THE RESPONSES OF SELECTED GARDEN ROSES (*ROSA* × *HYBRIDA*) TO DROUGHT AND SALT STRESSES

A Dissertation

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ABSTRACT

Water shortage and poor water quality are critical problems for agriculture in many regions of the world. Roses (*Rosa* × *hybrida* L.) are some of the most common garden plants in the world. Despite their popularity, however, they can present challenges to gardeners, particularly in relation to their responses to environmental stresses, such as those caused by arid and semiarid regions. The objectives of the present study were to investigate the tolerance of selected garden roses by evaluating their growth and physiological responses under drought and salt stress conditions.

Four experiments were conducted to evaluate 25 garden rose cultivars, including 22 Earth-Kind® rose cultivars. In Expt. 1, four garden roses were subjected to two watering treatments: well-irrigated and cyclic drought stress. 'RADrazz' was the most drought tolerant among the cultivars investigated. With lower gas exchange and greater reduction in flower numbers at low substrate moisture content (SMC), 'Marie Pavie' was the least drought tolerant. In Expt. 2, two Earth-Kind® rose cultivars were subjected to four constant SMC of 10, 20, 30, and 40 % by utilizing an automatic irrigation system. Plants at 30 and 40 % SMC maintained the highest shoot and root dry weight (DW), flower number, midday leaf water potential, and photosynthetic rate. Plants had excellent performance at 30 % SMC and acceptable growth and quality at 20 % SMC. The 10 % SMC led to significant growth reduction, poor quality, and 25 % mortality. In Expt. 3, six garden rose cultivars were subjected to three salinity levels at electrical conductivity (EC) of 1.5 (control), 4.0 or 8.0 $dS \cdot m^{-1}$. 'New Dawn' was considered to be

the most salt tolerant, while ‘Caldwell Pink’, ‘Marie Pavie’, and ‘The Fairy’ were the least salt tolerant with their greater reductions in flower number and shoot DW. In Expt. 4, 18 Earth-Kind® rose cultivars were subjected to two salinity levels at EC of 1.2 (control) and 10.0 $dS \cdot m^{-1}$. By comparing the growth and physiological responses at high saline solution among the 18 cultivars, ‘Belinda’s Dream’, ‘Climbing Pinkie’, ‘Mrs. Dudley Cross’, ‘Reve d’Or’, and ‘Sea Foam’ were the most salt tolerant, while Cecile Brunner’, ‘Else Poulsen’, ‘Madame Antoine Mari, ‘Perle d’Or, ‘Spice’, and ‘Souvenir de St. Anne’s’ were the least salt tolerant among the cultivars investigated.

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CHAPTER I

INTRODUCTION

1.1 Introduction

Water shortage and poor water quality are critical problems for agriculture in many regions of the world because they can negatively affect their growth, performance or even survival in landscape environments (Cregg, 2004; Zhu, 2002). Decreased water resources and increased population in urban areas have increased the competition for fresh water among agriculture, industry, and municipal/residential users. Using alternative water sources for agriculture and landscape irrigation is a potentially important approach to conserve fresh water. However, alternative water sources often have high sodium content which cause salt damage on sensitive plants. Therefore, the selection and use of drought and salt tolerant plants becomes increasingly important for the development of sustainable landscapes.

Roses (*Rosa* × *hybrida* L.) are some of the most common garden plants in the world. Despite their popularity, however, they can present challenges to gardeners, particularly in relation to their responses to environmental stresses, such as those caused by arid and semiarid regions. Texas A&M AgriLife Extension has designated a group of rose cultivars which perform well relative to other roses in the different climatic and soil conditions throughout Texas as Earth-Kind® (Aggie Horticulture, 2014). Earth-Kind® rose cultivars require minimum fertilizer, water, and pesticides while growing in gardens or landscapes. These rose cultivars exhibit consistent and superior pest tolerance combined with outstanding landscape performance, making them among the best garden plants to preserve and protect the natural resources and environment (MacKay et al., 2008). However, there is little science-based knowledge about the responses of Earth-Kind® rose cultivars to drought and salt stresses. Knowing how Earth-Kind® rose cultivars respond to environmental stresses will provide useful information that breeders can use in developing adaptable rose cultivars and lead to a better understanding of how certain cultivars withstand unfavorable environmental conditions better than others.

1.2 The Effects of Drought Stress on Ornamental Plants

Water deficit can affect plants in different ways, such as stomatal closure, growth inhibition, and reduced transpiration rates and photosynthesis (Yordanov et al., 2003). In nature, plants can either be subjected to short-term water deficits or long-term water deficits. Under conditions of slowly developing water deficits, plants can escape dehydration by shortening their life cycle, minimizing water loss or exhibiting metabolic protection in the case of rapid dehydration (Chaves et al., 2003). In addition, plant regulation of water loss and uptake can be induced by mild drought, which could allow the maintenance of their leaf relative water content (RWC) (Yordanov et al., 2003).

Previous research found that flower development of 'Madelon' rose was very sensitive to drought stress prior to petal and stamen initiation, thus affecting the quantity and quality of the flowering shoots (Chimonidou-Pavlidou, 1999; Chimonidou-Pavlidou, 2004). Abortion and malformation of flower buds occurred when drought stress was applied during stamen initiation. These malformed buds had no carpels, and the stamens were tightly packed on the center of the receptacle (Chimonidou-Pavlidou, 2004). When drought stress was applied at stage 1 (earliest bud break and internal formation of leaf primordia) and stage 4 (flower bud diameter 5-7 mm), the percentage of malformed buds was between 0 and 10%, but increased up to 25% and 35% when drought application was in stage 2 to 4 (the second three-leaflet leaf clearly separated and before the separation of the five-leaflet leaf) and stage 4 to stage 6 (the second five-leaflet leaf clearly separated and the bud not yet separated), respectively. The number of flowers that reached the marketable stage was not affected by drought stress that was imposed at stage 1 (Chimonidou-Pavlidou, 2004).

In a study by Niu and Rodriguez (2009), the growth and physiological responses of four rose rootstocks, *R.* × *hybrida* 'Dr. Huey', *R.* × *fortuniana*, *R. multiflora*, and *R. odorata*, to drought stress were compared. Compared to *R. odorata*, *R.* × *fortuniana* had greater shoot growth and leaf area during a cyclic drought stress period. Leaf RWC decreased as substrate moisture content decreased. Leaf net photosynthesis rate, transpiration rate and stomatal conductance decreased as substrate moisture content decreased to below 25% (Niu and Rodriguez, 2009). Similar results were found with oleander (*Nerium oleander* L.) where drought-tolerant clones had greater shoot growth than those less tolerant to drought under water deficit conditions (Niu et al., 2008b).

Bedding plants have the highest wholesale value of all floriculture crops in the United States, but little work has been done to optimize irrigation management in their production (Nemali and van Iersel, 2008). In a study of physiological responses of bedding plants [salvia 'Bonfire Red' (*Salvia splendens* Sellow ex Roemer & J.A. Schultes), vinca 'Cooler Peppermint' (*Catharanthus roseus* L. G. Don.), petunia

‘Lavender White’ (*Petunia* × *hybrida* Hort ex. Vilm.), and impatiens ‘Cherry’ (*Impatiens walleriana* Hook F.)] to different substrate water contents, optimal range of substrate water content was determined. With decreased substrate water content (0.32, 0.22, 0.15, and 0.09 $m^3 \cdot m^{-3}$), leaf water potential, photosynthesis and stomatal conductance of these species were lowest at 0.09 $m^3 \cdot m^{-3}$ water content. The substrate water content for optimum gas exchange of these studied bedding plants was in the range of 0.15 and 0.22 $m^3 \cdot m^{-3}$ (Nemali and van Iersel, 2008). Niu et al. (2006b) also reported that photosynthesis and stomatal conductance declined rapidly in the drought sensitive bedding plants as substrate moisture content decreased.

In a study by Henson et al. (2006), nine drought-tolerant bedding plant species were determined by evaluating their growth, visual quality, and stress responses at different irrigation levels [0, 25, 50, 75, and 100% evapotranspiration (ET_0)]. They found the species, periwinkle (*Catharanthus roseus* L.G. Don ‘Peppermint Cooler’), gloriosa daisy (*Rudbeckia hirta* L. ‘Indian Summer’), dusty miller (*Senecio cineraria* D.C. ‘Silver Dust’), marigold (*Tagetes erecta* L. ‘Inca Yellow’ and *T. patula* L. ‘Bonanza Gold’), zinnia (*Zinnia angustifolia* Kunth.), mealycup sage (*Salvia farinacea* Benth. ‘Rhea Blue’), petunia (*Petunia x hybrid* hort. ex. E. Vilm. ‘Merlin White’) and mock vervain (*Glandularia* J.F. Gmel. ‘Imagination’), had relatively high leaf percent cover and plant biomass under 25% ET_0 or less, thus suggesting that these species can be well adapted for low-water landscape installations (Henson et al., 2006). Staats and Klett (1995) reported that 50% ET_0 was the optimum irrigation level in Kentucky bluegrass (*Poa pratensis* L. ‘Challenger’), while spring cinquefoil (*Potentilla tabernaemontani*

Asch.) required irrigation at 75% ET_0 to maintain acceptable visual quality and *Sedum acre* L. did well at 25% ET_0, which indicated substantial variation in water requirements for ornamental plants.

Groundcovers are often recommended by the nursery industry to replace grass due to their low water requirement to maintain high visual quality (Pittenger et al., 2001). Pittenger et al. (2001) reported the effects of real-time reference ET_0 (20, 30, 40, and 50% ET_0) on six widely used species of landscape groundcovers [dwarf coyote bush (*Baccharis pilularis* DC. 'Twin Peaks'), ice plant (*Drosanthemum hispidum* L. Schwant.), large periwinkle (*Vinca major* L.), trailing gazania (*Gazania rigens* L. Gaertn. v. *leucolaena* 'Yellow Cascade'), potentilla (*Potentilla tabernaemontanii* Asch.), and English ivy (*Hedera helix* L. 'Needlepoint')]. They found that dwarf coyote bush, ice plant, and English ivy could maintain acceptable visual quality with a minimum of 20% ET_0, while large periwinkle required a minimum of 30 % ET_0. There was no acceptable visual quality observed in trailing gazania and potentilla at any treatment. These species with potential adaptation to drought were selected for use in the area with less irrigation levels (Pittenger et al., 2001). Starman and Lombardini (2006) found that growth and development of four ornamental herbaceous perennials, lantana (*Lantana camara* L.), cardinal flower (*Lobelia cardinalis* L.), mealy sage (*Salvia farinacea* Benth.), and fan flower (*Scaevola aemula* R. Br.), continued under low substrate water content (0.13 $mm^3 \cdot mm^{-3}$), which was sufficient for greenhouse production. They reported increased leaf-level water use efficiency (WUE) of these species at different drought cycles, and little effect of drought stress on photochemical efficiency of photosystem II. Additionally, plant height and leaf area of *S. farinacea,* and dry weight of *S. aemula* were reduced by drought stress, while there was no morphological change in *L. cardinalis* and *L. camara* (Starman and Lombardini, 2006).

Stabler and Martin (2000) found patterns of osmotic regulation as the substrate dried in southwest landscape plants, red bird of paradise (*Caesalpinia pulcherrima* L.) and blue palo verde (*Cercidium floridum* Benth.). Under three irrigation regimens (frequent, moderate, and infrequent), these two species had more negative shoot osmotic potential at infrequent and moderate irrigation than those at frequent irrigation. Final shoot and root dry weight of frequently irrigated blue Palo Verde were greater than those irrigated at the moderate or infrequent intervals, while they were not affected by irrigation frequency treatment in red bird of paradise. In addition, WUE was lowest in infrequently irrigated red bird of paradise and highest in infrequently irrigated blue Palo Verde. WUE in red bird of paradise might be improved by moderate irrigation frequency to avoid excessively wet or dry soils, while shorter and more frequent irrigation may be required to optimize WUE in blue Palo Verde. Responses of growth and flower yield to water stress were studied for the Big Bend bluebonnet (*Lupinus havardii* Wats.), a specialty cut flower native to southwest Texas (Niu et al., 2007b). Plants irrigated at 25% or greater volumetric soil moisture content (VMC) had maximum growth and cut flower production. Growth index, leaf greenness, cut raceme yield, and dry weight of the plants were lower at an irrigation level less than 25% VMC (Niu et al., 2007b).

1.3 The Effects of Salt Stress on Ornamental Plants

Soil salinity is a major constraint to crop yield, and salinity inhibition of plant growth is the result of osmotic and ionic effects. Different plant species have developed different mechanisms to tolerate salt stress (Munns, 2002). Osmotic potential in salt-stressed plants decreased due to inorganic ion and compatible organic solute accumulations (Hasegawa et al., 2000). Osmotic adjustment is an important mechanism of salt tolerance in plants because it contributes to the maintenance of water uptake and cell turgor, allowing stomatal opening, photosynthesis, and cell expansion (Serraj and Sinclair, 2002). Plants employ various strategies in response to elevated salt stress, including salt exclusion and inclusion, maximizing sodium (Na^+) efflux from root and its recirculation out of the shoot, intercellular compartment, maintaining a relatively high cytosolic potassium/sodium (K^+/Na^+) ratio, and increasing enzymatic and non-enzymatic antioxidant defense system (Hasegawa et al., 2000; Tester and Devenport, 2003).

Sodium (Na^+) and chloride (Cl^-) are usually the most prevalent ions in saline water, which has deleterious effects of necrosis and leaf tip burn. High soil ionic concentrations lower the soil water potential, which interferes with the plants' ability to extract water from the soil and maintain turgor. High soil salinity could also disturb membrane integrity and function, causing nutritional deficiency symptoms in plant growth (Grattan and Grieve, 1999), because high sodium chloride (NaCl) uptake competes with the uptake of other nutrient ions, such as K^+, calcium (Ca^{2+}), nitrogen (N), and phosphate (P) (Grattan and Grieve, 1999).

Roses have been reported to be sensitive to salinity, and there are appreciable yield losses at electrical conductivity (EC) levels up to 3 $dS \cdot m^{-1}$ (Bernstein et al., 1972). There is limited research comparing the salt tolerance of various rose rootstocks. In a study by Cabrera et al. (2009), the responses of yield, quality and ion accumulation to increasing salinity stress were evaluated in roses grafted on various rootstocks. Salt stress negatively affected the biomass, cut flower production, and foliage quality of 'Red France', 'Manetti', 'Natal Briar', and the scion 'Bridal White' grafted on 'Manetti', *R. odorata* (Andr.) Sweet, 'Natal Briar', and 'Dr. Huey'. By sustaining better absolute and relative biomass and flower yields and less tissue Na^+ and Cl^- accumulation, 'Manetti' was more salt tolerant. Based on the responses of productivity (biomass and flower yields), greenhouse roses could withstand up to an EC of 3.0 ± 0.5 $dS \cdot m^{-1}$, while they can tolerate up to 1 ± 0.2 $dS \cdot m^{-1}$ if considering their aesthetic responses. Niu and Rodriguez (2008) reported different responses of four rose rootstocks ['Dr. Huey', *R. fortuniana* Lindl., *R. multiflora* Thunb., and *R. odorata* (Andr.) Sweet] to salinity and dominant salt type in growth and ion uptake. They found the growth and visual quality of all rootstocks decreased with elevated salinities (7.9 to 8.2 $dS \cdot m^{-1}$ EC), and there was no effect of salinity treatment on daily ET rate per unit leaf area. Growth responses between chloride-dominated and sulfate-dominated salinity at moderate salinity (3.9 $dS \cdot m^{-1}$ EC) were similar, but chloride-dominated salinity led to the most severe foliar salt damage in rootstocks with a lower threshold tissue chloride (Cl) concentration (Niu and Rodriguez, 2008). Wahome et al. (2001) reported that *R. rubiginosa* L. was more tolerant to salt stress than *R. chinensis* Jacq. 'Major'. Comparisons of the responses of rose cultivars to salinity will be helpful to understand the mechanism of salt tolerance of major commercial rose rootstocks, which may be used as a reference for selecting salt-tolerant rose rootstocks for salt-affected areas.

Demand for salt-tolerant bedding plants is increasing in arid and semiarid regions as more low-quality water is used for landscape irrigation. Niu et al. (2010c) observed the responses of ten bedding plant species to saline water irrigation [angelonia (*Angelonia angustifolia* Benth.) 'Lavender Pink', 'Purple', 'White', ornamental pepper (*Capsicum annuum* L.) 'Calico', 'Black Pearl', 'Purple Flash', helenium (*Helenium amarum* Raf. H. Rock.), licorice plant (*Helichrysum petiolatum* L. DC.), plumbago (*Plumbago auriculata* Lam.), and vinca (*Catharanthus roseus* L.) 'Rose']. These plants had different salinity thresholds of irrigation water in which growth reduction occurred. Less salt-tolerant plants had increased leaf Cl concentrations at elevated salinities (0.8, 2.8, 4.0, 5.1, and 7.4 $dS \cdot m^{-1}$), and they had more reductions in shoot dry weight compared to more salt-tolerant plants. In general, plants were moderately tolerant to salt stress, and they may have little reduction in aesthetical appearance with irrigation of saline water up to 4.0 $dS \cdot m^{-1}$ (Niu et al., 2010c).

A study by Niu and Rodriguez (2006a) reported the performance of five landscape plants [yarrow (*Achillea millefolium* L.), gaillardia (*Gaillardia aristata* Foug.), sage (*Salvia coccinea* Juss ex J.), mosquito plant (*Agastache cana* Hook. Woot. & Standl.) and purple coneflower (*Echinacea purpurea* L. Moench)] irrigated by water with elevated salinity. After salinity treatment, salt tolerant plants had an aesthetically acceptable appearance for landscape performance. In both summer and fall studies, leaf osmotic potential of these plants decreased at increasing salinity levels (0.8, 2.0, and 4.0 $dS \cdot m^{-1}$ EC) (Niu and Rodriguez, 2006a). They also reported salt tolerance of two native landscape woody ornamentals, Texas mountain laurel [*Sophora secundiflora* (Ortega) Lag. Ex DC.] and Mexican redbud (*Cercis canadensis* var. *Mexicana*) (Niu et al., 2010b). Texas mountain laurel had no symptoms of foliar damages such as leaf drop, leaf curl, and edge burn and therefore was considered more salt tolerant, while these symptoms were observed in the less salt tolerant Mexican redbud. In a addition, with elevated salinity of irrigation water (3.0 and 6.0 $dS \cdot m^{-1}$ EC), Mexican redbud had greater reduction in shoot growth, leaf photosynthesis rate, and leaf stomatal conductance than Texas mountain laurel (Niu et al., 2010b). Based on the results, Mexican redbud is not recommended for poor-quality water areas, while Texas mountain laurel may be a good selection in high soil salinity areas, as long as soils are well drained.

The responses of Big Bend bluebonnet (*Lupinus havardii* Wats.) and Texas bluebonnet (*Lupinus texensis* Hook.) to various salinity levels (1.6, 3.7, 5.7, 7.6, and 9.4 $dS \cdot m^{-1}$ EC) were compared (Niu et al., 2007b). *Lupiinus texensis* had reduced shoot growth as salinity levels increased, but visual quality was acceptable when irrigated with salinity levels less than 7.6 $dS \cdot m^{-1}$ EC, while *L. harvardii* had only 7% plant survival at 7.6 $dS \cdot m^{-1}$ EC. In addition, *L. harvardii* had greater reduction in growth, plant height, and cut raceme at elevated salinity levels as compared to *L. texensis* (Niu et al., 2007b). A study was also reported the reductions of shoot dry weight and plant height in blanketflower (*Gaillardia aristata* Pursh.) at elevated salinity levels (0.8, 2.0, and 4.0 $dS \cdot m^{-1}$ EC) (Niu et al., 2007d). In a study by Niu et al. (2010d), salt tolerance of 20 genotypes of chile peppers (*Capsicum frutescens* L.) was determined at elevated salinity levels (1.4, 3.0, and 6.0 dS·m^{-1} EC). 'Ancho 1', 'Ancho 2', 'Cayenne 1', 'Early Jalapeno', and 'AZ-20' had 100% survival regardless of salinity treatment, and therefore had relatively high tolerance based on high survival and visual quality, while no plants of 'TAM Mild Habanero' survived with irrigation of saline water. Although there were less than half the genotypes surviving in the salinity treatment, the pepper genotypes were ranked for salt tolerance based on seedling survival, visual quality, and shoot growth (Niu et al., 2010d).

There is limited research comparing the salt tolerance among Earth-Kind® roses. Identifying the physiological mechanisms is important for efficient screening methods of salt-tolerant plants, and will be helpful for combining high quality and yielding ability with salt tolerance. Many researchers have used yield or yield components as selection criteria for salt tolerance; however, the physiological trait should also be considered as an useful screening method.

1.4 Objectives

The objectives of this study were to: (1) determine the association of growth and physiological traits of roses (such as shoot and root dry weight, flower number, visual quality, ion contents in leaves and stems, photosynthetic parameters, stomatal conductance, transpiration, SPAD value, chlorophyll fluorescence, and water relation parameters) with drought and salt tolerance, to ascertain reliable and multiple physiological traits that can be utilized as quick, easy and economical techniques to screen for drought and salt tolerance; (2) understand and elucidate underlying mechanisms in drought and salt stress and tolerance, more specifically the effect of drought and salt stress on water relations, photosynthesis, stomatal conductance, and ion toxicity; (3) identify the important growth and physiological parameters associated with drought and salt tolerance in different rose cultivars; and, (4) quantify the minimal irrigation requirement for garden roses.

CHAPTER II

RESPONSE OF SELECTED GARDEN ROSES TO DROUGHT STRESS*

2.1 Synopsis

A greenhouse study was conducted to evaluate the response of four garden roses (*Rosa* × *hybrida* L.), 'RADrazz', 'Belinda's Dream', 'Old Blush', and 'Marie Pavie' to drought stress. Plants grown in containers were subjected to two watering treatments, well-irrigated [water as needed: around 35% substrate moisture content (SMC) at re-watering] and cyclic drought stress (withholding irrigation until plants exhibit incipient wilting: around 10% SMC, then re-watering to field capacity for subsequent dry down). Shoot growth and flower number were reduced in the drought treatment compared to the well-irrigated plants in all cultivars, with least reduction in 'RADrazz'. Drought stress reduced root growth in 'Belinda's Dream' and 'Marie Pavie', while there was no difference in root growth in 'RADrazz' and 'Old Blush'. Decreased SMC induced the reduction in net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), and mid-day leaf water potential (ψ). Leaf water use efficiency (WUE) increased as SMC decreased in all cultivars. However, the relationship between these physiological parameters and SMC differed among the cultivars. At SMC between 10% and 20%, 'RADrazz' had higher P_n, g_s, E, and WUE compared to the other three cultivars. Therefore, 'RADrazz' was the most drought tolerant during container production among

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* Reprinted with permission from Cai, X., T. Starman, G. Niu, C. Hall, and L. Lombardini. 2012. Response of selected garden roses to drought stress. HortScience 47:1400-1403.

the cultivars investigated. With lower gas exchange rates and greater reduction in flower number at low SMC, ‘Marie Pavie’ was less drought tolerant compared to the other three cultivars.

2.2 Introduction

Water shortage and poor water quality are critical challenges to gardening and landscaping in many regions of the world. Population growth and increased urbanization have increased competition for fresh water among agriculture, industry, and municipal water users (Lea-Cox and Ross, 2001). Therefore, water conservation and the improvement of irrigation efficiency are important in landscape water management (Nicolas et al., 2008; Niu et al., 2006a). With watering restrictions, the effect of drought stress is exacerbated on plant establishment and survival, and the selection of drought tolerant plants becomes increasingly important for the development of sustainable landscapes.

Roses (*Rosa* × *hybrida* L.) are some of the most common garden plants in the world, and garden roses are one of the most popular and widely cultivated flowering plants. Moreover, some are valued for having ornamental fruit, and they can be used as hedges, screens and groundcovers. Their cultivation, however, presents several challenges to gardeners, because of their limited resistance to diseases, temperature extremes, drought and salt stresses. Garden rose cultivars are well adapted to temperate climates, but there is little science-based knowledge about their tolerance to different climatic and soil conditions throughout the world. The Texas AgriLife Extension Service has established a brand designating a group of rose cultivars as Earth-Kind®, and these roses provide the consumers with garden plants that require minimum fertilizer, water, and pesticides while growing in gardens or landscapes (McKay et al., 2008).

Knowing the responses of rose plants to environmental stresses is becoming an important concern in arid and semiarid regions. Niu and Rodriguez (2009) reported that *Rosa* × *fortuniana* had greater shoot growth and leaf area under cyclic drought stress and was considered to be more tolerant to drought than the other three cultivars (*R*. × *hybrida* 'Dr. Huey', *Rosa multiflora*, and *Rosa odorata*) in the study. Reduction of leaf area by drought is caused by reduced cell expansion and cell division, leaf rolling, and death of apical parts of leaves and whole leaves (Blum, 1996). Plants with a larger leaf area have a higher ratio of transpiration to evaporation, which results in higher water use efficiency (WUE) (Kingeman et al., 2005). In a study of five low-maintenance rose cultivars under water stress conditions, 'Pink Meidiland' had smallest leaf surface area, while 'Ferdy' had largest root-to-shoot ratio and lowest leaf area ratio, which may contribute to larger leaf water reserve and moisture uptake for better adaptation to drought conditions (Henderson et al., 1991).

In a study by Niu and Rodriguez (2009), *R. odorata*, which was considered to be the least drought tolerant cultivar, had lower leaf net photosynthetic rate (P_n), transpiration rate (E) and stomatal conductance (g_s) than the other three cultivars investigated under soil moisture content (SMC) between 10 and 20%. Similar results were found with oleander (*Nerium oleander* L.) wherein drought-tolerant clones under water deficit conditions had greater gas exchange rates than those less tolerant to drought (Niu et al., 2008b). In a study on two miniature roses ('Poulhappy Charming Parade' and

‘Poulbian Bianca Parade’), g_s and P_n in drought-treated plants were restored to rates comparable to well-irrigated plants after re-watering, although E, g_s and P_n were reduced under drought-stressed conditions (Williams et al., 1999). They also concluded that drought-stressed miniature roses produced more dry matter per volume of water consumed compared to well-irrigated plants, which might result in improvement of plant drought tolerance by utilizing water more efficiently. Egilla et al. (2005) found that relative water content, E, and g_s were decreased under drought-stressed conditions in Chinese hibiscus (*Hibiscus rosa-sinensis* L.). In another study on ponderosa pine (*Pinus ponderosa* Dougl.) and big sagebrush (*Artemisia tridentata* Nutt.), plants grown under drought had greater WUE than those under well-irrigated conditions (DeLucia et al., 1989).

Knowing how garden roses respond to stress will provide useful information that breeders can use in developing adaptable rose cultivars thus leading to a better understanding of why certain cultivars withstand unfavorable environmental conditions better than others. Earth-Kind® is a special designation given to select rose cultivars by the Texas AgriLife Extension Service through the Earth-Kind® landscaping program. It is based on the results of extensive research and field trials and is awarded only to those roses demonstrating superior pest tolerance combined with outstanding landscape performance (McKay et al., 2008). Our objectives were to investigate the relative drought tolerance in ‘Marie Pavie’ and three Earth-Kind® garden roses (‘Old Blush’, ‘RADrazz’, ‘Belinda’s Dream’) observed to withstand heat and drought stresses in southern landscapes. We evaluated the response of growth, water relations, and gas exchange rates of these rose cultivars to drought stress. We postulated that greenhouse studies could provide insight about the physiology of plants under drought-stressed conditions, which is a basis for species selection in dry landscapes.

2.3 Materials and Methods

2.3.1 Plant Materials and Culture

A total of 200 stem cuttings of garden roses, 'Belinda's Dream', 'RADrazz', 'Marie Pavie', and 'Old Blush', were collected from stock plants and rooted in a mist bed. Rooted cuttings were transplanted to 10 - cm pots filled with Sunshine LC1 (Canadian Sphagnum peat moss, perlite, starter fertilizer charger, dolomitic limestone and a long-lasting wetting agent) (Bellevue, WA) on 2 Oct. 2010 in an unshaded glass greenhouse at College Station, TX. Plants were transplanted to 11.4-L pots with the substrate of Sunshine Professional Growing Mix 4 (Canadian sphagnum peat moss, perlite, starter fertilizer charge, a controlled-release fertilizer and dolomite limestone) (Bellevue, WA) on 7 Dec. 2010. During this establishment stage, plants were pruned once a week to remove flower buds and improve plant shape, and they were irrigated as needed using a nutrient solution containing 300 $mg \cdot L^{-1}$ 15N-1.1P-6.2K and reverse osmosis (RO) water. The plants were periodically watered with 5.15 $g \cdot L^{-1}$ Sequestrene 138 (6% iron chelate) (Becker Underwood, Inc., Ames, IA) to prevent iron deficiency.

Although the cultivars used for the study are relatively pest-free in the landscape, in this greenhouse study, plant foliage was washed with soapy water periodically to control spider mites. Effort was also made to control powdery mildew disease by applying Daconil Ultrex (chlorothalonil) (Syngenta Crop Protection, Inc., Greensboro, NC) fungicide weekly commencing mid-Feb. 2011. Greenhouse temperatures were controlled by a pad-and-fan cooling system during the summer and by a natural gas heating system during the winter. The average temperature in the greenhouse was 22.8 °C day/18.1 °C night, which was measured and logged by temperature sensors (HOBOs, Onset Computer Corp., Bourne, MA). The average daily light integral (DLI) and the average relative humidity (RH), measured using RH/PAR sensors (Watchdogs, Spectrum Technologies), were 19.3 mol·m^{-2}·d^{-1} and 55.6%, respectively (Fig. 1).

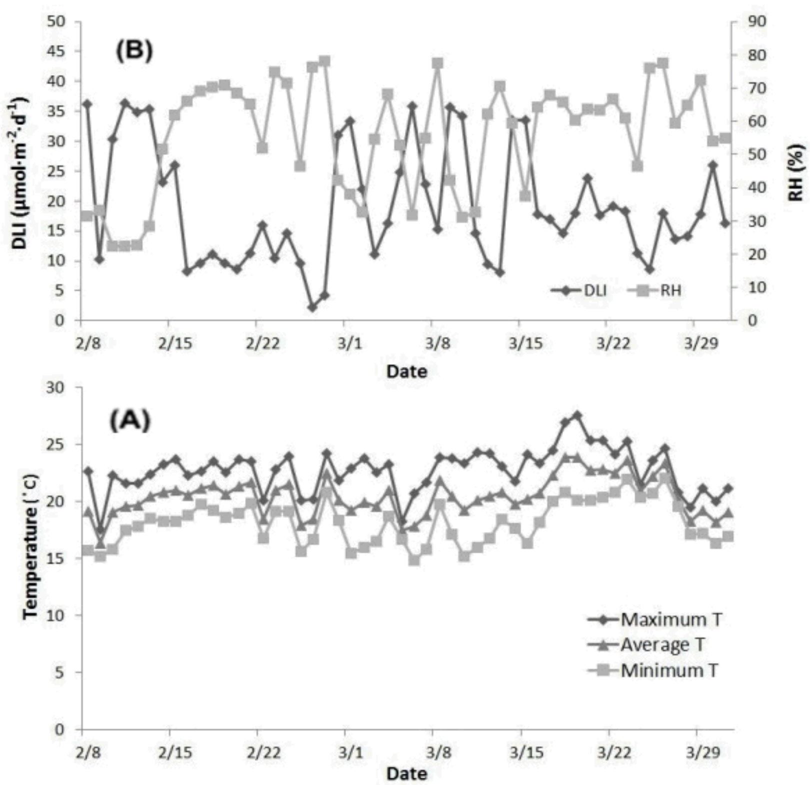

Figure 1. Variation of temperature (T) (°C) (maximum, minimum, and average) (A), daily light integral (DLI) (mol·m^{-2}·d^{-1}), and relative humidity (RH) (%) (B) in the greenhouse during the experimental period in 2011. Day and night air temperature and light in the greenhouse was measured every hour and averaged into daily measurements at plant canopy level.

2.3.2 Drought Treatment

Uniform plants of each cultivar were selected and divided into two irrigation treatment groups: well-irrigated and cyclic drought stress. Each treatment contained 10 plants per cultivar. The plants in each cultivar were pruned to uniform heights and rotated around the bench weekly to reduce variability. Irrigation treatment was initiated on 8 Feb. and ended on 31 Mar., 2011. At the beginning of the experiment, an extra plant was dried to wilting point, and SMC was then determined. Cyclic drought stress was imposed by withholding irrigation until the incipient wilting occurred (around 10% SMC). Plants were then re-watered to field capacity, and allowed to dry to 10% SMC again. During that period (about 7 days), plant gas exchange and mid-day leaf water potential (ψ) were measured every other day. The regime was repeated 3 more times. The control plants were well-irrigated (watered as needed to keep minimal SMC around 35%) throughout the experiment. The irrigation frequency for drought-treated plants ranged from every other week to once per week, varying with plant size and greenhouse environmental conditions. Well-irrigated plants (control) were watered two or three times per week. The same nutrient solution was applied to all plants at each irrigation event.

2.3.3 Measurements

Mid-day ψ was measured on young, fully expanded leaves using a pressure bomb (Soil Moisture Equipment Corp., Santa Barbara, CA) every other day during each dry down cycle. Instantaneous leaf gas exchange parameters (P_n, g_s, and E) were measured on six plants for each cultivar every other day during each dry down cycle. Between

10:00 and 12:00 HR, a young, fully expanded leaflet was put into the leaf chamber (cuvette) of a portable infrared gas exchange analyzer (LI-6400XT, LI-COR Inc., Lincoln, NE) with cuvette conditions set at 25 °C, 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF, and 400 $\mu mol \cdot mol^{-1}$ CO_2. Data were recorded when the environmental conditions and gas exchange parameters in the cuvette became stable. Instantaneous WUE was determined as P_n/E. Substrate moisture content was measured with a calibrated theta probe (type HH2, Delta-T Devices, Cambridge, U.K.). Flower number was recorded at the end of the experiment and plant growth was determined by harvesting plant shoots and roots. Plants roots were harvested by shaking out and washing away the soil media in roots. Dry weight (DW) of shoots and roots was measured after being oven-dried at 80 °C for 72 h.

2.3.4 Experimental Design and Statistical Analysis

The experiment utilized a split-plot design with irrigation treatment as the main plot and four cultivars as the subplot with 10 replications per treatment for each cultivar. A two-way analysis of variance (ANOVA) procedure was used to test the effects of drought stress and cultivar on plant growth. Means were separated in two treatments of each cultivar by least significant difference (LSD) t-test when there was an interaction between treatment and cultivars. Linear or quadratic regression analyses were performed to determine the nature and significance of association between Pn, E, gs, ψ, and WUE and SMC during the four dry-down cycles, which were selected based on the significance of quadratic correlation. When the quadratic correlation was not significant, a linear regression was chosen. General linear model (GLM) procedure analyses were performed to determine the differences of two lines by comparing the slope and intercepts. All statistical analyses were performed using SAS (version 9.1.3; SAS Institute, Cary, NC) to determine treatment differences at the 0.05 level of significance.

2.4 Results and Discussion

2.4.1 Growth

There were interactions between irrigation treatment and rose cultivar for all growth parameters. Compared to well-irrigated plants, flower number of ‘Old Blush’, ‘RADrazz’, ‘Belinda’s Dream’, and ‘Marie Pavie’ was reduced by 37%, 41%, 47%, and 60%, respectively, while shoot DW of these four cultivars was reduced by 33%, 21%, 36%, and 36%, respectively, under drought stress conditions (Table 1). Drought stress did not affect the root DW of ‘Old Blush’ and ‘RADrazz’, whereas root DW of ‘Belinda’s Dream’ and ‘Marie Pavie’ was reduced by 42% and 43%, respectively (Table 1). No difference was found in root-to-shoot ratio between the drought and well-irrigated plants in any cultivar.

Drought stress was reported to reduce plant growth in many studies (Eakes et al., 1991; Niu et al., 2008b; Niu and Rodriguez, 2009). In oleander, shoot growth, leaf area, and specific leaf mass were reduced under water deficit conditions, while root DW was not affected by drought treatment (Niu et al., 2008b). Maintaining root growth under drought stress may aid in water uptake; therefore, plants may have better adaptation to stressed environment. Additionally, root-to-shoot ratio was higher under drought stress, which resulted from a relatively larger decrease in shoot growth than in root growth (Niu et al., 2008b). Increased root-to-shoot ratio is a characteristic of many drought tolerant species, which indicates a greater carbon allocation to a larger root system for moisture uptake under water stress conditions (Henderson et al., 1991). Reduced shoot dry weight and total leaf area were found under moisture stress condition in bonfire salvia (*Salvia splendens* F. Sellow), which was due to loss of turgor during dry-down cycles (Eakes et al., 1991). Turgor loss has been reported to cause reductions in cell division and elongation, as well as essential metabolic processes required for plant growth and development (Hsiao, 1973). In bigtooth maples (*Acer grandidentatum* Nutt.), drought-stressed plants were less efficient in accumulating dry matter than well-irrigated plants (Bsoul et al., 2006). The lower growth reduction observed in 'RADrazz' under drought stress showed its better adaptation to water deficit conditions compared to the other three cultivars (Table 1). Greater reductions in flower number and leaf size were observed in 'Marie Pavie' under drought stress, which presented as minimized water loss via drought avoidance. In these four cultivars, no difference was found in root-to-shoot ratio in drought and well-irrigated plants.

Table 1. Flower number, shoot and root dry weight (DW) of four rose (*Rosa* × *hybrida* L.) cultivars, 'Old Blush', 'RADrazz', 'Belinda's Dream', and 'Marie Pavie', grown under well-irrigated cycle or subjected to cyclic drought stress for seven weeks (n = 10) [Irrigation treatment at different substrate moisture content (SMC)].

Cultivar	Irrigation treatment (SMC)	Flower Number	Shoot DW (g)	Root DW (g)
Old Blush	Well-irrigated (35-40 %)	41.0a[z]	68.5a	1.8a
	Drought (10-40 %)	25.8b	46.2b	1.4a
RADrazz	Well-irrigated (35-40 %)	18.9a	48.2a	2.2a
	Drought (10-40 %)	11.2b	38.1b	1.8a
Belinda's Dream	Well-irrigated (35-40 %)	6.6a	38.8a	1.2a
	Drought (10-40 %)	3.5b	24.9b	0.7b
Marie Pavie	Well-irrigated (35-40 %)	30.8a	42.2a	1.4a
	Drought (10-40 %)	12.2b	27.1b	0.8b

[z] Values in a column followed by different letters differ significantly at $P = 0.05$ for the same cultivar.

2.4.2 Gas Exchange

During the dry down, as SMC decreased, P_n, g_s, and E for ‘Old Blush’, ‘RADrazz’, ‘Belinda’s Dream’ and P_n for ‘Marie Pavie’ decreased quadratically, while g_s and E for ‘Marie Pavie’ decreased linearly (Fig. 2). In the range of 20% to 40% SMC, ‘Old Blush’ and ‘RADrazz’ generally had greater P_n, g_s and E compared with those of ‘Belinda’s Dream’ and ‘Marie Pavie’ (Fig. 2). At SMC of 10% to 20%, ‘Marie Pavie’ generally had lower P_n, g_s, and E compared with the other three cultivars, while ‘RADrazz’ had greater P_n, g_s, and E than the other three cultivars (Fig. 2). During the dry down, as SMC decreased, the instantaneous leaf WUE for the four cultivars increased quadratically (Fig. 3). As SMC decreased from 20% to 10%, WUE increased more rapidly in ‘RADrazz’ than the other three cultivars (Fig. 3).

Water deficit can affect plants in different ways, such as inducing stomatal closure, thus reducing transpiration rates and photosynthesis, and ultimately, inhibiting growth and performance (Yordanov et al., 2003). There were gas exchange differences among cultivars in response to decreased SMC, including higher rates of gas exchange for ‘RADrazz’. Decreased gas exchange rates indicate stomatal closure under low SMC, which is one of the primary defense mechanisms protecting plants from desiccation (Chaves, 1991). Maintaining high gas exchange rates at low SMC is important in regulating carbon dioxide (CO_2) uptake and water loss from plants. In ‘Bonfire’ salvia (*Salvia splendens* Sellow), Eakes et al. (1991) reported that E decreased due to lower g_s in plants under drought stress, which could account for reduced water loss. In a study on four bedding plants, Nemali and van Iersel (2008) found that P_n and g_s decreased at lower SMC. On the days after re-watering, $P_{n,}$ g_s, and E were low in all cultivars (Fig. 2), because gas exchange rates took days to recover from drought stress.

The physiological parameter of crop WUE is important to describe the relationship between plant water use and dry matter production. With increased WUE, there is a greater biomass production per water quantity transpired and less water is needed for growth and development (Nemali and van Iersel, 2008). In several studies (Araus et al., 2002; McKay et al., 2003), it has been reasoned that plants having high WUE at low g_s in response to drought stress are more drought resistant. In a study on clover (*Trifolium alexandrinum* L.), increased instantaneous WUE due to lowered water loss was observed in plants under drought stress, which were induced by decreased transpiration rate and leaf area (Lazaridou and Koutroubas, 2004). In our study, higher leaf WUE was observed under drought stress in all four cultivars (Fig. 3).

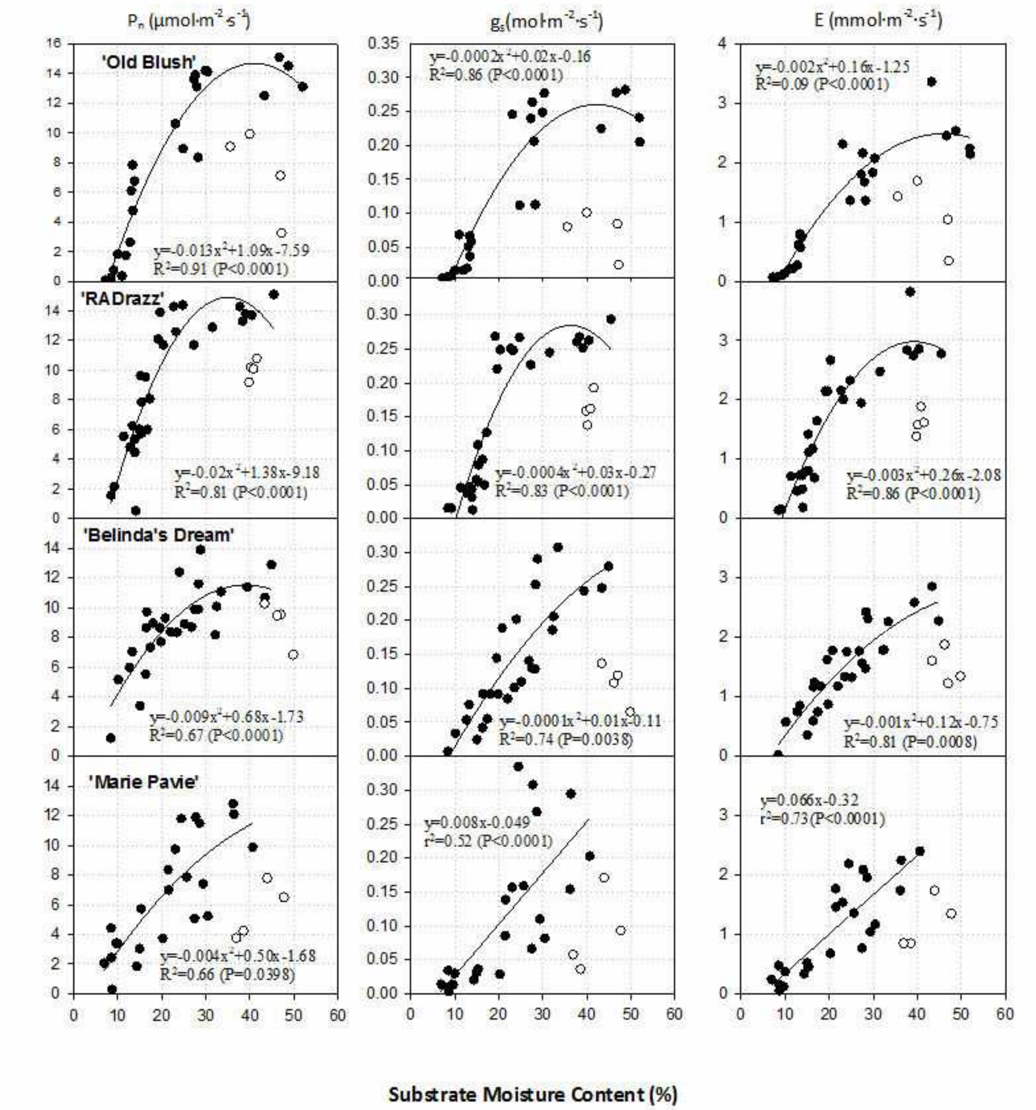

Figure 2. Relationship between leaf net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E) and the substrate moisture content (SMC) for four rose (*Rosa × hybrida* L.) cultivars, 'Old Blush', 'RADrazz', 'Belinda's Dream', and 'Marie Pavie', measured during dry down. The open symbols represent data measured on the second day of the dry down when plants were not fully recovered from the previous drought stress. The regression analysis excluded these data.

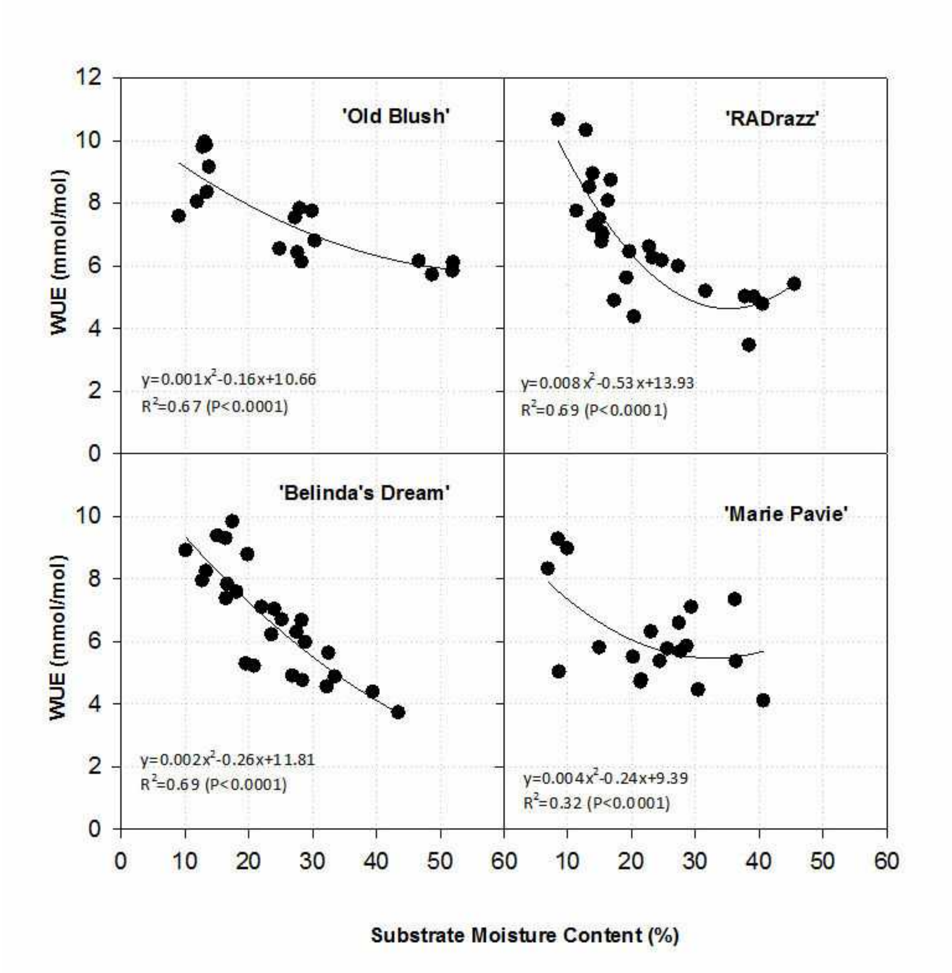

Figure 3. Relationship between leaf water use efficiency (WUE) and the substrate moisture content (SMC) for four rose (*Rosa* × *hybrida* L.) cultivars, 'Old Blush', 'RADrazz', 'Belinda's Dream', and 'Marie Pavie', measured during dry down.

2.4.3 Leaf Water Potential

During the dry-down, mid-day ψ at SMC between 20% and 35% was similar among all cultivars (Fig. 4). At SMC above 35% or below 10%, mid-day ψ was more negative in 'Old Blush' when compared with those of the other three cultivars (Fig. 4). These results may have been due to an infestation of powdery mildew in 'Old Blush' that occurred two weeks after treatment initiation. The humidity was very high (70%) compared with the first two weeks of the experiment (Fig. 1), and 'Old Blush' is susceptible to this disease. The mid-day ψ was less negative in 'Marie Pavie' than the other three cultivars at SMC less than 10% (Fig. 4).

Leaf ψ is an important parameter to indicate the level of plant water stress, and water availability to roots could be limited by low ψ. Niu and Rodriguez (2009) reported that predawn ψ decreased rapidly when SMC decreased to a critical value (around 20%) in *R.* × *hybrida* 'Dr. Huey', *R.* × *fortuniana*, *R. multiflora*, and *R. odorata*, with the most negative ψ in *R. odorata*. In the present study, mid-day ψ decreased rapidly at SMC less than 20% in 'Old Blush' and 'RADrazz' (Fig. 4). Based on the present results and those reported in the literature (Niu et al., 2007b; Niu et al., 2008b; Niu and Rodriguez, 2009), 20% SMC may be the critical threshold to cause steep declines in leaf ψ in many ornamental plants. Niu and Rodriguez (2009) suggested that frequent measurements of ψ are necessary to identify the critical SMC to detect differences among plants in leaf ψ responses to substrate drying. Decreased g_s resulting from drought stress was reported to aid in maintaining midday leaf ψ in seedlings of tropical rainforest species (Bonal and Guehl, 2001). Plant drought avoidance is related to maintenance of high tissue water potential under water stress conditions to reduce water loss from plants (Stoddard et al., 2006), which can maximize soil moisture use, resulting in lower WUE (Blum, 2005). At low SMC, ‘Marie Pavie’ had lower gas exchange rates (P_n, g_s, E, and WUE) compared to the other three cultivars, however, midday leaf ψ was relatively higher, which may be explained as drought avoidance to reduce water loss.

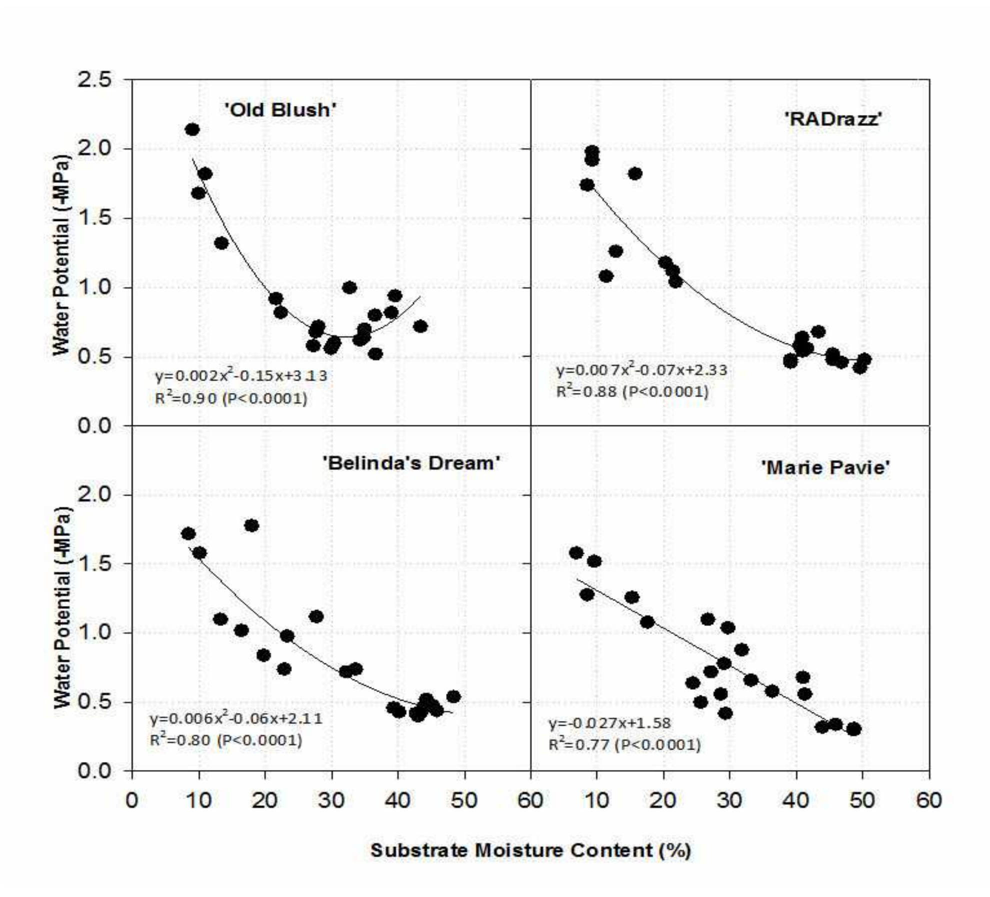

Figure 4. Relationship between mid-day leaf water potential (ψ) and the substrate moisture content (SMC) for four rose (*Rosa* × *hybrida* L.) cultivars, ‘Old Blush’, ‘RADrazz’, ‘Belinda’s Dream’, and ‘Marie Pavie’, measured during dry down.

2.5 Conclusion

In all four rose cultivars investigated, growth was reduced in drought-treated plants compared to well-irrigated plants but the reduction in ‘RADrazz’ was the least among the four cultivars. In response to decreasing SMC, P_n, g_s, E, and mid-day ψ decreased, while leaf WUE increased in all cultivars. The differences in the physiological responses to drying substrate among the cultivars resulted in their growth differences. ‘RADrazz’ was more drought tolerant, followed by ‘Old Blush’ and ‘Belinda’s Dream’, with its higher $P_{n,}$ g_s, E, and leaf WUE at SMC between 10% and 20% and smaller growth reduction compared to the other three cultivars. ‘Marie Pavie’ had the greatest reduction in flower number and lowest gas exchange rates under water deficit conditions, and it was the least drought tolerant among the cultivars investigated. Further research is needed to investigate the differences in recovery ability of gas exchange rates from drought stress among these cultivars.

CHAPTER III

THE EFFECT OF SUBSTRATE MOISTURE CONTENT ON GROWTH AND PHYSIOLOGICAL RESPONSES OF TWO LANDSCAPE ROSES*

3.1 Synopsis

A greenhouse study was conducted to quantify the irrigation requirements of two rose (*Rosa* × *hybrida* L.) cultivars, 'RADrazz' and 'Belinda's Dream', which are widely valued for their ease of maintenance in landscapes, grown at four constant volumetric substrate moisture contents (SMC) of 10, 20, 30, and 40%. In both cultivars, there were no differences in growth and physiological responses between 30% and 40% SMC. In 'RADrazz', shoot dry weight (DW) was reduced by 25% and 86%, root DW was reduced by 27% and 71%, and flower number was reduced by 27% and 86% at 20 and 10% SMC, respectively, compared to 30% SMC. Midday leaf water potential (ψ), photosynthesis (P_n), stomatal conductance (g_s), and transpiration (E) were highest at 30 and 40% SMC, and they were lowest at 10% SMC. In 'Belinda's Dream', shoot DW was reduced by 30% and 87%, root DW was reduced by 35% and 81%, and flower number was reduced by 42% and 75% at 20 and 10% SMC, respectively, compared to 30% SMC. Midday ψ was least negative at 40% SMC, while it was most negative at 10% SMC. There were no significant differences in midday ψ between 20 and 30% SMC. Photosynthetic rate (P_n), g_s, and E were highest at 30 and 40% SMC and lowest at 10%

SMC. In summary, plants at 30 and 40% SMC maintained the highest shoot and root DW, flower number, midday ψ, P_n, g_s and E. Water applied at 30% and 20% SMC was reduced by 31% and 70%, compared to 40% SMC, with excellent performance at 30% SMC and acceptable growth and quality at 20% SMC. The 10% SMC led to significant growth reduction, poor quality, and 25% mortality.

3.2 Introduction

Due to decreasing water resources and increasing population and urbanization, water conservation and development of more efficient irrigation systems are critical in greenhouse and landscape water management (Nicolas et al., 2008; Niu et al., 2006a). Additionally, many crops are over-irrigated in greenhouse production, which results in runoff and leaching of water and nutrients from the greenhouse into the environment. To optimize water use in greenhouse production, a thorough understanding of the amount of water needed to produce quality plants is vital. Although water requirements of food crops and turfgrass have been enumerated, data quantifying the irrigation requirements of ornamental landscape plants is minimal at present. By irrigating plants based on their water requirement, water use could be reduced, and plants may acclimate for drought tolerance in the landscape (Kozlowski and Pallardy, 2002).

Roses (*Rosa* × *hybrida* L.) are some of the most popular garden plants in the world. 'RADrazz' and 'Belinda's Dream' rose cultivars are well adapted to various climatic and soil conditions, and provide the consumers with garden plants that require minimum fertilizer, water, and pesticides while growing in gardens or landscapes (MacKay et al., 2008). However, there is little science-based knowledge about the minimal irrigation required for plant growth and their responses to different substrate moisture contents (SMC). Most drought studies utilize the dry down method to determine drought tolerance. In a cyclic drought study by Cai et al. (2012), 'RADrazz' and 'Belinda's Dream' roses had significant reduction in photosynthesis (P_n), stomatal conductance (g_s), and transpiration (E) as SMC decreased from 20% to 10%. Plant responses to such cyclic drought stresses may differ from the responses to a continuous drought at stable SMC. The constant SMC can be maintained by the use of sensor technology in greenhouse production.

Conserving water and reducing the environmental impact of runoff are two important issues confronting container production in greenhouses and nurseries (Warsaw et al., 2009). With increasing cost of water and stringent legislation, and decreasing water availability, the development of efficient irrigation technology that conserves water and reduces runoff without adversely affecting crop quality is becoming increasingly important for success of container nurseries. Applying irrigation based on plant water requirement is a key concept in water conserving irrigation scheduling (Warsaw et al., 2009). Using a real-time sensing technology to detect the substrate water status and control irrigation is a promising approach for improving sustainability of irrigation management (van Iersel et al., 2010).

The substrate volumetric water content (θ) is the most valuable environmental factor for automatic irrigation control (Jones, 2007). To study the growth and photosynthetic physiology of begonia (*Begonia semperflorens* L.) at six SMCs, Miralles-Crespo and van Iersel (2011) used the time domain transmissometry sensors (TDT) in multiple containers to control the irrigation based on container-specific θ thresholds. The six SMCs were ranging from 13.6 to 47.2%. The results showed that shoot dry weight (DW) of begonia increased as SMC increased, and plants had similar shoot DW at SMC higher than 34.8%. The total evapotranspiration increased linearly with SMC. With decreased SMC, begonia had significant reduction in leaf size, P_n, and g_s (Miralles-Crespo and van Iersel, 2011).

Burnett and van Iersel (2008) reported that there was an increase in water use efficiency and reduction in stem length and branch numbers of gaura (*Gaura lindheimeri Engelm. & Gray)* with decreasing SMC (45% to 10%). In a study by van Iersel et al. (2010), a substrate moisture sensor-controlled irrigation system was developed to quantify the daily water use of petunia (*Petunia* × *hybrida* Hort ex. Vilm) in SMCs from 5 to 40%. Lower SMC resulted in a decrease in shoot DW, leaf water potential (ψ) and osmotic potential (ψ_s). There was only slight additional growth above 25% SMC. Similarly, at four constant SMC (9%, 15%, 22%, and 32%), Nemali and van Iersel (2008) found that gas exchange, chlorophyll fluorescence, and leaf water potential were similar between 32% and 22% SMC for impatiens (*Impatiens wallerana* Hook.) and salvia (*Salvia splendens* Sell ex Roem. & Schult).

The objectives of the current study were to determine minimum water requirements and quantify the growth and physiological responses of two popular landscape roses, 'RADrazz' and 'Belinda's Dream', grown at four different SMCs using the TDT sensors in multiple containers to control the irrigation based on container-specific θ thresholds.

3.3 Materials and Methods

3.3.1 Plant Materials and Culture

Rooted cuttings of two landscape rose cultivars, ‘RADrazz’ and ‘Belinda's Dream’, were purchased from Greenheart Farm (Greenheart, Arroyo Grande, CA), and transplanted to 23.5 - L (28.1 cm wide, 45.7 cm long, 18.3 cm height) plastic containers (Iris USA Inc., Pleasant Prairie, WI) on 21 Jan., 2012 in a glass greenhouse at College Station, TX. Containers were filled with Fafard 52 mix (bark, Canadian sphagnum peat moss, perlite, vermiculite, dolomitic limestone, wetting agent) (Fafard, Inc., Anderson, SC). The mix was evenly amended with 54 grams of a slow release fertilizer 15N-3.9P-9.9K Osmocote (Peters Professional, Scotts-Sierra, Marysville, OH). After transplanting, plants were manually irrigated with reverse osmosis (RO) water to container capacity and drenched evenly with broad-spectrum fungicide (Banrot®, Scotts-Sierra Crop Protection Company, Marysville, OH) until runoff to prevent root rot. During the establishment stage (35 days), plants were pruned once a week to remove flower buds and improve plant shape.

Although the cultivars used for the study are relatively pest-free in the landscape, in this greenhouse study, plant foliage was washed with soapy water periodically to control spider mites. Greenhouse temperatures were controlled by a pad-and-fan cooling system during the summer and by a natural gas heating system during the winter. The average temperature in the greenhouse was 26.4 °C day/22.0 °C night, which was measured and logged by temperature sensors (HOBOs, Onset Computer Corp., Bourne, MA). The average daily light integral (DLI) and the average relative humidity (RH), measured using RH/PAR sensors (Watchdogs, Spectrum Technologies), were 27.5 $mol \cdot m^{-2} \cdot d^{-1}$ and 70.0%, respectively (Fig. 5).

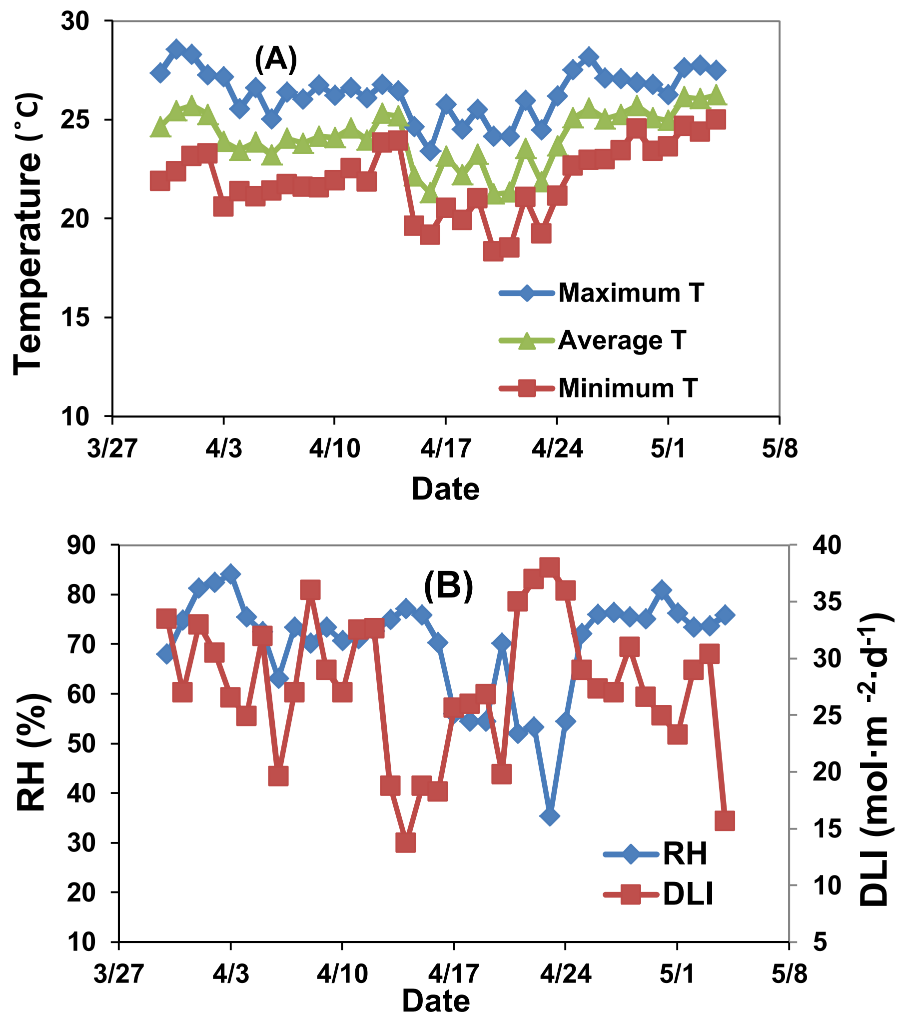

Figure 5. Variation of temperature (T) (°C) (maximum, minimum, and average) (A), daily light integral (DLI) ($mol \cdot m^{-2} \cdot d^{-1}$), and relative humidity (RH) (%) (B) in the greenhouse during the experimental period in 2012. Day and night air temperature and light in the greenhouse were measured every hour and averaged into daily measurements at plant canopy level.

3.3.2 Irrigation System

The irrigation system consisted of Acclima Time Domain Transmissometry (TDT) sensors (5.4 cm wide, 20.3 cm long, 1.4 cm height) (Acclima Inc., Meridian, ID), solenoid valves, and a polyethylene header pipe placed in the center and down the entire length of the bench. The Acclima TDT sensors were placed in the center of the container, lying flat. Two dribble ring emitters were placed on top of the root substrate and around the plants and secured with pins. Polyethylene pipe (Silver-line Plastics, Asheville, NC) was cut to fit between the header and each container and attached to the header with a tee fitting (Lasco Fittings Inc., Brownsville, TN). A 10-cm section of pipe was attached to each tee with a PVC threaded male adapter (Lasco Fittings Inc., Brownsville, TN) and Teflon tape (LG Sourcing Inc., North Wilkesboro, NC) that was attached to the solenoid valve (N-100F-H; Weathermatic, Dallas, TX). Containers were arranged in two 3 × 1 m greenhouse benches with 51 - cm between containers. Two dribble ring emitters (61 cm lead, 15 cm diameter) (Dramm Corporation, Manitowoc, WI) were evenly spaced and connected to the 25 - cm polyethylene pipe by a microtube to deliver water to the plants. A garden hose was used to connect the system to a pressure regulator (25 PSI; Mister Landscaper, Dundee, FL) at the greenhouse water main faucet. All sensors were placed and an extension cord (Chicago Electrical Power Tools, distributed by Harbor Freight Tools, Camarillo, CA) was cut into 86 cm pieces to connect each sensor. The irrigation system utilized in this greenhouse study measured SMC in multiple containers and irrigated the plants based on container-specific SMC thresholds (θ). A laptop computer was connected to the irrigation system once a day to monitor the SMC and check for problems. When a sensor failed, the computer was programmed to make the irrigation of that container depend on another Acclima sensor programmed to the same θ.

3.3.3 Substrate Moisture Content (SMC) Treatment

Uniform plants of each cultivar were selected and divided into four SMC treatment groups: 10, 20, 30, and 40%. The 40% SMC treatment corresponded to well-irrigated plants, while the 10% SMC treatment corresponded to drought stress. There were two cultivars in each container and four replications of the container (total 16 containers and 32 plants). Substrate moisture content (SMC) treatment was initiated on 13 Mar. and ended on 4 May 2012. Prior to the initiation of the treatment, the Acclima sensors were calibrated. After calibration, the 10, 20, 30, and 40% SMC treatments equaled to the Acclima readings of 1.1, 8.2, 15.2 and 22.3%, respectively (Fig. 6). Once the SMC dropped below the set point in each treatment, the solenoid valves would stay open until the SMC reached the expected value. Due to severe drought at 10% SMC, two plants senesced in one container. Plants at 10% SMC were then irrigated manually to container capacity for recovery (Fig. 6).

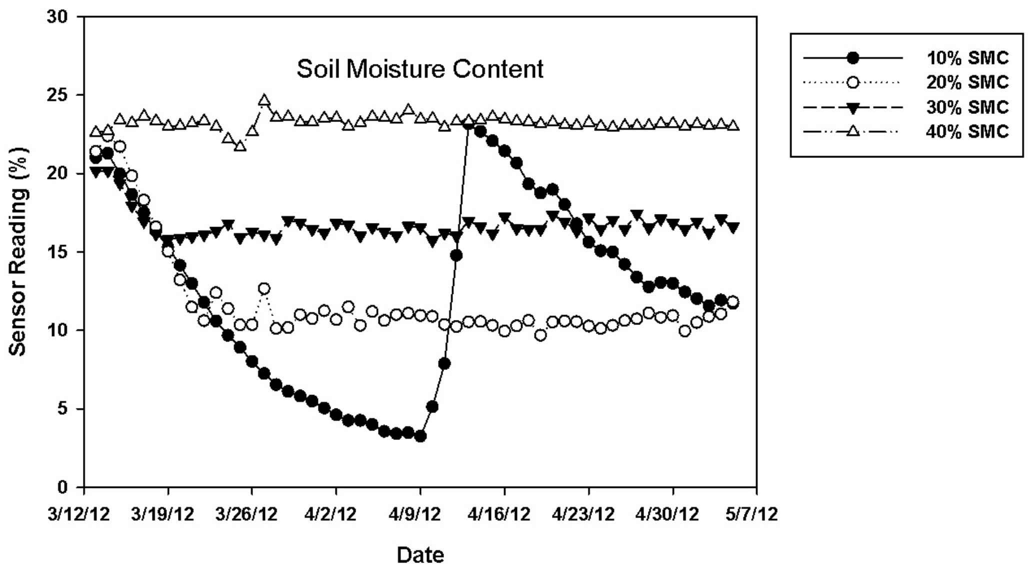

Figure 6. Responses of the substrate moisture content (SMC) thresholds for the Acclima system at 10, 20, 30, and 40% SMC treatments during the experimental period in 2012.

The total amount of water applied in each container was determined by multiplying the flow rate by the total watering time from the beginning to the end of the experiment. The flow rates of the dribble ring emitters were determined by opening one valve and placing the two dribble rings into a bucket to collect the water. The amount of water emitted per second was calculated by measuring the ratio of the volume of the water to the time (in seconds) the dribble rings were on. After calculation, the flow rate of a dribble ring was 5.3 mL per second.

3.3.4 Measurements

Midday leaf water potential (ψ) was measured on young, fully expanded leaves using a pressure bomb (Soil Moisture Equipment Corp., Santa Barbara, CA) every other day after four weeks of the treatment. Instantaneous leaf gas exchange parameters (P_n, g_s, and E) were measured on 4 plants per cultivar per treatment every week during the treatment period. Between 10:00 and 12:00 HR, a young, fully expanded leaflet was put into the leaf chamber (cuvette) of a portable infrared gas exchange analyzer (LI-6400XT, LI-COR Inc., Lincoln, NE) with cuvette conditions set at 25 °C, 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF, and 400 $\mu mol \cdot mol^{-1}$ CO_2. Data were recorded when the environmental conditions and gas exchange parameters in the cuvette became stable. At the end of the experiment, flower number was recorded. Dry weight (DW) of shoots and roots were determined after being oven-dried at 80 °C to constant weights.

3.3.5 Experimental Design and Statistical Analysis

The experiment utilized a two-factorial experiment design (cultivar × SMC). A two-way analysis of variance (ANOVA) procedure was used to test the effects of SMC and cultivar on plant growth and physiological responses. When there was an interaction between SMC and cultivars, means were separated into four SMC of each cultivar by Student-Newman-Keuls multiple comparison at $P = 0.05$. When the interaction was not significant, data were pooled across SMC or cultivar. All statistical analyses were performed using SAS (version 9.1.3; SAS Institute, Cary, NC).

3.4 Results and Discussion

3.4.1 Substrate Moisture Content (SMC) and Total Water Application

Although there were increases in plant size and large fluctuations in DLI and RH (Fig. 5), the sensor-controlled automated irrigation system was able to maintain the stable SMC (Fig. 6), which has been reported by Nemali and van Iersel (2006) and van

Iersel et al. (2010). However, due to severe drought at 10% SMC, two plants were dead in one container on 9 April, 27 days after the initiation of the treatment. All plants at 10% SMC were then watered manually to field capacity, and allowed to dry down again (Fig. 6). On 27 Mar. 2012, the 20 and 40% SMC treatments slightly exceeded the thresholds (Fig. 6). This was caused by heavy rain resulting in some leakage through the greenhouse glazing into the containers before it was stopped. The total amount of water applied during the treatment period decreased with decreasing SMC (Fig. 7). There was no applied irrigation water leached from the containers. Burnett and van Iersel (2008), Nemali and van Iersel (2006) and van Iersel et al. (2010) also reported that the total irrigation volume increased with increasing SMC thresholds. During the two-month treatment period, total water applied in each container was 5.7 L, 16.6 L, 39.3 L, and 57.2 L at 10, 20, 30, and 40% SMC, respectively. Compared to 40% SMC, there was 90%, 71%, and 31% reduction in water application at 10%, 20%, and 30% SMC, respectively (Fig. 7).

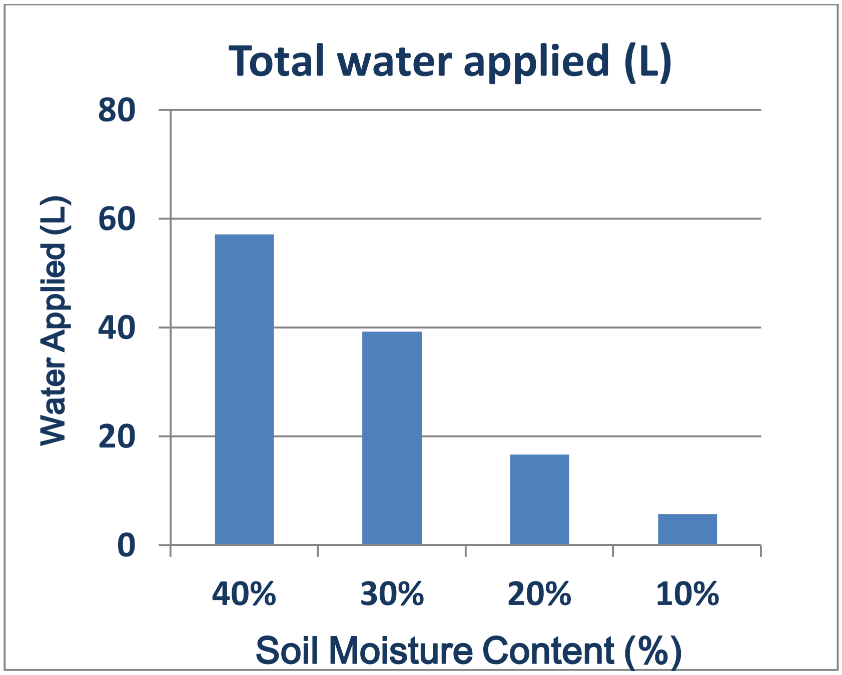

Figure 7. Total amount of water applied at 10, 20, 30, and 40% substrate moisture content (SMC) treatments after the two month experimental period.

3.4.2 Dry Weight (DW) of Shoots and Roots, and Flower Number

There were interactions between SMC treatment and rose cultivar for shoot and root DW and flower number. For both cultivars, there were no significant differences in shoot and root DW and flower number between 30% and 40% SMC (Fig. 8, 9, 10). In 'RADrazz', shoot DW was reduced by 25% and 86%, root DW was reduced by 27% and 71%, and flower number was reduced by 27% and 86% at 20 and 10% SMC, respectively, compared to 30% SMC. In 'Belinda's Dream', shoot DW was reduced by 30% and 87%, root DW was reduced by 35% and 81%, and flower number was reduced by 42% and 75% at 20 and 10% SMC, respectively, compared to 30% SMC (Fig. 8, 9, 10).

In both cultivars, shoot DW increased as the SMC increased from 10% to 30% with no significant effect at higher SMC set point (Fig. 8). Burnett and van Iersel (2008) reported that there was an increase in shoot DW as the SMC increased from 10 to 25% with no effects at higher SMC set points in gaura. Van Iersel et al. (2010) found that there was a quadratic relationship between the SMC and shoot DW in petunia; shoot DW increased as the SMC increased from 5% to 25% with no additional increase at higher SMC. In a study by Niu et al. (2007b), cut raceme yield and shoot and root DW of big bend bluebonnet (*Lupinus bavardii*) decreased as SMC decreased from 33% to 12% or 15%, and plants required 25% SMC or greater to maintain the maximum plant growth and cut flower production. In this study, at 20% SMC, lower reductions of shoot and root DW and flower number were observed in 'RADrazz', compared with 'Belinda's Dream' (Fig. 8, 9, 10), which was consistent with a previous study where 'RADrazz' had less growth reduction than 'Belinda's Dream' under drought stress (Cai et al., 2012). In a cyclic drought study (SMC decreased from 40% to 10%, then increased to 40% after re-watering), Cai et al. (2012) reported that shoot DW was reduced by 21% and 36% and flower number was reduced by 41% and 47% in 'RADrazz' and 'Belinda's Dream', respectively, compared to control plants. Drought stress did not affect the root DW of 'RADrazz', whereas root DW of 'Belinda's Dream' was reduced by 42%. In the current study, shoot DW was reduced by 86% and 87%, flower number was reduced by 86% and 75%, and root DW was reduced by 71% and 81% in 'RADrazz' and 'Belinda's Dream', respectively, under continuous drought at 10% SMC (Fig. 8, 9, 10).

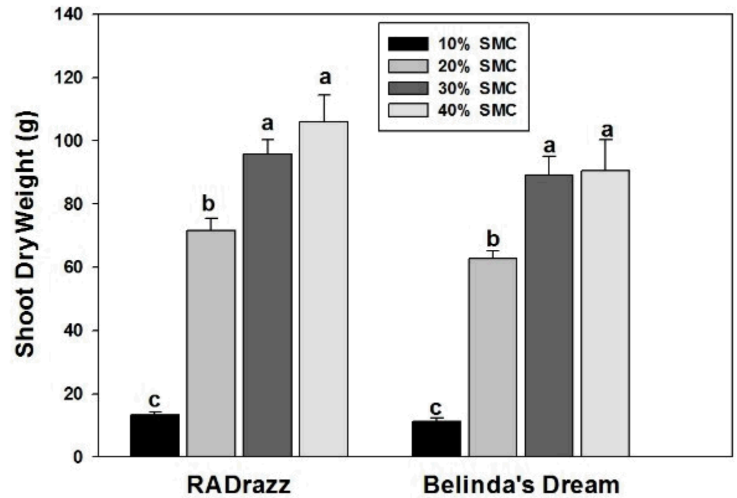

Figure 8. Effect of substrate moisture content (SMC) (10, 20, 30, and 40% SMC) on shoot dry weight (DW) of 'RADrazz' and 'Belinda's Dream'. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. Vertical bars represent standard error (SE).

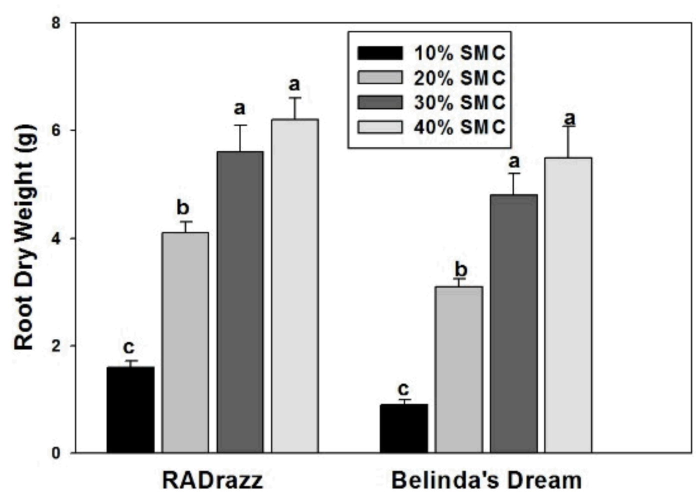

Figure 9. Effect of substrate moisture content (SMC) (10, 20, 30, and 40% SMC) on root dry weight (DW) of 'RADrazz' and 'Belinda's Dream'. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. Vertical bars represent standard error (SE).

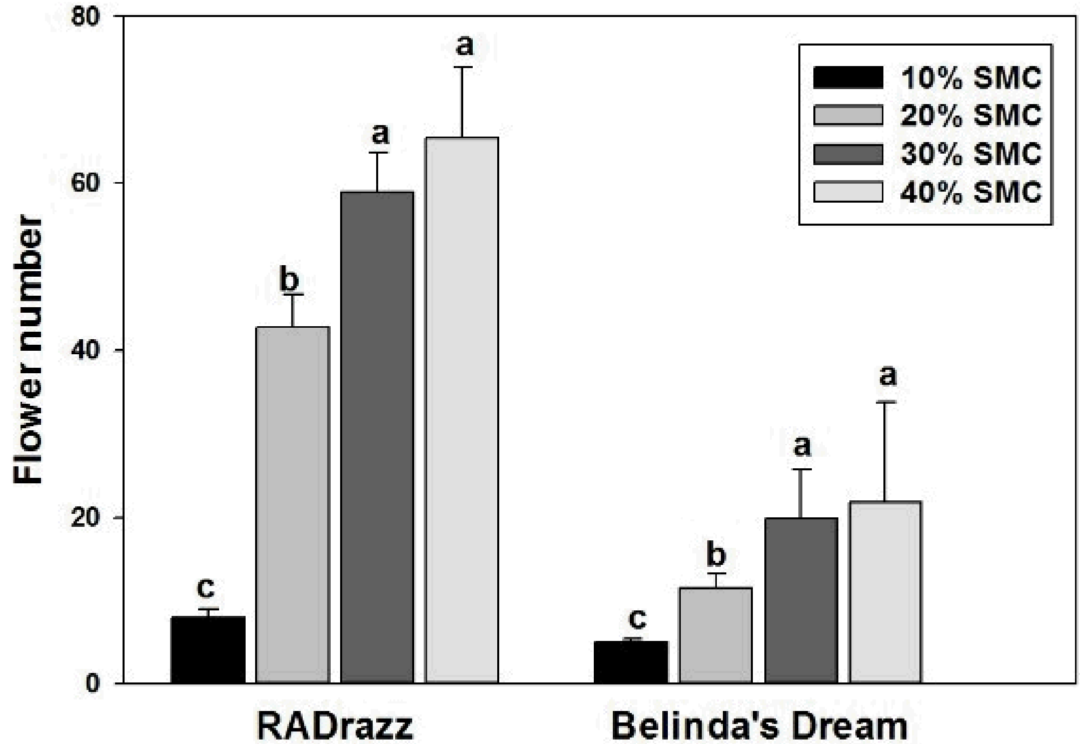

Figure 10. Effect of substrate moisture content (SMC) (10, 20, 30, and 40% SMC) on flower number of 'RADrazz' and 'Belinda's Dream'. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. Vertical bars represent standard error (SE).

3.4.3 Photosynthesis, Stomatal Conductance, and Transpiration

There were interactions between SMC treatment and rose cultivar for P_n, g_s and E. In ‘RADrazz’, there was no significant difference in P_n between 30 and 40% SMC at 17 days after treatment (DAT), while the P_n was reduced by 25% and 75% at 20 and 10% SMC, respectively, compared to 40% SMC (Fig. 11). At 24 DAT, P_n was highest at 40% SMC, and there was no significant difference in P_n between 20% and 30% SMC, while it was reduced by 82% at 10% SMC, compared to 40% SMC. At 31 DAT, there was no significant difference in P_n between 30% and 40% SMC, while the P_n was reduced by 21% and 83% at 20% and 10% SMC, compared to 40% SMC. At 38 DAT, P_n was highest at 40% SMC, and there was no significant difference in P_n between 20% and 30% SMC, while it was reduced by 79% at 10% SMC, compared to 40% SMC. At 45 DAT, there was no significant difference in P_n between 30% and 40% SMC, while the P_n was reduced by 28% and 62% at 20% and 10% SMC, respectively, compared to 40% SMC (Fig. 11). In ‘Belinda’s Dream’, there was no significant difference in P_n between 30% and 40% SMC during the treatment period, while the P_n was reduced by 21% to 37% at 20% SMC, and by 53% to 92% at 10% SMC, compared to 40% SMC (Fig. 11). The responses of g_s and E to different SMCs in two cultivars are similar to those of P_n (Fig. 11).

Decreasing moisture content causes reductions in plant gas exchange rates, which is one of the primary defense mechanisms protecting plants from desiccation (Chaves, 1991). In the current study, P_n, g_s and E decreased as SMC decreased from 40% to 10% with little or no difference between 30% and 40% or 30% and 20% SMC in ‘RADrazz’ and ‘Belinda’s Dream’ depending on time of measurement (Fig. 11). Reduced P_n may be due to the reduction in leaf photosynthetic area at decreasing SMC. In a study by Cai et al., (2012), they reported that P_n, g_s, and E for ‘RADrazz’ and ‘Belinda’s Dream’ decreased quadratically as SMC decreased from 40% to 10%, and ‘RADrazz’ had greater P_n, g_s and E than those of ‘Belinda’s Dream’ during drying down. Similarly, in the current study, the P_n, g_s and E were higher in ‘RADrazz’ than ‘Belinda’s Dream’ under continuous drought at 10% SMC. In a study on four bedding plants [salvia ‘Bonfire Red’, vinca ‘Cooler Peppermint’ (*Catharanthus roseus* L. G. Don.), petunia ‘Lavender White’, and impatiens ‘Cherry’], Nemali and van Iersel (2008) found that leaf P_n was lowest at 9% SMC and there was no difference in P_n among SMC of 15, 22, and 32%. Miralles-Crespo and van Iersel (2011) reported that leaf size, P_n , and stomatal conductance of begonia were lowest at 13.6% SMC as SMC decreased from 47.2% to 13.6% and there were no significant differences among the other SMCs (21%, 28.1%, 34.8%, 41.2%, and 47.2% SMC). Similarly, in the current study, P_n was lowest at 10% SMC, and there was no significant difference between SMC of 20% and 30% or between 30% and 40% SMC. Both cultivars can maintain the acceptable photosynthetic rates at 20% SMC (Fig. 11).

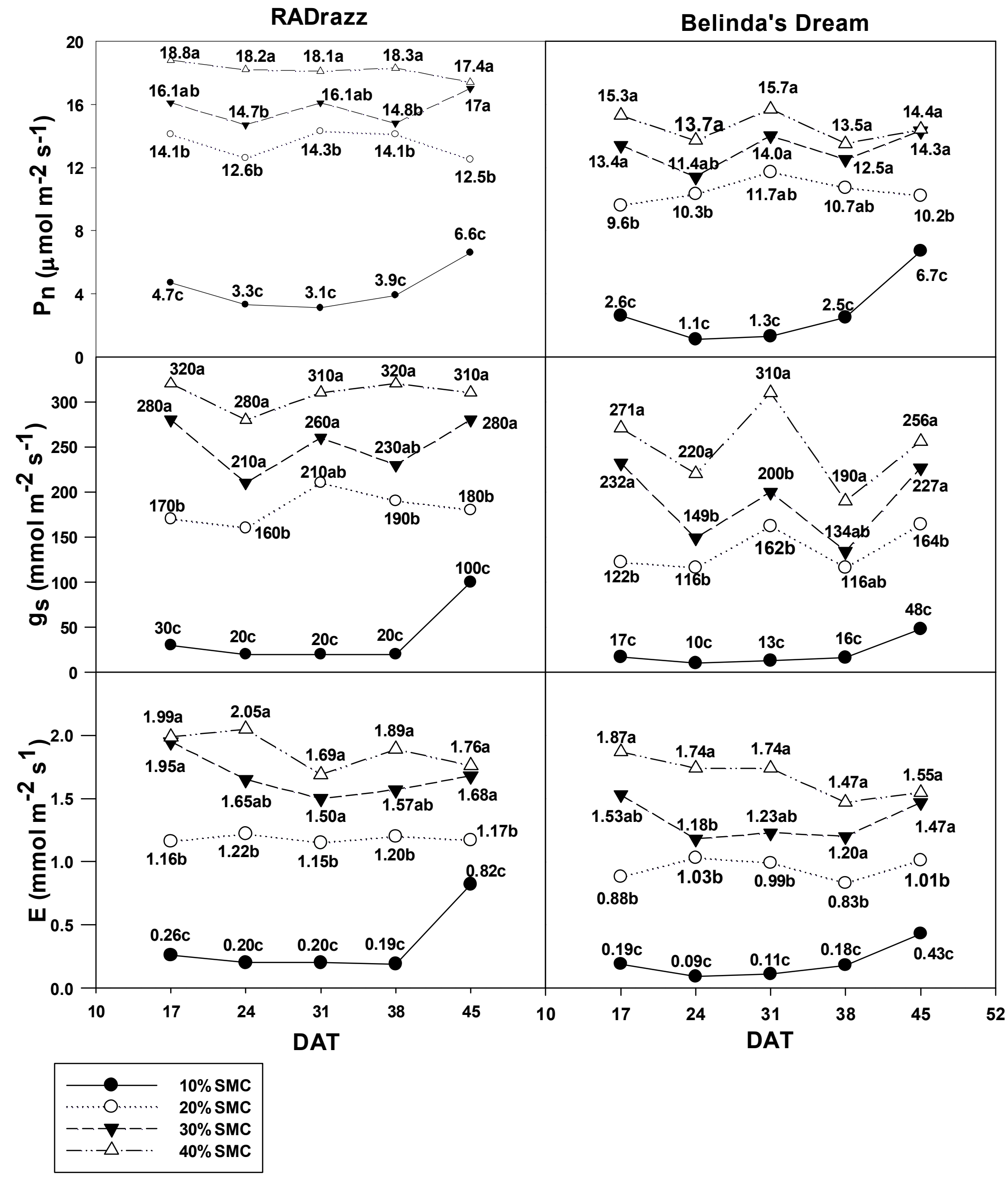

Figure 11. Responses of leaf net photosynthesis (P_n), stomatal conductance (gs), transpiration (E) to different substrate moisture contents (SMCs) (10, 20, 30, and 40% SMC) in 'RADrazz' and 'Belinda's Dream' during the treatment period. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. DAT represents days after treatment.

3.4.4 Midday Leaf Water Potential (ψ)

There were interactions between SMC treatment and rose cultivar for midday ψ. In 'RADrazz', midday ψ increased as SMC increased from 10% to 40%, and there were no significant differences in midday ψ between 30 and 40% SMC. Compared to 40% SMC, midday ψ was 0.57 MPa and 1.11MPa lower at 20 and 10% SMC, respectively (Fig. 12). In 'Belinda's Dream', there were no significant differences in midday ψ between 20 and 30% SMC. Midday ψ was least negative at 40% SMC, while it was most negative at 10% SMC. Compared to 40% SMC, midday ψ was 0.19 MPa and 0.60 MPa lower at 20 and 10% SMC, respectively (Fig. 12).

Leaf ψ is an important parameter to indicate the level of plant water stress. In the present study, midday ψ significantly decreased as SMC decreased from 20% to 10% in 'RADrazz' and 'Belinda's Dream', indicating severe drought stress at 10% SMC (Fig. 12). In a cyclic drought study, Cai et al. (2012) found that midday ψ decreased rapidly at SMC less than 20% in 'RADrazz' and 'Belinda's Dream. Some studies have reported that 20% SMC may be the critical threshold to cause steep declines in leaf ψ in big bend bluebonnet, *R.* × *hybrida* 'Dr. Huey', *R. fortuniana*, *R. multiflora*, and *R. odorata* (Niu et al., 2007b; Niu and Rodriguez, 2009). Van Iersel et al. (2010) reported that a quadratic relationship between SMC and leaf ψ in petunias; leaf ψ increased as the SMC increased from 5% to 15% with no additional increase at higher SMC. In a study by Nemali and van Iersel (2008), regardless of species (impatiens, petunia, salvia, and vinca), midday ψ was lowest at 9% SMC and did not differ among the other three SMC levels (15, 22, and 32% SMC). Similarly, there was no difference of midday ψ among the plants irrigated with amounts varying from 50% to 100% of evapotranspiration in olive (*Olea europea* L.) plants.

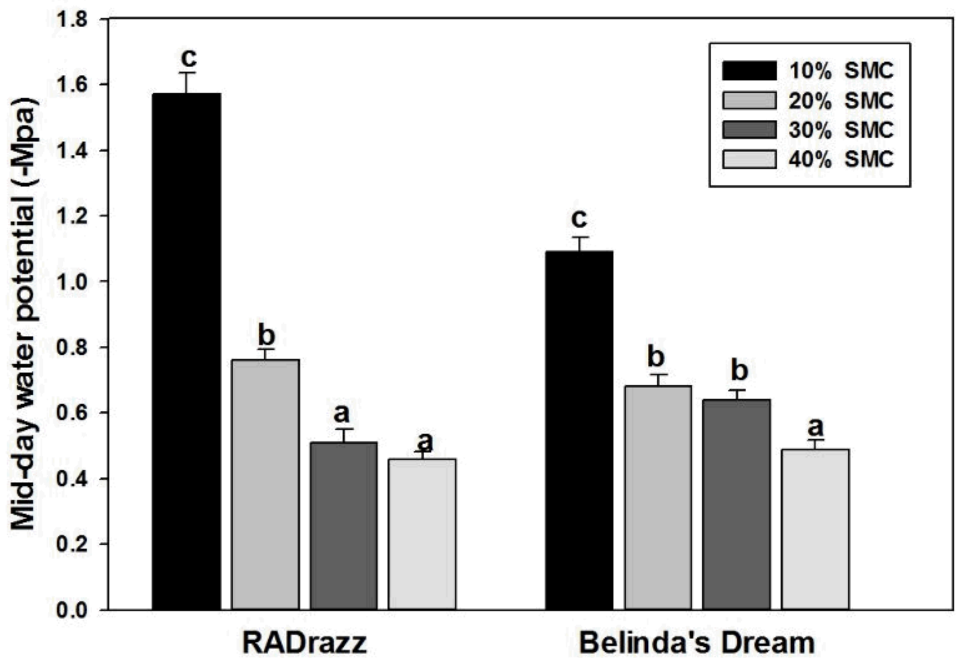

Figure 12. Effect of substrate moisture content (SMC) (10, 20, 30, and 40% SMC) on midday leaf water potential (ψ) of 'RADrazz' and 'Belinda's Dream'. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. Vertical bars represent standard error (SE).

3.5 Conclusion

Drought stress caused reductions in plants' growth, which could be used as a cultural control method for excessive plant growth. It is important for commercial greenhouse growers to predict plant growth responses to different SMCs, thereby determining their minimal water requirement. In two landscape roses investigated, all plants survived at four SMCs ranging from 10 to 40% except for two plants in one container (one for RADrazz and one for Belinda's Dream) at 10% SMC. In both cultivars, plants at 30 and 40% SMC maintained the highest shoot and root DW, flower number, midday ψ, and P_n. However, due to excessive irrigation at 40% SMC, algal growth observed on substrate surfaces would negatively impact plant aesthetic appearance and cause other management issues. There was no significant difference in P_n between SMC of 20% and 30% and between 30% and 40%, which could be due to the lack of differences in midday ψ among these SMCs. Total water applied at 30%, 20% and 10% SMC was reduced by 31%, 70%, and 90%, respectively, compared to 40% SMC. Plants had excellent performance at 30% SMC and acceptable growth and quality at 20% SMC. The 10% SMC led to significant growth reduction, poor visual quality (leaf wilt and drop), and 25% mortality. Results showed that soil moisture sensor based automatic irrigation systems may be utilized to conserve water consumption in greenhouse container production and quality plants of these two garden roses can be grown at reduced SMC during greenhouse production.

CHAPTER IV

RESPONSE OF SIX GARDEN ROSES TO SALT STRESS*

4.1 Synopsis

A greenhouse study was conducted to evaluate six garden roses ('Caldwell Pink', 'Carefree Delight', 'Marie Pavie', 'New Dawn', 'RADrazz', and 'The Fairy') in response to salt stress. Plants grown in containers were subjected to three salinity levels at electrical conductivity (EC) of 1.5 (control, nutrient solution), 4.0 or 8.0 $dS{\cdot}m^{-1}$ (moderate and high salinity levels). Compared to the control, shoot growth at moderate and high salinity levels decreased in all cultivars except for 'New Dawn'. 'Caldwell Pink', 'Marie Pavie', and 'The Fairy' had greatest shoot growth reduction (65% to 69%) at 8.0 $dS{\cdot}m^{-1}$ EC, followed by 'Carefree Delight' and 'RADrazz'. Flower numbers decreased at high and moderate salinity levels in all cultivars except 'New Dawn' and 'RADrazz'. In 'Marie Pavie', 'RADrazz', and 'The Fairy', no differences were found in leaf stomatal conductance between 1.5 and 4.0 $dS{\cdot}m^{-1}$ EC, and it was reduced at 8.0 $dS{\cdot}m^{-1}$ EC in all cultivars. 'Caldwell Pink' and 'The Fairy' had greatest reductions in leaf stomatal conductance at 8.0 $dS{\cdot}m^{-1}$ EC, followed by 'Carefree Delight', 'Marie Pavie', 'New Dawn', and 'RADrazz'. The maximal photo-chemical efficiency of photosystem II (PSII) decreased as salinity increased to 8.0 $dS{\cdot}m^{-1}$, which was highest in 'New Dawn' and lowest in 'Caldwell Pink'. As salinity increased, leaf sodium (Na^+) and chloride (Cl^-) concentrations increased in all cultivars, and they were highest in

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‘Caldwell Pink’, ‘Marie Pavie’ and ‘The Fairy’. The six rose cultivars responded differently to increasing salt stress. ‘New Dawn’ was considered to be more salt tolerant, while ‘Caldwell Pink’, ‘Marie Pavie’, and ‘The Fairy’ were less tolerant to salt compared to the other cultivars.

4.2 Introduction

Soil salinity is a major constraint to common economic crops in many arid and semi-arid regions of the world, which affects plants through osmotic, specific ion and oxidative stresses (Pitman and Lauchli, 2002). Salt stress could affect plant growth in different ways, such as decreasing water uptake, accumulating ions to toxic levels, and reducing nutrient availability (Tunçtürk et al., 2011). With increasing concern for reduction in plant productivity due to salinity, screening and identifying salt tolerant plant species is becoming increasingly important for breeders to incorporate desirable traits into economically useful plants. Many plant species have developed different mechanisms to tolerate salt stress, including osmotic stress tolerance, sodium (Na^+) or chloride (Cl^-) exclusion, and the tolerance of tissue to accumulated Na^+ or Cl^- (Munns and Tester, 2008).

Garden roses (*Rosa* × *hybrida* L.) are some of the most popular flowering plants in the world, and their widespread cultivation and versatility led to the diversity in color and forms of flowers, plant habit, and environmental adaptability. Roses are generally sensitive to salinity that exceeds 3.0 $dS \cdot m^{-1}$ electrical conductivity (EC), but some rose cultivars can tolerate up to an EC of 3.5 $dS \cdot m^{-1}$ without reduction in yield and quality (Cabrera, 2003). Irrigation waters with high salinity are dominated primarily by Na^+ and

Cl^-. Many salinity studies on horticultural crops have widely used sodium chloride (NaCl) as the sole salinizing agent, but calcium (Ca^{2+}) can also be a major contributor to the total solution EC (Grattan and Grieve, 1999). The excessive concentration of ions in saline soil is toxic to vegetative growth, root development, and flowering.

Some researchers have studied the responses of rose rootstocks and greenhouse cut roses to salt stresses (Cabrera and Perdomo, 2003; Cabrera et al., 2009; Niu and Rodriguez, 2008; Wahome et al., 2001). Wahome et al. (2001b) found that *Rosa chinensis* 'Major' and *R. rubiginosa* had leaf injury as EC increased from 0 to 3.0 $dS \cdot m^{-1}$. High concentrations of Cl^- in the leaves caused leaf injury, because the transport of chloride ions occurred in the transpiration stream (Marschner, 1993). As a result, plant growth decreased due to reduced photosynthetic active area. Four rose rootstocks ['Dr. Huey' (*Rosa* × *hybrida* L.), *R.* × *fortuniana* Lindl., *R. multiflora* Thunb., and *R. odorata* (Andr.) Sweet] showed growth reductions with elevated salinities in the irrigation water (Niu and Rodriguez, 2008). At high salinities (7.9 to 8.2 $dS \cdot m^{-1}$ EC), all rootstocks had salt damage on lower and older leaves, with high leaf Cl^- concentration. High salinity also reduced relative chlorophyll concentration and maximal photo-chemical efficiency of photosystem II (PSII) (F_v/F_m, $F_v = F_m - F_o$; F_m = maximum fluorescence F_o = minimum fluorescence) under elevated salinities (Niu and Rodriguez, 2008). In a study by Cabrera et al. (2009), greenhouse roses grafted on various rootstocks showed decreased biomass, cut flower production, and foliage quality as salt level increased. Compared with 'Bridal White' grafted on *R. odorata*, 'Natal Briar', and 'Dr. Huey', the 'Bridal White' grafted on 'Manetti' was more tolerant to salt stress with its higher biomass and flower yields and showed less toxicity symptoms under elevated salinity levels. Based on the aesthetic responses and harvested foliage, they reported that the greenhouse roses could withstand Na^+ and Cl^- concentrations up to 10 ± 2 mmol·L^{-1} (Cabrera et al., 2009). Cabrera and Perdomo (2003) also found that flower and dry weight of 'Bridal Pink' budded on 'Manetti' were not significantly affected by salinity concentrations ranging from 0 to 10 mmol·L^{-1} NaCl.

Using salt tolerant garden roses is important in urban landscapes in arid and semiarid areas where soil salinity is high due to poor quality irrigation water, high evaporation, and insufficient rainfall for leaching. In the western part of the United States, many landscapes have switched to or plan to use recycled wastewater or non-potable saline waters for irrigation (Qian et al., 2005), and the salinity is usually two to three times higher than potable water (Khurram and Miyamoto, 2005). Low quality irrigation water has been used for golf courses and some horticultural productions, which could result in salt damage on some sensitive plants (Niu et al., 2010c). Our objectives were to investigate the relative salt tolerance of six garden roses ('Caldwell Pink', 'Carefree Delight', 'Marie Pavie', 'New Dawn', 'RADrazz', and 'The Fairy') by evaluating the response of growth, leaf stomatal conductance, chlorophyll fluorescence, and tissue mineral content of these rose cultivars to a range of salt stress. Such evaluations could help breeders and bioengineers improve salt tolerance of garden roses.

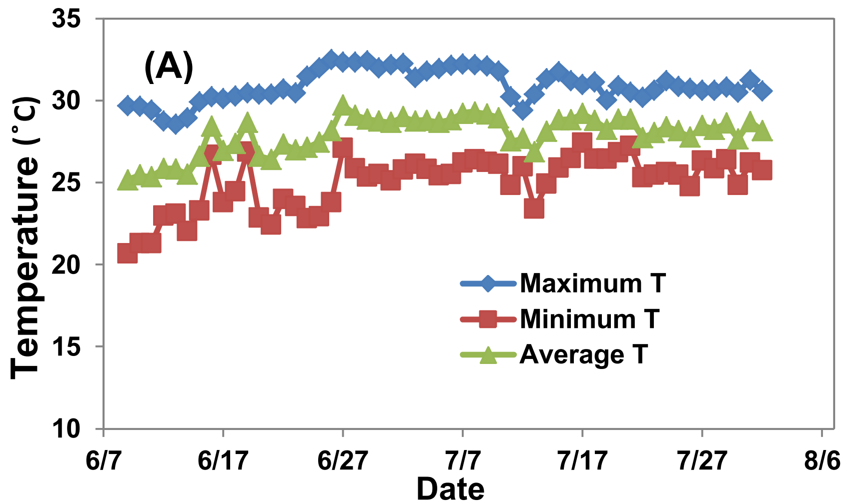
4.3 Materials and Methods

4.3.1 Plant Materials and Culture

Rooted cuttings of 'Caldwell Pink' (small shrub, 1.9 to 2.5 cm diameter flower size with light pink coloration), 'Marie Pavie' (small shrub, around 4.4cm diameter flower size with soft-pink to white coloration), 'New Dawn' (vigorous climber, around 7.6 cm diameter flower size with pink coloration), 'The Fairy' (dwarf shrub, 1.9 to 2.5 cm diameter flower size with light pink coloration), 'RADrazz' (medium shrub, medium flower size with cherry red coloration), and 'Carefree Delight' (spreading shrub, 3.8 to 5.1 cm diameter flower size with deep rich pink coloration) were purchased from Greenheart Farm (Greenheart, Arroyo Grande, CA). Cuttings were transplanted to 2.6-L plastic pots (15.4 cm diameter) filled with a 1:1 mix (volume) of Sunshine Professional Growing Mix No. 4 (SunGro Horticulture, Bellevue, WA) and composted mulch (Western Organics, Tempe, AZ). The media was amended with 5 $kg{\cdot}m^{-3}$ dolomitic limestone (Carl Pool Earth-Safe Organics, Gladewater, TX) and 1 $kg{\cdot}m^{-3}$ Micromax (Scotts, Marysville, OH) on 23 May, 2011. During the establishment stage (21 days), plants were grown in the greenhouse and well irrigated with a nutrient solution, which was made by adding 0.75 $g{\cdot}L^{-1}$ of 15N-2.2P-12.5 K (Peters 15-5-15; Scotts, Marysville, Ohio) to tap water.

Although the cultivars used for the study are relatively pest-free in the landscape, in this greenhouse study, plant foliage was washed with soapy water periodically to control spider mites. Greenhouse temperatures were controlled by a pad-and-fan cooling system. During the experiment period, the average air temperature in the greenhouse was

30.9±1.1 °C during the day and 25.1±1.6 °C at night. The average daily light integral (DLI) was 18.9±2.1 mol·m^{-2}·d^{-1}, and the average relative humidity (RH) was 38.9±9.9% during the experimental period (Fig. 13).



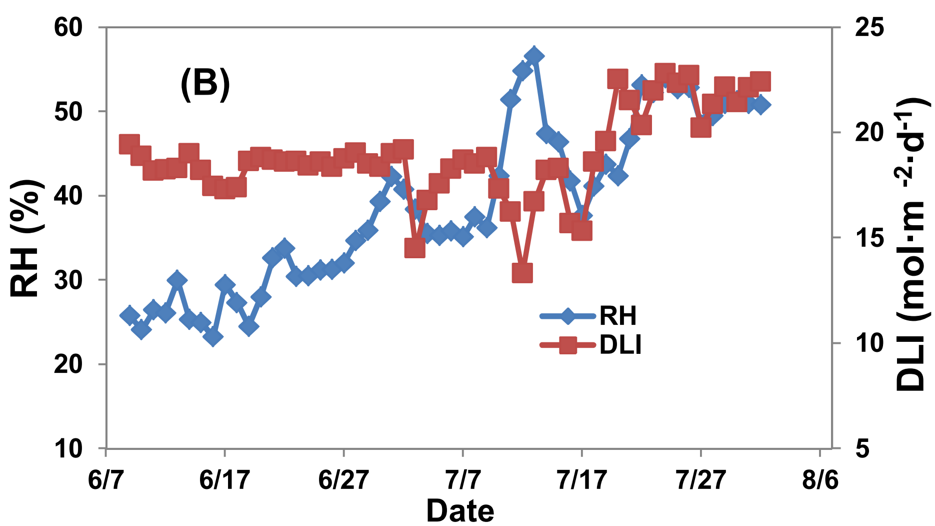


Figure 13. Variation of temperature (T) (°C) (maximum, minimum, and average) (A), daily light integral (DLI) (mol·m^{-2}·d^{-1}), and relative humidity (RH) (%) (B) in the greenhouse during the experimental period in 2011. Day and night air temperature and light in the greenhouse was measured every hour and averaged into daily measurements at plant canopy level.

4.3.2 Salt Treatment

Uniform plants of each cultivar were selected and divided into three irrigation treatment groups: EC=1.5 $dS \cdot m^{-1}$ (nutrient solution, control), EC = 4.0 and 8.0 $dS \cdot m^{-1}$. Saline solutions were prepared by adding NaCl and calcium chloride ($CaCl_2$) at 2:1 molar ratio to a nutrient solution. The nutrient solution was made by adding 0.75 $g \cdot L^{-1}$ of 15N-2.2P-12.5K to tap water. The major ions in the tap water were Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} at 184.0, 52.0, 7.5, 223.6, and 105.6 $mg \cdot L^{-1}$, respectively. A 100-L tank of saline solution was prepared each time with confirmed EC for each treatment. Each treatment contained 8 plants per cultivar. Before the initiation of treatments, plants in each cultivar were pruned to uniform heights and rotated around the bench weekly to reduce variability. Irrigation treatment was initiated on 9 Jun., 2011 and ended on 1 Aug., 2011. Plants were irrigated manually with 1000 mL treatment solution for all plants in the same treatment, which resulted in a leaching fraction of approximately 30 %. Irrigation frequency was three or four times a week during the treatment period depending on weather, treatment, and growth stage.

4.3.3 Measurements

The EC of leachate was determined by pour-through methods on 4 plants per treatment per cultivar using a salinity meter (Model B-173; Horiba, Ltd., Kyoto, Japan) every week (Wright, 1986). Leaf stomatal conductance was measured on young mature leaves using a leaf-porometer (SC-1 Decagon Devices, Inc., Pullman, WA) every week during the treatment period. This measurement was performed between 10:00 and

13:00 $_{HR}$. Leaf chlorophyll fluorescence was estimated in the morning on young mature leaves using a Plant Efficiency Analyzer (Handy PEA; Hansatech Instruments Ltd., Kings Lynn, U.K.) one week before the end of the experiment. Minimal fluorescence (F_o), maximum fluorescence (F_m), and the maximal photochemical efficiency of PSII (F_v/F_m, $F_v = F_m – F_o$) were measured to examine the effect of elevated salinity on leaf photosynthetic apparatus among the cultivars.

At the end of the experiment, leaf and stem fresh weights were determined, and flower number was recorded. Dry weight (DW) of leaves and stems was determined after being oven-dried at 70 °C to constant weights. To analyze leaf Na^+ and Cl^- concentrations, three of the 8 samples of leaves per treatment per cultivar were randomly selected. Dried tissue was ground with a stainless Wiley mill and submitted to the Soil, Water, and Air Testing laboratory (Las Cruces, NM) for Na^+ and Cl^- analyses.

4.3.4 Experimental Design and Statistical Analysis

The experiment utilized a split-plot design with the salinity treatment as the main plot and six cultivars as the subplot with 8 replications per treatment for each cultivar. A two-way analysis of variance (ANOVA) procedure was used to test the effects of soil salinity and cultivar on plant growth. When there was an interaction between treatment and cultivar, means were separated in three treatments of each cultivar by Student-Newman-Keuls multiple comparison at $P = 0.05$. When the interaction was not significant, data were pooled across salinity treatment or cultivar. All statistical analyses were performed using SAS (SAS Institute, 2009).

4.4 Results and Discussion

4.4.1 Pruned Dry Weight (DW), Flower Number, and Leachate Electrical Conductivity

There were interactions between salinity treatment and rose cultivar for shoot DW and flower number. Compared to control (1.5 $dS{\cdot}m^{-1}$) plants, shoot DW decreased in all cultivars except 'New Dawn' as salinity level increased to 4.0 and 8.0 $dS{\cdot}m^{-1}$(Fig. 14). At moderate salinity level (4.0 $dS{\cdot}m^{-1}$), 'Caldwell Pink', 'Carefree Delight', 'Marie Pavie', 'RADrazz', and 'The Fairy' had reductions of 40.8%, 35.7%, 40.3%, 27.9%, and 22.5% in shoot DW, respectively. At high salinity level (8.0 $dS{\cdot}m^{-1}$), shoot DW of these five cultivars was reduced by 67.9%, 40.9%, 69.3%, 51.9%, and 63.1%, respectively (Fig. 14). At high and moderate salinity levels, flower number decreased in all cultivars except 'New Dawn' and 'RADrazz' (Fig. 15). At high salinity levels, 'Caldwell Pink', 'Carefree Delight', 'Marie Pavie', and 'The Fairy' had flower reductions of 76.6%, 55.3%, 42.3%, and 72.7%, respectively (Fig. 15).

Cultivar did not affect leachate EC, and data were pooled across cultivars (Fig. 16). Fourteen days after the initiation of the treatment, the leachate EC for control (1.5 $dS{\cdot}m^{-1}$) plants was 3.0 $dS{\cdot}m^{-1}$ higher than that of the irrigation solution for control, while it was 5.0 and 10.0 $dS{\cdot}m^{-1}$ higher than that of the irrigation solution for 4.0 and 8.0 $dS{\cdot}m^{-1}$ treated plants, respectively (Fig. 16). Because plants at 5.0 and 10.0 $dS{\cdot}m^{-1}$ were irrigated with treatment solution three times a week during the first two weeks of the treatment, excessive salts were accumulated in the root zone, which caused the acute increases in leachate EC in plants at moderate and high salinity levels (Fig. 16). All plants were then irrigated with nutrient solution for the next two irrigation cycles to prevent excessive salt accumulation. Four weeks after the initiation of the treatment, the leachate EC was similar to the irrigation solution in all treatments, and it increased linearly during the experimental period (Fig. 16).

Most crops showed plant growth reductions at salinity levels above threshold levels (Maas, 1986), and the threshold varies with species and cultivars. In a study by Niu et al. (2008a), *R.* × *fortuniana* had smaller shoot growth reductions than *R. multiflora* and *R. odorata* at the elevated salinities. In the current study, shoot growth and flower production did not decrease for 'New Dawn' at moderate and high salinity levels (4.0 and 8.0 $dS{\cdot}m^{-1}$), while 'Caldwell Pink', 'Marie Pavie', and 'The Fairy' had greatest growth reduction at 8.0 $dS{\cdot}m^{-1}$ (Fig. 14, 15). In a study by Cabrera and Perdomo (2003), flower and dry weight of 'Bridal Pink' (on *R.* 'Manetti' rootstock) roses were not affected by EC up to 7.0 $dS{\cdot}m^{-1}$. After long exposure to moderate and high salinity levels, many crop species showed severe salt damage symptoms of scorching and necrosis around the leaf margins and ultimate leaf drop, causing plant death eventually (Marschner, 1993). We also observed severe foliar damage in 'Marie Pavie' and 'The Fairy' at 4.0 and 8.0 $dS{\cdot}m^{-1}$ (data not shown). In miniature roses (*Rosa* × *hybrida* L. 'Red Imp') cultivated in vitro, flower numbers decreased significantly at EC of 5.0 and 10.0 $dS{\cdot}m^{-1}$, which was due to their susceptibility to salt during reproductive developmental processes (Cha-um and Kirdmanee, 2010). Because of the negative effect of salt stress on the osmotic potential in the culture media, the uptake of water and some mineral nutrients were restricted. Thus, plant growth and development were inhibited, as well as a series of metabolic functions. In the current study, the least growth reduction observed in ‘New Dawn’ under high salinity level, which showed its better adaptation to salt stress compared to the other five cultivars. Greatest reductions in shoot growth and flower number were observed in ‘Caldwell Pink’, ‘Marie Pavie’, and ‘The Fairy’ under salt stress (Fig. 14, 15). Our results agreed with those in Niu et al. (2013). They reported large reductions of shoot DW and flower number in ‘Caldwell Pink’ at 6.4 $dS \cdot m^{-1}$ EC, and less tolerance to salinity at 4.4 and 6.4 $dS \cdot m^{-1}$ EC as evidenced by shoot DW reduction and leaf tip burn in ‘Marie Pavie’ and ‘The Fairy’ compared to ‘Basye’s Blueberry’, ‘Iceberg’, ‘Little Buckaroo’, ‘Rise N Shine’, and ‘Sea Foam’. With reduced flower numbers at elevated salinity levels in all rose cultivars except ‘New Dawn’ and ‘RADrazz’, they were still marketable, because no changes of flower size, coloration, and longevity were observed in plants treated with salinity stress (data not shown).

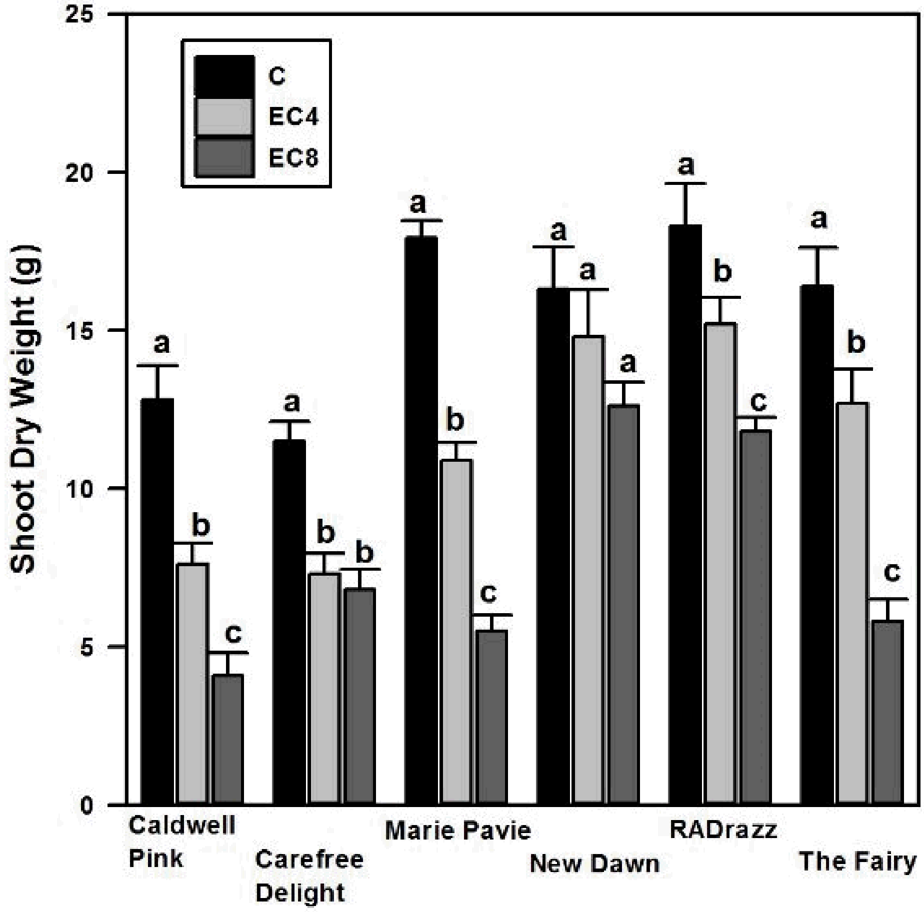


Figure 14. Effect of electrical conductivity (EC) of irrigation water on shoot dry weight (DW) of 'Caldwell Pink', 'Carefree Beauty', 'Marie Pavie', 'New Dawn', 'RADrazz', and 'The Fairy'. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. Vertical bars represent standard error (SE). C represents EC at 1.5 $dS{\cdot}m^{-1}$; EC4 represents EC at 4.0 $dS{\cdot}m^{-1}$; EC8 represents EC at 8.0 $dS{\cdot}m^{-1}$.

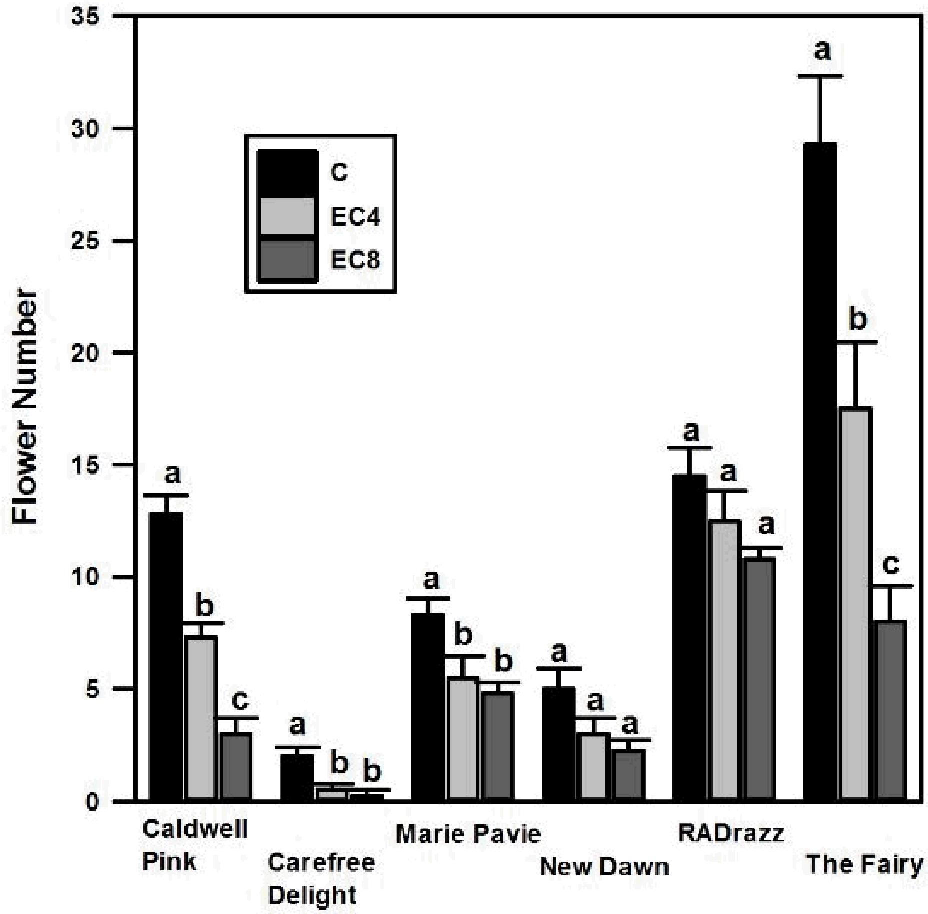


Figure 15. Effect of electrical conductivity (EC) of irrigation water on flower number of 'Caldwell Pink', 'Carefree Beauty', 'Marie Pavie', 'New Dawn', 'RADrazz', and 'The Fairy'. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. Vertical bars represent standard error (SE). C represents EC at 1.5 $dS \cdot m^{-1}$; EC4 represents EC at 4.0 $dS \cdot m^{-1}$; EC8 represents EC at 8.0 $dS \cdot m^{-1}$.

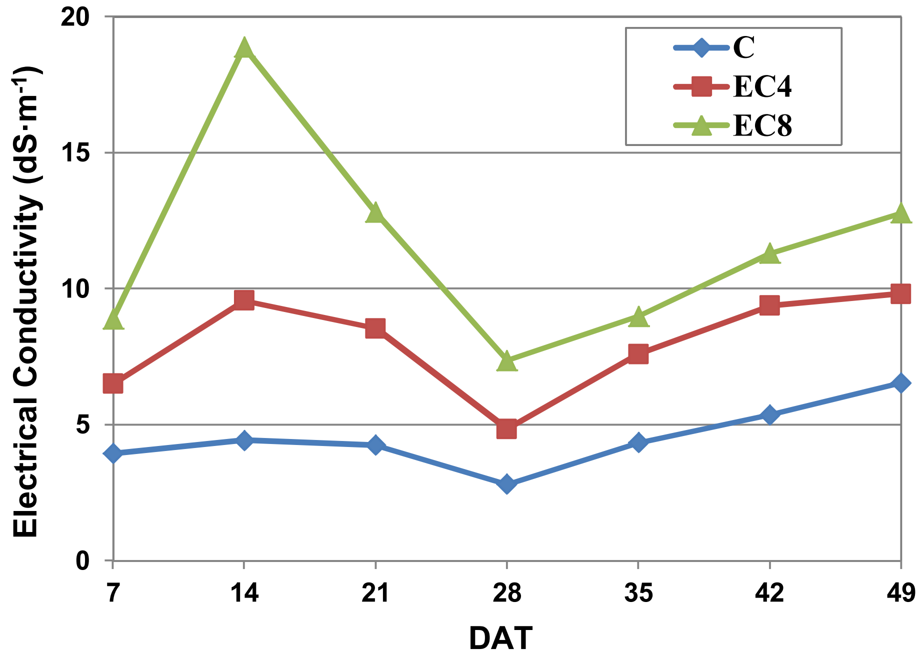


Figure 16. Variation of weekly leachate electrical conductivity (EC) during the experimental period in 2011. DAT represents days after treatment. C represents EC at 1.5 dS·m^{-1}; EC4 represents EC at 4.0 dS·m^{-1}; EC8 represents EC at 8.0 dS·m^{-1}.

4.4.2 Leaf Stomatal Conductance and Chlorophyll Fluorescence

There was an interactive effect between salinity treatment and cultivar on leaf stomatal conductance. As salinity levels increased to 8.0 dS·m^{-1}, stomatal conductance of 'Caldwell Pink', 'The Fairy', 'Marie Pavie', 'Carefree Delight', 'New Dawn', and 'RADrazz' was reduced by 35.9%, 32.9%, 26.5%, 23.1%, 22.6% and 19.4%, respectively (Fig. 17). No significant difference in stomatal conductance was found between 1.5 and 4.0 dS·m^{-1} in 'RADrazz', 'Marie Pavie', and 'The Fairy' (Fig. 17).

No interactive effect between salinity treatment and cultivar was found for F_m and F_v / F_m. Among the six cultivars, 'New Dawn' had greatest F_m and F_v / F_m , and

‘Caldwell Pink’ had lowest values of F_m and F_v / F_m at elevated salinity levels; however, F_v / F_m of ‘Caldwell Pink’ was only 2.2% lower than that of ‘New Dawn (Fig. 18). There was no significant difference in F_v / F_m between 1.5 and 4.0 $dS \cdot m^{-1}$, while the reduction of F_v / F_m was only 1.1% at 8.0 $dS \cdot m^{-1}$ (Fig. 18). Photochemical damage is reflected in decreases in F_m or F_v / F_m (Thomas and Turner, 2001). In this study, there was a decrease of 1.1% in F_v / F_m at high salinity level. Niu et al. (2008a) also reported that the F_v / F_m was reduced by 2.4% compared to control in *Rosa* × *fortuniana*, *R. multiflora*, and *R. odorata* at 3.0 and 6.0 $dS \cdot m^{-1}$. The small decrease in photochemical activity at elevated salinity stress may be due to the measurement on the fully expanded new leaf, instead of the same leaf on different days. The effectiveness of this measurement depends on species and salinity levels. Zribi et al. (2009) found that there was no reduction in F_v / F_m in tomato (*Solanum lycopersicum* L.) leaves treated with salinity up to 200 mM NaCl in dark-adapted leaves, while the F_v / F_m was reduced in salt-affected plants in light adapted leaves.

Stomatal conductance is a sensitive indicator of osmotic stress due to a rapid initial response of stomatal closure to salt stress (James et al., 2008). Severe reduction in stomatal conductance is an adaptive mechanism to tolerate excessive salt (Koyro, 2006). There is a close relationship between stomatal conductance and growth rates (James et al., 2008; Rahnama et al., 2010). Rahnama et al. (2010) reported that salt tolerant genotypes of wheat (*Triticum aestivum* L.) showed higher growth rate and stomatal conductance at elevated salinity compared to salt-sensitive genotypes, indicating their higher assimilation rate. In a study by Niu et al. (2010a), stomatal conductance decreased in all varieties of chile peppers (*Capsicum annuum* L.) except for 'NuMex Sweet' and 'Santa Fe Grande' at high salinity levels. Lycoskoufis et al. (2005) also reported that stomatal conductance of *C. annuum* 'Elisa' was reduced at high salinity (8.0 dS·m^{-1}), thereby inhibiting photosynthesis.

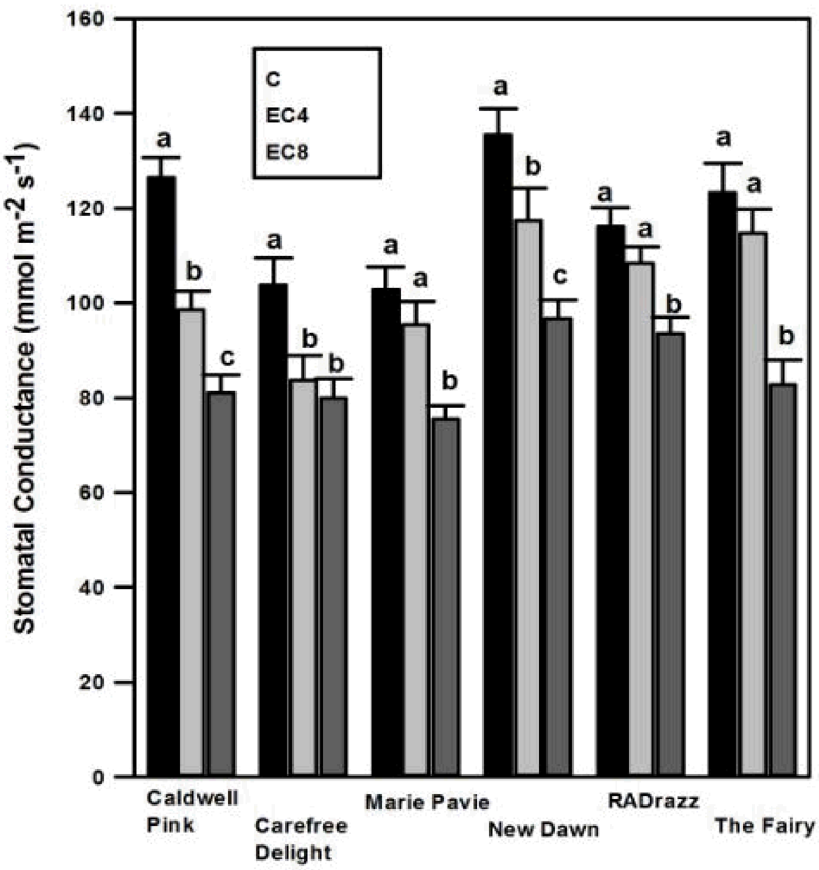


Figure 17. Effect of electrical conductivity (EC) of irrigation water on leaf stomatal conductance of 'Caldwell Pink', 'Carefree Beauty', 'Marie Pavie', 'New Dawn', 'RADrazz', and 'The Fairy'. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. Vertical bars represent standard error (SE). C represents EC at 1.5 dS·m^{-1}; EC4 represents EC at 4.0 dS·m^{-1}; EC8 represents EC at 8.0 dS·m^{-1}.

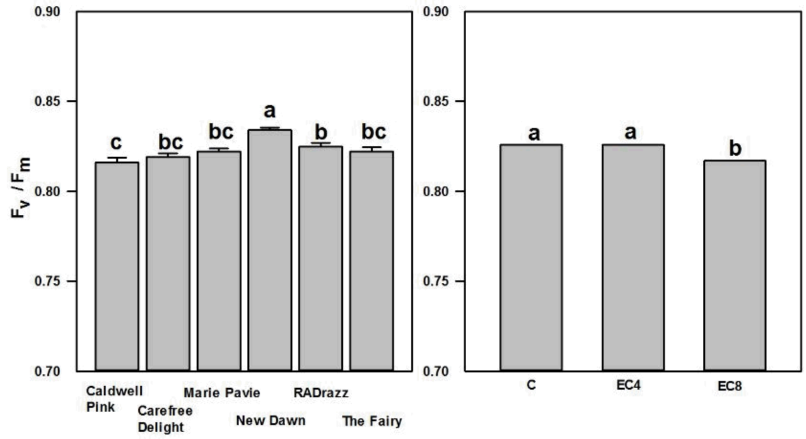


Figure 18. Effect of electrical conductivity (EC) of irrigation water on chlorophyll fluorescence of 'Caldwell Pink', 'Carefree Beauty', 'Marie Pavie', 'New Dawn', 'RADrazz', and 'The Fairy'. Left chart shows data obtained from control, EC4 and EC8 treatments across all cultivars, and right chart shows average data of six cultivars across 3 treatments. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. Vertical bars represent standard error (SE). C represents EC at 1.5 dS·m^{-1}; EC4 represents EC at 4.0 dS·m^{-1}; EC8 represents EC at 8.0 dS·m^{-1}.

4.4.3 Leaf Sodium and Chloride Uptake

There were interactive effects of salinity treatment and cultivar on leaf Na^+ and Cl^- concentrations. Leaf Na^+ and Cl^- concentrations increased in all cultivars as salinity levels increased. At EC of 8.0 dS·m^{-1}, 'Caldwell Pink', 'The Fairy', and 'Marie Pavie' had greatest leaf Na^+ and Cl^- concentrations (Fig. 19). The leaf Cl^- concentration was much higher than that of the Na^+ concentration (Fig.19). As the salinity of the irrigation solution increased, 'Carefree Delight', 'New Dawn', and 'RADrazz' had lower leaf Na^+ and Cl^- concentrations compared to the other cultivars (Fig. 19). There were no significant differences of leaf Ca^{2+} concentrations among the cultivars and treatments (data not shown).

Salt stress enhances the accumulation of leaf Na^+ and Cl^- ions, thereby reducing plant growth rate. Minimizing the ion uptake by the roots and ion accumulation in the shoots are important mechanisms of salt tolerance. Al-Karaki et al. (2009) reported that a more salt tolerant sweet pepper cultivar, 'Fla-viano', had lower Na^+ and Cl^- concentrations, compared to two other cultivars 'Sonar' and 'Alzado', indicating its higher ability to exclude Na^+ and Cl^-. In this study, 'Caldwell Pink', 'The Fairy', and 'Marie Pavie' had lower Na^+ exclusion ability than the other cultivars evidenced by higher Na^+ concentrations in leaves (Fig. 19). These three cultivars also had highest Cl^- concentrations among the six cultivars, which may be the cause of the severe leaf injury we observed (data not shown). 'Carefree Delight', 'New Dawn' and 'RADrazz' showed less leaf Na^+ and Cl^- concentrations as salt level increased, indicating their higher ability to exclude Na^+ and Cl^- (Fig. 19).

The mechanisms of salt tolerance in rose cultivars have been reported in some studies (Cabrera and Perdomo, 2003; Niu et al., 2008a; Wahome et al., 2001). Cabrera and Perdomo (2003) reported that leaf Na^+ concentrations were not affected by NaCl application, while leaf Cl^- concentrations increased significantly with increased salt addition and ranged from 1.0 to 17.5 $g{\cdot}kg^{-1}$ in 'Bridal Pink' roses (budded on *R. manetti* rootstock). Niu et al. (2008a) found that the salt tolerant *R.* × *fortuniana* had less leaf Na^+ and Cl^- concentrations than *R. multiflora* and *R. odorada*, while less salt-tolerant *R. multiflora* had higher leaf Cl^- concentration under elevated salinity levels. Wahome et al. (2001) reported that the lower leaves of less salt-tolerant *R. chinensis* 'Major' had higher Na^+ concentration than in all other parts. Salt tolerant *R. rubiginosa* retained Na^+ in the roots, thus preventing the ion from accumulating in the stems and leaves (Wahome et al., 2001).

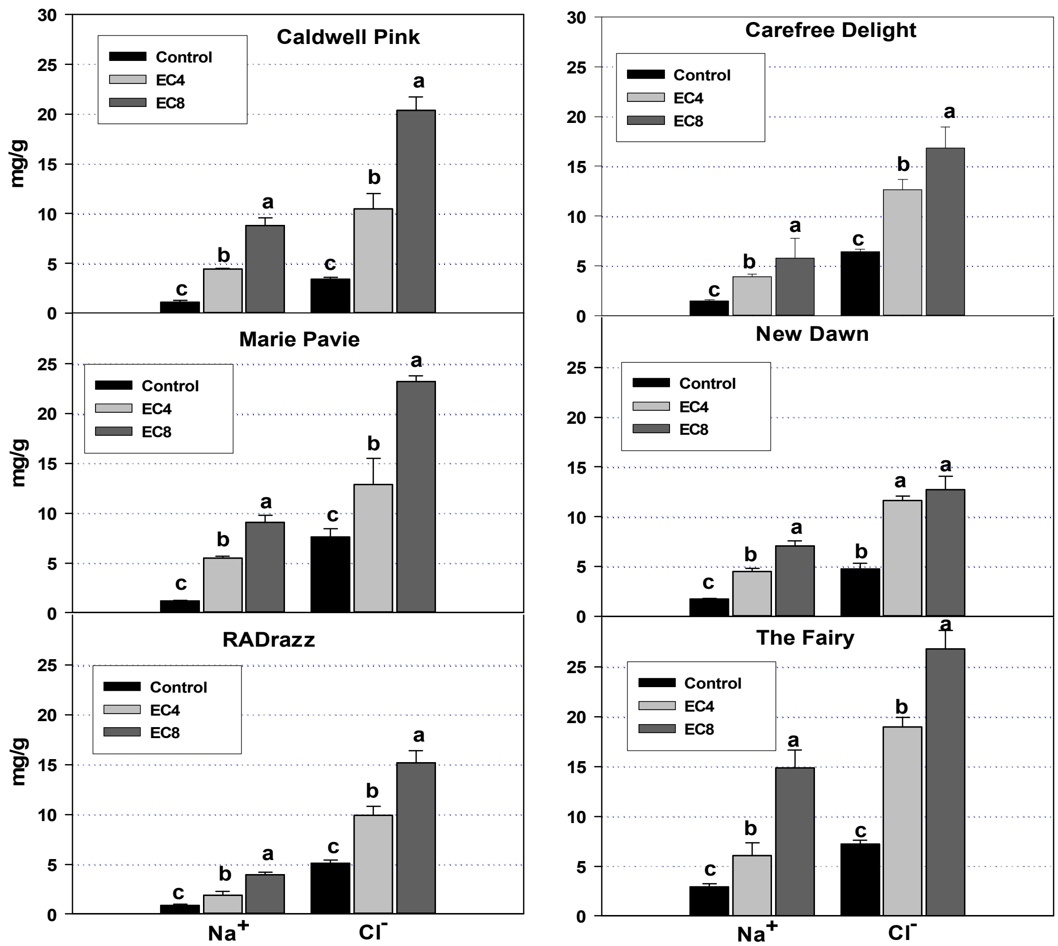


Figure 19. Effect of electrical conductivity (EC) of irrigation water on leaf sodium (Na^+) and chloride (Cl^-) concentrations 'Caldwell Pink', 'Carefree Beauty', 'Marie Pavie', 'New Dawn', 'RADrazz', and 'The Fairy'. Means within each cultivar followed by the same letter are not significantly different, tested by *Student–Newman–Keuls* (SNK) multiple comparison at $P = 0.05$. Vertical bars represent standard error (SE). Control represents EC at 1.5 $dS \cdot m^{-1}$; EC4 represents EC at 4.0 $dS \cdot m^{-1}$; EC8 represents EC at 8.0 $dS \cdot m^{-1}$.

4.5 Conclusion

At elevated salinity levels, plants had reductions in shoot growth and flower number in all cultivars except 'New Dawn'. 'Caldwell Pink', 'Marie Pavie', and 'The Fairy' had greatest growth reductions as salinity levels increased. In response to high salinity levels, stomatal conductance decreased in all cultivars. The differential accumulation of Na^+ and Cl^- in leaf tissues in response to elevated salt levels among the cultivars resulted in their growth differences. 'New Dawn' was more salt tolerant, followed by 'RADrazz' and 'Carefree Delight', with its least reductions in shoot DW and flower number and the lowest leaf Na^+ and Cl^- concentrations at elevated salt levels. 'Caldwell Pink', 'Marie Pavie', and 'The Fairy' had the greatest reduction in shoot growth, flower number, stomatal conductance and the highest leaf Na^+ and Cl^- concentrations at elevated salt levels, and they were the least salt tolerant among the cultivars investigated.

CHAPTER V

RESPONSES OF 18 EARTH-KIND® ROSE CULTIVARS TO SALT STRESS*

5.1 Synopsis

Earth-Kind® is a special designation given to select rose cultivars with superior stress tolerance (heat, drought and pest tolerance) and outstanding landscape performance. The responses of Earth-Kind® roses to high salinity stress are unknown. A greenhouse study was conducted to evaluate 18 Earth-Kind® rose cultivars (‘Belinda’s Dream’, ‘Cecile Brunner’, ‘Climbing Pinkie’, ‘Ducher’, ‘Duchesse de Brabant’, ‘Else Poulsen’, ‘Georgetown Tea’, ‘La Marne’, ‘Madame Antoine Mari’, ‘Marie Daly’, ‘Monsieur Tillier’, ‘Mrs. Dudley Cross’, ‘Mutabilis’, ‘Perle d’Or’, ‘Reve d’Or’, ‘Sea Foam’, ‘Souvenir de St. Anne’s’, and ‘Spice’) in College Station and 10 out of the same 18 cultivars in El Paso in response to two salinity levels at electrical conductivity (EC) of 1.2 (control, nutrient solution) and 10.0 $dS\cdot m^{-1}$ (EC 10). In both locations, ‘Belinda’s Dream’ and ‘Climbing Pinkie’ in EC 10 had no or little reduction in shoot growth, flower number, and leaf SPAD readings. The net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration (E) did not decrease in these two cultivars at EC 10 in El Paso. In College Station, ‘Mrs. Dudley Cross’, ‘Reve d’Or’, and ‘Sea Foam’ in EC 10 also had no or little reduction in shoot growth, flower number , and leaf SPAD readings. In both locations, ‘Cecile Brunner’ and ‘Else Poulsen’ in EC 10 had severe

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visual foliar salt damage, and they had the greatest reductions in shoot growth and flower number. In addition to these two cultivars, the lowest relative shoot dry weight (DW) and flower number were observed in ‘Madame Antoine Mari’, ‘Perle d’Or’, ‘Spice’, and ‘Souvenir de St. Anne’s’ in College Station. In summary, ‘Belinda’s Dream’, ‘Climbing Pinkie’, ‘ Mrs. Dudley Cross’, ‘Reve d’Or’, and ‘Sea Foam’ were the most salt tolerant cultivars, while ‘Cecile Brunner’, ‘Else Poulsen’, ‘Madame Antoine Mari’, ‘Perle d’Or’, ‘Spice’, and ‘Souvenir de St. Anne’s’ were the least salt tolerant among the cultivars investigated.

5.2 Introduction

With rapid increases in urban populations and industrial development, the availability of fresh water for landscape irrigation will be limited in the future. Therefore, alternative water sources, such as reclaimed water, are becoming commonly used to irrigate urban landscapes and agricultural crops (Niu and Rodriguez, 2008). Reclaimed water contains high levels of soluble salts, inducing salt stress to plants. High soil salinity is the result of low rainfall and high evapotranspiration in arid and semi-arid regions, while it is due to de-icing salts in northern areas (Niu et al., 2013). Increasing soil salinity negatively affects plant physiological and biochemical mechanisms that are associated with plant growth and development. Thus, screening and identifying salt tolerant plant species is becoming increasingly important, which could permit the use of low quality water and conserve higher quality water for other purposes.

Sodium (Na^+) and chloride (Cl^-) are usually the most prevalent ions in saline water, which may cause deleterious effects in plants such as necrosis and leaf edge burn

(Wahome et al., 2001). Excessive Na^+ and Cl^- uptake competes with the uptake of other nutrient ions, such as potassium (K^+), calcium (Ca^{2+}), or nitrogen (N), resulting in nutritional disorders and reduced yield and plant quality (Grattan and Grieve, 1999). High soil salinity also causes reduction in soil water potential, inhibiting plants' ability to extract water from the soil and maintain turgor. In addition, high ionic concentration can disturb membrane integrity and function, internal solute balance, and nutrient uptake, and it affects plant growth, water relations, and photosynthesis (Grattan and Grieve, 1999). Increased sodium chloride (NaCl) levels resulted in a reduction in shoot, root, and leaf biomass and an increase in root/shoot ratio, which were reported in cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr.], and alfalfa (*Medicago sativa* L.) (Berstein and Ogata, 1966; Kant et al., 1994; Meloni et al., 2001).

Plants had various salt tolerance mechanisms, including ion exclusion, maximizing Na^+ efflux from roots and its recirculation out of shoots and intercellular compartments, maintaining high cytosolic K^+/Na^+ ratio, or accumulation of optimal amount of compatible solute (Tester and Devenport, 2003). Salt tolerant plants usually have less adverse effects on foliar salt injury and growth and yield reduction at elevated salinity. The relative salt tolerance among multiple cultivars based on their growth and physiological responses at elevated salinity levels has been studied in greenhouse and garden roses, and rose rootstocks (Cabrera et al., 2009; Niu and Rodriguez, 2008; Niu et al., 2008a; Niu et al., 2013). As irrigation salinity increased from 1.4 to 6.4 $dS \cdot m^{-1}$, there was no or little visual damage in salt tolerant rose cultivars, 'Little Buckaroo', 'Sea Foam', and 'Rise N Shine', and shoot dry weight (DW) of these cultivars was not affected by salt stress (Niu et al., 2013). In a study by Niu et al., (2008a), *R ×fortuniana* was relatively more salt tolerant than the other two rootstocks, *R. odorata* and *R. multiflora,* with smaller growth reductions and higher visual quality at elevated salinities.

Garden roses (*Rosa* × *hybrida* L.) are some of the most economically important flowering shrubs in the world. Generally, roses are salt sensitive species with reduction in yield and quality at salinity levels that exceed electrical conductivity (EC) of 3.0 $dS \cdot m^{-1}$ (Urban, 2003). Earth-Kind® is a special designation given to select rose cultivars by the Texas AgriLife Extension Service through the Earth-Kind® landscaping program. These roses are trialed in large outdoor field plots in a location with typical conditions (MacKay et al., 2008). During years of testing, no pesticides and chemical or organic were applied to the research and trial roses. Based on actual recorded field data, best rose cultivars are selected by conformational trials throughout the region in various soil types, ranging from acid sands to highly alkaline clays (MacKay et al., 2008). The Earth-Kind® Rose Trials help to serve the horticulture community by identifying the most adaptable landscape roses (Harp et al., 2009). These roses exhibit consistent and superior pest tolerance combined with outstanding landscape performance, with minimum fertilizer, water, and pesticides. However, salt stress was not a factor considered during the evaluation process. There is little science-based knowledge about the responses of the Earth-Kind® roses to high salinity levels.

Using salt tolerant garden roses is important in urban landscapes in areas where soil salinity is high or irrigation water quality is poor. Our objectives were to compare the relative salt tolerance in 18 Earth-Kind® rose cultivars (‘Belinda’s Dream’, ‘Cecile Brunner’, ‘Climbing Pinkie’, ‘Ducher’, ‘Duchesse de Brabant’, ‘Else Poulsen’, ‘Georgetown Tea’, ‘La Marne’, ‘Madame Antoine Mari’, ‘Marie Daly’, ‘Monsieur Tillier’, ‘Mrs. Dudley Cross’, ‘Mutabilis’, ‘Perle d’Or’, ‘Reve d’Or’, ‘Sea Foam’, ‘Souvenir de St. Anne’s’, and ‘Spice’) in College Station, TX and 10 out of the same 18 Earth-Kind® rose cultivars replicated in El Paso, TX, and to determine the visual quality, shoot growth, flower number and DW, chlorophyll content, and gas exchange of these rose cultivars to elevated salinity in two locations, College Station and El Paso, TX.

5.3 Materials and Methods

5.3.1 Plant Materials and Culture

The study was conducted in two locations, College Station and El Paso, TX. In College Station, 18 cultivars of rose plants, ‘Belinda’s Dream’, ‘Cecile Brunner’, ‘Climbing Pinkie’, ‘Ducher’, ‘Duchesse de Brabant’, ‘Else Poulsen’, ‘Georgetown Tea’, ‘La Marne’, ‘Madame Antoine Mari’, ‘Marie Daly’, ‘Monsieur Tillier’, ‘Mrs. Dudley Cross’, ‘Mutabilis’, ‘Perle d’Or’, ‘Reve d’Or’, ‘Sea Foam’, ‘Souvenir de St. Anne’s’, and ‘Spice’, were shipped as potted plants from Chamblee’s Rose Nursery (Chamblee Rose Nursery, Inc., Tyler, TX) on 29 Jan. 2013. Larger growth habits of ‘Mrs. Dudley Cross’, ‘Mutabilis’, and ‘Reve d’Or’ required 7.57 - L plastic pots and the other 15 cultivars were in 3.79 - L plastic pots. Due to greenhouse space limitation in El Paso, 10 of the same 18 cultivars, ‘Belinda’s Dream’, ‘Cecile Brunner’, ‘Climbing Pinkie’, ‘Ducher’, ‘Duchesse de Brabant’, ‘Else Poulsen’, ‘Georgetown Tea’, ‘La Marne’, ‘Marie Daly’, and ‘Monsieur Tillier’ in 3.79 - L pots from the same nursery were used for the salinity tolerance study. The root substrate was a mix of sand, composted bark, and ground bark (1:1:1 by volume). Before initiating treatments, plants were grown in the greenhouse and well irrigated with a nutrient solution which was made by adding 1 $g \cdot L^{-1}$ of 15N-7.1P-14.1K (Peters 15-16-17; Scotts, Marysville, OH) or 1 $g \cdot L^{-1}$ 15N-2.2P-12.5K (Peters 15-5-15 Ca-Mg Special®; Scotts, Marysville, OH) to reverse osmosis (RO) water in College Station and El Paso, respectively. In addition, the plants in College Station were periodically watered with 5.15 $g \cdot L^{-1}$ Sequestrene® 138 (6% iron chelate) (Becker Underwood, Inc., Ames, IA) to prevent iron deficiency.

Although the cultivars used for the study are relatively pest-free in the landscape, in this greenhouse study, plant foliage was washed with soapy water and M-pede® (49% potassium salts of fatty acids; Dow AgroSciences LLC, Indianapolis, IN) periodically to control spider mites in College Station. In addition, AVID® 0.15 EC (2% Abamectin; Syngenta Crop Protection Inc., Greensboro, NC) and Talstar® (0.2% Bifenthrin; FMC Corporation Agricultural Products Group, Philadelphia, PA) were applied periodically to control spider mites in El Paso. Marathon® 1% G (1% Imidacloprid; OHP, Inc., Mainland, PA) was also applied to control aphids in both locations. Greenhouse temperatures were controlled by a pad-and-fan cooling system and gas heating system. During the experimental period in College Station, the average air temperature in the greenhouse was 24.9 °C during the day and 19.3 °C at night, the average daily light integral (DLI) was 21.6 $mol \cdot m^{-2} \cdot d^{-1}$, and the average relative humidity (RH) was 54.9%. In El Paso, the average air temperature in the greenhouse was 25.5 °C during the day and 21.7 °C at night. The average DLI was18.5 $mol \cdot m^{-2} \cdot d^{-1}$, and the average RH was 23.8%

during the experiment period. Prior to initiation of the treatment, all plants were pruned to a uniform height of 12.5 - cm and rotated around the bench weekly to reduce variability.

5.3.2 Salt Treatment

In both locations, uniform plants of each cultivar were selected and divided into two irrigation treatment groups: (1) control solution: EC = 1.2 $dS{\cdot}m^{-1}$ and, (2) saline solution: EC = 10.0 $dS{\cdot}m^{-1}$ (EC 10). By choosing this high salinity level, plants could have quick response to salt stress in a short-term study. There were 10 plants per treatment per cultivar, a total of 360 and 200 plants in College Station and El Paso, respectively. The 10.0 $dS{\cdot}m^{-1}$ saline solution was prepared by adding sodium chloride (NaCl) and calcium chloride ($CaCl_2$) at 2:1 molar ratio to the 1.2 $dS{\cdot}m^{-1}$ nutrient solution, with confirmation of EC by use of a salinity meter (Model B-173; Horiba, Ltd., Kyoto, Japan) . Irrigation treatment was initiated on 25 Feb. and ended on 8 Apr. 2013. Irrigation or saline solution was applied manually with 500 mL treatment solution for plants in 3.79 - L pots and 1000 mL for plants in 7.57 - L pots in the same treatment, which resulted in a leaching fraction of 30 to 50%. By irrigating consistent salinity solution, excessive salts were accumulated around root zone, which could cause severe root damage and leaf necrosis in a short term. To prevent excessive salt accumulation and make proper measurement, saline solution was applied only once a week with control solution at all other times.

5.3.3 Measurements

In both locations, the EC of leachate was determined on 2 or 3 plants per treatment per cultivar every week. At the end of the experiment, visual quality of the plants was assessed based on visual foliar salt damage (leaf edge burn, leaf necrosis, and leaf discoloration) on 7 or 10 plants per treatment per cultivar. Each plant was given a score of 1 to 5, where 1 = over 50% foliar damage (salt damage: burning and discoloring) or dead; 2 = moderate (25% to 50%) foliar damage; 3 = slight (<25%) foliage damage; 4 = good quality with little foliar damage (acceptable as landscape performance); 5 = excellent without foliar damage.

Leaf greenness index was measured by the non-destructive handheld chlorophyll meters [measured as the optical density, SPAD - 502 reading (Minolta Camera Co., Osaka, Japan)] at the end of the experiment on 4 or 6 leaves at similar positions for 5 or 10 plants per treatment per cultivar. The SPAD value ranged from 0 to 100 to estimate leaf chlorophyll content by measuring the light transmission at the wavelengths of 650 and 940 nm (Markwell et al., 1995). All plants had been watered prior to measurement.

Shoots were severed at the substrate surface at the end of the experiment. Total shoot length was determined by measuring all shoots of 7 or 10 plants per treatment per cultivar. Flower number was recorded, including buds, flowers, and dead flowers. Flowers, leaves and stems were harvested and dry weights (DW) of shoots and flowers were determined after oven-drying at 80 °C to a constant weight. Flower DWs were determined by collecting flower buds, open and dead flowers just below the hypanthium at the end of the experiment. To compare the effect of salt stress on the reduction of shoot growth and flowers, a relative value to the control treatment was calculated for each plant in the salt treatment. That is, relative total shoot length was calculated as:

$$Relative\ total\ shoot\ length = 100\% \times \frac{Total\ shoot\ length\ in\ salt\ treatment}{Averaged\ total\ shoot\ length\ in\ control}$$

Similarly, relative flower numbers, and shoot and flower DW were calculated.

In El Paso, instantaneous leaf gas exchange parameters including net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) were measured on 5 or 7 plants per treatment per cultivar at the end of the experiment. The measurement was taken by clipping a young, fully expanded leaflet into the leaf chamber (cuvette) of a portable gas exchange system (CIRAS-2; PP Systems, Amesbury, MA). The environmental conditions in the cuvette were controlled at 25 °C, 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF, and 375 $\mu mol \cdot mol^{-1}$ CO_2. Data were recorded when the environmental conditions and gas exchange parameters in the cuvette became stable. These measurements were taken between 10:00 and 12:00 $_{HR}$.

5.3.4 Experimental Design and Data Analysis

The experiment utilized a split-plot design with the salinity treatment as the main plot and cultivars as the subplot with 10 replications per treatment at both locations. A two-way analysis of variance (ANOVA) procedure was used to test the effects of salinity and cultivar on plant growth. When there was an interaction between treatment and cultivar, means were separated into two treatments of each cultivar by Student-Newman-Keuls multiple comparison at $P = 0.05$. When the interaction was not significant, data were pooled across salinity treatment or cultivar. Visual scores were analyzed by PROC

NPARIWAY, which was designed for non-parametric tests. All statistical analyses were performed using SAS (version 9.1.3; SAS Institute, Cary, NC).

5.4 Results and Discussion

5.4.1 Leachate Electrical Conductivity (EC)

In both locations, cultivar did not affect leachate EC, and data were pooled across cultivars. The leachate EC slightly increased for plants treated with control solution (EC of irrigation solution at 1.2 dS·m^{-1}), and it ranged from 1.6 to 3.2 dS·m^{-1} and 1.6 to 2.9 dS·m^{-1} from beginning to end of the experiment in College Station and El Paso, respectively (Fig. 20). When plants were treated with 10.0 dS·m^{-1}, the leachate EC was 5.8 to 9.3 dS·m^{-1} and 5.9 to 10.0 dS·m^{-1} in College Station and El Paso, respectively, during the first two weeks of the treatment (Fig. 20). After three weeks of the treatment, the leachate EC for plants treated with10.0 dS·m^{-1} was 10.6 to 13.7 dS·m^{-1} and 10.3 to 13.4 dS·m^{-1} in College Station and El Paso, respectively (Fig. 20).

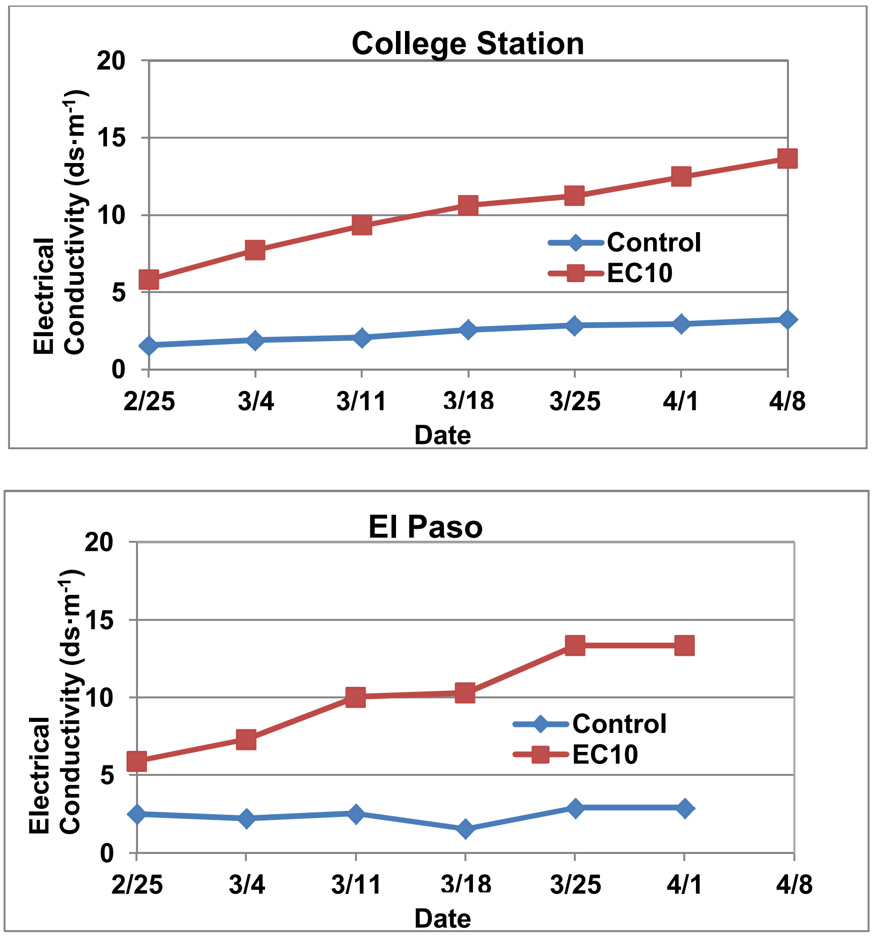

Figure 20. Variation of weekly leachate electrical conductivity (EC) during the experimental period in College Station and El Paso, TX in 2013. Control represents EC at 1.2 dS·m^{-1}; EC10 represents EC at 10.0 dS·m^{-1}.

5.4.2 Relative Shoot Dry Weight (DW) and Total Shoot Length

There was a strong positive correlation of relative shoot DW in 10 same rose cultivars between College Station and El Paso, TX, and the correlation coefficient is 0.90 (P=0.0004). In College Station, compared with the control solution, the saline solution did not reduce the relative shoot DW of ‘Reve d’Or’, and it had the highest relative total shoot length of 92% (Table 2). ‘Belinda’s Dream’, ‘Climbing Pinkie’, ‘Mrs. Dudley Cross’, and ‘Sea foam’ had a slight shoot DW reduction of 18%, 21%, 22%, and 20%, respectively (Table 2). In El Paso, ‘Belinda’s Dream’ and ‘Climbing Pinkie’ had the lowest shoot DW reduction of 25% and 22% compared with that of control, respectively (Table 3). ‘Climbing Pinkie’ also had the highest relative total shoot length of 89% (Table 3).

In College Station, ‘Madame Antoine Mari’ had the lowest relative shoot DW of 18%, indicating a shoot DW reduction of 82% compared with that of the control (Table 2). The great shoot DW reductions were also observed in ‘Cecile Brunner’, ‘Else Poulsen’, ‘Perle’ d’Or’, ‘Spice’, and ‘Souvenir de St. Anne’s’, and they had the relative shoot DW of 29%, 31%, 30%, 32%, and 36%, respectively (Table 2). ‘Cecile Brunner’ and ‘Souvenir de St. Anne’s’ had the lowest relative total shoot length of 23% and 31%, respectively (Table 2). In El Paso, ‘Cecile Brunner’ and ‘Else Poulsen’ had the lowest relative shoot DW of 35% and 38%, indicating a shoot DW reduction of 65 and 62% compared with that of the control (Table 3). These two cultivars also had the lowest relative total shoot length of 50% and 59% (50% and 41% reduction), respectively (Table 3).

Many studies have reported that shoot DW reductions were less in salt tolerant cultivars compared to salt sensitive ones at elevated salinity (Cassaniti et al., 2009; Niu and Rodriguez, 2006b; Niu and Rodriguez, 2008; Niu et al., 2008a). Niu et al. (2008a) found *R.* × *fortuniana* had smaller shoot growth reductions than *R. multiflora* and *R. odorata* as salinity increased from 1.6 to 6.0 $dS \cdot m^{-1}$. In a study by Marosz (2004), major shoot length and shoot DW of *Cotoneaster horizontalis* and *Potentilla fruticosa*

‘Longacre’ were not affected by the highest concentration of NaCl solution (EC=12 dS·m^{-1}), while there was a significant growth reduction in *C.* ‘Ursynow’ as salinity increased from 1.5 to 12.0 dS·m^{-1}. In the current study in two locations, there was no or little reduction of shoot DW and total shoot length for ‘Belinda’s Dream’ and ‘Climbing Pinkie’, while ‘Cecile Brunner’ and ‘Else Poulsen’ had the greatest shoot growth reduction at EC of 10.0 dS·m^{-1} (Tables 2, 3).

5.4.3 Relative Flower Number and Flower Dry Weight (DW)

There was a strong positive correlation of relative flower number in 10 same rose cultivars between College Station and El Paso, TX, and the correlation coefficient is 0.89 (P=0.0005). In College Station, ‘Climbing Pinkie’ had the highest relative flower number of 92%, indicating a flower number reduction of 8% compared with that of the control (Table 2). ‘Belinda’s Dream’ and ‘Sea Foam’ had the second highest relative flower number of 88% and 86%, respectively. These two cultivars also had the highest relative flower DW of 89% and 93%, indicating a flower DW reduction of 11% and 7% compared with that of the control, respectively (Table 2). With saline solution, ‘Mrs. Dudley Cross’ and ‘Reve d’Or’ had little reduction in flower number, representing a flower number reduction of 27% and 23%, respectively (Table 2). In El Paso, compared with the control, saline solution at EC of 10.0 dS·m^{-1} did not reduce the relative flower number and flower DW of ‘Climbing Pinkie’ (Table 3). ‘Belinda’s Dream’ had the relative flower number and flower DW of 83% and 97%, respectively (Table 3).

In College Station, the lowest relative flower number was observed in ‘Cecile Brunner’, ‘Spice’ and ‘Souvenir de St. Anne’s’, representing a flower number reduction of 57%, 62% and 61%, respectively (Table 2). ‘Cecile Brunner’, ‘Else Poulsen’, and ‘Souvenir de St. Anne’s’ had the lowest relative flower DW of 35%, 30%, and 38%, respectively (Table 2). In El Paso, the lowest relative flower number and flower DW were observed in ‘Cecile Brunner’ and ‘Else Poulsen’ (Table 3).

Under high salinity levels, flower buds may fail to open, or meristems and branches may die in flowering woody shrubs and trees (Azza Mazher et al., 2007). In a study by Niu et al. 2013, the number of flowers and buds was not affected by salinity treatment in ‘Belinda’s Dream’, ‘Rise N Shine’, and ‘Sea Foam’, while there was a significant reduction of flower numbers in ‘Basye’s Blueberry’, ‘Bucbi’, ‘Winter Sunset’, and ‘Marie Pavie’ as salinity level increased from 1.4 to 6.4 $dS{\cdot}m^{-1}$. Marosz (2004) found that flowering of *P. fruticosa* ‘Longacre’ was not affected by salinity treatment, while *C.*‘Urysynow’ and *C. horizontalis* did not flower at EC of 6.0 and 12.0 $dS{\cdot}m^{-1}$. Cabrera and Perdomo (2003) reported that flower number of *R. hybrida* ‘Bridal Pink’ (grafted on *R.* ‘Manetti’ rootstock) was not affected by EC up to 7.0 $dS{\cdot}m^{-1}$. Cut flower yield did not decrease for *R. hybrida* ‘Long Mercedes’ grafted on rootstock *R. indica* at EC of 2.5 $dS{\cdot}m^{-1}$ (Nirit et al., 2006). In the current study, flower number was not or little affected by saline solution for ‘Belinda’ Dream’, ‘Climbing Pinkie’, ‘Mrs. Dudley Cross’, ‘Reve d’Or’ and ‘Sea Foam’ in College Station, and for ‘Belinda’s Dream’ and ‘Climbing Pinkie’ in El Paso (Tables 2, 3). ‘Cecile Brunner’, ‘Spice’, and ‘Souvenir de St. Anne’s’ had the greatest reductions in flower number at EC of 10.0 $dS{\cdot}m^{-1}$ in College Station, while ‘Cecile Brunner’ and ‘Else Poulsen’ had the greatest reductions in flower number in El Paso (Tables 2, 3).

Table 2. Relative shoot dry weight (DW), total shoot length, flower number and flower DW of 18 Earth-Kind® rose cultivars (classified as salt tolerant, moderately tolerant, and intolerant) irrigated with saline solution at electrical conductivity (EC) of 10.0 $dS \cdot m^{-1}$ in College Station (n = 7).

Cultivars	Relative shoot DW (%)		Relative total shoot length (%)		Relative flower DW (%)		Relative flower DW (%)		Salt Tolerance
Belinda's Dream	82	ab[z]	67	c-f	88	ab	89	a	Tolerant
Climbing Pinkie	79	ab	79	bc	92	a	74	ab	Tolerant
Mrs. Dudley Cross	78	ab	64	c-f	73	abc	71	ab	Tolerant
Reve d'Or	100	a	92	a	77	abc	82	ab	Tolerant
Sea Foam	80	ab	71	b-e	86	ab	93	a	Tolerant
Ducher	41	b-h	51	d-g	65	abc	39	b	Moderate
Duchesse de Brabant	58	b-g	57	c-f	73	abc	61	ab	Moderate
Georgetown Tea	45	b-h	55	d-g	58	abc	49	ab	Moderate
La Marne	54	b-g	52	d-g	53	abc	35	b	Moderate
Mutabilis	55	b-g	54	c-f	72	abc	53	ab	Moderate
Marie Daly	42	b-g	54	d-g	55	abc	52	ab	Moderate
Monsieur Tillier	47	b-h	65	c-f	61	abc	59	ab	Moderate
Cecile Brunner	29	f-h	23	h	43	bc	35	b	Intolerant
Else Poulsen	31	f-h	43	e-h	48	abc	30	b	Intolerant
Madame Antoine Mari	18	h	39	f-h	50	abc	50	ab	Intolerant
Perle d'Or	30	f-h	42	e-h	67	abc	58	ab	Intolerant
Spice	32	e-h	47	e-h	38	c	51	ab	Intolerant
Souvenir de St. Anne's	36	d-h	31	gh	39	c	38	b	Intolerant

[z]Means with the same letters were not different tested by Student-Newman-Keuls multiple comparison at $P = 0.05$.

Table 3. Relative shoot dry weight (DW), total shoot length, flower number and flower DW of 10 Earth-Kind® rose cultivars (classified as salt tolerant, moderately tolerant, and intolerant) irrigated with saline solution at electrical conductivity (EC) of 10.0 dS·m^{-1} in El Paso (n = 10).

Cultivars	Relative shoot DW (%)		Relative total shoot length (%)		Relative flower number (%)		Relative flower DW (%)		Salt Tolerance
Belinda's Dream	75	a[z]	68	ab	83	ab	97	ab	Tolerant
Climbing Pinkie	78	a	89	a	100	a	100	a	Tolerant
Ducher	48	ab	68	ab	48	b	45	b	Moderate
Duchesse de Brabant	66	ab	75	ab	75	ab	79	ab	Moderate
La Marne	45	ab	76	ab	52	b	41	b	Moderate
Marie Daly	44	ab	69	ab	50	b	38	b	Moderate
Monsieur Tillier	64	ab	73	ab	69	ab	60	b	Moderate
Cecile Brunner	35	b	50	b	47	b	36	b	Intolerant
Else Poulsen	38	b	59	b	49	b	38	b	Intolerant

[z]Means with the same letters were not different tested by Student-Newman-Keuls multiple comparison at $P = 0.05$.

5.4.4 Visual Quality and Leaf Chlorophyll Content (SPAD Readings)

There was a strong positive correlation of visual quality score in 10 same rose cultivars between College Station and El Paso, TX, and the correlation coefficient is 0.85 (P=0.002). There were interactions between salinity treatment and rose cultivar for visual quality score and SPAD readings in both locations. With saline solution treatment, severe foliar salt damage such as leaf burn and necrosis on lower and old leaves was observed in many cultivars. In College Station, the plants irrigated with control solution did not have any foliar damage except 'La Marne', 'Mutabilis', 'Marie Daly', and 'Perle d'Or' (Table 4), because these four cultivars had some foliar damage from spider mites (data not shown). With saline solution, 'Mrs. Dudley Cross' and 'Reve d'Or' had the least foliar salt damage with an average visual score of 4.0 and 4.3, respectively (Table 4). 'Belinda's Dream', 'Climbing Pinkie', and 'Sea Foam' had an average visual score of 3.6, 3.6, and 3.7, respectively (Table 4). Relative chlorophyll contents measured as SPAD readings were not reduced by saline solution in 'Belinda's Dream', 'Climbing Pinkie', 'Mrs. Dudley Cross', 'Reve d'Or', and 'Sea Foam' (Table 4). In El Paso, with saline solution, 'Belinda's Dream' and 'Climbing Pinkie' had an average visual score of 3.3 and 3.7, respectively (Table 5). Leaf SPAD readings were not or little affected by saline solution for 'Belinda's Dream' and 'Climbing Pinkie' (Table 5).

In College Station, 'Cecile Brunner', 'Perle d'Or', and 'Spice' had the most severe foliar salt damage with an average visual score of 1.8, 1.2, and 1.4, respectively (Table 4). 'Else Poulsen', 'Madame Antoine Mari', and 'Souvenir de St. Anne's' had severe foliar salt damage with an average visual score of 2.4, 2, and 2.4, respectively (Table 4).

In El Paso, 'Cecile Brunner' and 'Else Poulsen' had severe foliar salt damage with an average visual score of 2.1 and 3, respectively (Table 5).

Plant salt tolerance can be assessed based on the degree of foliar salt damage (Niu and Cabrera, 2010). Elevated salinity stress leads to foliar injury, which causes reduction in visual quality. In a study by Niu et al. (2013), the rose cultivars of 'Carefree Beauty', 'Folksinger', and 'Winter Sunset' had severe visual damage at EC of 3.1 $dS{\cdot}m^{-1}$, while 'Belinda's Dream', 'Little Buckaroo', 'Rise N Shine', and 'Sea Foam' showed little or no visual damage at EC up to 6.4 $dS{\cdot}m^{-1}$. Niu et al. (2012a) found that there was little or no visual foliar damage at EC of 8.1 $dS{\cdot}m^{-1}$ in 'NuMex Cinco de Mayo', 'NuMex Thanksgiving', and 'NuMex Twilight' ornamental chile peppers (*Capsicum annuum*), while 'NuMex Memorial Day' had the most severe foliar damage at elevated salinity. In the current study, saline solution caused little visual salt damage in 'Mrs. Dudley Cross' and 'Reve d'Or' in College Station. We also observed severe foliar salt damage in 'Cecile Brunner' and 'Else Poulsen' in College Station and El Paso with saline solution treatment (Tables 4, 5).

Salt stress stimulates chlorophyll degradation, resulting in leaf chlorosis (Santos, 2004). Although there were no relationships between chlorophyll content and SPAD readings for rose species in previous studies, leaf SPAD readings have been a useful parameter in salinity tolerance evaluation (Niu et al., 2008a). Plants treated with saline solution were starting to senesce as a result of high salinity, evidenced by lower SPAD readings. In ten herbaceous perennials and groundcovers, SPAD readings were not affected by salinity treatment in *Gaillardia aristata* Pursh, *Lantana* × *hybrida*, *Lonicera* *japonica* Thunb., and *Verbena macdougalii* Heller, while less salt tolerant species, *Lantana montevidensis* (Spreng.) Brig. and *Glandularia.* × *hybrida* (Gronland & Rumpler) G.L. Nesom & Pruski, had reduced SPAD readings at elevated salinity stress (Niu et al., 2007b). Increasing salinity stress was also shown to decrease leaf SPAD readings in two cherry (*Prunus cerasus* L.) rootstocks (Sotiropoulos et al., 2006). In a study with rose by Niu et al. (2008a), leaf SPAD readings decreased as salinity level increased from 1.6 to 6.0 $dS \cdot m^{-1}$. The salt tolerant cultivar, *R.* × *fortuniana*, had higher leaf SPAD readings compared with two other rootstocks. In the current study, with saline solution treatment, leaf SPAD readings were not or little affected by saline solution for 'Belinda's Dream', 'Climbing Pinkie', 'Mrs. Dudley Cross', 'Reve d'Or', and 'Sea Foam' in College Station, or for 'Belinda's Dream' and 'Climbing Pinkie' in El Paso, indicating that their leaf chlorophyll contents were not affected by salinity. Therefore, these cultivars could maintain green leaves under saline solution treatment (Tables 4, 5).

Table 4. Visual foliar salt damage ratings and SPAD readings of 18 Earth-Kind® rose cultivars (classified as salt tolerant, moderately tolerant, and intolerant) irrigated with saline solution at electrical conductivity (EC) of 1.2 (control, nutrient solution) and 10.0 $dS \cdot m^{-1}$ in College Station (n = 7).

Cultivars	Visual score[y]		SPAD		Salt Tolerance
	Control	EC 10	Control	EC 10	
Belinda's Dream	4.6	3.6	49.7 a[z]	48.0 a	Tolerant
Climbing Pinkie	4.3	3.6	44.5 a	42.1 a	Tolerant
Mrs. Dudley Cross	4.7	4	57.1 a	55.6 a	Tolerant
Reve d'Or	5	4.3	52.5 a	50.6 a	Tolerant
Sea Foam	4.9	3.7	55.4 a	57.0 a	Tolerant
Ducher	4.8	2.6	49.8 a	40.8 b	Moderate
Duchesse de Brabant	4.9	3.5	53.4 a	44.6 b	Moderate
Georgetown Tea	4.8	3.2	52.8 a	42.3 b	Moderate
La Marne	3.7	2.4	54.1 a	40.8 b	Moderate
Mutabilis	3.8	3.2	52.6 a	41.2 b	Moderate
Marie Daly	3.9	2.2	50.9 a	37.6 b	Moderate
Monsieur Tillier	4.8	3.5	61.9 a	53.1 b	Moderate
Cecile Brunner	4.4	1.8	52.4 a	40.6 b	Intolerant
Else Poulsen	4.2	2.4	47.8 a	34.4 b	Intolerant
Madame Antoine Mari	4.6	2	54.5 a	45.4 b	Intolerant
Perle d'Or	3.9	1.2	45.7 a	36.8 b	Intolerant
Spice	4.2	1.4	49.5 a	44.2 b	Intolerant
Souvenir de St. Anne's	4.5	2.4	53.5 a	43.1 b	Intolerant

[y]1= over 50 % foliar damage (salt damage: burning and discoloring) or dead; 2 = moderate (25 %–50 %) foliar damage; 3 = slight (<25 %) foliage damage; 4 = good quality with little foliar damage (acceptable as landscape performance); 5 = excellent without foliar damage.

[z]Means with the same letters between treatments were not different tested by Student-Newman-Keuls multiple comparison at $P = 0.05$.

Table 5. Visual foliar salt damage ratings and SPAD readings of 10 Earth-Kind® rose cultivars (classified as salt tolerant, moderately tolerant, and intolerant) irrigated with saline solution at electrical conductivity (EC) of 1.2 (control, nutrient solution) and 10.0 dS•m^{-1} in El Paso (n=10).

Cultivars	Visual score[y]		SPAD		Salt Tolerance
	Control	EC 10	Control	EC 10	
Belinda's Dream	5	3.3	51.2 a[z]	47.3 b	Tolerant
Climbing Pinkie	5	3.7	41.5 a	37.2 a	Tolerant
Ducher	5	2.9	49.8 a	43.0 b	Moderate
Duchesse de Brabant	5	4.4	51.3 a	48.0 a	Moderate
Georgetown Tea	5	3.9	52.9 a	46.4 b	Moderate
La Marne	4.7	2.4	53.7 a	42.3 b	Moderate
Marie Daly	4	1.4	49.1 a	36.8 b	Moderate
Monsieur Tillier	5	3.9	56.6 a	51.6 a	Moderate
Cecile Brunner	4.5	2.1	51.9 a	38.1 b	Intolerant
Else Poulsen	4.9	3	45.0 a	37.0 b	Intolerant

[y]1= over 50 % foliar damage (salt damage: burning and discoloring) or dead; 2 = moderate (25 %–50 %) foliar damage; 3 = slight (<25 %) foliage damage; 4 = good quality with little foliar damage (acceptable as landscape performance); 5 = excellent without foliar damage.
[z]Means with the same letters between treatments were not different tested by Student-Newman-Keuls multiple comparison at $P = 0.05$.

5.4.5 Gas Exchange Rates

There were interactive effects of salinity treatment and cultivar on leaf gas exchange, net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E). At saline solution of 10.0 $dS{\cdot}m^{-1}$, the P_n, g_s, and E did not decrease in 'Belinda's Dream' and 'Climbing Pinkie' (Table 6). Compared with the control, the saline solution significantly reduced the P_n, g_s and E in 'Cecile Brunner', while 'Else Poulsen' had reduction in P_n with saline solution (Table 6).

At elevated soil salinities, leaf gas exchange decreased for most crops. At high salinity, leaf photosynthesis can be reduced by lowered stomatal conductance caused by toxic ions (Munns, 2002; Netondo et al., 2004). In a study by Niu et al. (2012b), salinity stress at EC of 8.0 $dS{\cdot}m^{-1}$ reduced leaf P_n, g_s, and E in four maize inbred lines (CUBA1, B73, B5C2, and BR1) and four sorghum hybrids (SS304, NK7829, Sordan79, and KS585). Herralde et al. (1998) reported that *Chrysanthemum coronopifolium* Vill had reduced stomatal conductance and photosynthetic rate under saline stress (15 days of exposure to 140 mm NaCl followed by a recovery period of 11 days), indicating that there was a toxic effect of salt concentration. In the current study in El Paso, high salinity at EC of 10.0 $dS{\cdot}m^{-1}$ did not decrease P_n, g_s, and E in 'Belinda's Dream' and 'Climbing Pinkie', while 'Cecile Brunner' had great reduction in P_n, g_s, and E (Table 6).

Table 6. Leaf gas exchange (net photosynthetic rate, P_n; stomatal conductance, g_s; transpiration rate, E) of 10 Earth-Kind® rose cultivars (classified as salt tolerant, moderately tolerant, and intolerant) irrigated with saline solution at electrical conductivity (EC) of 1.2 (control, nutrient solution) and 10.0 $dS \cdot m^{-1}$ in El Paso.

Cultivars	P_n		g_s		E		Salt
	Control	EC 10	Control	EC 10	Control	EC 10	Tolerance
Belinda's Dream	11.4 a[z]	11.1 a	183.3 a	240.7 a	2.9 a	3.3 a	Tolerant
Climbing Pinkie	11.9 a	9.9 a	164.6 a	140.8 a	2.7 a	2.6 a	Tolerant
Ducher	11.2 a	7.9 b	149.2 a	138.6 a	2.3 a	2.5 a	Moderate
Duchesse de Brabant	14.9 a	12.8 a	256.8 a	182.4 b	3.3 a	2.8 a	Moderate
Georgetown Tea	15.3 a	13.7 a	254.0 a	258.2 a	3.6 a	3.2 a	Moderate
La Marne	16.0 a	11.1 b	271.2 a	179.7 b	3.5 a	3.1 a	Moderate
Marie Daly	10.3 a	5.9 b	181.2 a	116.2 b	2.8 a	2.1 b	Moderate
Monsieur Tillier	18.0 a	15.3 b	362.2 a	335.0 a	4.3 a	4.4 a	Moderate
Cecile Brunner	11.7 a	6.2 b	234.3 a	119.3 b	3.1 a	2.3 b	Intolerant
Else Poulsen	13.1 a	9.5 b	205.0 a	197.2 a	3.1 a	3.1 a	Intolerant

[z]Means with the same letters between treatments were not different tested by Student-Newman-Keuls multiple comparison at $P = 0.05$.

5.5 Conclusion

In summary, salt tolerance of Earth-Kind® rose cultivars was consistent in two locations, with strong positive correlations of relative shoot DW, flower number, and visual quality score in 10 same cultivars between two locations.. In College Station and El Paso, 'Belinda's Dream' and 'Climbing Pinkie' had the highest relative shoot DW and flower number, and they had little or no reduction in SPAD readings at EC of 10.0 $dS{\cdot}m^{-1}$. In addition to these two cultivars, 'Mrs. Dudley Cross', 'Reve d'Or', and 'Sea Foam' also had the highest relative shoot DW and flower number in College Station. In both locations, 'Cecile Brunner' and 'Else Poulsen' had the lowest relative shoot DW and flower number. The lowest relative shoot DW and flower number were also observed in 'Madame Antoine Mari', 'Perle d'Or', 'Spice', and 'Souvenir de St. Anne's' in College Station. By comparing the growth and physiological responses at high concentrations of saline solution among the 18 cultivars in College Station and 10 cultivars in El Paso, 'Belinda's Dream', 'Climbing Pinkie', 'Mrs. Dudley Cross', 'Reve d'Or', and 'Sea Foam' were the most salt tolerant, followed by 'Duchesse de Brabant', 'Mutabilis', 'Monsieur Tillier', 'Georgetown Tea', 'Marie Daly', 'La Marne', and 'Ducher'. 'Cecile Brunner', 'Else Poulsen', 'Madame Antoine Mari, 'Perle d'Or, 'Spice', and 'Souvenir de St. Anne's' were the least salt tolerant among the cultivars investigated. Many landscapes have switched to use reclaimed water or no potable saline waters for irrigation. The typical salinity levels in reclaimed water are 1.3 to 2.0 $dS{\cdot}m^{-1}$. In areas with high soil salinity due to poor quality irrigation water, high evaporation, and insufficient rainfall for leaching, 'Belinda's Dream', 'Climbing Pinkie', 'Mrs. Dudley

Cross’, ‘Reve d’Or’, and ‘Sea Foam’ would be good selections for planting in landscapes, while ‘Cecile Brunner’ and ‘Else Poulsen’ are not recommended. Although Earth-Kind® roses are designated as rose cultivars with superior stress tolerance and outstanding landscape performance, some cultivars are not tolerant to poor quality irrigation water, such as ‘Cecile Brunner’, ‘Else Poulsen’, ‘Madame Antoine Mari, ‘Perle d’Or, ‘Spice’, and ‘Souvenir de St. Anne’s’. Because there was some foliage damage from spider mites in ‘La Marne’, ‘Mutabilis’, ‘Marie Daly’, and ‘Perle d’Or’ in College Station, further study may be needed to confirm the salt tolerance of these cultivars.

CHAPTER VI

SUMMARY OF FINDINGS

- Drought stress caused reductions in shoot and root dry weight (DW), flower numbers, photosynthetic rate (P_n), stomatal conductance (g_s), transpiration (E), and mid-day leaf water potential (ψ) in all four rose cultivars, 'RADrazz', 'Belinda's Dream', 'Old Blush', and 'Marie Pavie'.
- At substrate moisture content (SMC) between 10% and 20%, 'RADrazz' had smaller growth reduction and higher P_n, g_s, and E compared to the other cultivars under cyclic drought stress.
- At SMC between 10% and 20%, 'Marie Pavie' had the greatest growth reduction and lowest gas exchanges rates under water deficit conditions.
- 'RADrazz' was the most drought tolerant, followed by 'Old Blush' and 'Belinda's Dream', while 'Marie Pavie' was the least drought tolerant.
- 'Belinda' Dream' and 'RADrazz' could survive at SMC as low as 10%. Water application was reduced by 31%, 70%, and 90%, respectively, compared to 40% SMC.
- 'Belinda' Dream' and 'RADrazz' maintained the highest shoot and root DW, flower number, middy ψ, and P_n at 30 and 40% SMC. However, algal growth was observed at 40 % SMC due to excessive irrigation.
- Plants had excellent performance at 30% SMC and acceptable growth and quality at 20% SMC.

- The 10% SMC led to significant growth reduction, poor quality, and 25% mortality in ‘RADrazz’ and ‘Belinda’s Dream’.
- Elevated salinity stress caused reductions in shoot growth, flower number, and stomatal conductance in ‘Caldwell Pink’, ‘Carefree Beauty’, ‘Marie Pavie’, ‘RADrazz’, and ‘The Fairy’ except ‘New Dawn’.
- ‘New Dawn was the most salt tolerant, followed by ‘RADrazz’ and ‘Carefree Delight’, with least reductions in shoot DW and flower number and the lowest leaf Na^+ and Cl^- concentrations at elevated salt levels.
- ‘Caldwell Pink’, ‘Marie Pavie’, and ‘The Fairy’ had the greatest reduction in shoot growth, flower number, stomatal conductance and the highest leaf Na^+ and Cl^- concentrations at elevated salt levels, and they were the least salt tolerant among the cultivars investigated.
- In College Station, compared with the control solution, the saline solution did not reduce the relative shoot DW of ‘Reve d’Or’, and it had the highest relative total shoot length. ‘Belinda’s Dream’, ‘Climbing Pinkie’, ‘Mrs. Dudley Cross’, and ‘Sea foam’ had a slight shoot DW reduction.
- In El Paso, with saline solution, ‘Belinda’s Dream’ and ‘Climbing Pinkie’ had the lowest shoot DW reduction. ‘Climbing Pinkie’ also had the highest relative total shoot length.
- In College Station, with saline solution, ‘Madame Antoine Mari’ had the lowest relative shoot DW. ‘Cecile Brunner’, ‘El Poulsen’, ‘Perle’ d’Or’, ‘Spice’, and

‘Souvenir de St. Anne’s’ had the second lowest relative shoot DW. ‘Cecile Brunner’ and ‘Souvenir de St. Anne’s’ had the lowest relative total shoot length.

- In El Paso, with saline solution, ‘Cecile Brunner’ and ‘Else Poulsen’ had the lowest relative shoot DW. These two cultivars also had the lowest relative total shoot length.
- In College Station, with saline solution, ‘Climbing Pinkie’ had the highest relative flower number. ‘Belinda’s Dream’ and ‘Sea Foam’ had the second highest relative flower number. These two cultivars also had the highest relative flower DW. With saline solution, ‘Mrs. Dudley Cross’ and ‘Reve d’Or’ had small reductions in flower number.
- In El Paso, compared with the control, saline solution at EC of 10.0 $dS \cdot m^{-1}$ did not reduce the relative flower number and flower DW of ‘Climbing Pinkie’. ‘Belinda’s Dream’ had high relative flower numbers and flower DWs of 83% and 97%, respectively.
- In College Station, with saline solution, the lowest relative flower number was observed in ‘Cecile Brunner’, ‘Spice’ and ‘Souvenir de St. Anne’s’. ‘Cecile Brunner’, ‘Else Poulsen’, and ‘Souvenir de St. Anne’s’ had the lowest relative flower DW.
- In El Paso, with saline solution, the lowest relative flower number and flower DW was observed in ‘Cecile Brunner’ and ‘Else Poulsen’.

- In College Station, relative chlorophyll contents measured as SPAD readings were not reduced by saline solution in ‘Belinda’s Dream’, ‘Climbing Pinkie’, ‘Mrs. Dudley Cross’, ‘Reve d’Or’, and ‘Sea Foam’.
- In El Paso, leaf SPAD readings were not or little affected by saline solution for ‘Belinda’s Dream’ and ‘Climbing Pinkie’. At saline solution of 10.0 $dS \cdot m^{-1}$, the net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) did not decrease in ‘Belinda’s Dream’ and ‘Climbing Pinkie’. Compared with the control, the saline solution significantly reduced the P_n, g_s and E in ‘Cecile Brunner’.
- In summary, ‘Belinda’s Dream’, ‘Climbing Pinkie’, ‘ Mrs. Dudley Cross’, ‘Reve d’Or’, and ‘Sea Foam’ were the most salt tolerant cultivars, while ‘Cecile Brunner’, ‘Else Poulsen’, ‘Madame Antoine Mari, ‘Perle d’Or’, ‘Spice’, and ‘Souvenir de St. Anne’s’ were the least salt tolerant among the cultivars investigated.

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