of different salt sources (NaCl, KCl and $NaNO_3$, at 60 $mmol \cdot L^{-1}$) on Valencia orange [*Citrus sinensis* (L.) Osbeck] budded on Cleopatra mandarin (*Citrus reticulata* Blanco) or trifoliate orange [*Poncirus*

trifoliata (L.) Raf.]. According to their findings Cl-based salts markedly reduced plant growth in both scion-root stock combinations whereas $NaNO_3$ had very little effect.

From a first impression it appears that in 'Manetti' the effects of salt stress on plant growth were due to osmotic effects and not to specific ion (toxicity) effects. In 'Natal Briar', it could be inferred that the detrimental effects were due to both general osmotic stress and specific ion toxicities. However, separating osmotic effects on whole plants from toxic effects is not a straightforward task, and in fact it may well be impossible (Ben-Gal et al., 2009). A study of the effects of increasing concentrations of NaCl and $CaCl_2$, either alone or in equinormal combination on three different species: bean (*Phaseolus vulgaris* L.), corn (*Zea mays* L.) and melon (*Cucumis melo* L.) showed that when yield response was related to the electrical charge concentration of the salts, i.e. salinity expressed in $meq \cdot L^{-1}$ or EC, the stress effects of Na and Ca appeared to be of different magnitudes (Ben-Gal et al., 2009). Plant growth was more sensitive to excess of Na than to excess of Ca and the effect of combined Na and Ca was intermediate. The effects of the two salts were, however, indistinguishable when salinity was expressed in terms of osmotic potential of the irrigation water. Growth inhibition appeared to depend only on the specific contribution of a particular salt to the total osmotic pressure, since for all three species the response curves of yield as a function of level of equipotential solutions of NaCl, $CaCl_2$ or combinations of the two salts practically overlapped (Ben-Gal et al., 2009). Apparent differences between the effects of excess Na and Ca on plant yield stemmed from the different valences of the ions. In solutions of equinormal concentrations of NaCl and $CaCl_2$, the number of Cl ions was the same but the number of Na ions was about twice that of Ca, i.e. the concentration of particles was greater and, consequently, the solution's osmotic potential was lower (Ben-Gal et al., 2009).

Consequently, the detrimental effects caused by salinity, particularly on plants grafted on 'Natal Briar', could be due more to the differential contribution of each particular salt to the total osmotic potential of the saline solution rather than to specific ion effects.

Sodium, Cl and other mineral nutrients accumulation in flowering shoot leaves

Sodium concentration in leaves of flowering shoots seemed to be greatly influenced by the Na^+-accompanying anion. While SO_4^{2-} did not affect leaf [Na^+], Cl^- and NO_3^- noticeably promoted increases in leaf [Na^+], especially NO_3^-, whose effect was even more marked on plants grafted on 'Natal Briar' (Fig. 4.14C). Awada et al. (1995) compared the effects of NaCl and Na_2SO_4 (at 0, 15, 30, 45 and 60 $mmol \cdot L^{-1}$) on snapbean (*Phaseolus vulgaris* 'Contender'). Interestingly, even though [Na^+] were effectively two-fold in the Na_2SO_4 treatments, (due to stoichiometry in solutions, as the comparative salt concentrations applied in the irrigation solution were in $mmol \cdot L^{-1}$), the authors found greater Na^+ contents in shoots of snapbean plants subjected to the NaCl treatments compared to those subjected to Na_2SO_4. Similar findings were reported by Renault et al. (2001) who studied the effects of 0, 25, 50 or 100 $mmol \cdot L^{-1}$ of NaCl or Na_2SO_4 salts on red-osier dogwood seedlings (*Cornus stolonifera* Michx); Na^+ tissue content was greater in plants treated with NaCl than those treated with Na_2SO_4, even though the concentration of Na^+ (in $meq \cdot L^{-1}$) in the irrigation solution was half in the NaCl treatments as well. In a study with four rose rootstocks [*Rosa* 'Dr. Huey', *R. x fortuniana*, *R. multiflora* and *R. x odorata* (Andrews) Sweet] subjected to Cl^- or SO_4^{2-} dominated salinity, leaf [Na] were similar between both salt types at moderate salinity levels (EC~3.9 $dS \cdot m^{-1}$) and rootstock dependant at high salinity levels (EC~7.9-8.2 $dS \cdot m^{-1}$) (Niu and Rodriguez, 2008b).

In plants grafted on 'Natal Briar' productivity and quality variables (total flowering DW and total FS harvested per plant, and total average LCI) were markedly and negatively affected by NO_3^- (Fig. 4.8B, Fig. 4.8D and 4.9B). Also, 'Natal Briar' plants subjected to this anion exhibited considerable defoliation. The noticeably greater [Na] in leaves of flowering shoots of those plants subjected to salt blends containing $NaNO_3$ might be the main cause for the more severe detrimental effects observed in this RS. The significantly greater [Na] found in leaves of flowering shoots of plants budded on the 'Natal Briar' RS may be due to both its lack of ability to restrict Na^+ transport to the leaves as observed in 'Manetti', and the greater requirement for tissue nitrogen compared to chloride and sulfur. According to Sadasivaiah and Holley (1973) the normal range for N in rose leaves is 30-35 $g \cdot kg^{-1}$ (3.0-3.5%), while for S it is 0.16-0.21 $mg \cdot kg^{-1}$, and for most plant species the Cl^- requirement for optimal plant growth is in the range of 0.2-0.4 $g \cdot kg^{-1}$ (Marschner, 1995). In the present experiment N was supplied in the salinized-nutrient solution in the NO_3^- form (as KNO_3 and $NaNO_3$). Anion uptake across the plasma membrane is normally an active process requiring co-transport with protons and, in general, the uptake of Na^+ is balanced with the uptake of Cl^- (a negatively charged ion) and efflux of K^+ (Tyerman and Skerrett, 1999). It is contended that sodium entered and translocated within the rose plant, as main counterion, during the process of NO_3^- uptake and long-distance transport to the upper part of plants. The antagonism of Na^+ versus K^+ in soil solution and plant uptake is well documented in the literature (Marschner, 1995; Grattan and Grieve, 1999). This contention is supported by the lower [K] observed in the leaf tissues of plants exposed to $NaNO_3$ salts.

Many crop species with relatively low salt tolerance are typical Na^+ excluders and capable, at low and moderate salinity levels, of restricting the transport of Na^+ into the leaves where it is highly toxic in salt sensitive species (Marschner, 1995). In the present and previous experiments (Chapters II and

III) tissue [Na] were greater in the lower plant organs, which confirms the plant's ability to restrict Na^+ transport to the upper leaves, as previously reported in roses by Bernstein et al. (2006), Cabrera (2003a), Cabrera and Perdomo (2003), Niu and Rodriguez (2008a), Sadasivaiah and Holley (1973); and in red-osier dogwood seedlings (*Cornus stolonifera* Michx; Renault, et al., 2001). In roses, however, this Na^+ exclusion is not general to all rootstocks (Baas and van den Berg, 1999; Cabrera, 2003a; Fernández-Falcón et al., 1986; Niu and Rodriguez, 2008a). In the present experiment even though both RS had relatively similar [Na] patterns relative to the organ position on the plant, 'Manetti' rootstock exhibited greater [Na] in roots and smaller concentrations in upper organs, supporting previous reports stating its superior capacity to restrict Na^+ transport to the leaves than other rose rootstocks (Cabrera and Perdomo, 2003; Sadasivaiah and Holley, 1973).

Similar to our previous experiments leaf [Cl] was progressive over time and reached far greater concentrations in leaves (both old and those on flowering shoots) compared to the lower plant parts (roots, main and stems) in both RS (Fig. 4.15 and Fig. 4.16). Greater [Cl] in the upper parts (shoots or leaves) have also been reported in NaCl-treated seedlings of red-osier dogwood (*Cornus stolonifera* Michx; Renault et al., 2001), rose rootstocks (*Rosa* 'Dr. Huey', *R. x fortuniana*, *R. multiflora* and *R. x odorata* (Andrews) Sweet*;* Niu and Rodriguez, 2008b), 'Bigarreau Burlat' and 'Tragana Edessis' cherry plants (*Prunus avium* L.; Papadakis et al., 2007) and hardy blue plumbago (*Ceratostigma plumbaginoides* Bunge)*,* purple iceplant [*Delosperma cooperi* (Hook.f.) L. Bolus], gazania [*Gazania rigens* (L.) Gaertn.], and germander (*Teucrium chamaedrys* L.; Niu and Rodriguez, 2006). With exception of the pure blend of NaCl, transport and deposition of Cl^- in the flowering shoot leaves of 'Natal Briar' plants was greater throughout the experimental period (Fig. 4.15A and 4.15B). Plants more tolerant to Cl^- absorb it more slowly, but the leaf-chloride level at which injury occurs tends to be similar for all susceptible plants

(Bernstein and Hayward, 1958). 'Manetti' plants seemed to be able to sequester some of the absorbed Cl^- in the lower plant organs (roots and main stems) and restrict its transport to the flowering shoot leaves to a greater extent than 'Natal Briar' plants (Fig. 4.15 and Fig. 4.16). In this experiment it was observed that EC_L collected from plants grafted on 'Manetti' continued to increase over time taking almost twice the time it took for those on 'Natal Briar' to reach a stable level on EC_L (Fig. 4.2A and Fig. 4.3A). Additionally, it was observed that [Cl] was slightly greater in leachates from 'Manetti' plants throughout the entire experimental period, especially in those plants subjected to the pure NaCl blend with the average [Cl] being 25% greater in this RS (Fig. 4.2B and Fig. 4.3B). Thus, in addition to restricting the transport of Cl^- to flowering shoots leaves, 'Manetti' plants could also be excluding it. Tolerance to chloride salinity may be significantly improved by using rootstocks that absorb chloride more slowly (Bernstein, 1975). By excluding and sequestering Cl^-, 'Manetti' plants may delay its transport to and accumulation in young plant tissues, thus preventing detrimental effects it imposes on them for a longer period of time.

Nitrate, as the Na^+-accompanying anion affected negatively leaf [P] and [K] in both RS. In 'Manetti' plants Cl^- and SO_4^{2-} had positive or no effects on [P] and [K], while in 'Natal Briar' Cl^- affected negatively [P] and [K] only when combined with NO_3^- (Fig. 4.17A-C; Fig. 4.18A-B). Interaction between salinity and phosphate (P) nutrition is highly dependant upon the plant species (or cultivar), composition and level of salinity and the concentration of P in the substrate (Grattan and Grieve, 1992). As for K, these results are incongruent with the role of K^+ as the dominant counterion for NO_3^- in long-distance transport in the xylem, as well as for storage in vacuoles (Marschner, 1995).

When combined in the tertiary blend, the three anions affected negatively leaf [Ca] in both RS, and in 'Natal Briar' plants those subjected to NaCl (at 100%) had much lower leaf [Ca] than the other two pure blends (Fig. 4.18C-D). Salinity dominated by Na-salts not only reduces Ca^{2+} availability but also its

transport and mobility to growing regions of the plant (Grattan and Grieve, 1999). Sodium chloride-based salinity has been reported to reduce foliar Ca^{2+} in citrus rootstocks [sour orange, *Citrus aurantium* (L.); Cleopatra mandarin, *Citrus reticulata* Blanco; and Carrizo citrange, *Citrus sinensis* (L.), Osbeck x *P. trifoliate* (L.) Ref.; Ruiz et al., 1997] and in corn (*Zea mays* L.; Maas and Grieve, 1987; Fortmeier and Schubert, 1995).

In both RS NO_3^- enhanced leaf [Mg] while SO_4^{2-} caused a reduction in leaf [Mg] in both RS (Fig. 4.19A). In sorghum [*Sorghum bicolor* (L.) Moench] sulfate salinization (Na_2SO_4) caused a decreased growth in part due to depression in the shoot contents of K^+ and Mg^{2+} (Boursier and Lauchli, 1990). In the present experiment Mg^{2+} was supplied to all saline solutions at equal concentrations. However, according to the SPECIES program calculations (Barak, 1990), those blends containing SO_4^{2-} had lower free ion concentrations of Mg^{2+} (average of 84% for the SO_4^{2-}-binary and tertiary blends, and 77% for the SO_4^{2-}-pure blend). Contrarily, in the NaCl and/or $NaNO_3$ blends concentrations of free ions of Mg^{2+} were on average 91%. Thus, increases in the proportion of the SO_4^{2-} anion caused a proportional reduction in the actual concentrations of available Mg^{2+} in solution, and consequently, in the observed leaf [Mg].

On the second harvest event (101 DAT) those plants grafted on 'Manetti' exhibited greater concentrations of S, P, K and Fe, and lower leaf concentrations of Ca and B than those grafted on 'Natal Briar'. For most of these mineral elements the findings are in agreement with those reported by Cabrera (2002), particularly on [B] which was considerably greater in leaf tissue of 'Bridal White' rose plants grafted on 'Natal Briar' compared to 'Manetti', *R. x odorata* (Andrews) Sweet and 'Dr. Huey' (91 $mg \cdot kg^{-1}$ versus 51, 56 and 62 $mg \cdot kg^{-1}$, respectively). In Cabrera's study rose plants were growing under non-salinized conditions. In the present experiment, by the second harvest (101 DAT) leaf [B] ranged between 36-43 $mg \cdot kg^{-1}$ in 'Manetti' and between 65-80 $mg \cdot kg^{-1}$ in 'Natal

Briar' (on average 87% greater than in 'Manetti'). By the fourth harvest (225 DAT) leaf [B] ranged between 92-142 $mg \cdot kg^{-1}$ in 'Natal Briar' plants while in 'Manetti' those plants subjected to NaCl and/or Na_2SO_4 in the salt blend had [B] of 60-70 $mg \cdot kg^{-1}$, only slighter above the normal range. Conversely, in those 'Manetti' plants subjected to $NaNO_3$ in the salt blend (regardless of the proportion or other blend components) leaf [B] ranged between 107-202 $mg \cdot kg^{-1}$, with the greatest leaf [B] corresponding to the pure blend of $NaNO_3$ (Fig. 4.19B). Apparently, in 'Manetti' $NaNO_3$ caused an unusual big uptake and translocation of B to the flowering shoots. According to Sadasivaiah and Holley (1973) the range of [B] in tissue considered normal for roses is between 40-60 $mg \cdot kg^{-1}$. Clearly, in plants growing on 'Natal Briar' the uptake and translocation of B goes beyond normal levels. Boron is quite toxic to most ornamental plants at very low concentrations, and as little as 0.8 $mg \cdot L^{-1}$ (in solution) can result in leaf-margin necrosis (Farnham, 1985). The tap water used to prepare the salinized nutrient solutions in the present experiment had [B] ~0.14 $mg \cdot L^{-1}$, which according to Petersen (1996) is considered as non-hazardous. In closely related species, genotypes susceptible to B toxicity generally have greater concentrations of B in leaves and shoots than do tolerant genotypes (Nable et al., 1997). The relatively great leaf [B] found on plants growing on 'Natal Briar' could indicate a greater B uptake/translocation inherent to this RS which would be probably exacerbated by the imposition of salinity stress.

CHAPTER V

EVALUATING THE INFLUENCE OF THE COUNTER CATION ON THE DETRIMENTAL EFFECTS IMPOSED BY CHLORIDE-BASED SALINITY ON ROSE (*ROSA* L. 'ERIN')

INTRODUCTION

Agricultural productivity is threatened by the depletion of groundwater and by water logging and salinization of soils from poorly managed or antiquated irrigation systems (Elashry, 1994). Irrigation has constituted the foundation of numerous civilizations; some have risen and fallen with the growth and decline of their irrigation systems, while others have maintained sustainable irrigation for thousands of years (van Schilfgaarde, 1994). Soil salinization is a common problem in areas with low rainfall and high evaporation rates, and when combined with irrigation and poor drainage it can lead to permanent soil fertility loss (FAO, 2005). Saline soils usually occur in areas that receive salts from other locations, and surface and ground waters used for irrigation are the primary carriers (Richards, 1954). Restricted drainage is a factor that usually contributes to salinization of soils and may involve the presence of high ground-water table or low permeability of the soil.

In saline soils, which by definition have electrical conductivities of the saturation extract (EC_e) greater than 4 $dS \cdot m^{-1}$ and exchangeable-sodium-percentages (ESP) less than 15% (Richards, 1954), salinity is usually caused by mixtures of salts rather than a single salt. The amount of soluble salts present controls the osmotic strength of the soil solution (Bernstein, 1975; Richards, 1954). Ions frequently found in excess in saline soils include Cl^-, SO_4^{2-}, HCO_3^-, Na^+, Ca^{2+}, Mg^{2+}, and less frequently K^+ and NO_3^- (Martin and Koebner, 1995).

Investigations on effects of salinity on plants have increased during the past few years (Maas and Grieve, 1987). However, a large percentage of salinity studies on horticultural or agronomic crops use NaCl as the sole salinizing agent (Grattan and Grieve, 1999). In saline substrates where Na^+ and Cl^- are the dominant ions, their concentrations exceed by far the demand, leading to toxicity in non salt-tolerant plants (Marschner, 1995). Under these circumstances, it is difficult to differentiate osmotic from specific ion effects, considering that both Na^+ and Cl^- may be directly toxic (Bernstein, 1975) and that a synergistic effect between them has been reported, with greater injury when both ions are present (Martin and Koebner, 1995).

In order to elucidate the effects of different ions on growth and gas exchange parameters, Bañuls et al. (1997) exposed 'Valencia' orange plants [*Citrus sinensis* (L.) Osbeck] to different salts (NaCl, $Ca(NO_3)_2$, KCl, and $NaNO_3$). Plant growth, photosynthesis and stomatal conductance were markedly reduced to a greater extent by chloride-salts (NaCl and KCl), whereas non-significant effects on these variables were observed in treatment with $NaNO_3$. Trajkova et al. (2006) exposed cucumber plants (*Cucumis sativus* L.) to low (3 $dS \cdot m^{-1}$) and moderate (5 $dS \cdot m^{-1}$) levels of salinity induced by addition of either NaCl or $CaCl_2$ at equal rates (on a chemical equivalent basis). According to their results, both vegetative growth and fruit yield of cucumber showed a greater susceptibility to NaCl compared to equal electrical conductivity (EC) levels of $CaCl_2$ salinity.

Niu and Rodriguez (2008b) subjected four rose rootstocks (*Rosa* L. 'Dr. Huey', *R. x fortuniana*, *R. multiflora* and *R. x odorata*) to two levels (moderate: 3.9 $dS \cdot m^{-1}$ and high: 8.1 $dS \cdot m^{-1}$) of chloride- or sulfate-dominated salinity. The effect of the dominant salt type on plant growth varied with rootstock and salinity level. However, it is noteworthy to mention that at both salt levels and in all salt combinations both anions (Cl^- and SO_4^{2-}) were always present at considerably high concentrations, especially Cl^- which was already present in the tap water

used for the experiment at concentrations of 224 $mg \cdot L^{-1}$ (~6.32 $meq \cdot L^{-1}$). Therefore, it would be difficult to differentiate the anion's specific effects from the osmotic and/or the synergestic effects imposed on the rose's growth.

Use of different salts in the nutrient solution permits an increase in Cl^- and Na^+ ions independently, offering a possibility to differentiate specific effects of both ions (Bañuls and Primo-Millo, 1992). Even though nutrient solutions for plant growth are made up of dissociated salts, plants need and absorb specific ions. This fact imposes the major constraint upon nutrient solutions, namely balance of charge: the sum of the cation equivalents must be equal to the sum of the anion equivalents (Schrevens and Cornell, 1993). This constraint is the major reason for the impossibility of using classical experimental designs (factorial-type designs) with nutrient solutions since use of a given anion, implies the use of a counter cation (Schrevens and Cornell, 1993). In a factorial experiment treatments consist of all possible combinations of the selected levels in two or more factors (Gomez and Gomez, 1984). Thus, the concentration of the final mixture or combination of ingredients will vary from one treatment to the next making it impossible to distinguish between effects due to varying osmotic pressure of blends and the individual component's effects. This problem can be dealt with by using the theory of mixture designs and model forms (Schrevens and Cornell, 1993; detailed explanation included in the Introduction section of Chapter IV). For mixture experiments, design factors are the proportions of components that sum to a constant, and response variables depend only on these component proportions (Goldfarb et al., 2004). These proportions must be nonnegative, and if expressed as fractions they must sum to unity (Schrevens and Cornell, 1993). The shape of the design space for these experiments depends on the number of components and the constraints on each (Goldfarb et al., 2004).

The present study was conducted to determine responses of rose plants to Cl-based salinity and the effects of the Cl^--counter cations.

MATERIALS AND METHODS

Plant culture and management

On January 20, 2006, 96 bare-rooted 'Erin' rose plants budded on *Rosa* L. 'Manetti' were transplanted into 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 $kg \cdot m^{-3}$ dolomitic limestone (Carl Pool Products, Gladewater, TX) and 0.6 $kg \cdot m^{-3}$ of both Micromax ® micronutrients fertilizer (The Scotts Company, Marysville, OH) and Aqua-GroG 2000 (The Scotts Company, Marysville, OH). Plants were placed on 5.5 m x 1.5 m x 0.4 m raised benches, with three plants abreast in each bed and spaced at 30 cm between centers. Plants were irrigated with a nutrient solution made with 15-5-15 Cal-Mag (The Scotts Company, Marysville, OH) and adjusted to deliver 150 $mg \cdot L^{-1}$ of nitrogen until salinized treatments were implemented. On March 08, 2006, plants underwent a hard pinch (removal of the terminal portion of a soft shoot, including two to four leaves; Langhans, 1987a). Throughout this experiment plants were managed following conventional pruning practices (Langhans, 1987b), inducing synchronized flushes of growth and flowering.

The experiment was conducted at the Texas A&M University Research and Extension Center, Dallas, TX, in a 6 m x 12 m glass-covered greenhouse fitted with exhaust fans, an evaporative wet-pad cooling system and heat provided by thermostatically-controlled gas burners. Greenhouse temperatures were set at 25°C day and 16°C night (actual values registered 38.5°C max, 13.0°C min and 25±0.06°C average for the 23-week experimental period). Temperature, humidity and photosynthetically active radiation were monitored with sensors connected to a Campbell CR510 Datalogger (Campbell Scientific Inc., Logan, UT).

Salinity treatments, consisting of a base nutrient solution supplemented with seven salt mixtures, were implemented on April 6, 2006. A modified ½ strength Hoagland formulation (Hoagland and Arnon, 1950) was used as a base solution containing (in $mmol \cdot L^{-1}$): 8.5 N (as NO_3^-), 0.5 P (as $H_2PO_4^-$), 3.5 K, 2.75 Ca, 1.0 Mg, 1.0 S (as SO_4^{2-}), 1.0 $mg \cdot L^{-1}$ Fe as Fe-EDDHA and half-strength Hoagland's micronutrient concentration. Seven saline solutions, in which the concentration of chloride [Cl] was held constant at 12 $mmol \cdot L^{-1}$, were used for this experiment using NaCl, $CaCl_2$ and KCl salts (Table 5.1). In pure blends 100% of the Cl came from a single salt source (NaCl, $CaCl_2$ or KCl), in binary blends 50% of the Cl came from each of two different salts, and in the tertiary blend 33.33% of the Cl derived from each of the three different salt sources.

In this experiment an additional control treatment (non-salinized base nutrient solution) was included as well. The calculated (expected) electrical conductivity (EC_E; sum of cations or anions, in $meq \cdot L^{-1}$, divided by 10) was approximately 2.3 $dS \cdot m^{-1}$ for all saline solutions and 1.1 $dS \cdot m^{-1}$ for the control treatment, plus an additional 0.89±0.02 $dS \cdot m^{-1}$ average contribution from the tap water used to prepare the solutions. Before adding any salts (base nutrient solution and salt treatments) pH of the tap water was adjusted to 6.0±0.03 using 6 M H_2SO_4.

Solutions were pumped from 150-L containers with submersible pumps (Model 2E-38N, Little Giant Pump Co., Oklahoma City, OK) feeding 1.3 cm (diameter) polyethylene irrigation lines that supported spray–stake Spot Spitter® emitters (Roberts Irrigation Products, San Marcos, CA), connected via 3.2 mm (diameter) spaghetti tubing. Each plant container was fitted with one calibrated emitter. Representative plants from selected treatments were routinely weighed to gravimetrically determine the evapotranspiration rate (ET). Total base irrigation volume consisted of ET plus an additional leaching target fraction of 25%.

Table 5.1. Salt composition, concentration and proportion of the cations in the saline blends added to a base nutrient solution (modified half-strength Hoagland solution). Chloride was held constant at a concentration of 12 mmol·L^{-1} (i.e. 12 meq·L^{-1}) in all saline blends.

Salt blend	Salt blend composition	Cation concentration (meq·L^{-1})				Cation proportion in the salt blend			Salt blend key*
		Na^+	Ca^{2+}	K^+	Sum of cations	Na^+	Ca^{2+}	K^+	
Pure	NaCl	12	0	0	12	1	0	0	1-0-0
Pure	$CaCl_2$	0	12	0	12	0	1	0	0-1-0
Pure	KCl	0	0	12	12	0	0	1	0-0-1
Binary	NaCl-$CaCl_2$	6	6	0	12	0.5	0.5	0	0.5-0.5-0
Binary	NaCl-KCl	6	0	6	12	0.5	0	0.5	0.5-0-0.5
Binary	$CaCl_2$-KCl	0	6	6	12	0	0.5	0.5	0-0.5-0.5
Tertiary	NaCl-$CaCl_2$-KCl	4	4	4	12	0.33	0.33	0.33	0.33-0.33-0.33

*Order of the cations is Na^+-Ca^{2+}-K^+.

Electrical conductivity (EC meter model 2052, VWR Scientific), pH (pH/mV/Ion meter AP63 accumet ® portable; Fisher Scientific) and Cl concentrations (Digital Chloridometer Model 4425000, Labconco Co., Kansas City, MO) were monitored on three leachate samples collected from all treatments on a bi-weekly basis. Chloride concentrations were determined according to Adriano and Doner (1982).

Data collection

Plant productivity and flowering shoots quality

There were a total of four harvests during this experiment (total duration of 23 weeks). Flowering shoots were harvested at commercial maturity, recording their dry weight (DW), number (FS), length (FSL) and leaf chlorophyll index (LCI; Chlorophyll meter, SPAD-502, Minolta Co. LTD, Japan) per plant. Harvested flowering shoots were put into paper bags and oven-dried at 70^{o}C. At the end of the experiment, immediately after the fourth harvest of flowering shoots, three whole plants per treatment were destructively harvested and analyzed for nutrient content and biomass partitioning. Plants were cut into four portions: roots, main stem (rootstock stem portion below the graft union), and scion old stems and old leaves.

Flowering shoot DW and FS per plant from each harvest were added to obtain total flowering shoot DW and total FS harvested per plant. For FSL and LCI the average per harvest and total average from the four harvests were used in the data analysis.

Water relations measurements

Relative water content (RWC) and stem water potential (SWP) were determined for three selected plants from each treatment during each harvest. For RWC three leaflets from one flower shoot per plant were cut and weighed to determine their fresh weight (FW); soaked in deionized water in petri dishes and refrigerated for a 24 hour period to determine their turgid weight (TW); and oven-dried at 70^{o}C for 48 hours, or until they recorded a stable weight, to determine their dry weight (DW). Relative water content was calculated by the formula RWC=[(HW-DW)/(TW-DW)*100] (Jiang and Huang, 2001). Stem water potential was measured with a pressure chamber (Model 610, PMS Instrument, CO., Corvallis, Oregon, USA). Leaves of flower stems were covered with an aluminized Mylar envelope to synchronize their water potential with that from the stems at least two hours before measuring (Kim et al., 2004). Sampling time for both variables was midday (between 12:00 PM and 1:30 PM) when the evaporative demand was at its peak. For leaf osmotic potential (LOP), the first five-leaflet leaf of a flowering shoot per plant was excised, covered with aluminum foil, placed in a plastic bag and transported to the laboratory in an ice cooler. After a 4-hour rehydration period with deionized water, petioles, rachis and petiolules were discarded and the leaf blades were frozen at -20^{o}C until analyzed. Before analyzing, samples were thawed for 15-18 min in plastic bags. Tissue sap was then extracted with a leaf press and collected on a filter disc which was immediately placed in a vapor pressure osmometer (Model 5520, Wescor, Inc., Logan UT). Osmometer readings in mmol·kg^{-1} were converted to osmotic potential values in MPa, based on the van't Hoff equation (Nobel, 1983).

Tissue analyses

During each harvest the three uppermost five-leaflet leaves from each flowering shoot were collected and pooled for each plant, dried and ground (to pass a 40-mesh screen). Samples from harvests II (91 DAT) and IV (159 DAT) were sent to the Louisiana State University AgCenter Soil Testing and Plant Analysis Laboratory to be analyzed for total nutrient concentration. Phosphorous, K, Ca, Mg, S, B, Cu, Fe, and Zn were measured by ICP procedures, and N with a Leco N analyzer. Analyses of chloride concentration ([Cl]) in leaves of flowering shoots from all regular harvests were made using a digital chloridometer (Model 4425000, Labconco Co., Kansas City, MO). Sodium concentration ([Na]) in leaves of flowering shoots from harvests II and IV (91 and 159 DAT) was measured by flame emission on a Fast Sequential Atomic Absorption Spectrometer (AA240FS, Varian, Inc. Australia). Similarly, [Cl] and [Na] were determined in roots, main stems, old stems and old leaves from the destructive harvest of whole plants at the end of the experiment.

Salt burn damage

After the last harvest event (161 DAT) a foliar salt injury rating evaluation was taken 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 burn damage). This evaluation was performed on the leaves remaining on the plant after harvesting the flowering shoots.

Experimental design and statistical analyses

The experimental design was a randomized complete block design (RCBD). There were a total of eight treatments (seven saline solutions, Table

5.1, and a non-salinized control) with 12 replications per treatment (one pot with one plant as a replication) for a total of 96 experimental units. Among the salinized treatments data were analyzed as a simplex-centroid design in a mixture experiment and when comparisons between the control treatment and the salinized treatments were needed, they were made by orthogonal contrasts. Also regression, correlation, general linear model, and Chi-square procedures were performed using SAS ® 9.1 for Windows (SAS Institute Inc., Cary, NC).

Most of the variables were evaluated at several points in time throughout the entire experimental period (with exception of those variables measured during the destructive harvest of plants at the end of the experiment). For all those variables evaluated over time a repeated measures procedure was performed including time (DAT) as a second factor [additionally to salt blend composition (SB)] to determine the pattern of the responses over time. For the total sums of flowering shoots DW and FS harvested per plant (sums of all four harvests), and all variables measured during the destructive harvest of plants, if effects due to SB were present data were analyzed with Design-Expert V. 6.0.11 (Stat-Ease, Inc. Minneapolis, MN.) as a simplex-centroid design in a mixture experiment with a total of seven salt mixtures or blends: three pure blends, three binary blends, and one tertiary (centroid) blend. For every response this program provides an analysis of variance (ANOVA), the regression equation (best fitted model), diagnostics for the set of data and the model graphs (contour and 3-D surface). To estimate the value of a response at a given mixture or blend, the component coefficients provided by the model must be multiplied by the proportion of the components in the blend. The number and type of coefficients provided by the model will depend on the type of the response (linear, quadratic or special cubic).

Physiology and productivity of roses are highly influenced by the season of the year and differences in yield may be caused by different environmental factors (Maas, 1990; Mastalerz, 1987). Due to these circumstances variance

heterogeneity that is independent of treatment effects is introduced. To overcome this problem, yields can be expressed on a relative basis (Mass, 1990). Hence, for some of the variables evaluated at harvests events over time (i.e. DW, FS, and FSL) data were first converted to a comparable scale (relative data) and then subjected to an arc sine transformation (Gomez and Gomez, 1984) to allow for a distinction of true treatment effects.

RESULTS

Leachate electrical conductivity (EC_L), Cl concentration ($[Cl_L]$) and pH (pH_L)

Volumes of irrigation solution applied were the same for all treatments throughout the entire experimental period with a total of 79 L/plant, except for the control treatment whose plants received a total of 83 L/plant. Leaching fractions varied similarly over time for all treatments and no differences among all solutions were found ($P>0.05$), with an overall average for the entire season of 33%.

Leachate electrical conductivity increased over time differently among treatments ($P<0.0001$) exhibiting linear, quadratic and cubic patterns (Fig. 5.1A). However, while most of the salinized solutions had relatively comparable values over time, leachates from plants subjected to KCl showed greater EC_L values than the rest of the salt treatments throughout the experimental period, with exception of the binary blend of NaCl-$CaCl_2$ (Fig. 5.1A). Leachates from the NaCl-$CaCl_2$ binary blend increased considerably in the last phase of the experimental period (Fig. 5.1A). In all treatments $[Cl_L]$ followed patterns very similar to those of EC_L (Fig. 5.1B. Control plants had (expected) lower EC_L and $[Cl_L]$ values compared to the salinized treatments (Fig. 5.1A and Fig. 5.1B).

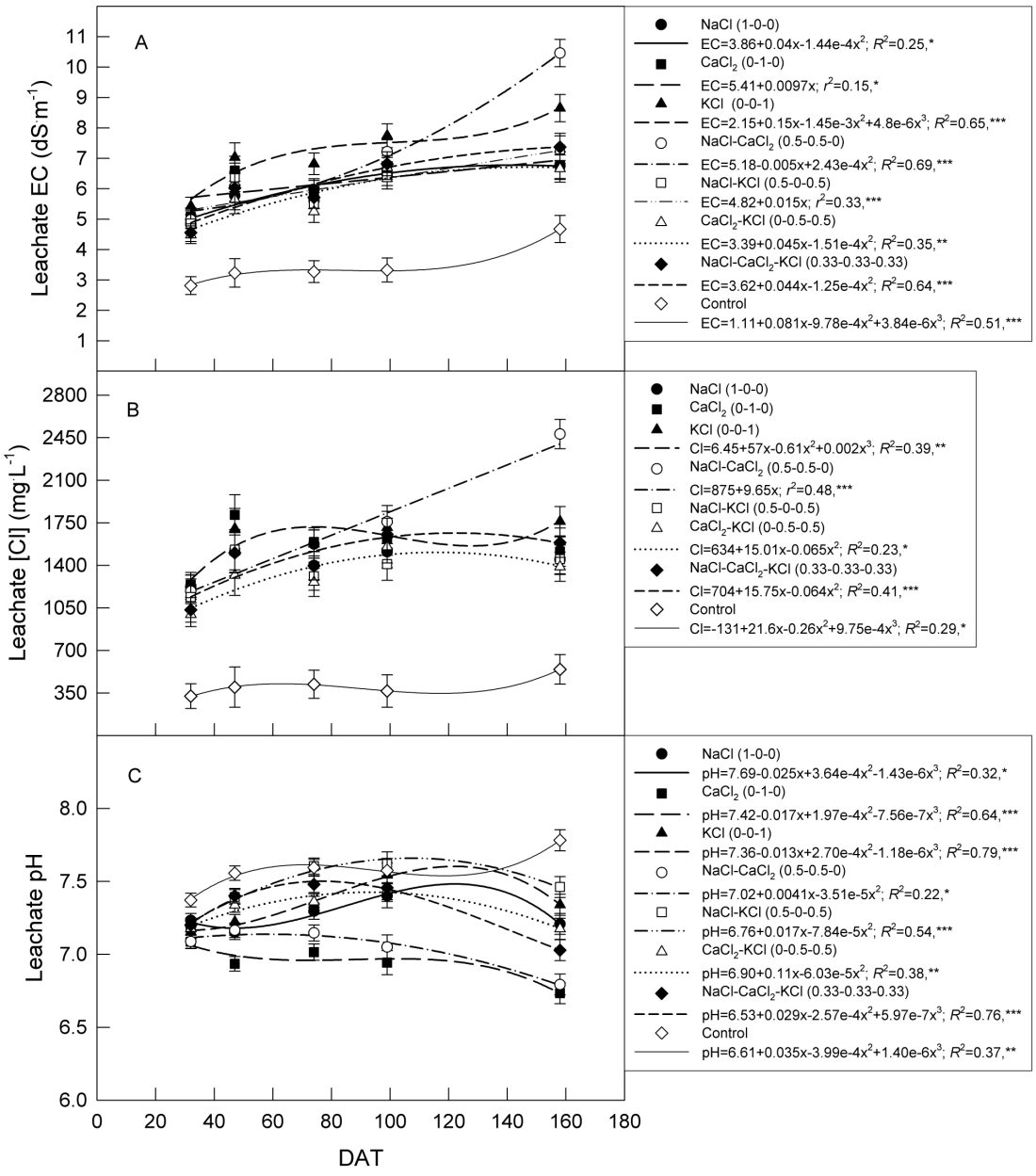

Fig. 5.1. (A) Electrical conductivity (EC), (B) Cl^- concentration [Cl], and (C) pH over time in leachates from 'Erin' roses subjected to a moderately high (12 mmol·L^{-1}) Cl^--based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. Symbols represent the mean ± standard error of six plants. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Leachate pH from most of the solutions tended to increase until approximately 100 DAT; afterwards, pH_L values decreased slightly in all treatments, except the control whose pH_L values were the greatest at the last leachate collection date registered (158 DAT; Fig. 5.1C). Contrary to the rest of the salinized treatments pH_L values in the blends of $CaCl_2$ (pure) and NaCl-$CaCl_2$ (binary) exhibited, in general, a decreasing tendency, reaching the lowest values at the last measurement date (158 DAT; Fig. 5.1C). In general terms, salt blends containing $CaCl_2$ tended to have the lowest pH_L averages (Fig. 5.1C).

In this experiment the supplemental salts were supplied at equinormal rates (i.e. $meq \cdot L^{-1}$), and therefore the EC_E of the resulting saline solutions were assumed to be similar (~3.2 $dS \cdot m^{-1}$). However, as in our previous experiment (Chapter IV), numerical differences were evident in the measured electrical conductivities of the saline solutions. Consequently, the same data analysis procedures used in the previous experiment were performed in this experiment to analyze the influence of salt sources on the measured pH (pH_{SS}) and EC (EC_{SS}) of the resulting saline solutions.

The type of salt used affected both pH_{SS} and pH_L (P=0.0171 and P<0.0001, respectively). Sodium had no significant effect on pH_{SS} as its proportion in the salt blend changed (Fig. 5.2A). Conversely, the anions Ca^{2+} and K^+ had linear effects. Calcium caused pH_{SS} to decrease as its proportion in the salt blend increased, while K^+ caused pH_{SS} to increase as its proportion in the salt blend increased (Fig. 5.2A). As for pH_L, although the shape of the surface response fits a quadratic model (Table 5.2), the cations' individual responses were similar to those from the pH_{SS} (Fig. 5.2A and 5.2B). There were positive synergistic effects on pH_L for the combinations (binary blends) of K^+-Ca^{2+} and K^+-Na^+ (Fig. 5.2B).

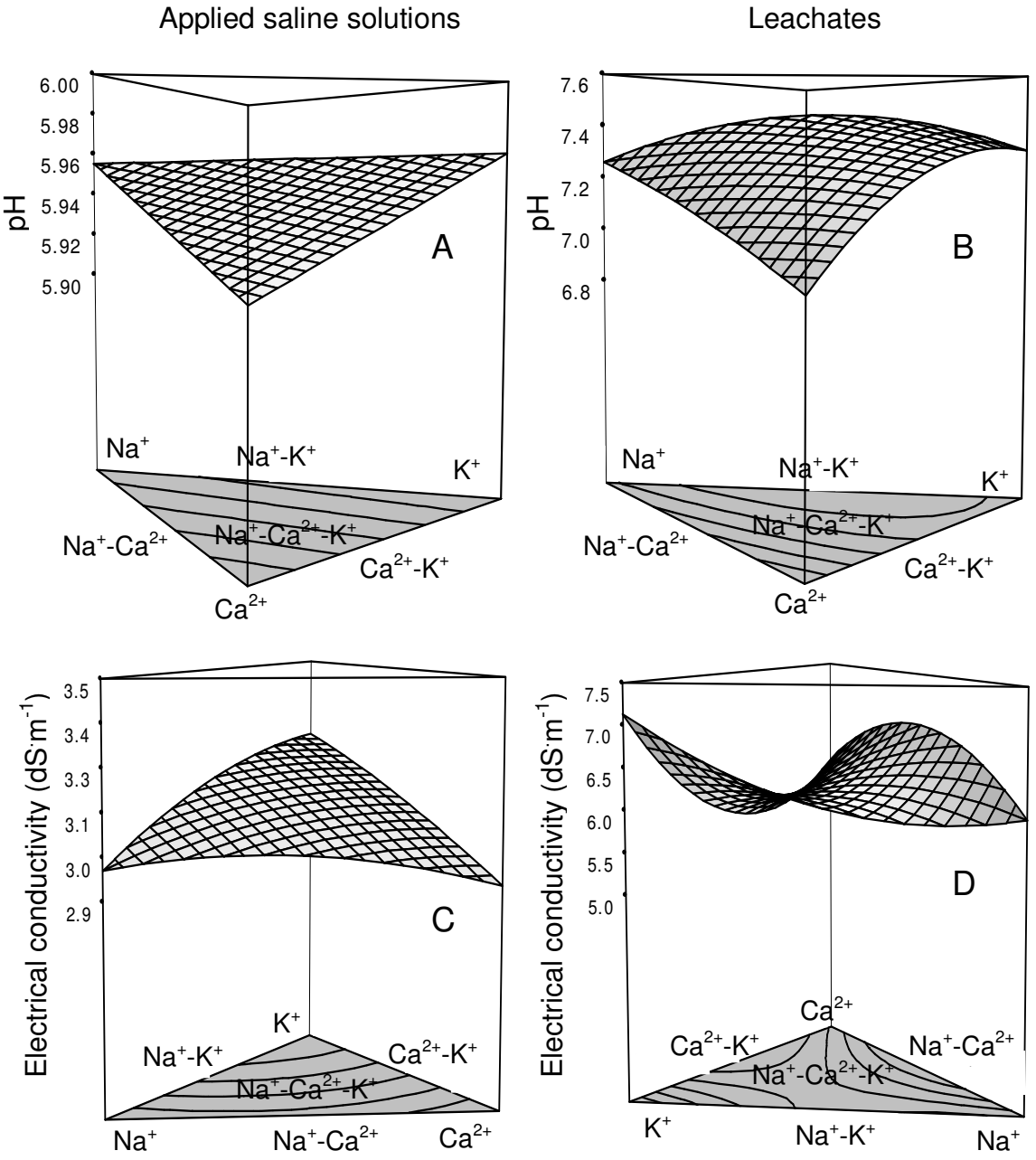

Fig. 5.2. pH (A and B) and electrical conductivity (C and D) of applied saline solutions and leachates from 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution with varying proportions of the counter cations Na^+, Ca^{2+} and K^+ in the salt mixture. Figures are 3-dimensional response surfaces. For fitted models see Table 5.2.

Table 5.2. Fitted models for pH and electrical conductivity (EC) of the saline solutions applied and of leachates from 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution with varying proportions of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

	pH	Electrical conductivity ($dS \cdot m^{-1}$)
Saline solution	pH_{ss}=5.96*(NaCl)+5.92*($CaCl_2$)+ 5.97*(KCl); r^2=0.18, P=0.0171	EC_l=2.99*(NaCl)+2.93*($CaCl_2$)+ 3.27*(KCl)+0.25*(NaCl*$CaCl_2$)+ 0.23*(NaCl*KCl)+0.092*($CaCl_2$* KCl); R^2=0.86, P<0.0001
Leachates	pH_l=7.26*(NaCl)+6.95*($CaCl_2$)+ 7.32*(KCl)+0.12*(NaCl*$CaCl_2$)+ 0.63*(NaCl*KCl)+0.62*($CaCl_2$* KCl); R^2=0.79, P<0.0001	EC_l=5.98*(NaCl)+6.21*($CaCl_2$)+ 7.14*(KCl)+3.09*(NaCl*$CaCl_2$)- 1.96*(NaCl*KCl)-3.78*($CaCl_2$* KCl); R^2=0.29, P=0.0242

Similarly, the composition of the salt blend affected EC_{SS} and EC_L, (P=0.0171 and P=0.0242, respectively). The cations' individual responses were similar to those from pH_{SS} and pH_L (Fig. 5.2A-D). As the proportion of Ca^{2+} in the salt blend increased, EC_{SS} tended to decrease while EC_{SS} increased with increasing proportions of K^+ (Fig. 5.2C). Varying proportions of Na^+ did not seem to affect EC_{SS} to the same extent the other two cations did (Fig. 5.2C). In EC_L the cation K^+ caused noticeable increases in EC_L compared to Na^+ and Ca^{2+} as well (Fig. 5.2D). The binary blend of Na^+-Ca^{2+} had a synergistic positive effect on EC_L (as depicted by the upward curvature on the Na^+-Ca^{2+} edge of the

response surface; Fig. 5.2D).

Leachate Cl concentration fitted a quadratic response (Fig. 5.3). However, the general model was not significant (Model P=0.1470), indicating that all salt mixtures rendered similar [Cl_L]. The shape of the response surface for [Cl_L] (Fig. 5.3) resembles that of EC_L (Fig. 5.2D).

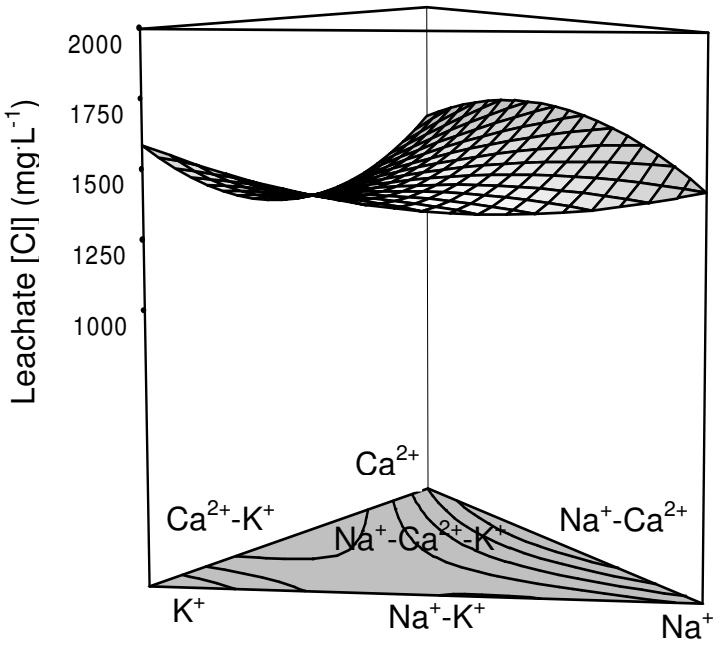

Fig. 5.3. Chloride concentration in leachates from 'Erin' roses subjected to a moderately high (12 mmol·L^{-1}) Cl^--based salinity in a half strength Hoagland's solution with varying proportions of the counter cations Na^+, Ca^{2+} and K^+ in the salt mixture. Figure is 3-dimensional response surface. Model: Leachate [Cl]=1452*(NaCl)+1554*($CaCl_2$)+1593*(KCl)+673*(NaCl*$CaCl_2$)-598*(NaCl*KCl)-1059*($CaCl_2$*KCl); R^2=0.20. To estimate the value of a leachate [Cl] at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

Even though the differential electrical charge of the salinizing cations (Na^+, Ca^{2+} and K^+) was equalized, i.e. applied on an equivalent basis, their

expression in a total molar basis (in $mmol \cdot L^{-1}$) differed among saline solutions, depending on the proportion and type of anion used in each salt blend (Table 5.3). Also, as in our previous experiment, different cations and their proportions had a noticeable influence on EC_{SS} (Fig. 5.2C). Thus, it was considered pertinent to determine the concentrations of free ions ([free ion]) and ion pairs ([ion pairs]) in all saline solutions using the program SPECIES (Barak, 1990). Free ion concentration data were used to estimate the osmotic potentials of the saline solutions (π_{ss}) with the van't Hoff's equation according to Nobel (1983) (Table 5.3).

Table 5.3. Applied salinity, electrical conductivities (EC), and osmotic potential of the saline solutions. The expected EC was 3.2 $dS \cdot m^{-1}$, which included salinities provided by the base nutrient solution (1.1 $dS \cdot m^{-1}$), supplemental salts (1.2 $dS \cdot m^{-1}$), and tap water (0.89 $dS \cdot m^{-1}$).

Salt source and salt blend key	Applied salinity ($mmol \cdot L^{-1}$)	Expected EC** ($dS \cdot m^{-1}$)	Measured EC ($dS \cdot m^{-1}$)	Osmotic potential (MPa)
NaCl (1-0-0)*	12	3.2	3.0 ± 0.049	-0.1235
$CaCl_2$ (0-1-0)	6	3.2	2.9 ± 0.042	-0.1076
KCl (0-0-1)	12	3.2	3.3 ± 0.032	-0.1239
NaCl-$CaCl_2$ (0.5-0.5-0)	9	3.2	3.0 ± 0.048	-0.1155
NaCl-KCl (0.5-0-0.5)	12	3.2	3.2 ± 0.047	-0.1237
$CaCl_2$-KCl (0-0.5-0.5)	9	3.2	3.1 ± 0.042	-0.1157
NaCl-$CaCl_2$-KCl 0.33-0.33-0.33	10	3.2	3.1 ± 0.047	-0.1183
Non-salinized control	0	2.0	1.9 ± 0.048	-0.0660

*Proportion of the cation (Na^+-Ca^{2+}-K^+) in the salt blend from a total of 12 $meq \cdot L^{-1}$.
**Sum of cations or anions, in $meq \cdot L^{-1}$, divided by 10 (it includes a 0.89 $dS \cdot m^{-1}$ contribution from the tap water).

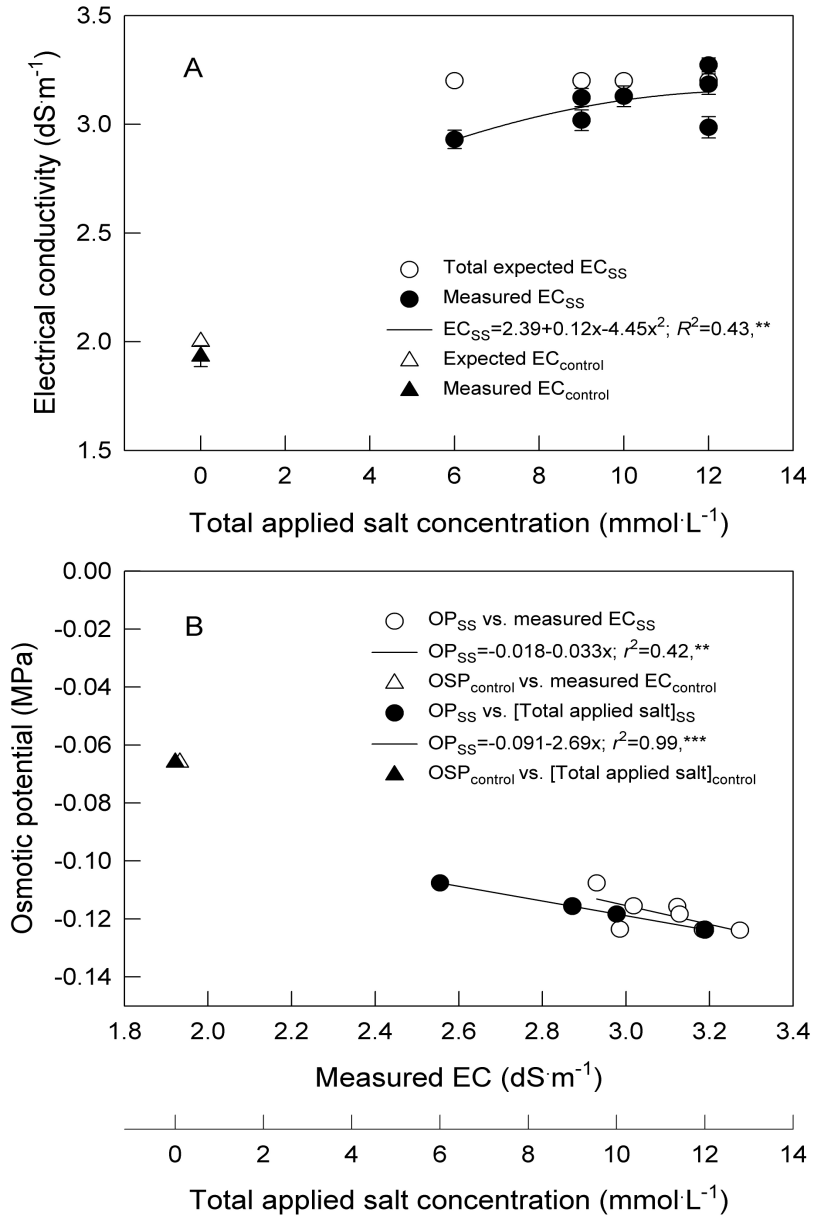

Fig. 5.4. (A) Electrical conductivities and (B) osmotic potential of the solutions applied to 'Erin' roses subjected to a moderately high (12 mmol·L^{-1}) Cl^--based salinity in a half strength Hoagland's solution with varying proportions of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,***=non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. $_{SS}$=saline solutions. Data from the control treatment are not part of the regression lines; they were included for reference purposes only.

A closer association was found between measured EC_{SS} and the solutions' total applied salt concentration expressed on a molar basis ($mmol \cdot L^{-1}$) than when expressed on a normal basis ($meq \cdot L^{-1}$) (Fig. 5.4A). However, significant differences were still observed for EC_{SS} among some saline solutions having the same salt concentration (in $mmol \cdot L^{-1}$) (Fig. 5.4A).

Osmotic potential of the saline solutions decreased, i.e. became more negative, as EC_{SS} increased (Fig. 5.4B). However, π_{ss} had a closer association with the total applied salt concentration of the solutions than with the measured EC_{SS} (Fig. 5.4B).

Biomass and flower productivity

Flowering shoots DW, FS and FSL responded differently to SB over time (P=0.0155, P=0.0166 and P=0.0282, respectively). At the first two harvest events (55 and 91 DAT) no differences among SB were present, but by the third and fourth harvests (124 and 159 DAT) differences among SB became apparent for all three variables evaluated (Fig. 5.5A-C). By the last harvest (159 DAT) plants subjected to KCl in the salt blend registered the lowest values in all three variables, especially those receiving 100% KCl (Fig. 5.5A-C). Variation in LCI was similar over time for all salt blends (P>0.05; results not shown).

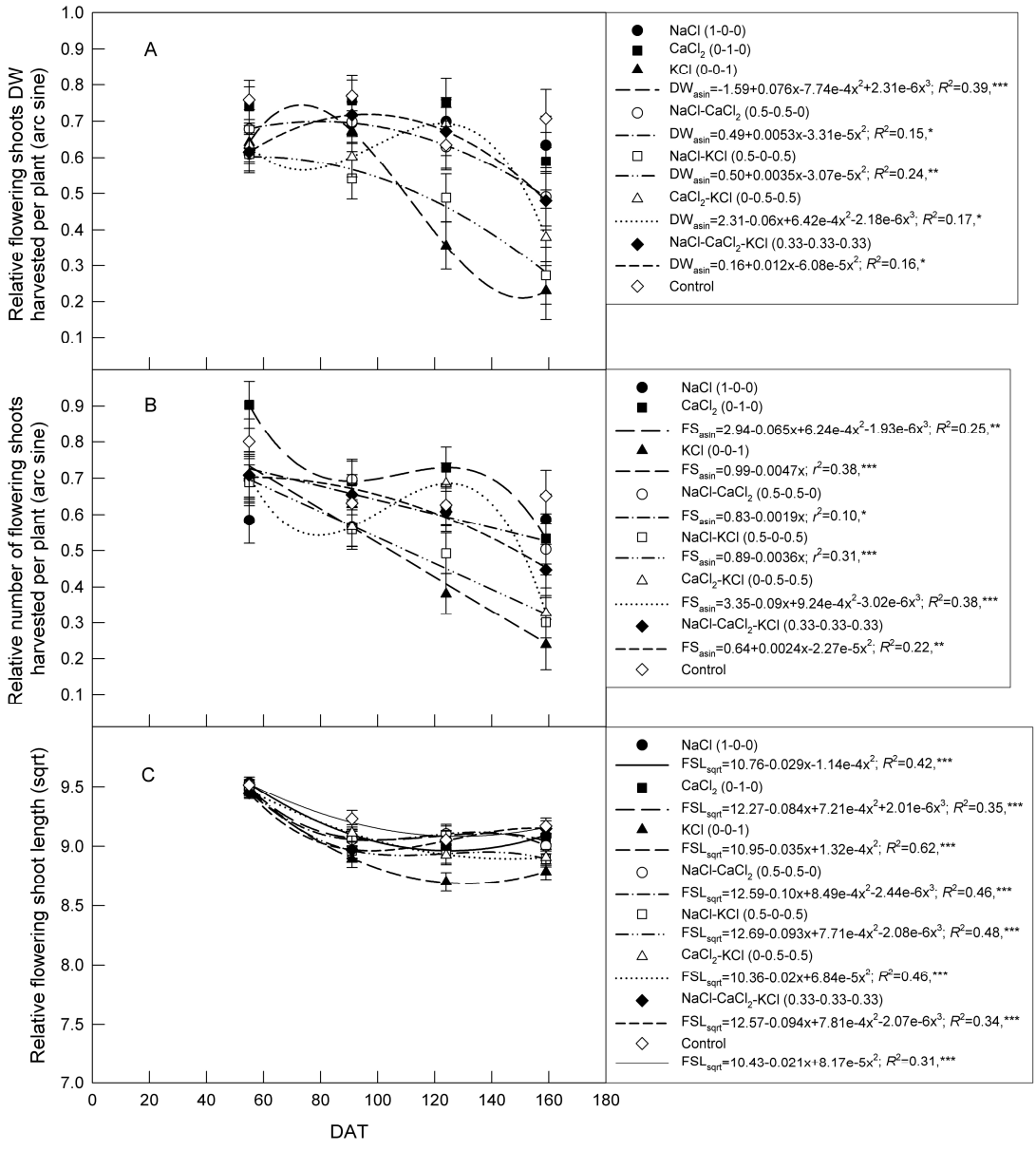

Fig. 5.5. (A) Relative flowering shoot dry weight (DW), (B) relative number of flowering shoots harvested per plant, and (C) relative flowering shoot length of 'Erin' roses subjected to a moderately high (12 mmol·L^{-1}) Cl$^-$-based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. Symbols represent the mean ± standard error of 12 plants. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Responses of total flowering shoot DW and total FS harvested per plant, and total average FSL to SB fitted linear models (P=0.0002, P<0.0001, and P=0.0007, respectively). For all three variables effects were positive for the cation Ca^{2+}, negative for K^+ and non-significant for Na^+ (Fig. 5.6A-C). Mean values of total flowering shoot DW, total FS and total average FSL increased as the proportions of Ca^{2+} in the salt blends increased, decreased as the proportion of K^+ increased, while no significant responses were observed when varying the proportions of Na^+ in the salt blend (Fig. 5.6 A-C; for fitted models see Table 5.4). The response of total average LCI to SB fitted a special cubic model (P=0.0024; Fig. 5.6D; Table 5.4). When all three components were present in the salt blend (tertiary or centroid), their effect on total average LCI was positive as depicted by the upward curvature in the center of the response surface (Fig. 5.6D). Plants subjected to the tertiary blend yielded a greater total average LCI values compared to the averages of their pure blends. As for the combination of two salt components, only that of Na^+ and K^+ affected significantly this response. When mixed together these two cations had a negative effect on total average LCI (downward curvature on their edge) with a lower value for their binary blend than when only one of them was present in the blend (pure blends; Fig. 5.6D).

When comparing only among the three pure blends those of $CaCl_2$ and NaCl had greater mean values for all four variables evaluated (total DW and FS harvested per plant, and total average FSL and LCI) than the KCl blend (P<0.05 for all comparisons; Fig. 5.6A-D). The $CaCl_2$ had also more total FS than the NaCl blend (P=0.0325), (Fig. 5.6B).

When comparing each salt blend against the control treatment, the non-salinized plants had greater total DW and FS than plants subjected to the blends of KCl and NaCl-KCl, 88 g versus 66 g, and 65 g, respectively for total DW, and 27 versus 20, and 21, respectively for total FS (P<0.05 for all comparisons). As for total average FSL, the non-salinized plants had longer flowering shoots than those from the KCl (40.2 cm versus 38.0 cm, respectively; P<0.05). In total

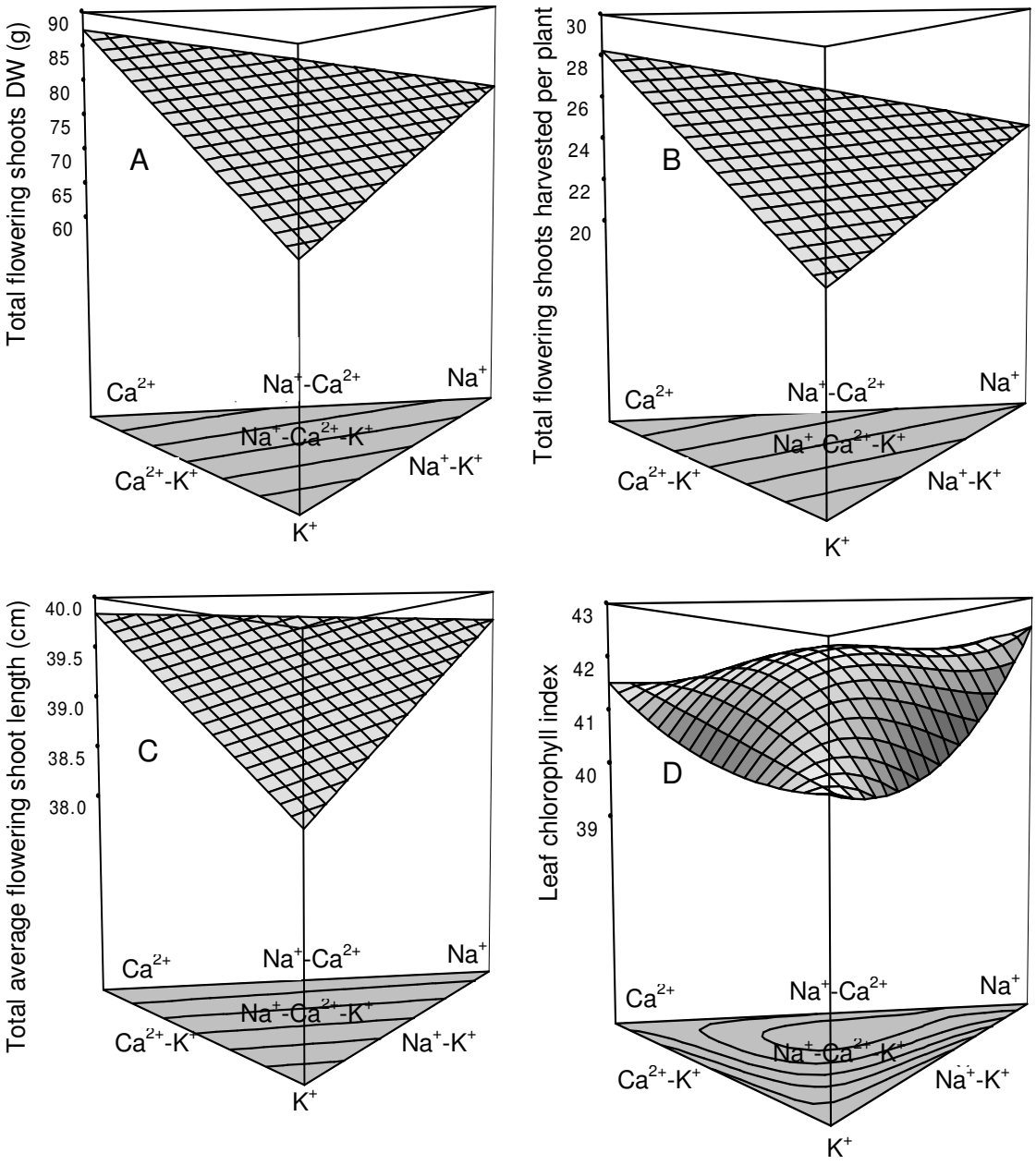

Fig. 5.6. Effect of varying proportions of Na^+, Ca^{2+} and K^+ as Cl^--counter cations on total flowering shoot dry weight (A), total number of flowering shoots harvested per plant (B), total average flowering shoot length (C) and total average leaf chlorophyll index (D) of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surfaces. For fitted models see Table 5.4.

Table 5.4. Fitted models for productivity variables (total flowering shoot dry weight, DW; total flowering shoots harvested per plant, FS; average flowering shoot length, FSL; and average leaf chlorophyll index, LCI) of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

Variable	Best fitted model
DW	=78.1*(NaCl)+87.4*($CaCl_2$)+63.0*(KCl); r^2=0.22, P=0.0002
FS	=24.2*(NaCl)+28.3*($CaCl_2$)+20.0*(KCl); r^2=0.29, P<0001
FSL	=39.7*(NaCl)+39.8*($CaCl_2$)+38.3*(KCl); r^2=0.19, P=0.0007
LCI	=41.9*(NaCl)+41.0*($CaCl_2$)+40.0*(KCl)-1.14*(NaCl*$CaCl_2$)-4.38*(NaCl*KCl)-1.96*($CaCl_2$*KCl)+40.3*(NaCl*$CaCl_2$*KCl); R^2=0.26, P=0.0024

average LCI there were not statistical differences between the control treatment and each of the salt blends (P>0.05 for all comparisons).

All four variables (total flowering shoot DW and total FS harvested per plant, and total average FSL and LCI) seemed to be influenced to a greater extent by the measured EC_{SS} than by the π_{ss} (Fig. 5.7A-D). All four variables were affected negatively by increasing EC_{SS} values, especially total DW and FS (Fig. 5.7A).

As for the organs evaluated during the destructive harvest of plants at the end of the experiment, the dry weights of roots, main stems and old stems were not affected by SB (P>0.05 for all three variables). On the other hand, old

leaves dry weight (OLDW), had a significant response to SB (P<0.0001). The pattern of the OLDW response was very similar to those of total DW, total FS and total average FSL presented in Fig. 5.6A-C, with positive linear effects for Ca^{2+}, negative linear effects for K^+, and no significant effects for varying proportions of Na^+ in the salt blend (Model: OLDW=3.80*(NaCl) +5.29*($CaCl_2$)+1.75*(KCl); r^2=0.55, P<0.0001).

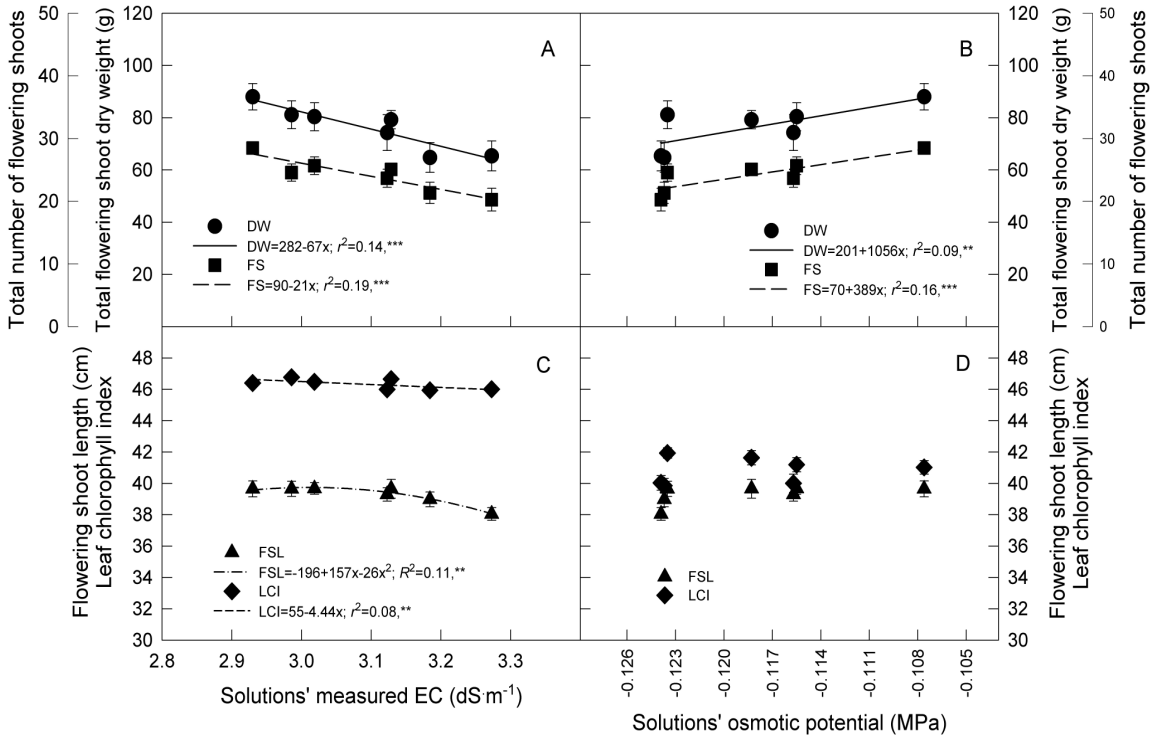

Fig. 5.7. Effect of electrical conductivity (EC) [A and C] and osmotic potential [B and D] of the saline solutions on total dry weight, total number and average length of flowering shoots, and leaf chlorophyll index of 'Erin' roses subjected to a moderately high (12 mmol·L^{-1}) Cl^--based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. Symbols represent the mean ± standard error of 12 plants. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at P≤0.05, P≤0.01, and P≤0.001, respectively.

When comparing each of the salt blends with the control treatment, no differences were found in roots and main stems DW (P>0.05 in both cases; results not shown). However, non-salinized plants had greater old stems DW than those plants subjected to the KCl and NaCl-KCl blends (28 g vs. 21 g and 20 g, respectively; P<0.05 for both comparisons), and greater OLDW than plants from the KCl pure blend (5.14 g vs. 1.77 g, respectively; P<0.05).

Foliar salt injury

Due to the cell size restrictions for the Chi-square test to be valid (see details in Chapter II) three adjacent salt burn categories were combined (0, 1 and 2, and 3, 4 and 5) yielding two final categories: 1) 0-40% of the foliage affected and 2) salt injury affecting between 41-100% of the foliage.

The pattern of leaf injury caused by salinity was different among salt blends (X=0.0154). In general terms, leaf injury was less pronounced in plants from the $CaCl_2$-pure blend, the tertiary or centroid blend and the control treatment, with greater percentage on plants falling in the first salt burn category (Fig. 5.8). Conversely, plants subjected to NaCl and/or KCl in the salt blend presented a greater leaf damage extent, particularly those exposed to the pure blend of KCl (Fig. 5.8).

Although no defoliation evaluation was performed, the plants subjected to salt blends containing KCl experienced considerable defoliation. Conversely, NaCl and $CaCl_2$ salts did not cause defoliation in plants.

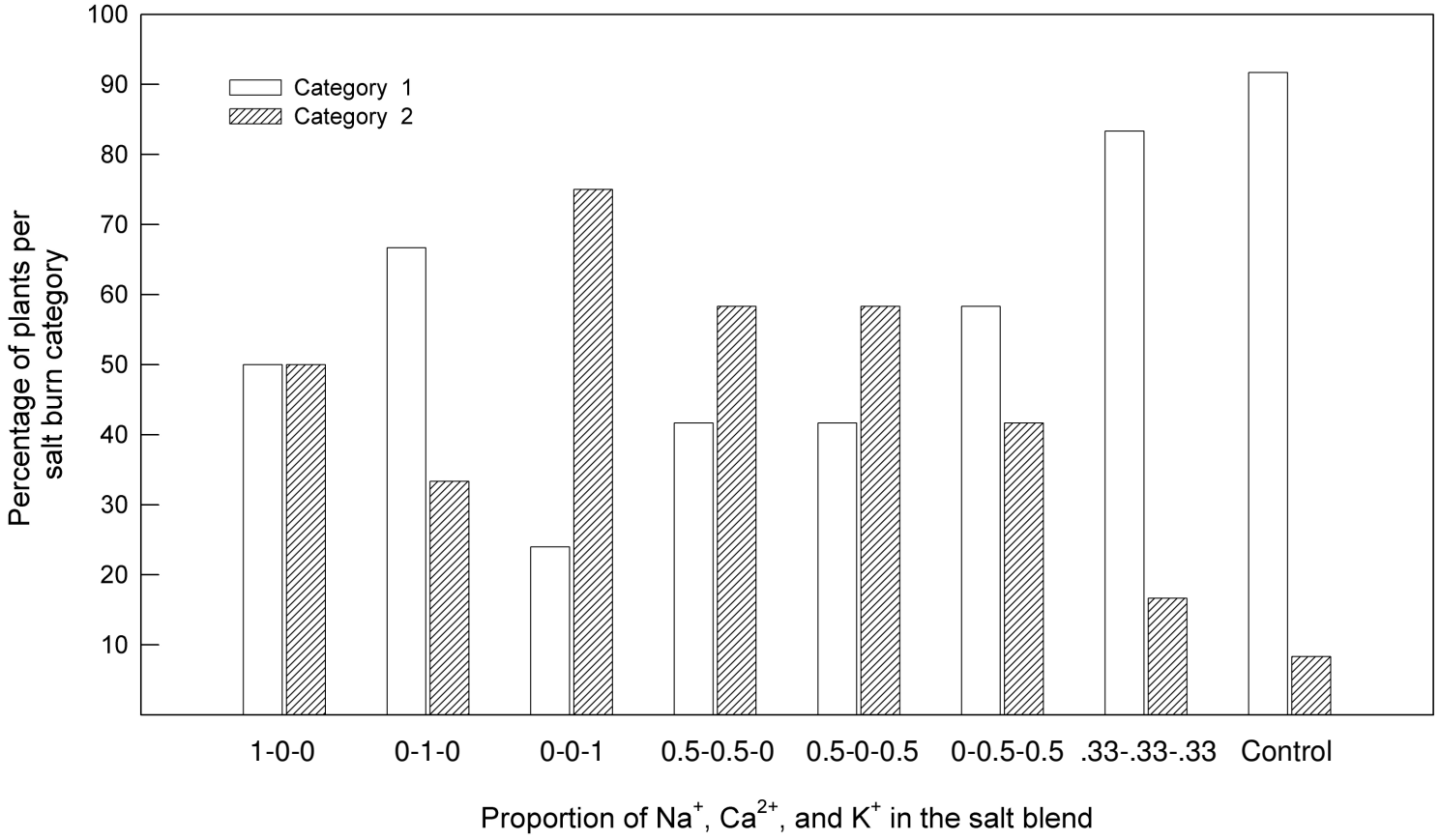

Fig. 5.8. Salt damage of foliage of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. Categories are classified according to the percentage of foliage exhibiting salt damage; Category 1 includes plants with 0-40% of the foliage exhibiting salt burn injury and Category 2 includes plants with 41-100% of foliage presenting salt burn injury. (n=96).

Water relations variables

The pattern of variation in RWC, SWP and LOP over time was similar for all salt blends (no interactions between SB and DAT were present for all three variables; $P>0.05$). Similarly, none of these three variables was affected by the SB ($P>0.05$ for all variables). The overall averages for RWC, SWP and LOP were 94%, -0.67±0.01 MPa, and -1.21±0.02 MPa, respectively.

When compared with the control treatment, differences were found only in SWP. Non-salinized (control) plants had greater SWP values (less negative) than plants subjected to the pure blends of NaCl and $CaCl_2$ and their binary blend (average of -0.58 MPa for control plants and -0.74 MPa, -0.71 MPa and -0.72 MPa for the NaCl, $CaCl_2$ and NaCl-$CaCl_2$ blends, respectively).

Tissue mineral nutrient content

Chloride [Cl], sodium [Na], calcium [Ca] and potassium [K] concentrations

Chloride [Cl]. Concentrations of chloride in leaves of flowering shoots increased at differential rates among all salinized solutions (interactive effects between SB and DAT were present; $P=0.0182$). For most of the salinized solutions the rate of increase in leaf [Cl] followed a linear pattern (Fig. 5.9). Conversely, in the pure blend of NaCl and the tertiary blend (all three components present at 33%), the increase in leaf [Cl] followed a cubic pattern with a greater increase rate between 91 and 124 DAT for the pure blend of NaCl and between 124 and 159 DAT for the tertiary blend (Fig. 5.9). By 91 DAT (first harvest event after plants were exposed to the salt stress) leaf [Cl] was similar among plants from all saline treatments (Fig. 5.9). However, by the second harvest event (91 DAT), those plants subjected to NaCl (pure blend) exhibited the lowest leaf [Cl] and, in general, they tended to have the lowest

concentrations for the rest of the experimental period (Fig. 5.9). Plants receiving the pure blend of KCl, on the contrary, tended to have the greatest leaf [Cl] throughout the experimental period (Fig. 5.9).

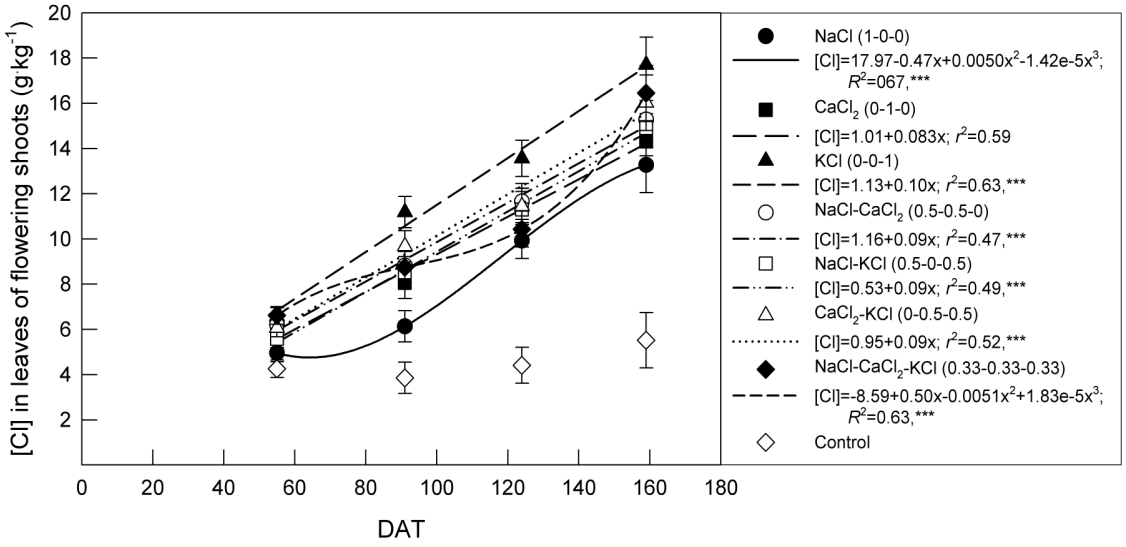

Fig. 5.9. Leaf chloride concentration over time in flower shoots of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. Symbols represent the mean ± standard error of 12 observations. Symbols obscure the error bars that are not apparent. Significance according to GLM: ns, *,**,*** non significant, and significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Compared to the control treatment, by 55 DAT leaf [Cl] was greater in all salinized solutions ($P<0.05$ for all comparisons), except for the NaCl and NaCl-KCl salt blends, which had similar concentrations to those non-salinized plants (Fig. 5.9). By all the next harvest events leaf [Cl] was greater for all salinized treatments compared to the control plants (Fig. 5.9).

Samples from the first and last harvest events (55 and 159 DAT) were analyzed to determine the effect of SB on leaf mineral element concentration at two points in time (beginning and end of the exposure to saline stress).

Leaf [Cl] was affected linearly by the cation proportions in the salt blend at both harvest events, 55 and 159 DAT (P=0.0460 and P=0.0502, respectively; Fig. 5.10A-B; Table 5.5). By 55 DAT Na^+ imposed a negative effect on leaf [Cl], causing a linear decrease in leaf [Cl] as the proportion of Na^+ in the salt blend increased (Fig. 5.10A). Calcium and K^+ had no significant effects on leaf [Cl] at 55 DAT (Fig. 5.10A). By 159 DAT, the response of leaf [Cl] was still negative for Na^+ while for K^+, on the other hand, it was positive, with increasing leaf [Cl] as the proportion of K^+ in the salt blend increased (Fig. 5.10B). Varying proportions of Ca^{2+} had no significant effects on leaf [Cl] at 159 DAT (Fig. 5.10B).

Chloride concentrations in roots, main stems and old leaves (evaluated at the end of the experimental period) were not affected by the composition of the salt blend (P<0.05 for all three organs) and averaged 7.46 $g \cdot kg^{-1}$, 7.05 $g \cdot kg^{-1}$and 20.4 $g \cdot kg^{-1}$, respectively. Conversely, in old stems [Cl] was affected by the cation proportions, exhibiting a linear response [Model: Cl ($g \cdot kg^{-1}$)=7.13*(NaCl) +7.70*($CaCl_2$)+8.51*(KCl); r^2=0.16, P=0.0336]. Sodium and K^+ had significant effects on [Cl] of old stems; increasing the proportion of Na^+ in the salt blend caused decreases in old stems [Cl], whereas as the proportion of K^+ in the salt mixture increased old stems [Cl] increased, denoted by its greater coefficient in the best fitted model. Varying proportions of Ca^{2+} had no significant effects on old stems [Cl].

Concentration of chloride in roots and main stems was similar when comparing the saline treatments against the control treatment (averages of 7.5 $g \cdot kg^{-1}$ and 6.9 $g \cdot kg^{-1}$ for roots and main stems; P>0.05 for both comparisons). In

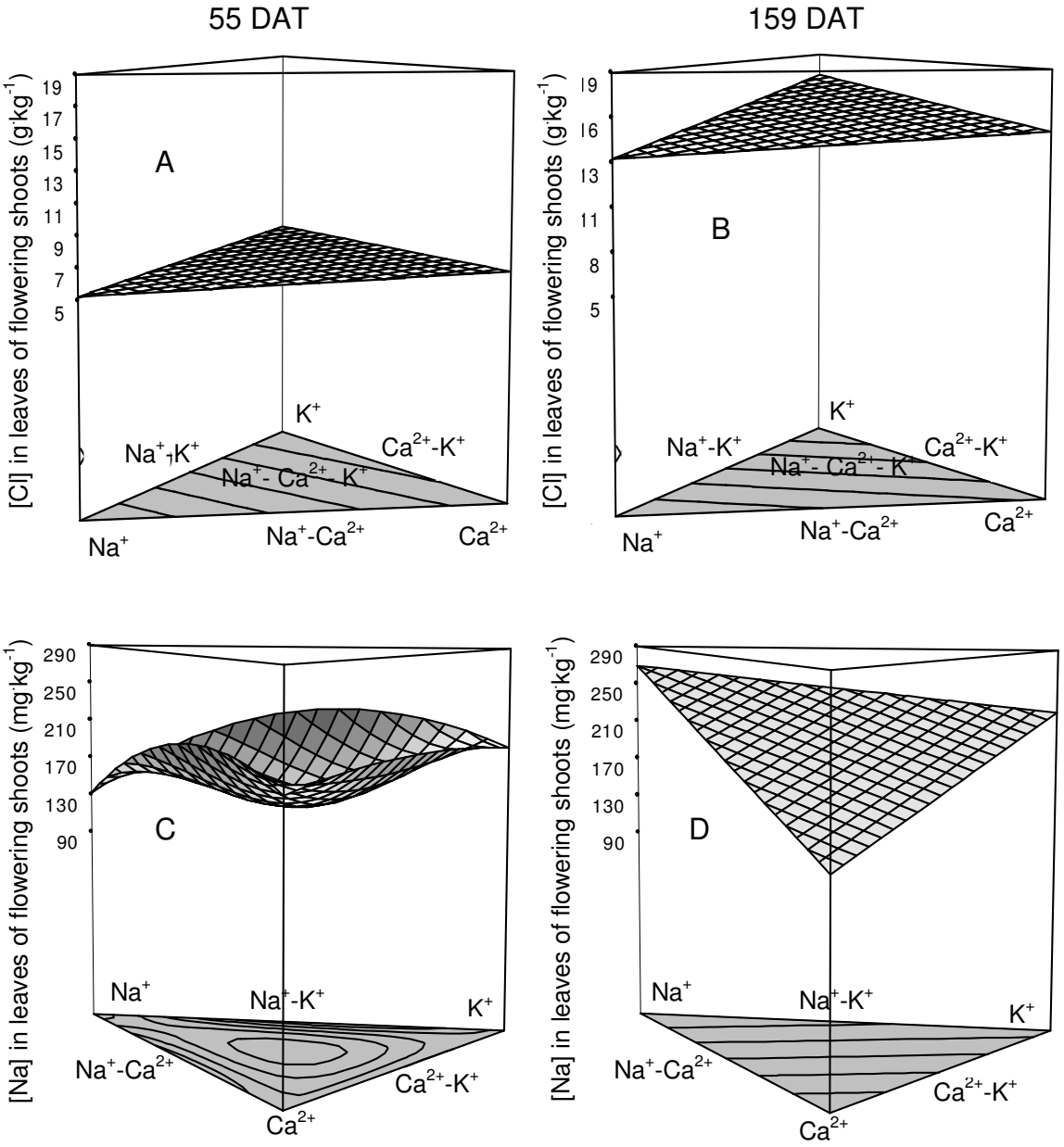

Fig. 5.10. Effect of varying proportions of Na^+, Ca^{2+} and K^+ as Cl^--counter-cations on chloride (A and B) and sodium (C and D) concentrations in leaves of flowering shoots of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surfaces. For fitted models see Table 5.5.

Table 5.5. Fitted models for chloride (Cl) and sodium (Na) concentrations in leaves of flowering shoots of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

Mineral element	Best fitted model
	55 DAT
Cl	Cl=5.25*(NaCl)+6.20*($CaCl_2$)+6.48*(KCl); r^2=0.08, *P*=0.0460
Na	Na=132*(NaCl)+175*($CaCl_2$)+188*(KCl)+196*(NaCl*$CaCl_2$)+249*(NaCl*KCl)+50*($CaCl_2$*KCl)-2198*(NaCl*$CaCl_2$*KCl); R^2=0.52, *P*=0.0012
	159 DAT
Cl	Cl=13.67*(NaCl)+15.02*($CaCl_2$)+17.50*(KCl); r^2=0.08, *P*=0.0502
Na	Na=270*(NaCl)+109*($CaCl_2$)+226*(KCl); r^2=32, *P*=0.0020

old stems [Cl] was greater for all salinized solutions (4.8 $g \cdot kg^{-1}$ in the control versus average of 7.8 $g \cdot kg^{-1}$ in the salt treatments; $P<0.05$ for all comparisons). In old leaves [Cl] was greater for all salinized treatments as well (8.3 $g \cdot kg^{-1}$ vs. average of 21.4 $g \cdot kg^{-1}$ for the salt treatments), except for the NaCl and NaCl-KCl salt blends, which had statistically similar concentrations to those in the non-salinized plants (8.3 $g \cdot kg^{-1}$ vs. 18.7 $g \cdot kg^{-1}$ and 17.4 $g \cdot kg^{-1}$, respectively; $P<0.05$).

Sodium [Na]. By 55 DAT the response of leaf [Na] fitted a special cubic model (P=0.0012; Fig. 5.10C: Table 5.5). Both Na^+-Ca^{2+} and Na^+-K^+ binary blends showed positive synergetic effects on leaf [Na] as their averages were greater than the averages of their respective pure blends (depicted by the upward curvatures on their edges of the triangle; Fig. 5.10C). When all three cations were present in the salt blend (tertiary blend) the response of leaf [Na] was negative (depicted by the downward curvature in the central region of the

surface response), rendering considerably lower [Na] averages than when only one cation was included in the salt blend (pure blends) (Fig. 5.10C).

By 159 DAT both Na^+ and Ca^{2+} had significant linear effects on the response of [Na], positive for Na^+ as leaf [Na] increased with increasing concentrations of NaCl in the salt blend, and negative for Ca^{2+} as leaf [Na] decreased when the proportion of $CaCl_2$ in the salt blend increased (Fig. 5.10D). In fact, while plants subjected the pure blends of NaCl and KCl had statistically similar leaf [Na], those receiving the pure blend of $CaCl_2$ had the lowest leaf [Na] means (Fig. 5.10D).

Comparing between plants from the non-salinized (control) solution and the salinized tratments, there were no differences in leaf [Na] by 55 DAT (P>0.05). By 159 DAT, on the other hand, the pure blend of NaCl, the binary blend of NaCl-KCl and the pure blend of KCl (with no supplemental Na in this blend) had greater leaf [Na] than those plants from the control treatment. All blends containing $CaCl_2$ had similar [Na] than the control (including the binary blend of NaCl-$CaCl_2$ which had 50% of supplemental NaCl; results not shown).

From the destructive harvest of whole plants the response of tissue [Na] to SB was linear in roots and main stems, quadratic in old stems and special cubic for old leaves (Table 5.6). In roots and main stems [Na] increased as the proportions of NaCl in the salt blend increased (greatest coefficient for the Na^+ cation in both fitted models, Table 5.6). In old stems, plants subjected to blends containing 50-100% of NaCl had the greatest [Na] as depicted by their greater coefficients in the fitted models (Table 5.6). Similarly, in old leaves, the pure blend of NaCl had greater [Na] than the other two pure blends (lower coefficients for the pure blends of $CaCl_2$ and KCl in the model, Table 5.6). When combined in the salt blend Na^+ and K^+ had positive synergetic effect on the [Na] in old leaves, causing increases of more than two-fold compared to the pure blend of NaCl (Table 5.6). Plants subjected to the tertiary blend rendered considerably lower old leaves [Na] averages compared to their pure blends (Table 5.6).

Table 5.6. Fitted models for sodium (Na) concentration ($g \cdot kg^{-1}$) in organs of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

Plant organ	Best fitted model
Roots	Na=3.33*(NaCl)+1.40*($CaCl_2$)+1.08*(KCl); r^2=0.87, P<0.0001
Main stems	Na=2.87*(NaCl)+1.10*($CaCl_2$)+0.89*(KCl); r^2=0.93, P<0.0001
Old stems	Na=1.70*(NaCl)+0.26*($CaCl_2$)+0.34*(KCl)-1.55*(NaCl*$CaCl_2$)-1.03*(NaCl*KCl)+0.0019*($CaCl_2$-KCl); R^2=0.91, P<0.0001
Old leaves	Na=0.53*(NaCl)+0.21*($CaCl_2$)+0.29*(KCl)-0.22*(NaCl*$CaCl_2$) +1.18*(NaCl*KCl)+0.0084*($CaCl_2$-KCl)-4.30*(NaCl*$CaCl_2$*KCl); R^2=0.85, P<0.0001

Comparing between salinized treatments and the control treatment, the NaCl pure and binary blends (NaCl-$CaCl_2$ and NaCl-KCl) had greater [Na] in roots and main stems. In old stems only the pure blend of NaCl and the binary blend of NaCl-KCl had greater [Na] than the control plants. In leaves only the binary blend of NaCl-KCl had greater [Na] than the control plants (results not shown).

Calcium [Ca]. At both harvest events (55 and 159 DAT) the response of leaf [Ca] fitted linear models (Fig. 5.11A-B; Table 5.7). At both dates Ca^{2+} had positive effects on leaf [Ca], causing it to increase as its proportion in the salt blend increased, i.e. the coordinate line for the Ca^{2+} cation increases linearly from a proportion of zero to a proportion of 1 (Fig. 5.11A-B). In contrast, at both dates as well, K^+ had negative linear effects, causing decreases in leaf [Ca] as its proportion in the salt blend increased (Fig. 5.11A-B). Varying the proportion

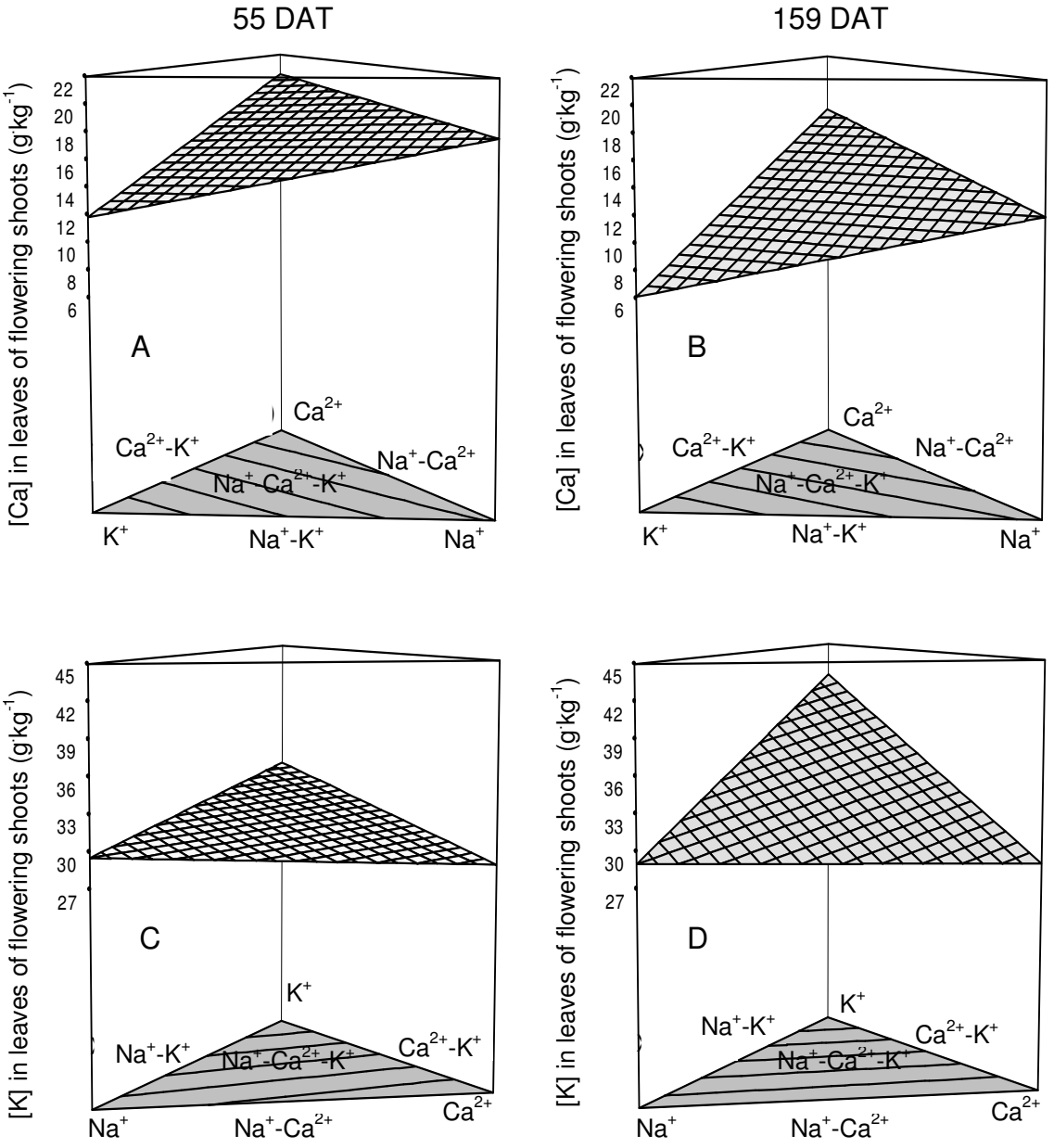

Fig. 5.11. Effect of varying proportions of Na^+, Ca^{2+} and K^+ as Cl^--counter cations on calcium (A and B) and potassium (C and D) concentrations in leaves of flowering shoots of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surfaces. For fitted models see Table 5.7.

Table 5.7. Fitted models for calcium (Ca) and potassium (K) concentrations in leaves of flowering shoots of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

Mineral element	Best fitted model
	55 DAT
Ca	$Ca=17.75*(NaCl)+20.39*(CaCl_2)+11.88*(KCl)$; $r^2=0.77$, $P<0.0001$
K	$K=29.57*(NaCl)+28.24*(CaCl_2)+33.98*(KCl)$; $r^2=0.61$, $P<0.0001$
	159 DAT
Ca	$Ca=12.26*(NaCl)+17.60*(CaCl_2)+6.13*(KCl)$; $r^2=0.82$, $P<0.0001$
K	$K=29.02*(NaCl)+28.23*(CaCl_2)+42.11*(KCl)$; $r^2=0.83$, $P<0.0001$

of Na^+ in the salt blend had no significant effects on the leaf [Ca] response at any of the two dates (Fig. 5.11A-B).

Potassium [K]. Similar to leaf [Ca], leaf [K] fit linear models at both harvest events (55 and 159 DAT) (Fig. 5.11C-D; for models see Table 5.7). Leaf [K] decreased as the proportion of Na^+ and Ca^{2+} in the salt blend increased (Fig. 5.11C-D). On the other hand, as the proportion of K^+ (KCl) in the salt blend increased, so did leaf [K] (Fig. 5.11C-D).

Leaf concentration of other mineral nutrients

Phosphorous [P]. Leaf [P] was affected by the cation proportion only at 55 DAT, fitting a linear model (Fig. 5.12A; Table 5.8). Sodium and Ca^{2+} had significant effects on the concentration of this element. Increasing the proportion of Na^{+} in the salt blend caused increases in leaf [P] (Fig. 5.12A), while the contrary happened with Ca^{2+}, as increasing its proportion caused reductions in leaf [P] (Fig. 5.12A). Varying proportions of K^{+} in the salt blend did not affect leaf [P] (Fig. 5.12A).

Magnesium [Mg]. Leaf [Mg] was affected linearly by the cation proportion at both harvest events, 55 and 159 DAT (Fig. 5.12B-C; Table 5.8). Both Na^{+} and Ca^{2+} had positive effects with leaf [Mg] increasing as the proportions of these two cations in the salt blend increased (Fig. 5.12B-C). Potassium, on the other hand, had a negative effect of the leaf [Mg] response, causing it to decrease when the proportion on KCl in the salt blend increased (Fig. 5.12B-C).

Sulfur [S]. Leaf [S] was affected by SB at 159 DAT, fitting a special cubic model (Fig. 5.12D; Table 5.8). When all three salt components were present in the salt blend (tertiary blend) leaf [S] average surpassed that of their pure blends (Fig. 5.12D). The binary blend of $CaCl_2$-KCl had synergetic negative effects on leaf [S] as the concentration of this element in plants subjected to this salt mixture rendered significantly lower leaf [S] values than the average of their pure blends (Fig. 5.12D).

Zinc [Zn]. Similar to leaf [S], leaf [Zn] was affected by the cation composition at 159 DAT only. The response of leaf [Zn] was similar to that showed by Mg, fitting a linear model (Fig. 5.12E; Table 5.8), with positive effects for Na^{+} and Ca^{2+} and negative for K^{+}. Leaf [Zn] increased as the proportions of Na^{+} and Ca^{2+} increased and decreased as the proportion of K^{+} increased (Fig. 5.12E).

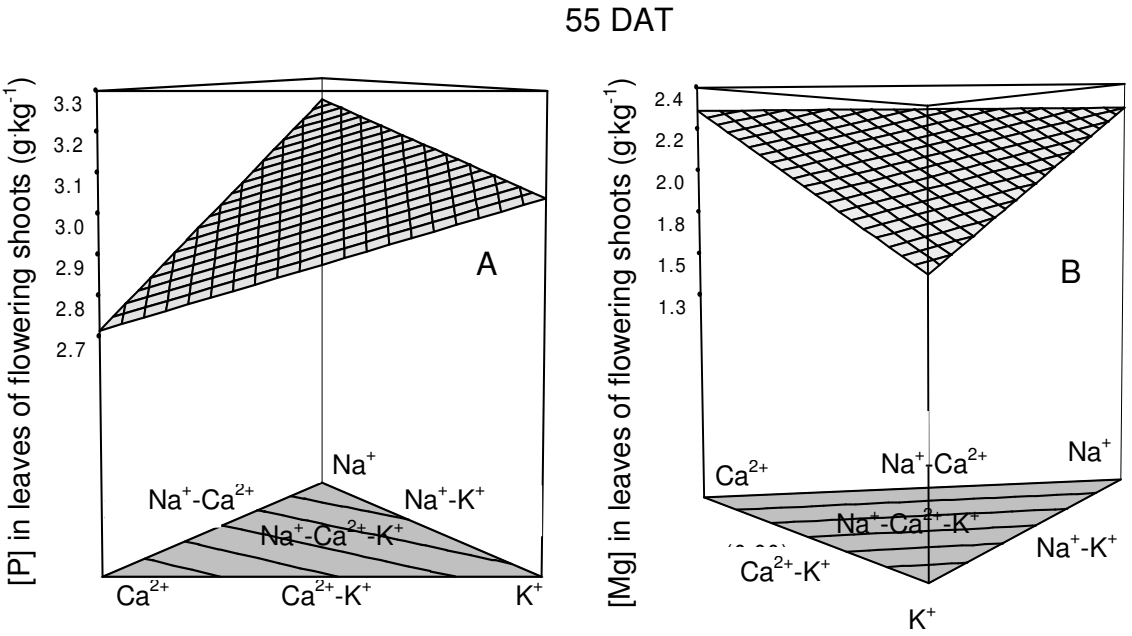

Fig. 5.12. Effect of varying proportions of Na^+, Ca^{2+} and K^+ as Cl^--counter cations on (A) phosphorous [P], (B and C) magnesium [Mg], (D) sulfur [S] and (E) zinc [Zn] concentrations in leaves of flowering shoots of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution. Figures are 3-dimensional response surfaces. For fitted models see Table 5.8.

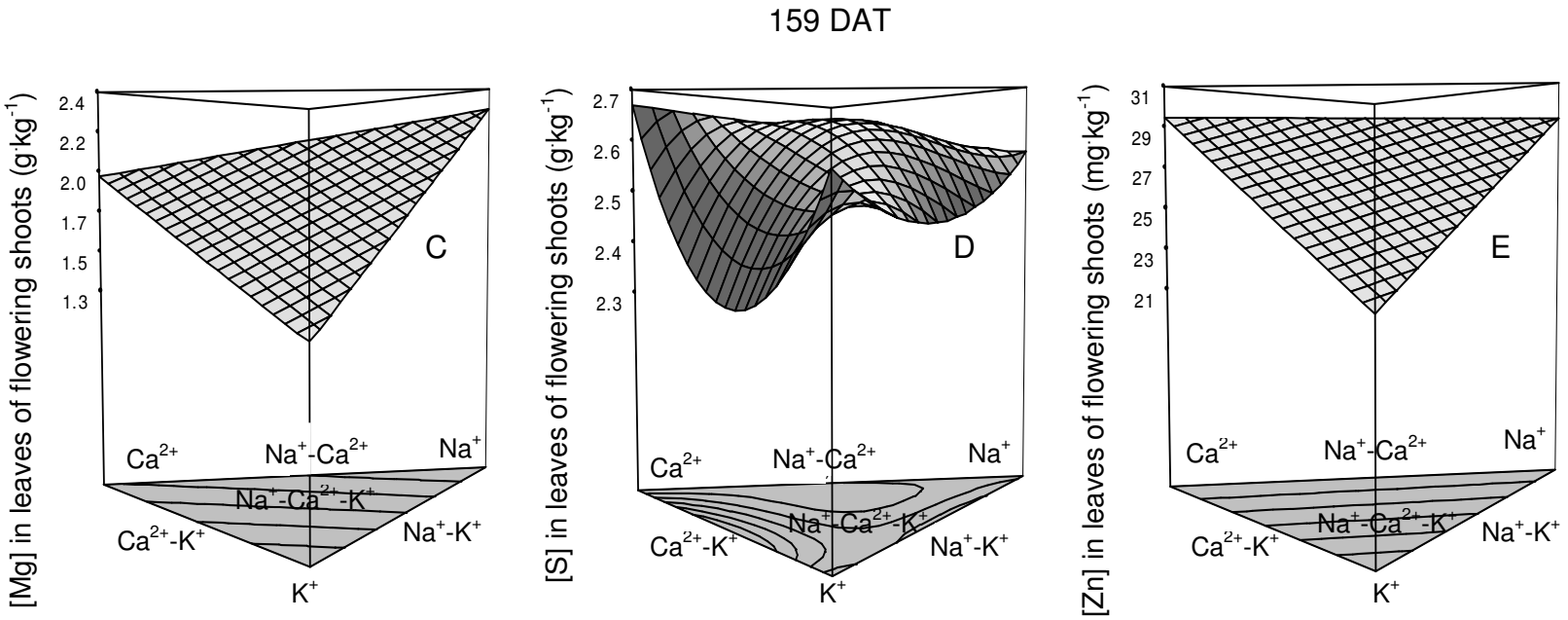

Fig. 5.12. Continued.

Table 5.8. Fitted models for phosphorous (P), magnesium (Mg), sulfur (S) and zinc (Zn) concentrations in leaves of flowering shoots of 'Erin' roses subjected to a moderately high (12 $mmol \cdot L^{-1}$) Cl^--based salinity in a half strength Hoagland's solution with varying concentrations of the accompanying cations (Na^+, Ca^{2+} and K^+) in the salt mixture. To estimate the value of a response at any given mixture, multiply the component's coefficient by the component's proportion (in parenthesis) in the salt blend.

Mineral element	Best fitted model
	55 DAT
P	P=3.24*(NaCl)+2.72*($CaCl_2$)+3.04*(KCl); r^2=0.31, P=0.0026
Mg	Mg=2.31*(NaCl)+2.32*($CaCl_2$)+1.65*(KCl); r^2=0.67, P<0.0001
	159 DAT
Mg	Mg=2.28*(NaCl)+1.94*($CaCl_2$)+1.29*(KCl); r^2=0.82, P<0.0001
S	S=2.59*(NaCl)+2.70*($CaCl_2$)+2.62*(KCl)-0.024*(NaCl*$CaCl_2$)- 0.50*(NaCl*KCl)-1.37*($CaCl_2$*KCl)+5.72*(NaCl*$CaCl_2$*KCl); R^2=0.37, P=0.0347
Zn	Zn=29.2*(NaCl)+29.5*($CaCl_2$)+22.2*(KCl); r^2=0.50, P<0.0001

DISCUSSION

Influence of the Cl^- accompanying cation on the salinized solutions' properties and the overall plant response to salinity stress

In all salt blends chloride was held constant at a concentration of 12 $mmo \cdot L^{-1}$, varying only the type and proportion of its counter-cations (Na^+, Ca^{2+}, and K^+). The EC_E for all saline solutions was expected to be ~3.2 $dS \cdot m^{-1}$ (contributions of 1.1, 1.2 and 0.89 $dS \cdot m^{-1}$ from the base nutrient solution, supplemental salts and tap water used to prepare the solutions, respectively). In other words, the saline stress imposed on the plants was expected to be equal for all salt blends. However, EC_{SS} varied slightly among solutions (Fig. 5.2C). In general, the KCl-blends had the greatest EC_{SS} values, followed by the NaCl-blends and the lowest EC_{SS} values observed were for the $CaCl_2$-blends (Table 5.3).

As in our previous experiment (Chapter IV), in the present experiment the total salinity applied to all seven solutions (on an equivalent basis) was the same, varying only the type and proportion of the salt blend components, specifically the Cl-accompanying cations. All solutions had a total of 12 molecular units of the monovalent anion Cl^-, but for every applied unit of the monovalent cations Na^+ and/or K^+, only half a unit of the divalent cation Ca^{2+} was applied. Thus, the total concentration of molecular particles (or units) was lower in the Ca^{2+}- than in the Na^+- and/or K^+-based salt blends. In addition, according to the calculations made with the SPECIES Program (Barak, 1990), blends containing $CaCl_2$ had the lowest concentrations of free ions ([free ion]) in solution with an average of 46.3 $mmol \cdot L^{-1}$ for the pure and binary blends. Sodium chloride- and KCl blends had similar [free ion] averages, 49.6 and 49.7 $mmol \cdot L^{-1}$, respectively. Consequently, π_{SS} followed a similar order, greater (less negative) for the $CaCl_2$ blends and lower for the NaCl and/or KCl blends (Table

5.3). This suggests a lower osmotic stress imposed on the plants by these $CaCl_2$ salt blends. Ion-association occurred to a greater extent in $CaCl_2$-blends, specifically with SO_4^{2-} (as $CaSO_4$), denoted by the lowest proportions of free SO_4^{2-} ions in these blends (0.70) compared to NaCl and KCl (0.77 for both components). According to Robinson and Stokes (1970), in solutions with ions of symmetrical valency (i.e. the absolute values of the signed units of charge are the same) of double or higher charge, an appreciable fraction of the ions are present as closely associated pairs. Such pairs will have no net charge. They will therefore make no contribution to the electrical conductivity of the solution, while their thermodynamic effects will be those of removing a certain number of ions from the solution and replacing them by half the number of dipolar 'molecules' (Robinson and Stokes, 1970). By forming dipolar molecules with no net charge, Ca^{2+} and SO_4^{2-} associations not only caused a decrease in the EC_{SS} but also caused an increase in the π_{SS}. Both variables, EC_{SS} and π_{SS}, had a closer association to the total applied salt concentration when it was expressed in $mmol \cdot L^{-1}$ (Fig. 5.4A and Fig. 5.4B). The lowest [free ion]'s and greater ion association occurrence in $CaCl_2$-blends would account for their greatest π_{SS}, and lowest EC_{SS}. Even though NaCl and KCl-blends had very similar [free ion]'s (results not shown), and both salts are composed of monovalent ions only, EC_{SS} from KCl-blends were slightly greater than in the NaCl-blends (Fig. 5.2C; Table 5.3).

Differences between NaCl- and KCl-blends' EC_{SS} could be explained by the concept of equivalent conductivity. Upon solution in water the molecules of certain substances dissociate into two or more portions, bearing equal charges of electricity of opposite sign (ions) (Millard, 1921). The conduction of electricity by these solutions is due to the motion of ions through the solution. Positively charged ions move toward the negative pole and give up their charges; negatively charged particles move toward the positive pole and give up their charges (Millard, 1921). When the solution has been diluted until ionization is

complete, each ion is free to move independently of the other, and the equivalent conductivity is the sum of two separate values which may be assigned to the separate ions. Under such complete ionization a limiting value of equivalent conductivity will be reached. Each salt approaches a limiting equivalent conductance as the concentration decreases. In very dilute solutions, where this limit is essentially reached, each ion is free to move about as if no other ions were present. It might seem at first thought that all ions move through a solution with the same velocity. However, this is not the case given that the equivalent conductivities of substances which are ionized to about the same extent are widely different, suggesting that there is a difference in ionic velocities (Millard, 1921). The equivalent conductance of each ion in a solution may be calculated from the ionic velocity ratio (velocity of one ion divided by the sum of the velocities of the two ions) and from the limiting conductance. At a temperature of 18^{o}C K^{+} shows a limiting conductance 47-49% greater than Na^{+} (Millard, 1921; Laidler and Meiser, 1982). At concentrations close to those used in the present experiment and at temperatures of the solution of 18^{o}C, KCl salts exhibited equivalent conductivities 19.5% greater than the NaCl salts (Millard, 1921).

Electrical conductivity in leachates collected from plants in most of the salinized solutions ranged, over time, between 4.3 and 10.5 $dS{\cdot}m^{-1}$ (Fig. 5.1A), which surpasses by far the maximum leachate (EC 1.4-1.8 $dS{\cdot}m^{-1}$; Brun and Settembrino, 1996) and soil solution salinity thresholds (EC's~2-3 $dS{\cdot}m^{-1}$ of the saturation extract; Bernstein et al.,1972; Davidson and Boodley, 1987; Hughes and Hanan, 1978) that have been recommended for roses in the past. Leachate [Cl] exhibited similar patterns to EC_L (Fig. 5.1A and Fig. 5.1B). This close relationship between both variables may be due to the fact that in all salt blends Cl alone constituted 52% of the calculated EC, therefore variations in $[Cl_L]$ would be strongly reflected in $[EC_L]$.

Similar to one of our previous experiments (Chapter III), the effects caused by salinity were more detrimental in the newly developing flowering shoots, with little or no negative effects on the lower, woody organs (old stems and roots). Productive variables such as total flowering shoot DW and total FS harvested per plant, total average FSL and foliar visual quality were markedly affected by the composition of the salt blend. Potassium and Ca^{2+} had significant effects on all these productivity/quality variables. Sodium had no significant effects on the first three but had negative effects on foliage visual quality. Salt blends containing KCl were the most detrimental, as all productivity and quality variables were markedly affected in plants exposed to these salt mixtures. Reductions in flowering shoot DW and FS harvested per plant were observed earlier in plants exposed to the pure KCl and NaCl-KCl binary blends than in the rest of the salinized treatments (Fig. 5.5A and Fig. 5.5B). In general terms, plants receiving the K^+ pure and binary blends rendered the lowest yields (in total flowering DW and total FS harvested per plant) and exhibited the lowest total average LCI means (Fig. 5.6A-D). Calcium was clearly the least harmful Cl^- counter-cation, whereas Na^+ was not as harmful as K^+, but it was more detrimental than Ca^{2+}. Similarly, in seedlings of sensitive 'Carrizo' citrange [*Citrus sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.] and tolerant 'Cleopatra' mandarin [*Citrus reshni* Hort. ex Tan.] chloride salts ($CaCl_2$, NaCl and KCl, at 15, 30 and 30 $mmol \cdot L^{-1}$, respectively) reduced growth and gas exchange parameters, increased leaf damage and abscission and produced anatomical disarrangements and mineral imbalances (Romero-Aranda et al., 1998). However, in both cultivars Ca^{2+} was more beneficial, and K^+ more detrimental for plant growth than Na^+ (Romero-Aranda et al., 1998). Potassium chloride was also more detrimental for growth compared to NaCl in cells lines of lucerne (*Medicago media* Pers. 'Rambler'; Chaudhary et al., 1997), olive plants (*Olea europaea* L.; Vigo et al., 2002), and in the halophytes *Atriplex nummularia* Lindl. (Ramos et al., 2004) and *Atriplex prostrata* Boucher ex CD (Egan and

Ungar, 1998). Trajkova et al. (2006) studied the comparative effects of low (4.1 $dS \cdot m^{-1}$) and moderate (6.33 $dS \cdot m^{-1}$) levels of NaCl and $CaCl_2$ salinity (equal rates on a chemical equivalent basis) on cucumber plants (*Cucumis sativus* L.). Fresh and dry weights of stems and leaves of cucumber plants were reduced only under conditions of high NaCl salinity, whereas root mass was not affected. Fruit yield decreased proportionately to increases in NaCl salinity, while $CaCl_2$ salinity caused a reduction in fruit yield only at the high EC level which was comparable to that caused by low the NaCl salinity. Based on their results, the authors concluded that at equal EC levels, $CaCl_2$ salinity effects were less detrimental compared to those caused by NaCl salinity. Similarly, Yokas et al. (2008) reported decreases in stomatal density, chlorophyll content, plant growth, and yield of tomato (*Lycopersicon esculentum* Mill.) subjected to increasing concentrations of NaCl (30, 60 and 90 $mmol \cdot L^{-1}$) and $CaCl_2$ (20, 40 and 60 $mmol \cdot L^{-1}$). Reductions in tomato fruit yield were greater in the NaCl treatments than in the $CaCl_2$ treatment, even though the levels of salinity were greater (on a chemical equivalent basis) in the $CaCl_2$ treatment. Sodium chloride and KCl salinities (60 $mmol \cdot L^{-1}$) markedly reduced plant growth on Valencia orange [(*Citrus sinensis* (L.) Osbeck] (Bañuls, et al., 1997). Similar to several of the experiments cited above, in our study the order of plant toxicity caused by salinity is as follows: KCl>NaCl>$CaCl_2$. Salts containing symmetrical, monovalent ions (like NaCl, and KCl) have greater ionization percentages than salts with ions of assymetrical charges (like Na_2SO_4 and $CaCl_2$) (Barak, 1990; Robinson and Stokes, 1970; Treadwell 1916; Millard, 1921). Greater ionization of a salt yields a greater number of molecules in solution, while with salts of low values of ionization the total number of free ions will be lower (due to lower ionization values and greater ion association) (Robinson and Stokes, 1970). This created a differential in the osmotic potential of the solutions to which plants were being subjected in the present experiment (NaCl, $CaCl_2$ and KCl), and that on Chapter IV (NaCl, Na_2SO_4 and $NaNO_3$).

Chloride, Na, Ca, K accumulation patterns over time and the influence of the Cl^- counter-cation on leaf [Cl]

Leaf [Cl] increased progressively, even though flowering shoots were removed at every harvest event. In the present study, and for most of the salinity treatments, leaf [Cl] increased linearly reaching concentrations that ranged between 12 and 18 $g \cdot kg^{-1}$ by the last harvest event recorded at 159 DAT (Fig. 5.9). This linear and cumulative increases of leaf [Cl] confirm our findings in the previous experiments (Chapters II, III and IV), which indicate that greater amounts of Cl^- are being absorbed and transported and, possibly, retranslocated to the developing flowering shoots. In general, and as in our previous experiments, [Cl] were considerably greater in non-woody tissues, with averages of 20 $g \cdot kg^{-1}$ and 15 $g \cdot kg^{-1}$ in old leaves and young leaves, respectively, in contrast with woody organs like roots, main stems and old stems, which averaged 7.5, 7.1 and 7.8 $g \cdot kg^{-1}$, respectively. Since chloride salts are readily soluble in soil solution, Cl^- mobility in the soil is high, it is readily taken up by plants, and its mobility in short- and long-distance transport is high (Grattan, 2002; Marschner, 1995). Leaf Cl accumulation could be influenced by factors like the organ's transpiration rate and age, i.e. time the organ has been transpiring, and therefore receiving this anion along with the water delivered by the xylem.

The Cl^- counter-cation had a very significant influence in leaf [Cl]. When Cl^- was accompanied by Na^+ (as NaCl), its concentration in leaves of flowering shoots was considerably reduced (Fig. 5.10A-B). On the contrary, when accompanied by K^+, leaf [Cl] was markedly increased, whereas when Ca^{2+} was the counter-cation, leaf [Cl] was lower than with K^+, but greater than with Na^+ (Fig. 5.10A-B). Our results are in agreement with those reported by Romero-Aranda et al. (1998) who subjected 'Carrizo' citrange [*Citrus sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.] and 'Cleopatra' mandarin (*Citrus reshni*

Hort. ex Tan.) to different chloride salts ($CaCl_2$, NaCl and KCl at 15, 30 and 30 $mmol \cdot L^{-1}$, respectively). According to the authors, in comparison with Na^+, both Ca^{2+} and K^+ increased leaf [Cl] (up to 25% and 69%, respectively). Similarly, in their comparative effects of NaCl and $CaCl_2$ salinity on cucumber (*Cucumis sativus* L.), Trajkova et al. (2006) found that at a high salinity level (24 $meq \cdot L^{-1}$), [Cl] in old and young leaves was significantly greater in plants subjected to the $CaCl_2$ source than in those with NaCl. Similar findings have been reported for young olive plants (*Olea europaea* L. 'Chondrolia Chalkidikis'; Vigo et al., 2002) and cell lines of lucerne (*Medicago media* Pers. 'Rambler'; Chaudhary et al. 1997). In the present experiment, compared to excess Na^+, the presence of high levels of K^+ and Ca^{2+} induced greater increases in leaf [Cl]. This is in agreement with the contention that fluxes of cations with greater permeability/selectivity than Na^+, such as Ca^{2+} and K^+, moving passively develop diffusion potentials favorable to a greater Cl^- uptake (Serrano, 1996).

Contrary to leaf [Cl], and like in our previous experiments (Chapters II, III and IV), leaf [Na] did not increase as markedly over time (Fig. 5.10C-D), and its concentration was considerably greater in the lower woody organs than in the leaves. These differences were more marked in roots and main stems, and for those plants receiving NaCl in the salt blends. It should be noted here that many crop species with relatively low salt tolerance are typical Na^+ excluders and capable, at low and moderate salinity levels, of restricting transport of Na^+ into the leaves where it is highly toxic in salt sensitive species (Marschner, 1995). Our results confirm the plant's ability to restrict Na^+ transport to the upper leaves, as previously reported in roses by Bernstein et al. (2006), Cabrera (2003a), Cabrera and Perdomo (2003), Niu and Rodriguez (2008a), Sadasivaiah and Holley (1973); and also in red-osier dogwood seedlings (*Cornus stolonifera* Michx; Renault, et al., 2001).

In roses, however, this Na^+ exclusion is not general to all rootstocks (Baas and van den Berg, 1999; Cabrera, 2003a; Fernández-Falcón et al., 1986;

Niu and Rodriguez, 2008a). In the present experiment the rootstock used was 'Manetti', and Cabrera and Perdomo (2003) and Sadasivaiah and Holley (1973) have reported a superior capacity of this rootstock to restrict Na^+ transport to its scion leaves compared to other rootstocks. Greater [Cl] in the upper parts (shoots or leaves) and/or greater [Na] in the lower parts (roots) of the plant were found in our previous studies (Chapters II, III and IV) and have also been reported in NaCl-treated seedlings of red-osier dogwood (*Cornus stolonifera* Michx; Renault et al., 2001), 'Mandelon' roses (Baas and van den Berg, 1999); *Crataegus opaca* Hook. & Arn (Picchioni and Graham, 2001), cucumber (*Cucumis sativus* L. 'Orlando') and melon (*Cucumis melo* L. 'Ananas') (Kaya et al. 2003b), snapdragon (*Antirrhinun majus* L. 'Monaco Rose'; Carter and Grieve, 2008), rose rootstocks *Rosa.* L. 'Dr. Huey', *R. x fortuniana*, *R. multiflora* and *R. x odorata* (Niu and Rodriguez, 2008b), and 'Bridal Pink' roses budded on the rootstock 'Manetti' (Cabrera and Perdomo, 2003).

Apparently, unlike Cl^-, Ca^{2+} did not seem to be retranslocated towards the new, growing tissues as the leaf [Ca] of flowering shoots decreased from 55 DAT to 159 DAT, in general, similarly for all SB (Fig. 5.11A-B). Decrease in leaf [Ca] over time was observed as well in two of our previous experiments (Chapters II and III). In all three experiments salt stress caused plant productivity to decrease over time. As a consequence, the reduction in dry mass production might have caused a reduction in the total plant transpiration. A study conducted by Baas et al. (2003) focused on Ca^{2+} distribution in cut roses and found a direct close association between transpiration rates and leaf [Ca]. The authors concluded that local [Ca] in the rose organs can be related to their respective transpiration rates.

In the present experiment, increasing the concentration of K^+ in the solution caused leaf [Cl] to increase, and [Mg], [Ca] and [Zn] to decrease. Romero-Aranda (1998) also reported reductions in [Ca] (up to 74%) and [Mg] (up to 62%) caused by KCl in 'Carrizo' citrange and 'Cleopatra' mandarin. When

the K^+ supply is abundant, a 'luxury consumption' often occurs, possibly causing interference with the uptake and physiological availability of Mg^{2+} and Ca^{2+} (Marschner, 1995). This negative effect on Ca^{2+} accumulation by K^+ oversupply would worsen the effects of the saline stress on plants, given that the particular role of Ca^{2+} in increasing the salt tolerance of plants is well documented (Marschner, 1995).

Leaf [K], like leaf [Cl], increased linearly in those treatments containing KCl in the salt blend at both sampling dates (55 DAT and 159 DAT) (Fig. 5.11C-D). Soluble salts that occur in soils consist mostly of various proportions of the cations Na^+, Ca^{2+}, and Mg^{2+} and the anions Cl^- and SO_4^{2-}, while constituents that ordinarily occur only in minor amounts are K^+, HCO_3^-, CO_3^{2-} and NO_3^- (Richards, 1954; Grattan and Grieve, 1999; Bernstein, 1975). Cations are transported 'downhill' along the electrical potential gradient across the plasma membrane (Marschner, 1995). For K^+, however, at low external concentrations (<1 mM) uptake is coupled to metabolic activity, where the high affinity uptake system operates against the prevailing electrochemical potential difference (Marschner, 1995). Roses, like several other plant species, as stated in our present and previous experiments, and other studies cited throughout this document, have developed mechanisms to exclude and/or restrict the uptake and transport of Na^+ to the shoot tissues. In contrast, being that K^+ is rarely found in saline substrates, plants have developed a highly selective uptake system of K^+ over Na^+ to absorb this cation (K^+) against electrochemical potential differences (Marschner, 1995). In our experiment external K^+ concentrations were increased artificially, which might have facilitated its passive/highly selective uptake by the root systems in those plants receiving KCl treatments. Additionally, K^+ is characterized by its high mobility in plants at all levels, within individual cells, within tissues, and in long-distance transport via the xylem and phloem (Marschner, 1995).

The standard range of leaf [K] in roses has been reported between 1.8 and 3.0% (18-30 $g \cdot kg^{-1}$) (White, 1987). In our study, at the beginning of the experiment (55 DAT) leaf [K] ranged between 28 and 34 $g \cdot kg^{-1}$ (Fig. 5.11C; Table 5.7), barely surpassing the standard range. However, by the end of the experimental period (159 DAT), while leaf [K] did not change in plants subjected to NaCl and/or $CaCl_2$ (Fig. 5.11D; Table 5.7), it increased linearly with increasing proportions of KCl in the salt blend, reaching concentrations up to 42 $g \cdot kg^{-1}$ in the pure KCl blend (Fig. 5.11D; Table 5.7), far more than the normal range reported in roses (White, 1987). Excessive amounts of K^+ may be detrimental to some plants (Grattan and Grieve, 1992). Potassium, the most abundant cation in the cytoplasm, and its accompanying anions make a major contribution to the osmotic potential of cells and tissues of glycophytic plant species (Marschner, 1995). Our results showed, however, no significant differences in all water relations variables in KCl-treated plants compared to the other salt sources or to the non-salinized (control) plants. This suggests that the excess K^+ found in leaves was either not used in osmotic adjustment or large variance in these leaf osmotic potential measurements did not allow for differential resolution. The more deleterious effects caused by KCl- compared to NaCl or $CaCl_2$-salts might be due to a combination of several factors such as lower osmotic potentials of the KCl-solutions (compared to $CaCl_2$), greater uptake and/or transport of Cl^- to the upper plant parts, ion imbalances, and probably an excess of K^+ in the cytosol (Ramos et al., 2004).

CHAPTER VI

SUMMARY

The 'Green' industry, which includes the floricultural, ornamental, turf and landscape maintenance industries, is one of the fastest growing segments of agriculture in the United States (Lea-Cox et al., 2004). Many greenhouse and container-nursery production operations can be classified as intensive agriculture because they use a combination of fertilizers, growth regulators, insecticides and fungicides to mass-produce landscape and ornamental plants in high volumes on small acreages (Lea-Cox et al., 2004). Under protected structures plants grow under environmental conditions that are closer to optimal, thus maintaining sustained production during extended seasons (Jovicich et al., 2007). These plants are grown in containers with soilless media requiring, therefore, greater amounts of water and nutrients per unit area than in the field. Still, greenhouse systems are more efficient in the use of water since production in greenhouses is much greater compared to open field (Jovicich et al., 2007). Furthermore, vegetables grown in greenhouses with closed irrigation systems use 30% to 50% less water per fruit weight than those produced with drain-to-waste (i.e. open irrigation) systems (Marfà, 1999). Therefore, potentially, irrigating with recycling solution could lead to savings of 50 and 60% with respect to field irrigated crops (Jovicich et al., 2007).

Among ornamental plants, roses (*Rosa* L.) are among the most popular garden shrubs, as well as the favorite cut flowers sold by florists. Mineral nutrition of this species has received much attention due to its high production costs and more recently salinity has become an important issue. Due to the scarcity of high quality water, continued and increased agricultural production will depend on utilization of marginal waters for irrigation (Bernstein, et al., 2006) and recycled effluents (Raviv and Blom, 2001). In contrast to agronomic species

and crops, when establishing permissible levels of salinity for ornamentals, aesthetic characteristics of the plant are as or more important than growth or yield. Loss or injury of leaves due to salt stress is unacceptable for ornamentals, even if their growth remains unaffected (Maas, 1990). In the past roses have been classified as fairly salt-sensitive (Bernstein et al., 1972; Davidson and Boodley, 1987; Hughes and Hanan, 1978; Brun and Settembrino, 1996). However, recent nutrition studies suggest that they may actually tolerate moderate to relatively high salinities (Cabrera and Perdomo, 2003; Wahome et al., 2000).

Rosa L. 'Manetti', one of the oldest clonal rootstocks, was selected in the nineteenth century and became very popular in southern Europe in a short time (De Vries, 2003a,b). It was and still is being used for garden roses and greenhouse cut roses in the Mediterranean area and in the USA (De Vries, 2003c). Despite their favorable characteristics, including proven tolerance to edaphic and environmental stresses, 'Manetti' and several other clonal rootstocks introduced in the late twentieth century, were replaced by 'Natal Briar', which has almost completely dominated the western European, North American and South American cut-rose industry from about 1990 onwards. (De Vries, 2003a,c). Originating in South Africa, 'Natal Briar' did not result from a breeding program, neither was it systematically selected from a population, but is just a genotype that became highly successful once it was accidentally tried out as a rootstock (De Vries, 2003b). Now it has practically displaced most other clonal rootstocks (De Vries, 2003b) even though its performance under poor soil conditions has never been fully investigated.

A series of experiments was carried out having as main objectives to reassess the limits of tolerance to salinity of roses, to establish the influence of the rootstock on the general response to salt stress, to determine if supplementing the salinized solution with Ca^{2+} alleviates the detrimental effects

caused by salinity, and to demarcate the influence of Na^+ and Cl^--counter ions on the harmful effects caused by these salinizing elements.

REASSESSING THE LIMITS OF TOLERANCE TO SALINITY OF ROSES AND THE INFLUENCE OF THE ROOTSTOCK

Flower productivity and quality parameters, and plant water relations were negatively affected by increasing salt concentrations in the nutrient solution. However, in general terms, there were no significant differences in the periodic and cumulative data collected in the salinity range of 0.0-6.0 $mmol \cdot L^{-1}$ NaCl-$CaCl_2$. It was only for the two greatest salt concentrations, 12.0 and 24.0 $mmol \cdot L^{-1}$ [electrical conductivity of the saline solutions (EC_{SS}) of 3.25 $dS \cdot m^{-1}$ and 4.85 $dS \cdot m^{-1}$, respectively; and electrical conductivity of the leachates (EC_L) of 6.3 $dS \cdot m^{-1}$ and 7.3 $dS \cdot m^{-1}$, respectively) that substantial reductions in all evaluated variables were found during regular flowering shoot harvests and at the destructive harvest of whole plants. Plants budded on the 'Manetti' rootstock were more vigorous, produced more and longer flowering shoots per plant, and their foliar salt burn injury was considerably less compared to those plants budded on 'Natal Briar'.

Even though flowering shoots were removed from the plants at each harvest event, their leaf chloride concentration [Cl] increased from one harvest to the next. This cumulative increase implies that over time greater amounts of Cl^- were absorbed from the growing substrate and/or retranslocated from lower portions of the plant to newly developing flowering shoots. By the first harvest of flowering shoots (31 DAT) 'Natal Briar' plants already had a leaf [Cl] that was 246% greater than in 'Manetti' plants (8.02 versus 2.32 $g \cdot kg^{-1}$, respectively). 'Manetti' plants registered their greatest mean leaf [Cl] at the last harvest (265 DAT; 8.82 $g \cdot kg^{-1}$), but it barely surpassed the mean concentration registered by 'Natal Briar' plants during the first harvest event at 31 DAT (8.02 $g \cdot kg^{-1}$).

Chloride concentrations were very low in old, woody tissues (roots and old stems) compared with young, non-woody tissues, i.e. leaves. In roots and old stems [Cl] was greater in 'Manetti' plants compared to roots and old stems from 'Natal Briar' plants.

Contrary to leaf [Cl], leaf sodium concentration [Na] remained steady in both rootstocks, averaging 43 $mg \cdot kg^{-1}$. It was only at the 24 $mmol \cdot L^{-1}$ salt level and for harvest V (192 DAT) that leaf [Na] increased considerably in leaves of 'Natal Briar' plants. Probably over time and after the 12.0 $mmol \cdot L^{-1}$ salt concentration this rootstock could not keep up the restriction in Na^+ uptake and/or transport to upper leaves. Sodium concentrations were much greater in roots, old stems and old leaves than in flowering shoots' leaves for both rootstocks.

In 'Natal Briar', rootstock with greater leaf [Cl] and leaf [Na], and greater leaf salt injury, the levels of leaf [B] where 142% greater than in 'Manetti'. Leaf concentrations of N, Mg, S and Fe decreased as the salinity in the irrigation water increased (S only in plants subjected to the greatest levels of salinity).

Given the closer, and negative correlations between tissue [Cl] with the productivity and quality variables evaluated, it seems that the reductions in flower yield and quality were due to a greater extent to Cl^- rather than to Na^+ toxicity.

Based on these results and other salinity studies (Cabrera and Perdomo 2003; Wahome et al., 2000), it is inferred that the NaCl or NaCl-$CaCl_2$ salinity tolerance limit for greenhouse roses, although highly influenced by the rootstock, is between 12 and 15 $mmol \cdot L^{-1}$.

EFFECTS OF SUPPLEMENTAL CALCIUM ON THE RESPONSE OF ROSES TO SALINITY STRESS

With exception of the non-salinized control, the stress imposed by the salinity treatments (12 $mmol \cdot L^{-1}$ NaCl supplemented with 0.0, 2.5, 5.0, 7.5, and 10.0 $mmol \cdot L^{-1}$ $CaSO_4$) in this experiment caused reductions in plant productivity [flowering shoot dry weight (DW) and number of flowering shoots (FS) harvested per plant] and affected the plants' water status [lower relative water content (RWC) and more negative stem water potential (SWP) and leaf osmotic potential (LOP)].

Detrimental effects caused by salinity were more evident on aerial parts of the plants causing reductions not only on flowering shoot DW and FS harvested per plant, but also on old leaves' DW from the plants destructively harvested at the end of the experiment. The DW in the lower plant organs (main stem and roots) were not affected to the same degree by salinity stress (osmotic and/or ion-specific) as the leaves.

After approximately 3.5 months of exposure to NaCl salinity treatments, the plants' visual appearances were also affected by the stress, exhibiting salt burn injury mostly on the plants' basal foliage (old leaves) and to a lesser degree on the basal leaves of cut-flower shoots. The extent of the damage increased as time of exposure to salinity increased. Salt burn injury on old leaves was less pronounced and no substantial injury was exhibited on flowering shoot leaves of plants subjected to Na_2SO_4.

In this experiment supplementing the saline solution with calcium (as $CaSO_4$) did not alleviate harmful effects caused by salinization with NaCl on both plant productivity, quality, and water relations. Several factors might influence the degree and nature of salinized plants' responses to supplemental Ca applications such as the genotypes' salt tolerance, levels of [Ca] found in the

substrate and irrigation water, composition of the salinizing agents and the supplemental Ca counter-anion (i.e. Cl^-, NO_3^-, SO_4^{2-}).

Reductions in plant productivity and leaf osmotic potential (LOP) seemed to be highly influenced by the Na^+ accompanying-anion. Plants exhibited more detrimental effects on their flowering shoot productivity, old leaves' DW and more negative LOP when exposed to NaCl-based salinity than when exposed to Na_2SO_4-based salinity (both at the 5 $mmol \cdot L^{-1}$ additional Ca level). Contrary to NaCl, the Na_2SO_4-salinized treatment did not have any negative effects on plant productivity, yielding similar total flowering shoot DW and total FS as the non-salinized control plants. Also, those plants subjected to Cl^- as the Na^+-accompanying anion exhibited salt damage to a greater extent on their foliage than those exposed to the counter anion SO_4^{2-}.

In general, Cl^- transport and deposition was progressive over time and more pronounced in leaves (both old leaves and flowering shoot leaves) than in roots, main stems and old stems. Contrary to leaf [Cl], leaf [Na] of flowering shoots did not change significantly over time, being similar between the control and the Na-salinized treatments (with either Cl^- or SO_4^{2-} as counter-anions), and its concentration in flowering shoot leaves was much lower compared to leaf [Cl]. Opposite to Cl^- as well, Na^+ transport and accumulation was greater in basal organs, particularly roots and main stems.

While leaf [Ca] and leaf [S] exhibited a positive association with plant productivity/quality variables [flowering shoot total DW, total FS, total average flowering shoot length (FSL) and total average leaf chlorophyll index (LCI)], leaf [Cl] showed a negative association with the first three. Sodium did not appear to be related positively or negatively to any of the productive variables mentioned above.

Results from the present and the previous experiment (Chapter II) indicate that Cl^- might be the major culprit in the reduction in rose plant productivity and quality caused by NaCl-salinity.

EVALUATING THE INFLUENCE OF THE COUNTER ANION ON THE DETRIMENTAL EFFECTS IMPOSED BY SODIUM-BASED SALINITY ON ROSES

In 'Manetti' plants the response of plant productivity/quality to salt stress was not as marked as it was in 'Natal Briar'. In 'Natal Briar' the counter-anion NO_3^- had a negative effect on flowering shoot total DW and total FS harvested per plant, and total average LCI, Cl^- had no effects on any these variables' responses, and SO_4^{2-}, on the other hand, had a positive effect.

Sodium concentrations in leaves of flowering shoots seemed to be greatly influenced by the Na^+-accompanying anion. While SO_4^{2-} did not affect leaf [Na], Cl^- and NO_3^- noticeably promoted increases in leaf [Na], especially NO_3^-, whose effect was even more marked on plants grafted on 'Natal Briar'. The noticeably greater [Na] in leaves of flowering shoots of those plants subjected to salt blends containing $NaNO_3$ might be the main cause for the more severe detrimental effects observed in this rootstock.

There were marked differences in EC_{SS} and EC_L among the different salt blends (SB), which were due apparently to the type and proportion of the anion present in the salt blend. Sulfate had a noticeable effect on lowering electrical conductivity of both saline solutions and, therefore, of leachates.

All SB had a total of 12 molecular units of the monovalent cation Na^+, but for every applied unit of the monovalent anions Cl^- and/or NO_3^-, only half a unit of the divalent anion SO_4^{2-} was applied. Thus, the total concentration of molecular particles (or units) was lower in the SO_4^{2-}- than in the Cl^-- and/or NO_3^--based salt blends. Also, in all solutions, independently of the type of supplemental salts used, only between 78% and 81% of the applied SO_4^{2-} was present as a free ion in solution, while 19% to 22% was associated (ion pairs), primarily with Ca^{2+} (13.30%), Mg^{2+} (3.71%), Na^+ (2.85%) and K^+ (1%). Conversely, 98.5% and 100% of the applied Cl^- and NO_3^-, respectively, were

present as free ions in solution. Sulfate-ion associations with Ca^{2+} and Mg^{2+} not only meant a decrease in the SO_4^{2-} [free ion], but also a decrease in Ca^{2+} and Mg^{2+} [free ion]'s. Ion-associations between SO_4^{2-} and Ca^{2+} and Mg^{2+} had a dual effect on the electrochemical properties of the solutions. First, by forming molecules with no net charge, they caused a decrease in EC_{SS}. Second, due to the ion pair formation, two ions (SO_4^{2-} and either Ca^{2+} or Mg^{2+}) were replaced by one dipolar molecule, reducing even more the initial lower number of ions present in SO_4^{2-}-salt blends (due to its divalent electrical charge) increasing, consequently, the saline solutions' osmotic potential (π_{SS}) for these SB (Ben-Gal et al., 2009). This suggests a lower osmotic stress imposed on plants by SO_4^{2-}-SB than with NaCl or KCl.

When the total salinity of the solutions was expressed in $mmol \cdot L^{-1}$, instead of $meq \cdot L^{-1}$, a close linear relationship was observed between total applied salinity and EC_{SS}. Consequently, the detrimental effects caused by salinity, particularly on plants grafted on 'Natal Briar', could be due more to the differential contribution of each particular salt to the total osmotic potential of the saline solution rather than to specific ion effects.

These observations point to a rarely considered and/or studied situation, namely the significance of both the specific and differential contribution of each ion in solution to the actual (resultant or measured) electrical conductivity (EC) and its effective osmotic strength (Ben-Gal et al., 2009). Therefore, it is contended that the common reporting of salinity on the equivalent or equinormal basis of EC alone might be masking or hiding effects that are effectively influencing soil solution chemistry and plant/crop responses. This also poses the challenging task of (re)interpreting results among both similar and dissimilar salinity studies by comparing them on a more level or integrative salt stress index or basis (i.e. solution's osmotic potential).

EVALUATING THE INFLUENCE OF THE COUNTER CATION ON THE DETRIMENTAL EFFECTS IMPOSED BY CHLORIDE-BASED SALINITY ON ROSES

Expected electrical conductivity (EC_E) for all saline solutions was expected to be ~3.2 $dS \cdot m^{-1}$ (contributions of 1.1, 1.2 and 0.9 $dS \cdot m^{-1}$ from the base nutrient solution, supplemental salts and tap water used to prepare the solutions, respectively). However, EC_{SS} varied slightly among SB. In general, the KCl-blends had the greatest EC_{SS} values, followed by NaCl-blends and the lowest EC_{SS} values were observed in the $CaCl_2$-blends.

Pure and binary $CaCl_2$-blends had the lowest concentrations of free ions ([free ion]) in solution (average of 46.3 $mmol \cdot L^{-1}$). Sodium chloride- and KCl-blends had similar [free ion] means, 49.6 and 49.7 $mmol \cdot L^{-1}$, respectively. Consequently, π_{SS} followed a similar order, greater (less negative) for $CaCl_2$ blends and lower for NaCl and KCl blends. This suggests a lower osmotic stress imposed on plants by these $CaCl_2$ salt blends. Ion-association occurred to a greater extent in $CaCl_2$-blends, specifically with SO_4^{2-} (as $CaSO_4$), denoted by the lowest proportions of free SO_4^{2-} ions in these blends (0.70) compared to NaCl and KCl (0.77 for both components). By forming dipolar molecules with no net charge, Ca^{2+} and SO_4^{2-} associations not only caused a decrease in EC_{SS} but also caused an increase in π_{SS}. Both variables, EC_{SS} and π_{SS}, had a closer association to the total applied salt concentration when it was expressed in $mmol \cdot L^{-1}$. Both, lowest [free ion] and greatest ion association occurrence in $CaCl_2$-blends compared to NaCl- or KCl-blends, would account for their greatest π_{SS}, and lowest EC_{SS}.

Salts containing symmetrical, monovalent ions (like NaCl, and KCl) have greater ionization percentages than salts with ions of assymetrical charges (like Na_2SO_4 and $CaCl_2$) (Barak, 1990; Robinson and Stokes, 1970; Treadwell 1916; Millard, 1921). Greater ionization of a salt yields a greater number of molecules

in solution, while with salts of low values of ionization the total number of free ions will be lower (due to lower ionization values and greater ion association) (Robinson and Stokes, 1970). This created a differential in π_{SS} to which plants were being subjected in the present experiment. Even though NaCl and KCl-blends had very similar [free ion] (results not shown), and both salts are composed of monovalent ions only, EC_{SS} from KCl-blends were slightly greater than in NaCl-blends. Differences between NaCl- and KCl-blends' EC_{SS} could be explained by the concept of equivalent conductivity. At concentrations close to those used in the present experiment and at temperatures of the solution of 18^{o}C, KCl salts exhibit equivalent conductivities 19.5% greater than the NaCl salts (Millard, 1921).

Productive variables such as flowering shoot total DW and total FS harvested per plant, total average FSL, and foliar visual quality were markedly affected by SB. Potassium and Ca^{2+} had significant effects on all these productivity/quality variables. Sodium had no significant effects on the first three but had negative effects on foliage visual quality. Salt blends containing KCl were the most detrimental, as all productivity and quality variables were markedly affected in plants exposed to these salt mixtures. Calcium was clearly the least harmful Cl^{-} counter-cation, whereas Na^{+} was not as harmful as K^{+}, but it was more detrimental than Ca^{2+}.

The Cl^{-} counter-cation had a very significant influence in leaf [Cl]. When Cl^{-} was accompanied by Na^{+} (as NaCl), its concentrations in leaves of flowering shoots were considerably reduced. On the contrary, when accompanied by K^{+}, leaf [Cl] was markedly increased, whereas when Ca^{2+} was the Cl^{-} counter-cation, leaf [Cl] was lower than with K^{+}, but greater than with Na^{+}.

By the end of the experimental period (159 DAT), leaf [K] did not change in plants subjected to NaCl and/or $CaCl_2$. In plants subjected to KCl, on the other hand, leaf [K] increased linearly as the proportion of KCl increased in the salt blend, reaching concentrations up to 42 $g \cdot kg^{-1}$ in the pure KCl blend, far

more than the normal range reported in roses (White, 1987). Excessive amounts of K^+ may be detrimental to some plants (Grattan and Grieve, 1992).

More deleterious effects caused by KCl-, compared to NaCl- or $CaCl_2$-salts might be due to a combination several factors such as lower osmotic potentials of KCl-solutions (compared to $CaCl_2$), greater uptake and/or transport of Cl^- to upper plant parts, ion imbalances, and probably an excess of K^+ in the cytosol (Ramos et al., 2004).

LITERATURE CITED

Adriano, D.C. and H.E. Doner. 1982. Bromine, chlorine, and fluorine, p. 449-483. In: A.L. Page, R.H. Miller, and D.R. Keeney (eds.). Methods of soil analysis, ASA Monog. No. 9. 2nd edition. Amer. Soc. Agron., Madison, WI.

Ashraf, M. and S. Ahmad. 2000. Influence of sodium chloride on ion accumulation, yield components and fibre characteristics in salt-tolerant and salt-sensitive lines of cotton (*Gossypium hirsutum* L.). Field Crops Research 66:115-127.

Awada, S., W.F. Campbell, L.M. Dudley, and J.J. Jurinak. 1995. Interactive effects of sodium chloride, sodium sulfate, calcium sulfate, and calcium chloride on snap bean growth, photosynthesis, and ion uptake. J. Plant Nutr. 18:889-900.

Baas, R. and D. van den Berg. 1999. Sodium accumulation and nutrient discharge in recirculation systems: a case of study with roses. Acta Hort. 507:157-164.

Baas, R., S.V. Oers, A. Silber, N. Bernstein, M. Ioffe, M. Keinan, and A. Bar-tal. 2003. Calcium distribution in cut roses as related to transpiration. J. Hort. Sci. and Biotech. 78:1-9.

Bailey, D.A. 1996. Alkalinity, pH, and acidification, p. 69-91. In: D.W. Reed (ed.). Water, media, and nutrition for greenhouse crops. Ball Publishing, Batavia, IL.

Bañuls, J. and E. Primo-Millo. 1992. Effects of chloride and sodium on gas exchange parameters and water relations of *Citrus* plants. Physiol. Plant. 86:115-123.

Bañuls, J., F. Legaz, and E. Primo-Millo. 1991. Salinity-calcium interactions on growth and ionic concentration of citrus plants. Plant and Soil 133:39-46.

Bañuls, J., M.D. Serna, F. Legaz, M. Talon, and E. Primo-Millo. 1997. Growth and gas exchange parameters of *Citrus* plants stressed with different salts. J. Plant Physiol. 150:194-199.

Barak, P. 1990. SPECIES: A spreadsheet program for modeling speciation of soil solution. J. Agron. Educ. 19:44-46.

Ben-Gal, A., H. Borochov-Neori, U. Yermiyahu, and U. Shani. 2009. Is osmotic potential a more appropriate property than electrical conductivity for evaluating whole-plant response to salinity? Environ. Exp. Bot. 65:232-237.

Bernstein, L. 1975. Effects of salinity and sodicity on plant growth. Ann. Rev. Phytopath. 13: 295-312.

Bernstein, L. and H. Hayward. 1958. Physiology of salt tolerance. Ann. Rev. Plant Physiol. 9:25-46.

Bernstein, L., L.E. Francois, and R.A. Clark. 1972. Salt tolerance of ornamental shrubs and ground covers. J. Amer. Soc. Hort. Sci. 97:550-556.

Bernstein, L., L.E. Francois, and R.A. Clark. 1974. Interactive effects of salinity and fertility on yields of grains and vegetables. Agron. J. 66:412-421.

Bernstein, N., B.T. Asher, F. Haya, S. Pini, R. Ilona, C. Amram, and I. Marina. 2006. Application of treated wastewater for cultivation of roses (*Rosa hybrida*) in soil-less culture. Sci. Hort. 108:185-193.

Bohn, H.L., B.L. McNeal, and G.A. O'Connor. 1985. Soil chemistry. 2nd edition. John Wiley & Sons Inc., New York.

Boursier, P. and A. Lauchli. 1990. Growth responses and mineral nutrient relations of salt-stressed sorghum. Crop Sci. 30:1226-1233.

Brun, R. and A. Settembrino. 1996. Production and quality of *Rosa hybrida* cv. Sonia in rockwool at three levels of electrical conductivity combined with two levels of relative humidity. Acta Hort. 424: 115-117.

Cabrera, R.I. 2000. Evaluating yield and quality of roses with respect to nitrogen fertilization and leaf nitrogen status. Acta Hort. 511:133-136.

Cabrera, R.I. 2001. Effect of NaCl salinity and nitrogen fertilizer formulation on yield and nutrient status of roses. Acta Hort. 547:255-260.

Cabrera, R.I. 2002. Rose yield, dry matter partitioning and nutrient status responses to rootstock selection. Sci. Hort. 95:75-83.

Cabrera, R.I. 2003a. Demarcating salinity tolerance in greenhouse roses. Acta Hort. 609: 51-57.

Cabrera, R.I. 2003b. Mineral nutrition, p. 573-580. In: A. Roberts, S. Gudin, and T. Debener (eds.). Encyclopedia of rose science. Academic Press, London, UK.

Cabrera, R.I. and P. Perdomo. 2003. Reassessing the salinity tolerance of greenhouse roses under soiless production conditions. HortScience 38:533-536.

Carter, C.T. and C.M. Grieve. 2008. Mineral nutrition, growth, and germination of *Antirrhinum majus* L. (snapdragon) when produced under increasingly saline conditions. HortScience 43:710-718.

Chaudhary, M.T., M.J. Merret, and S.J. Wainwright. 1997. Growth, ion content and proline accumulation in NaCl-selected and non-selected cell lines of Lucerne cultured on sodium and potassium salts. Plant Sci. 127:71-79.

Cockshull, K.E. and J.S. Horridge. 1977. Apical dominance and flower initiation in rose. J. Hort. Sci. 52:421-427.

Cornell, J. 2002. Experiments with mixtures. Designs, models, and the analysis of mixture data. John Wiley & Sons, Inc., New York.

Cornell, J.A. 1973. Experiments with mixtures: A review. Technometrics 15:437-455.

Cornell, J.A. and J.M. Harrison. 1997. Models and designs for experiments with mixtures. Part II. Exploring a subregion of the simplex and the inclusion of other factors in mixture experiments. Univ. of Florida. Agric. Exp. Station. Institute of Food and Agric. Sci. Bul. 899.

Cornell, J.A. and S.B. Linda. 1991. Methods and designs for experiments with mixtures. Part I. Exploring the whole simplex subregion. Univ. of Florida. Agric. Exp. Station. Institute of Food and Agric. Sci. Bul. 879.

Davison, W. and J. Boodley. 1987. Physiological disorders, p. 245-266. In: R.W. Langhans (ed.). Roses. A manual on the culture, management, diseases and insects of greenhouse roses. Roses, Inc., Haslett, MI.

Daya, S. 2001. Understanding statistics. Chi-square test for larger (rxc) contingency tables. Evidence-based Obstetrics and Gynecology 3:59-60.

De Vries, D.P. 2003a. Clonal rootstocks, p. 651-656. In: A.V. Roberts, T. Debener, and S. Gudin (eds.). Encyclopedia of rose science. Elsevier Inc., San Diego, CA.

De Vries, D.P. 2003b. Rootstock breeding, p. 639-645. In: A.V. Roberts, T. Debener, and S. Gudin (eds.). Encyclopedia of rose science. Elsevier Inc., San Diego, CA.

De Vries, D.P. 2003c. Usage of rootstocks, p. 645-651. In: A.V. Roberts, T. Debener, and S. Gudin (eds.). Encyclopedia of rose science. Elsevier Inc., San Diego, CA.

Ebert, G., J. Eberle, H. Ali-Dinar, and P. Ludders. 2002. Ameliorating effects of $Ca(NO_3)_2$ on growth, mineral uptake and photosynthesis of NaCl-stressed guava seedlings (*Psidium guajaba* L.). Sci. Hort. 93:125-135.

Egan, T.P. and I.A. Ungar, 1998. Effect of different salts of sodium and potassium on the growth of *Atriplex prostrata* (Chenopodiaceae). J. Plant Nutr. 21:2193-2205.

Ehret, D.L., R.E. Redmann, B.L. Harvey, and A. Cipywnyk. 1990. Salinity-induced calcium deficiencies in wheat and barley. Plant and Soil 128: 143-151.

Elashry, M.T. 1994. Water-resources management for the next century. Interciencia 19:117-119.

Elliot, A.C. and W.A. Woodward, 2007. Statistical analysis. Quick reference guidebook with SPSS examples. Sage Publications, Inc. Thousand Oaks, CA.

Epstein, E. 1972. Mineral nutrition of plants: Principles and perspectives. John Wiley & Sons, Inc., New York.

Farnham, D.S., R.F. Hasek, and J.L. Paul. 1985. Water quality: Its effects on ornamental plants. Univ. of California. Coop. Ext. Leaflet 2995.

Ferguson, L. and S.R. Grattan. 2005. How salinity damages citrus: Osmotic effects and specific ion toxicities. HortTechnology 15:95-99.

Fernández-Falcón, M., C.E. Álvarez-González, V. García, and J. Baez. 1986. The effect of chloride and bicarbonate levels in irrigation water on nutrient content, production and quality of cut roses 'Mercedes'. Scientic Hort. 29:373-385.

Flowers, T.J. 1988. Chloride as a nutrient and as an osmoticum, p. 55-78. In: B. Tinker and A. Läuchli (eds.). Advances in plant nutrition. Vol. 3.

Cambridge University Press, New York.

Food and Agriculture Organization of the United Nations. 2005. Field guide. 20 things to know about the impact of salt water on agricultural land in Aceh Province. Draft publication RAP.

Fortmeier, R. and S. Schubert. 1995. Salt tolerance of maize (*Zea mays* L.): The role of sodium exclusion. Plant, Cell and Environ. 18:1041-1047.

Gilliam, J.W. 1971. Rapid measurement of chlorine in plant materials. Soil Sci. Soc. Amer. Proc. 35:512-513.

Goldfarb, H.B., C.M. Anderson-Cook, C.M. Borror, and D.C. Montgomery. 2004. Fraction of design space plots for assessing mixture and mixture-process designs. J. Quality Technology 36:169-179.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd edition. John Wiley & Sons, Inc., New York.

González-Real, M.M. and A. Baille. 2000. Changes in leaf photosynthetic parameters with leaf position and nitrogen content within a rose plant canopy (*Rosa hybrida*). Plant, Cell and Environ. 23:351-363.

Gorham, J. 2007. Sodium, p. 569-583. In: A.V. Barker and D.J. Pilbeam. (eds.) Handbook of plant nutrition. CRC Press, Taylor & Francis Group, New York.

Grattan, S.R. 2002. Irrigation water salinity and crop production. Water quality fact sheet. Division of Agriculture and Natural Resources. University of California. UC DANR electronic publication #8066.

Grattan, S.R. and C.M. Grieve. 1992. Mineral element acquisition and growth response of plants grown in saline environments. Agric. Ecosys. Environ. 38:275-300.

Grattan, S.R. and C.M. Grieve. 1999. Salinity-mineral nutrient relations in horticultural crops. Sci. Hort. 78:127-157.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 31:149-190.

Handreck, K. and N. Black. 2002. Growing media for ornamental plants and turf. 3rd edition. UNSW Press, Sydney, Australia.

Hiyama, T., K. Kochi, N. Kobayashi, and S. Sirisampan. 2005. Seasonal variation in stomatal conductance and physiological factors observed in a secondary warm-temperate forest. Ecol. Res. 20:333-346.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Univ. of California, California Agricultural Experiment Station, Circ. 347.

Hu, Y. and U. Schmidhalter. 1997. Interactive effects of salinity and macronutrient level on wheat. II. Composition. J. Plant Nutr. 20:1169-1182.

Hughes, H.E. and J.J. Hanan. 1978. Effect of salinity in water supplies on greenhouse rose production. J. Amer. Soc. Hort. Sci. 103:694-699.

Janzen, H.H. and C. Chang. 1987. Cation nutrition of barley as influenced by soil solution composition in a saline soil. Can. J. Soil Sci. 67:619-629.

Jiang, Y. and B. Huang. 2001. Osmotic adjustment and root growth associated with drought preconditioning-enhanced heat tolerance in Kentucky bluegrass. Crop Sci. 41:1168-1173.

Jovicich, E., D.J. Cantliffe, E.H. Simonne, and P.J. Stoffella. 2007. Comparative water and fertilizer use efficiencies of two production systems for cucumbers. Acta Hort. 731:235-241.

Kafkafi, U., N. Valoras, and J. Letey. 1982. Chloride interaction with nitrate and phosphate nutrition in tomato (*Lycopersicon esculentum* L.) J. Plant Nutr. 5:1369-1385.

Kaya, C., B.E. Ak, and D. Higgs. 2003a. Response of salt-stressed strawberry plants to supplementary calcium nitrate and/or potassium nitrate. J. Plant Nutr. 26:543-560.

Kaya, C., D. Higgs, H. Kirnak, and I. Tas. 2003b. Ameliorative effect of calcium nitrate on cucumber and melon plants drip irrigated with saline water. J. Plant Nutr. 26:1665-1681.

Kaya, C., H. Kirnak, D. Higgs, and K. Saltali. 2002. Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high (NaCl) salinity. Sci. Hort. 93: 65-74.

Kim, S.-H., K.A. Shackel, and J.H. Lieth. 2004. Bending alters water balance and reduces photosynthesis of rose shoots. J. Amer. Soc. Hort. Sci.

129:896-901.

Kozlowski, T.T. and S.G. Pallardy. 1997. Physiology of woody plants. 2nd edition. Academic Press, New York.

Laidler, K.J. and J.H. Meiser. 1982. Physical chemistry. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA.

Lambers, H., F.S. Chapin III, and T.L.Pons. 1998. Plant physiological ecology. Springer-Verlag, New York.

Langhans, R.W. 1987a. Building young plants, p. 61-63. In: R.W. Langhans (ed.). Roses. A manual on the culture, management, diseases and insects of greenhouse roses. Roses Inc., Haslett, MI.

Langhans, R.W. 1987b. Timing, pruning and supporting, p. 65-69. In: R.W. Langhans (ed.). Roses. A manual on the culture, management, diseases and insects of greenhouse roses. Roses Inc., Haslett, MI.

Lea-Cox, J.D., D.S. Ross, and K.M. Teffeau. 2004. Developing water and nutrient management plans for container nursery and greenhouse production systems. Acta Hort. 633:373-379.

Lorenzo, H., M.C. Cid, J.M. Siverio, and M.C. Ruano. 2000. Effects of sodium on mineral nutrition in rose plants. Ann. Appl. Biol. 137:065-072.

Maas, E.V. 1990. Crop salt tolerance, p. 262-304. In: K.K. Tanji (ed.). Agricultural salinity assessment and management. Amer. Soc. of Civil Engineers, ASCE Manuals and reports on engineering practice No. 71., New York.

Maas, E.V. and C.M. Grieve. 1987. Sodium-induced calcium deficiency in salt-stressed corn. Plant, Cell and Environ. 10:559-564.

Marcelis-van Acker, C.A.M. 1994. Effect of assimilate supply on development and growth potential of axillary buds in roses. Annals of Botany 73:415-420.

Marfà, O. 1999. Soiless culture and fertilizer efficiency. Proc. IFA Agricultural Conference on Managing Plant Nutrition, Barcelona, Spain.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd edition. Academic Press, London, UK.

Martin, P.K. and R.M.K. Koebner. 1995. Sodium and chloride ions contribute synergistically to salt toxicity in wheat. Biol. Plant. 37:265–271.

Martinez, V. and A. Cerdá. 1989. Influence of N source on rate of Cl, N, Na, and K uptake by cucumber seedlings grown in saline conditions. J. Plant Nutr. 12:971-983.

Mastalerz, J.W. 1987. Environmental factors light, temperature, carbon dioxide, p. 147-170. In: R.W. Langhans (ed.). Roses. A manual on the culture, management, diseases and insects of greenhouse roses. Roses Inc., Haslett, MI.

Millard, E.B. 1921. Physical chemistry for colleges. A course of instruction based upon the fundamental laws of chemistry. McGraw-Hill Book Company, Inc. New York.

Miyamoto, S. and J.M. White. 2002. Foliar salt damage of landscape plants induced by sprinkler irrigation. TWRI TR-1202.

Montesano, F. and M.W. van Iersel. 2007. Calcium can prevent toxic effects of Na^+ on tomato leaf photosynthesis but does not restore growth. J. Amer. Soc. Hort. Sci. 132:310-318.

Munns, R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant, Cell and Environ. 16:15-24.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant, Cell and Environ. 25: 239-250.

Nable, R.O., G.S. Bañuelos, and J.G. Paull. 1997. Boron toxicity. Plant and Soil 193:181-198.

Navarro, J.M., C. Garrido., M. Carvajal, and V. Martínez. 2002. Yield and fruit quality of pepper plants under sulphate and chloride salinity. J. Hort. Sci. & Biotech. 77:52-57.

Navarro, J.M., V. Martinez, and M. Carvajal. 2000. Ammonium, bicarbonate and calcium effects on tomato plants grown under saline conditions. Plant Sci. 157:89-96.

Neumann, P. 1997. Salinity resistance and plant growth revisited. Plant, Cell and Environ. 20:1193-1198.

Niu, G. and D.S. Rodriguez. 2006. Relative salt tolerance of selected

herbaceous perennials and groundcovers. Sci. Hort. 110:352-358.

Niu, G. and D.S. Rodriguez. 2008a. Effect of saline water irrigation on growth and physiological responses of three rose rootstocks. HortScience 43:1479-1484.

Niu, G. and D.S. Rodriguez. 2008b. Responses of growth and ion uptake of four rose rootstocks to chloride- or sulfate-dominated salinity. J. Amer. Soc. Hort. Sci. 133:663-669.

Nobel, P.S. 1983. Biophysical plant physiology and ecology. W.H. Freeman and Company, New York.

Obiol, R. and J. Cardús. 1974. Influence of rootstocks on rose culture. Acta Hort. 43:197-200.

Papadakis, I.E., G. Veneti, C. Chatzissavvidis, T.E. Sotiropoulus, K.N. Dimassi and N. Therios. 2007. Growth, mineral composition, leaf chlorophyll and water relationships of two cherry varieties under NaCl-induced salinity stress. Soil Sci. and Plant Nutr. 53:252-258.

Petersen, F.H. 1996. Water testing and interpretation, p. 31-49. In: D.W. Reed (ed.). A grower's guide to water, media, and nutrition for greenhouse crops. Ball Publishing, Batavia, IL.

Picchioni, G.A. and C.J. Graham. 2001. Salinity, growth, ion uptake selectivity of container-grown *Crataegus opaca*. Sci. Hort. 90:151-166.

Quist, T.M. and C.F. Williams. 1999. Irrigation water quality and ion balance in leaves of deciduous ornamental trees. J. Plant Nutr. 22:1011-1019.

Ramos, J., M.J. López, and M. Benlloch. 2004. Effect of NaCl and KCl salts on the growth and solute accumulation of the halophyte *Atriplex nummularia*. Plant and Soil 259:163-168.

Raviv, M. and T.J. Blom. 2001. The effect of water availability and quality on photosynthesis and productivity of soilless-grown cut roses. Sci. Hort. 88:257-276.

Reed, D.W. 1996. Combating poor water quality with water purification systems, p. 51-67. In: D.W. Reed (ed.). A grower's guide to water, media, and nutrition for greenhouse crops. Ball Publishing, Batavia, IL.

Renault, S., C. Croser, J.A. Franklin, and J.J. Zwiazek. 2001. Effects of NaCl

and Na_2SO_4 on red-osier dogwood (*Cornus stolonifera* Michx) seedlings. Plant and Soil 233:261-268.

Rengel, Z. 1992. The role of calcium in salt toxicity. Plant, Cell and Environ. 15:625-632.

Richards, L.A. 1954. Diagnosis and improvement of saline and alkali soils. United States Dept. Agric., Washington, DC.

Robinson, R.A. and R.H Stokes. 1970. Electrolyte solutions. 2nd edition. Butterworth & Co., London, UK.

Romero-Aranda, R., J.L. Moya, F.R. Tadeo, F. Legaz, E. Primo-Millo, and M. Talon. 1998. Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: beneficial and detrimental effects of cations. Plant, Cell and Environ. 21:1243-1253.

Ruiz, D., V. Martínez, and A. Cerdá. 1997. Citrus response to salinity: Growth and nutrient uptake. Tree Physiol. 17:141-150.

Sadasivaiah, S.P. and W.D. Holley. 1973. Ion balance in nutrition of greenhouse roses. Bull. Suppl. Roses, Inc., Haslett, MI.

Schrevens, E. and J. Cornell. 1993. Design and analysis of mixture systems: Applications in hydroponic, plant nutrition research. Plant and Soil 154:45-52.

Serrano, R. 1996. Salt tolerance in plants and microorganisms. Toxicity targets and defense responses. Intl. Rev. Cytology 165:1-52.

Singh, B.P. and S.D. Chitkara. 1984. Effect of different salinity and sodicity levels on establishment and budtake performance of various rose rootstocks. Haryana J. Hort. Sci. 13:88-90.

Takeda, T. and K. Takahashi. 1998. Seasonal changes of macro element absorption in solution cultured roses. J. Japanese Soc. Hort. Sci. 67:116-122.

Terada, M., Y. Kageyama, and K. Konishi. 1997. The relationship between growth of a rose plant and its nutrient and water uptake in hydroponic culture. J. Japanese Soc. Hort. Sci. 66:149-155.

Trajkova, F., N. Papadantonakis, and D. Savvas. 2006. Comparative effects of NaCl and $CaCl_2$ salinity on cucumber grown in a closed hydroponic

system. HortScience 41:437-441.

Treadwell, F.P. 1916. Analytical chemistry. I. Qualitative analysis. 5th edition. John Wiley & Sons, Inc. New York.

Tyerman, S.D. and I.M. Skerrett. 1999. Root ion channels and salinity. Sci. Hort. 78:175-235.

Valdez-Aguilar, L.A., D.W. Reed, and J.A. Cornell. 2008. Quantitative separation of the bicarbonate from the counter-cation effect in bean plants. J. Amer. Soc. Hort. Sci. 133:468-475.

van Schilfgaarde, J. 1994. Irrigation - a blessing or a curse. Agric. Water Mgmt. 25:203-219.

Vigo, C., I.N. Therios, A. Patakas, M. Karatassou, and A. Nastou. 2002. Changes in photosynthetic parameters and nutrient distribution of olive plants (*Olea europaea* L.) cultivar 'Chondrotia Chanlkidikis' under NaCl, Na_2SO_4, and KCl salinities. Agrochimica 46:33-46.

Wahome, P.K., H.H. Jesch, and I. Grittner. 2000. Effect of NaCl on the vegetative growth and flower quality of roses. J. Applied Bot. 74:38-41.

Wahome, P.K., H.H. Jesch, and I. Grittner. 2001. Mechanisms of salt stress tolerance in two rose rootstocks: *Rosa chinensis* 'Major' and *R. rubiginosa*. Sci. Hort. 87:207-216.

White, J.W. 1987. Fertilization, p. 87-142. In: R.W. Langhans. (ed.). Roses. A manual on the culture, management, diseases and insects of greenhouse roses. Roses Inc., Haslett, MI.

Wright, G.C., K. D. Pattern, and M.C. Drew. 1992. Salinity and supplemental calcium influence growth of rabbiteye and southern highbush blueberry. J. Amer. Soc. Hort. Sci. 117:749-756.

Yaron, B., N. Zieslin, and A.H. Halevy. 1969. Response of Baccara roses to saline irrigation. J. Amer. Soc. Hort. Sci. 94:481-484.

Yokas, I., A.L. Tuna, B. Burun, H. Altunlu, F. Altan., C. Kaya. 2008. Responses of the tomato (*Lycopersicon esculentum* Mill.) plant to exposure to different salt forms and rates. Turk. J. Agric. For. 319-329.

VITA

Name: Alma Rosa Solís Pérez

Address: Texas A&M Research and Extension Center at Dallas, Dallas, TX 75252

Email Address: almasolis@tamu.edu; almarosa_solis@yahoo.com.mx

Education:
B.S., Plant Science, Universidad Autónoma Chapingo (México), 1997
M.S., Fruit Science, Colegio de Postgraduados en Ciencias Agrícolas (México), 2001
Ph.D., Horticulture, Texas A&M University (USA), 2009