

PARTIAL ROOTZONE DRYING IN FLORIDA CITRUS:  
PHYSIOLOGICAL RESPONSES

By

AYAKO KUSAKABE

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To my mom and dad

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## LIST OF ABBREVIATIONS

ABA	abscisic acid
[ABA]	abscisic acid concentration
$A_{CO_2}$	net assimilation of $CO_2$ ( $\mu\text{mol } CO_2/m^2/s$ )
BHT	2,6-di- <i>tert</i> -butylphenol
CHO	carbohydrates
$^{\circ}C$	degree Celsius
$C_i$	intercellular $CO_2$ concentration
$cm^2$	square centimeter
DAT	day after treatment
DW	dry weight
ELISA	enzyme-linked immunosorbent assay
$ET_c$	crop evapotranspiration
ET	evapotranspiration
FL	Florida
FW	fresh weight
FW/DW	fresh weight to dry weight ratio
g	gram
$g_s, g_{lf}$	stomatal conductance ( $\text{mmol } H_2O /m^2/s$ ), leaf conductance ( $\text{mmol } H_2O /m^2/s$ )
h	hour
HLB	Huanglongbing
L	liter
m, $m^2$	meter, square meter
ml	milliliter
mg	milligram

mM	millimolar
NaCl, Na <sup>+</sup> , Cl <sup>-</sup>	sodium chloride, sodium ion, chloride ion
N, N <sub>la</sub> , N <sub>m</sub>	nitrogen, N concentration on a mg cm <sup>-2</sup> leaf area (la) basis, N concentrations on a % dry mass basis
nmol	nanomolar
P-K	Phosphorous, and Potash (Potassium)
PRD, APRD, FPRD	partial rootzone drying, alternate partial rootzone drying, fixed partial rootzone drying
PAR	photosynthetically active radiation
pH	the power of hydrogen (a measure of the acidity or alkalinity of a solution)
PRS	partial rootzone drying with saline water
RDI	restricted deficit irrigation
RT:SH	root to shoot
SRL	specific root length (m/g)
TR	leaf transpiration
VPD	leaf-to-air vapor pressure difference (kPa)
WUE	water use efficiency
WW	well-watered controls
X-[ABA]	abscisic acid concentrations in xylem sap
Ψ, Ψ <sub>stem</sub> , Ψ <sub>lf</sub> , Ψ <sub>soil</sub>	water potential, stem water potential (MPa), leaf water potential (MPa), soil water potential
SS	salt stress
TR	transpiration (mmol H <sub>2</sub> O/m <sup>2</sup> /s)
%	percent

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Ayako Kusakabe

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Chair: James P. Syvertsen  
Cochair: Rebecca L. Darnell  
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The overall objective of the application of partial root zone drying (PRD) was to evaluate whether this mild drought stress could improve whole plant water use efficiency (WUE) and leaf WUE in potted citrus. Knowledge of how citrus trees cope with PRD can be the basis for successfully applying deficit irrigation. Therefore, the feasibility, risks or potential benefits of PRD with saline water or with good quality water were evaluated to determine the scope for water savings, changes in plant water relations, growth, WUE and nutritional responses. Results demonstrated that PRD with saline and with good quality water from the spring season to early summer, saved water and restricted plant growth while leaf nitrogen (N) was not limiting regardless of treatments. Roots in wet soil of PRD-treated plants did not enable PRD plants to maintain water status relative to well-watered (WW) plants. Whole plant WUE and root abscisic acid (ABA) at the end of the study were similar across treatments. Higher leaf ABA in PRD with saline water may have partly contributed to the lower stomatal conductance ( $g_s$ ), transpiration (TR), and water consumption compared with WW plants. Rates of net CO<sub>2</sub> assimilation ( $A_{CO_2}$ ) were reduced more than those of leaf TR in PRD-treated plants resulting in decreased leaf WUE. Therefore, PRD-treated plants resulted in water savings but drought stress reduced leaf function.

## CHAPTER 1 INTRODUCTION

### **Deficit Irrigation**

Drought stress (or water deficit) is one of the most important environmental factors inhibiting photosynthesis and limiting tree growth, crop yield and quality (Arbona et al., 2005). Most citrus production depends on inputs of irrigation water to obtain optimum tree growth, crop yield and quality. Irrigated agriculture is regarded as a major water user, equivalent to two thirds of the total fresh water used for human consumption (Fereres and Evans, 2006). Increased water utilization, mismanagement, and pollution are increasing the scarcity of good quality water (Petit et al., 1999). Therefore, efficient water use has become an important issue in recent years (Tang et al., 2005). Improvement in growth or yield per unit of water used, which is crop water use efficiency (WUE), is an ultimate goal in many agricultural systems. Shortages of available water call for optimum irrigation management and dictate the maximization of WUE for sustainable agricultural output (Wakrim et al., 2005). An instantaneous measure of WUE, leaf or photosynthetic WUE, focuses on the short-term efficiency of carbon gain per water loss or net  $\text{CO}_2$  assimilation ( $A_{\text{CO}_2}$ ) per leaf transpiration (TR). To the extent that  $A_{\text{CO}_2}$  can be related to yield and TR can be related to crop water use, leaf WUE ( $A_{\text{CO}_2}/\text{TR}$ ) has been used as an instantaneous surrogate estimate of longer term crop WUE (Bacon, 2004; Syvertsen et al., 2010). In general, a higher leaf WUE under mild water deficits results from partial stomatal closure with a concomitant relatively small reduction in  $A_{\text{CO}_2}$  (Chaves and Oliveira, 2004).

### **The Concept of Partial Rootzone Drying and Its Physiological Background**

One practice to conserve water is partial root zone drying (PRD), which is a deficit irrigation technique using controlled drought stress to improve WUE. PRD relies on a simple spatial separation of dry and wet roots (Liu et al., 2007; Shahnazari et al., 2007) that can be

easily maintained during an entire season and enables plants to grow with partially reduced stomatal conductance ( $g_s$ ) without great losses due to severe drought stress (Santos et al., 2003; Kang and Zhang, 2004). The PRD theory is based on 1) the utilization of a root stress-generated signal of abscisic acid (ABA) to regulate stomatal behavior, 2) a modification of plant growth, and 3) the prevention of physiological effects associated with severe drought stress (Davies and Zhang, 1991; Stoll et al., 2000; Davies et al., 2002; Kang and Zhang, 2004; Tahi et al., 2007). PRD methods are based on the assumption that half the root system in the dry side senses soil drying and produces ABA-mediated chemical signals traveling through the xylem from the roots to shoots that reduce  $g_s$ , and thereby limit vegetative growth (Davies et al., 2002; Bravo, 2005). Thus, partial stomatal closure can bring about a reduction in TR due to the linear relationship between TR and  $g_s$  (Kang et al., 2001). PRD plants can restrict water loss, simultaneously sustain  $A_{CO_2}$  rate at equivalent levels to well-watered (WW) plants (De Souza et al., 2003; Chaves and Oliveira, 2004) and perhaps increase leaf WUE (Bravdo, 2005) due to the non-linear relationship between photosynthesis and  $g_s$  (Düring et al., 1996).

In citrus, vegetative and fruit growth occur simultaneously and are not independent (Hutton et al., 2007). Long-lived citrus trees in subtropical climates can have continuous vegetative growth throughout the fruit production cycle. Therefore, reproductive development can compete with vegetative growth (Kredemann and Barrs, 1981). Severe plant water deficits can reduce water flow through the soil-plant system and can influence physiological and developmental processes including fruit production (Hsiao, 1973). Plant water deficits can limit growth due to inhibited cell division, cell expansion and photosynthetic capacity through stomata closure (Hutton et al., 2007). Using precise control of irrigation water, mild water stress maintained through PRD irrigation can reduce excess shoot vigor and competition for

carbohydrates (CHO) between growing shoot tips, roots and fruit and thereby, promote a shift in the CHO partitioning towards reproductive tissues. These changes in plant metabolism also may positively affect fruit yield and quality (Loveys et al., 2000).

If yields are not reduced during PRD, it offers the potential for higher WUE of crops compared to full irrigation (Stoll et al., 2000; Dos Santos et al., 2003). Although PRD irrigated crops require frequent applications of water to only part of the root zone (Stoll et al., 2000; Dos Santos et al., 2003; Leib et al., 2006), in many field studies, PRD irrigated roots also can experience partial soil drying with infrequent irrigation (Kirda et al., 2004).

From the viewpoint of citrus pest management, regulating vegetative growth using mild drought stress could aid in pest and disease control. Healthy citrus trees can grow an excess of leaves, more than that required to produce a good fruit crop (Yuan et al., 2005). New leaf flushes are susceptible to infection by citrus canker, feeding by citrus leaf miner and by the Asian citrus psyllid (*Diaphorina citri* Kuwayama), the vector of Huanglongbing (HLB, citrus greening). Fewer new flushes can limit Asian citrus psyllid reproduction and development (Rogers and Stansly, 2006). Thus, with limited new flushes, PRD may help keep pest populations under control and make it possible to use reduced doses of pesticides for pest suppression.

PRD can be either alternate PRD (APRD), a regular alternation of the wet and dry root zones, or fixed PRD (FPRD) where the wet and dry sides of the rootzone are consistently maintained during the growing season (Dodd, 2007). Typically, the most effective PRD irrigation can be usually achieved using APRD because it is often considered that an alternation of irrigation between the wet and dry soil columns is necessary for the maintenance of a continuously strong ABA signal from drying roots (Stoll et al., 2000; Liu et al., 2008).

Preferential water uptake from the wet soil and a corresponding decline in water uptake from the dry soil has been found during PRD irrigation (Liu et al., 2008). The drying roots in the APRD dry side diminished water uptake at a low soil water potential ( $\Psi_{\text{soil}}$ ), while the wet roots in the APRD wet side drastically increased water uptake (Yao et al., 2001). Therefore, the TR stream may decrease the ABA concentrations in xylem sap ([X-ABA]) due to the interaction of both effects (Green and Clothier, 1995; Yao et al., 2001). A rapid shift of preferential water uptake by the wet roots, which were previously drier roots in APRD, was observed in kiwifruit vines (Green and Clothier, 1995), bell pepper (Yao et al., 2001) and potato plants (Liu et al., 2008). Lower  $g_s$  was found in APRD-irrigated potato tubers compared to WW (Liu et al., 2006a) while  $g_s$  and X-ABA were negatively correlated in PRD irrigated potatoes during soil drying (Liu et al., 2005).

Soil columns should not be too thoroughly dried in order to avoid adverse effects on root physiology due to excessively low  $\Psi_{\text{soil}}$  and to sustain an active synthesis of ABA (Dodd, 2006). In PRD-treated grapevines, ABA signals were diminished due to prolonged drying of one side of the rootzone and  $g_s$  recovered to levels similar to the WW treatments (Stoll et al., 2000). Therefore, the stimulation of maximum ABA signalling would be important to achieve a maximum WUE through the optimization of irrigation techniques like APRD (Dodd et al., 2006). However, the timing still remains uncertain as to when the irrigation should be shifted between wet and dry sides.

PRD has only been successfully practiced in areas of low rainfall where soil water availability can be effectively controlled using irrigation (Hutton, 2004). Florida has summer rainfall in excess of citrus evapotranspiration (ET) requirements in summer whereas drought stress is common during the relatively dry spring and fall seasons (Garcia-Sanchez et al., 2007).

Therefore, the extent to which PRD can be beneficial in Florida citrus during the spring, fall and winter irrigation seasons needs to be investigated. Even though the high summer rainfall in Florida makes controlling soil water almost impossible during that time, there may be a potential to use fixed PRD where one half of the root zone is consistently irrigated depending on rainfall, while the other half receives no water in order to impose a limited amount of drought stress during summer.

### **The Use of Deficit Irrigation in Citrus Trees**

The effects of PRD irrigation have been investigated on different field crops (Kang and Zhang, 2004; Liu et al., 2006a, b; Shahnazari et al., 2007), fruit trees (Gencoglan et al., 2006; Spreer et al., 2007), and vegetables (Kirda et al., 2004; Dorji et al., 2005). There is a great potential for the enhancement of WUE and the maintenance of yield under PRD irrigation (Davies and Hartung, 2004). The improvement of yield and quality in fruits, vegetable and other crops by PRD has also been demonstrated (Sepaskhah and Kamgar-Haghighi, 1997; Dry et al., 2000; Zegbe-Dominguex et al., 2003; Zegbe et al., 2006; Leib et al., 2006; Shahnazari et al., 2007; Spreer et al., 2007). The effects of regulated deficit irrigation (RDI), where insufficient water is applied to the entire rootzone during the least sensitive periods of the phenological cycle (Chalmers et al., 1981; McCarthy et al., 2002; Loveys et al., 2004; Cameron et al., 2006), have been reported in citrus under Mediterranean-like climate conditions (Hutton et al., 2007; Perez-Perez et al., 2008; Perez-Perez et al., 2009).

An RDI strategy was compared to a WW control in southern Spain in a drip-irrigated orchard of mature 'Lane late' sweet orange trees grafted on Cleopatra (Cleo) and Carrizo rootstocks (Perez-Perez et al., 2008). RDI was used during phase I (initial fruit-growth period, just after bloom and fruit set) and phase III (final fruit ripening) of fruit development by

completely stopping irrigation. The RDI irrigated citrus trees saved 28 to 32% of irrigation water while there was only a marginal reduction in yield during the first two seasons. However, there was a significant yield reduction of about 16% in the final third season. Cleo has a lower hydraulic conductivity (Syvertsen and Graham, 1985) with a deeper and more densely branched root system than Carrizo (Hearn and Hutchinson, 1977) and may have had an earlier acclimation to drought stress than Carrizo (Castle and Krezdorn, 1977). In addition, Carrizo has more fibrous roots restricted to a smaller volume of soil profile than Cleo. Thus, Cleo rootstocks can be more drought-tolerant than Carrizo and can mitigate negative effects on vegetative development, yield, and fruit quality when DI is applied in semi-arid regions (Perez-Perez et al., 2008).

In 6-month-old potted citrus seedlings of Swingle citrumelo in the greenhouse, FPRD-50 (50%  $ET_c$ ) plants saved about 22% of water compared to WW plants (Melgar et al., 2010). The FPRD-50 treatment had no effect on plant growth except reduced stem DW. FPRD-50 plants had a greater root length than WW plants. Since FPRD-50 plants had lower values of leaf gas exchange than WW plants throughout the experiment.

A recent study in mature sweet orange grafted on Carrizo in southern Spain, used RDI treatments only during phase III (final fruit-growth period, ripening) to reduce water use by 14 to 20% compared to WW trees and had no significant effect on yield, fruit number or crop WUE (Perez-Perez et al., 2009). Similar results were reported for RDI irrigated 'Clemenules' mandarin (Ginester and Castle, 1996; Gonzalez-Altozano and Castel, 1999).

In mature 'Valencia' orange trees under southern Australian conditions, RDI treatments with irrigation intervals from 3 to 17 days during phase II (fruit enlargement stage) and phase III of fruit growth, saved about 15 to 36% of water relative to WW trees irrigated at 100%  $ET$  (Hutton et al., 2007). In general, vegetative growth in the Mediterranean climate of southern

Australia occurs only in late spring and in autumn. Both yield and fruit quality could be maintained using water deficits with the combination of high frequency irrigation during spring-early summer (every 3 days) and with low frequency of irrigation in late summer and autumn (every 17 days). The promotion of more blossoms in the subsequent spring for DI trees compared to WW trees, resulted in higher fruit set (Hutton et al., 2007). It appears that DI during summer reduced vegetative growth and water use, but there was a risk for a small reduction in fruit size. A number of authors have reported negative influences on fruit growth capacity when RDI was applied during phase II of fruit development (Goldhamer et al., 2002; Girona et al., 2005). The lack of consistent results in RDI experiments in citrus may be related to a lack of understanding of how water deficits influence physiological responses. There are still many physiological processes related to phenological sensitivity to water stress that are not fully understood (Marsal et al., 2006). A better understanding of the physiological basis behind PRD can facilitate adoption of more efficient irrigation practices, and therefore, offer a better understanding of the potential utility of PRD in FL citrus production. There is a lack of understanding of physiological and growth advantages or disadvantages of seasonal drought stress.

### **Irrigation Water Quality**

Although PRD may have the potential to improve WUE in citrus production wherever it is grown, PRD has only been beneficial when irrigation water is of good quality with low salinity (Dos Santos et al., 2003). The risks and/or potential benefits of PRD using saline irrigation water have not been studied. Salt stress has an adverse effect on crop water availability due to osmotic stress and toxicity of salt ions (Syvertsen et al., 1989). Any salt stress may induce differences in physiological responses to drought under PRD irrigation. PRD-grown plants may respond differently to salinity if one half of the root zone is consistently irrigated with saline water in contrast to part of the root system being irrigated with good quality water.

Recent FPRD experiments using slightly saline water on 15-year-old avocado trees grown in a commercial orchard in Australia (Neuhaus et al., 2009), showed that even though the FPRD saline treatments were only applied in the 1<sup>st</sup> season during phase I through III of fruit growth, the FPRD irrigated avocado trees did not flower strongly. These trees retained functioning leaves but had much fruit drop, and therefore, a reduced number of fruit per tree and reduced yields for two years. Two months after saline treatments began patterns of midday leaf conductance ( $g_{lf}$ ) of WW and FPRD treatments appeared similar. Both treatments had increased midday  $g_{lf}$  from 10:00 h to 14:00 h and  $g_{lf}$  fell to 25  $\text{mmol m}^{-2} \text{s}^{-1}$  at 1700 h. WW leaves increased the midday  $g_{lf}$  to 250  $\text{mmol m}^{-2} \text{s}^{-1}$  compared to 150 to 200  $\text{mmol m}^{-2} \text{s}^{-1}$  in FPRD leaves. Leaves above the FPRD wet side and FPRD dry side behaved similarly. Therefore, there apparently was no sectoring of water relations characteristics in the canopy above the contrasting irrigation treatments in the rootzone. These WW and FPRD trees showed the same water potential ( $\Psi$ ) of leaves, shoots and fruit at dawn. After two months, WW and FPRD trees had similar shoot growth, equivalent leaf tissue water content and fruit tissue water content at maturity. Leaf Na concentration in FPRD trees was 2 to 4 times higher than in WW, but was well below the toxic excess range. In contrast, both WW and FPRD trees had excessive leaf water Cl. Interestingly, leaf water Cl in FPRD dry side was slightly higher than FPRD wet side so soil water deficit for 7 months with saline water may have negatively impacted roots in FPRD. Although no  $A_{CO_2}$  and leaf WUE was reported in this study, periodically measured leaf photosynthetic WUE and physiological responses using PRD with and without saline water could confirm the risks and/or potential benefits of PRD under these conditions. There is no information on PRD irrigated citrus in Florida with either high quality or saline water. Prior to

achieving increased WUE without the risk of yield loss during water deficit stress in the field, the potential scope for water savings needs to be understood.

### **Objectives**

The overall objective of the application of partial root zone drying (PRD) was to evaluate whether this mild drought stress could improve whole plant and leaf WUE in potted citrus. Knowledge of how citrus trees cope with PRD can be the basis for successfully applying deficit irrigation. Therefore, the feasibility, risks or potential benefits of PRD with and without saline water was evaluated to determine the scope for water savings, plant water relations, growth, WUE and nutritional responses. It was hypothesized that roots in wet soil can maintain a favorable plant water status while the remaining roots in dry soil will promote the synthesis of ABA signals leading to reduced water loss, reduced vegetative growth and increased WUE with saline and /or with good quality water compared to well-watered control plants.

## CHAPTER 2 PARTIAL ROOTZONE DRYING AND SALINITY

### Overview

Efficient water use by crops has become an important issue in recent years (Tang et al., 2005). Improvement in growth or yield per unit of water used, which is crop water use efficiency (WUE), is an ultimate goal in many agricultural systems. Shortages of available water call for optimum irrigation management and dictate the maximization of WUE of field crops for sustainable agricultural output (Wakrim et al., 2005). Among the deficit irrigation strategies to conserve water, partial rootzone drying (PRD) is an irrigation technique successfully used in other crops that uses controlled drought stress to increase WUE. PRD relies on a simple spatial separation of dry and wet roots (Liu et al., 2007; Shahnazari et al., 2007) that can be easily maintained during an entire season. PRD can be either alternated (APRD), where the wet and dry root zones are regularly alternated, or fixed (FPRD), where the wet and dry sides of the root zone are consistently maintained during the growing season (Dodd, 2007). PRD enables plants to grow with partially reduced stomatal conductance ( $g_s$ ) without great losses due to severe drought stress (Santos et al., 2003; Kang and Zhang, 2004).

Using precise control of irrigation water applications, mild drought stress maintained through PRD irrigation may reduce excess shoot vigor and competition for carbohydrates (CHO) between growing tips, roots and fruit and thereby, promote a shift in the CHO partitioning towards reproductive tissues. These changes in plant metabolism also may positively affect fruit yield and quality (Loveys et al., 2000). If yields are not reduced during PRD, it offers the potential for higher WUE of crops compared to full irrigation as has been reported in grapevines (Stoll et al., 2000; Dos Santos et al., 2003). Although PRD-irrigated crops require frequent applications of water to only part of the root zone (Stoll et al., 2000; Dos Santos et al., 2003;

Leib et al., 2004), PRD irrigated roots can experience partial soil drying with infrequent irrigation (Kirda et al., 2004).

From the viewpoint of citrus pest management, regulating vegetative growth using moderate drought stress could aid in pest and disease control. Healthy citrus trees can grow an excess of leaves, more than that required to produce a good fruit crop (Yuan et al., 2005). New leaf flushes are susceptible to infection by citrus canker and also to feeding by the citrus leaf miner and the Asian citrus psyllid (*Diaphorina citri* Kuwayama), the vector of Huanglongbing (HLB, citrus greening). Fewer new flushes can limit Asian citrus psyllid reproduction and development (Rogers and Stansly, 2006) thus, with limited new flushes, PRD may help keep pest populations under control and make it possible to use reduced doses of pesticides for pest control.

PRD has only been successfully practiced in areas of low rainfall where soil water availability can be effectively controlled using irrigation (Hutton, 2004). Florida has summer rainfall in excess of citrus evapotranspiration (ET) requirements in summer whereas drought stress is common during the relatively dry spring and fall seasons (Garcia-Sanchez et al., 2007). Therefore, the extent to which PRD can be beneficial in Florida citrus during the spring, fall and winter irrigation seasons needs to be investigated. Since the high summer rainfall in Florida makes controlling soil water almost impossible during that time, there may be potential to use fixed PRD in order to impose a limited amount of drought stress during summer.

Although PRD may have the potential to improve WUE in citrus production regions, PRD has only been beneficial when irrigation water is of good quality with low salinity (Dos Santos et al., 2003). Irrigation water from some sources may contain high salt contents especially in areas with scarce water resources. Salt stress has an adverse effect on water availability to

crops due to osmotic stress and toxicity of salt ions (Syvertsen et al., 1989). Any salt stress may induce differences in physiological responses to drought of crops under PRD irrigation. PRD-grown plants may respond differently to salinity, allowing one half of the root zone to be consistently irrigated with saline water while the other receives no water in contrast to the mechanisms underlying plant responses when part of the root system receives good quality water.

There is no information on PRD irrigated citrus in Florida either with high quality or saline water. Therefore, the objectives of this experiment were to evaluate the feasibility, risks or potential benefit of FPRD with saline and with good quality water in potted citrus seedlings in terms of the scope for water savings, plant water relations, growth, WUE and nutritional responses. It was hypothesized that roots in wet soil can maintain a favorable plant water status while the remaining roots in dry soil will promote the synthesis of abscisic acid (ABA) signals leading to reduced water loss, reduced vegetative growth and increased WUE under FPRD both with and without saline water.

## **Materials and Methods**

### **Plant Material and Growing Conditions**

This study was conducted at the University of Florida Citrus Research and Education Center (Lake Alfred, Florida, USA) from February to May 2007. The woody tap roots of 24, uniform (similar sized), six-month-old, Swingle citrumelo (*Citrus paradisi* Macfad. × *Poncirus trifoliata* (L.) Raf.) seedlings were divided into equal halves. Each half of the split root was positioned between two separate but attached 2.4 liter-square plastic pots and transplanted into in an autoclaved Candler fine sandy soil (USDA, 1990). The spatial separation of the root systems allowed maintaining different water conditions on each root half while both supplied a single shoot. Both root halves were well-watered and fertilized (as described below) for 3 weeks to

allow the root systems to become established. The plants were grown in a temperature-controlled greenhouse under natural photoperiods of about 11h with maximum photosynthetically active radiation (PAR; LI-170; LICOR, Inc., Lincoln, NE) at plant level of  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Average day/night temperature was 36/21 °C and relative humidity varied diurnally from 40 to 100%.

### **Irrigation Treatments**

Twenty-five days after transplanting, plants were subjected to one of four irrigation treatments: (1) well-water control (WW) where both root halves were irrigated with 50%  $\text{ET}_c$  each (receiving 100%  $\text{ET}_c$  in total), (2) partial root-zone drying (PRD) where one half of the root system was irrigated with 100%  $\text{ET}_c$  while the other half received no water, (3) partial root-zone drying with saline water (PRS) where one half of the root system was irrigated with 100%  $\text{ET}_c$  water plus 50 mM Sodium chloride (NaCl) while the other half received no water, or 4) salt stress (SS) where both sides were irrigated with 50%  $\text{ET}_c$  plus 50 mM NaCl. There were six replicate seedlings per treatment. To avoid osmotic shock, NaCl was applied in the irrigation water in increments of 15 to 20 mM over the course of the first two irrigations until the final concentration of 50 mM was reached. Treatments were continued for nine weeks (66 days) from March to May. Water plus a dilute solution of a liquid complete citrus fertilizer (8N - 2P - 8K% by weight) was supplied to the wet sides at about three to eight day irrigation intervals based on the crop evapotranspiration ( $\text{ET}_c$ ) measured by weight loss from the pots. All plants received this dilute fertigation throughout the experiment such that about 7 mg of nitrogen (N) per week was applied to each plant. The total amount of fertilizer applied varied by treatments with the amount of the irrigation water in this experiment. For salt stress treatments, plants were irrigated with the same nutrient solution supplemented with NaCl. Total average plant water use per plant was calculated as the sum of the  $\text{ET}_c$  applied after 9 weeks of treatments.

## **Gas Exchange Measurements**

Net assimilation of CO<sub>2</sub> ( $A_{CO_2}$ ), stomatal conductance ( $g_s$ ), leaf transpiration (TR), intercellular CO<sub>2</sub> concentration ( $C_i$ ), and leaf water use efficiency (leaf WUE =  $A_{CO_2} TR^{-1}$ ) were measured on single mid-stem leaves of four replicate plants per treatment during the experiment using a portable photosynthesis system (LI-6200; LI-COR Inc., Lincoln, NE) with a 0.25 L cuvette. Gas exchange measurements were taken at 44, 46, 50, 57, and 64 days after treatment (DAT) began, corresponding to 1 to 4 days after irrigation. Since there were no differences attributable to time, values were averaged over 5 days. In addition, some of the non-destructive leaf measurements were duplicated on leaves on opposite sides of the shoot to determine if leaves above the different root half treatments differed. All gas exchange measurements were made in the morning from 08:00 h to 10:00 h to avoid high temperatures and low humidity in the afternoon. During all measurements, PAR exceeded 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Leaf temperature was  $34 \pm 2$  °C and leaf-to-air vapor pressure difference (VPD) was  $3 \pm 0.4$  kPa within the cuvette.

## **Plant Water Relations**

Midday stem water potential ( $\Psi_{\text{stem}}$ ) was determined in mature, fully expanded leaves in the middle of four replicate plants per treatment at the end (66 DAT) of the experimental periods using a pressure chamber (PMS instrument, Corvallis, Oregon, USA) according to Scholander et al. (1965).  $\Psi_{\text{stem}}$  was measured in leaves that were enclosed within foil-covered plastic and aluminum envelopes at least 1 h before the measurement (McCutchan and Shackel, 1992). This procedure gave an estimate of the water potential in the xylem of the shoot, at the point of attachment of the petiole of the leaf (Garnier and Berger, 1985).

## **Leaf and Root Abscisic Acid (ABA) Analysis**

66 DAT began, healthy mature leaves and tips of roots (1 g of fresh weight; FW) were harvested from each seedling between 09:00 and 12:00 h. Each tissue sample was immediately

frozen in liquid N<sub>2</sub> and stored at -18 °C until analysis. Frozen leaf and root tissues were finely ground and extracted overnight at 4 °C with 20 ml of ABA extraction solvent (80% acetone + 100 mg/L 2,6-di-*tert*-butylphenol (BHT) + 500 mg/L of citric acid). The ABA concentration ([ABA]) of the extract was measured by an enzyme-linked immunosorbent assay (ELISA, Phytodtek ABA, Agdia Inc., Elkhart, IN) using a monoclonal antibody for ABA (Liu et al., 2003). All assays were made with a duplicate from sub-samples of each tissue. [ABA] was quantified using a series of standards of known non-radioactive [ABA] in the assay such that sample counts can be calibrated from the resultant standard curve and calculated by interpolation with the logit transformation of the ABA standard curve following by the protocol of Quarrie et al. (1988).

### **Plant Growth and Tissue Nitrogen (N) Analysis**

Sixty-six DAT, all seedlings were harvested, dissected into leaves, stems, and roots, and weighed to obtain FW. The total leaf area was measured with a LI-COR 300 leaf area meter (LI-COR Inc., Lincoln, NE, USA). Root length in each pot was estimated using a line-intercept method (Tennant, 1975) after gently brushing sand from the roots and oven-dried at 60 °C for 48 to 72 h. Both root halves in the PRD-treated plants were weighed and measured individually. All leaves were briefly rinsed with deionized water, oven-dried at 60 °C for 48 to 72 h. Dry weight (DW) of leaves, stems, and roots was determined to estimate growth and patterns of biomass accumulation such as root to shoot (RT: SH) ratio and total plant DW. FW to DW ratios (FW/DW) in leaves and roots were calculated to estimate variation in moisture contents. Leaf DW per unit leaf area, an index of leaf thickness, was calculated as the ratio between leaf DW and leaf area. Specific root length (SRL), an index of root thinness, was calculated as the ratio between root length and root DW. Whole plant WUE was calculated as the ratio between TPDW and total plant water use. For tissue nitrogen (N) analysis, the dried leaves, stems, and roots were

grounded to a fine powder and analyzed for N concentration (%) by a commercial lab (Waters Agricultural Lab, Camilla, GA). Leaf N concentration ( $\text{mg cm}^{-2}$ ) was calculated as the tissue nutrient concentrations (in  $\text{mg g}^{-1}$ ) \*Leaf DW per unit leaf area (in  $\text{g cm}^{-2}$ ). N contents in leaves, stems, and roots (in mg) were calculated as the tissue nutrient concentrations (in  $\text{mg g}^{-1}$ )\*tissue DW. When no differences between root halves were found in the PRD-treated plants, weights, lengths, and N concentration for the roots in both pots, the values of both pots were added together.

### **Statistical Analysis**

The treatment effects were evaluated by one-way analysis of variance (ANOVA, SPSS statistical package, Chicago, IL) with four treatments and 4-6 replicates. Means were separated by Duncan's multiple range tests at  $P < 0.05$ .

## **Results**

### **Plant Water Use**

Crop evapotranspiration ( $ET_c$ ) of PRD and PRS plants, both grown with 100% evapotranspirational loss applied to one half of the root system, were significantly lower compared to the SS and WW control plants from 14 DAT through the final irrigation (63 DAT; Figure 2-1). Throughout the experiment, the average plant water consumption of PRS and PRD plants was 64 and 72% of WW plants, respectively, while plant water use of SS plants was 95% of WW plants (Table 2-1). Based on the total water consumption over the 63 day experiment, application of PRD and PRS saved an average of 28 and 36% of irrigation water, respectively, compared to the WW plants. The SS plants, however, used the same amount of water as the WW plants. SS, PRD, and PRS plants decreased mean stem water potential ( $\Psi_{\text{stem}}$ ) by 21, 21, and 34%, respectively, compared to WW plants (Figure 2-2).

## **Plant Growth**

PRD and PRS treatments significantly reduced total plant dry weight (DW) by 23% and 36%, respectively, compared to the WW plants (Figure 2-3). A decrease in TPDW of SS plants of 12% was not significantly different from WW and PRD plants. SS, PRD, and PRS plants significantly reduced shoot DW 23, 33, and 45%, respectively. Root DW in PRS plants was reduced by 18% of WW with no significant changes in root DW of PRD and SS plants. All stressed plants significantly increased root to shoot (RT: SH) ratio compared with the WW plants.

SS, PRD, and PRS treatments significantly reduced leaf DW 20, 36 and 47%, respectively (Table 2-1), stem DW 27, 31, and 38%, and total leaf area 28, 43, and 51%, respectively, compared to WW plants. Since leaf DW and leaf area changed in parallel, there were no treatment effects on leaf DW per unit of leaf area. PRD and PRS treatments significantly reduced root length 40 and 41%, and SRL 23 and 30% compared to WW plants. SS plants had similar root length and SRL to WW plants. There were no treatment differences in leaf FW to DW ratio (FW/DW) but root FW/DW of PRD and PRS plants were lower than root FW/DW of WW and SS plants. Whole plant WUE (total DW per water use) of WW and the other three were similar but SS plants had lower whole plant WUE than PRD plants.

## **Leaf and Root Nitrogen (N)**

Leaf and stem N concentrations (expressed on a % dry mass basis,  $N_m$ ), in PRD plants increased 9 and 38% respectively, compared to WW plants, while PRS plants were unchanged from WW plants (Table 2-2). SS reduced leaf  $N_m$  13% but had similar stem  $N_m$  to WW plants. Root  $N_m$  was not significantly different among treatments. PRD increased leaf N 28%, expressed on a  $\text{mg cm}^{-2}$  leaf area basis ( $N_{la}$ ) compared to WW plants, while  $N_{la}$  of PRS and SS plants were unchanged.

### **Abscisic Acid (ABA) Concentrations in Leaves and Roots**

Mean ABA concentration ([ABA]) in PRS leaves plants was more than two fold higher than in WW and SS leaves (Table 2-3). Root [ABA] was a small fraction of leaf [ABA] but was not significantly different among treatments.

### **Gas Exchange**

Rates of  $g_s$  and leaf TR were significantly lower in SS and PRS plants than in WW plants, while  $g_s$  and leaf TR in PRD were not affected (Figure 2-4A and B). PRD, PRS, and SS treatments reduced  $A_{CO_2}$  14, 48, 54%, respectively, compared to WW (Figure 2-4C). The significant decrease of  $A_{CO_2}$  and the relatively small variation in TR in PRD plants resulted in a decrease of leaf water use efficiency (WUE) by 7% below WW plants. The decreased  $A_{CO_2}$  and TR in both PRS and SS plants reduced leaf WUE 41% compared to WW plants (Figure 2-4D).

## **Discussion**

### **Plant Water Use**

Over the 14 irrigation cycles, there was a tendency for higher water savings by PRS plants than PRD plants throughout the study. PRD and PRS plants undoubtedly saved more evaporative water loss from the soil surface than SS plants, because half their root system was left dry. Soil evaporation was previously reduced by 40% using PRD in a cotton field as evaporation occurred mostly from the wet side of the irrigation (Tang et al., 2010). In this study from spring to early summer (mid-March to Mid-May), the irrigation amounts of PRD-treated plants were equivalent to 58 to 68%  $ET_c$  of WW plants. Thus, the present results confirmed the hypothesis of water savings under PRD both with saline water and with good quality water.

$\Psi_{stem}$  is an estimate of the water status of the tree's xylem (Dzikiti et al., 2010) and can be influenced by availability of soil water, hydraulic conductivity of roots and soil along with whole plant TR (Chone et al., 2001). Therefore,  $\Psi_{stem}$  can be more representative of tree water status

than  $\Psi_{lf}$  since  $\Psi_{stem}$  is less fluctuating diurnally (Dzikiti et al., 2010). All stressed plants had lower  $\Psi_{stem}$  than WW plants even though the lowered  $\Psi_{stem}$  of stressed plants did not change greatly in response to the different levels of water consumption per plant or to the uneven distribution of soil water (Figure 2-2).

PRD relies on a spatial separation of dry and wet roots (Liu et al., 2007; Shahnazari et al., 2007), and, therefore, takes advantage of plant physiological responses to an uneven distribution of soil water (Kang and Zhang, 2004). In theory, the well-watered half of the root system under PRD maintains high shoot water status (Dry and Loveys, 1999; Rodrigues et al., 2008). Our results contrasted with the study on grapevine where PRD did not show a significant decrease of  $\Psi_{stem}$  but  $g_s$  was decreased compared to WW plants (Rodrigues et al., 2008). Similar greenhouse results to those of this study were reported for split-root potted PRD Swingle citrumelo seedlings where  $\Psi_{stem}$  was decreased 72% along with decreased  $g_s$  and  $A_{CO_2}$  compared to WW plants, however there were no differences in plant growth (Melgar et al., 2010). The present results did not support the hypothesis that roots in wet soil of PRD-treated plants can maintain a favorable plant water status relative to WW plants.

The reductions of  $\Psi_{stem}$  in the PRD-treated plants may have been indicative of an increased flow resistance in soil (Steudle, 2000; Boursiac et al., 2005) or from the soil to roots (Hutton et al., 2007) due to the limited water supply under PRD and PRS. Critical threshold  $\Psi_{stem}$  at which some important physiological processes are inhibited ranges from -2.0 to -2.3 MPa for citrus trees and a decrease in the daily total sap flow of 10% in stressed Satsuma mandarin trees was associated with a drop in  $\Psi_{stem}$  of at least 1.0 MPa (Dzikiti et al., 2010). Therefore, the stress-induced decrease in  $\Psi_{stem}$  probably did not maintain a higher hydraulic conductance in the xylem (Schultz, 2003; Vandeleur et al., 2009) under stress conditions.

Although  $\Psi_{\text{stem}}$  in all stressed plants was not significantly different, the small numerical differences could be explained by different levels of osmotic adjustment (Downton, 1983; Patakas and Noitsakis, 1997) from salt and/or drought treatment or through changes in cell wall elasticity (Patakas and Noitsakis, 1997). SS leaves had a more negative osmotic potential and a 74% increase in turgor over the other three treatments (data not shown) which could have been due to the presence of compatible solutes (osmoregulation). This increase in turgor in SS plants would have allowed growth to continue as root growth was only reduced in PRS plants (Figure 2-3).

### **Plant Growth**

Both PRD and PRS reduced growth (Figure 2-3). Whole plant WUE of all stressed plants was equivalent to WW plants (Table 2-1). Whole plant WUE in PRD plants was 13 % higher than in SS plants which may have been due to the smaller growth reduction and/or higher irrigation consumption from SS plants compared to PRD plants. The present results did not support the hypothesis that WUE at the whole plant level was increased by PRD-treated plants relative to WW plants.

One of the important water stress response is increased biomass allocation to roots, leading to a higher root to shoot (RH: SH) ratio (Lovisolo et al., 2010), even if total plant biomass decreases with water stress (Poorter and Nagel, 2000). This is due to the lower sensitivity of root growth than shoot growth to water stress, which has been attributed to rapid root osmotic adjustment and maintenance of water uptake in response to the decrease in soil water content (Hsiao and Xu, 2000; Sharp et al., 2004). This increased proportional allocation below ground may provide a more favorable water balance (Taiz and Zeiger, 2006) and nutrient transport to the shoots (Lovisolo et al., 2010) under stress conditions.

All stressed plants increased RT: SH ratios by 51 to 59% (Figure 2-3). Although PRS plants significantly reduced both shoot and root DW compared to WW plants, the decrease in root DW was less than shoot DW as observed by PRD and SS plants, resulting in an increased RT:SH ratio. The present results support the hypothesis that PRD-treated plants limited vegetative growth. Leaf thickness (leaf DW per unit of leaf area), however, remained similar across all treatments as well as comparable leaf FW/DW ratios. Such similar leaf morphology and no change in leaf succulence indicated that all stressed plants probably had similar amounts of carbon investment in leaves (Simioni et al., 2004) regardless of the drought and/or salinity stress.

The accumulation of final root DW in PRD and SS plants was equivalent to WW plants (Figure 2-3). Root growth of SS plants was not affected by salinity stress. By exposing some roots of PRD plants to drying soil, they had a decrease of root length (Table 2-1). PRS plants, however, had less root biomass and less root length, as reflected in the decreased SRL after long-term exposure of some roots to drying soil while other roots received saline water. Both PRD and PRS plants developed short thick roots (i.e. lower SRL), indicating more structural investment in roots than in WW and SS plants. The thicker or high root tissue density in both PRD-treated plants may have indicated mechanisms to allow roots to better penetrate drying soils and to promote better water transport (Cornelissen et al., 2003). Root FW/DW ratios were affected in PRD-treated plants, which indicated a decrease in shoot and root water contents, implying turgor loss in expanded cells (Sharma et al., 2004), but not by SS plants. The differences in root FW/DW between PRD-treated and SS plants could be explained by osmotic adjustment, which can reduce growth sensitivity to water stress (Cutler et al., 1980) in SS plants or less growth under water stress by conserving turgor (Sharmal et al., 2004) in SS plants.

There were no significant differences on root biomasses or SRL between wet and dry compartments of PRD and PRS plants (data not shown). This homogeneous root distribution and root structure in the wet and dry sides may reflect the potential for internal hydraulic redistribution at night (Bauerle et al., 2008) or hydraulic lift (Dawson, 1993; Harton and Hart, 1998). Such redistribution can alleviate drought conditions, replenish any root xylem embolism (McCully, 1999), and maintain plant root function (Williams et al., 1993) and viability (Huang, 1999). Experimental evidence under extremely heterogeneous field conditions has shown that internal hydraulic redistribution may assist in sustaining water uptake of the entire root system by rehydrating roots in dry soil (Hultine et al., 2003). A water redistribution process from wet to dry roots in response to water potential gradients can contribute to a decrease of ABA biosynthesis (Lovisolo et al., 2010). This may have contributed to the lack of PRD treatment effects on root ABA since dry roots may not have experienced sufficient drought stress.

### **Leaf and Root Nitrogen (N), ABA and Gas Exchange**

Although leaf  $N_m$  was increased by PRD, but reduced in SS relative to WW plants, all leaf  $N_m$  in all plants were higher than the critical leaf N concentrations of 2.5% (Obreza and Morgan, 2008). Moreover, rates of  $A_{CO_2}$  do not increase with increasing leaf N above leaf N concentrations of 2.3% in citrus (Syvertsen, 1987). Thus, leaf N was not limiting regardless of treatment.

The levels of [ABA] in leaves were much higher than in roots at the end of the study (Table 2-3). This result was similar to that obtained in one-year-old citrus seedlings under a rapid and drastic water stress treatment where there was approximately 14 times higher [ABA] in leaves than in roots 24 h after the onset of water stress (Gómez-Cadenas et al., 1996). This may be partly explained by rapid responses in leaves to water stress (Norman et al., 1990) from a rapid drop in humidity in air surrounding leaves (Xie et al., 2006), and to changes in pH of either

the apoplast or the symplast (Beis and Patakas, 2010) which increase local [ABA] synthesis in leaves, or to lower rates of ABA degradation (Beis and Patakas, 2010). [ABA] in citrus leaves and roots decreased drastically after rehydration and became similar to non-stressed plants 18 h after rewatering in leaves and after 12 h in roots (Gomez-Cadenas et al., 1996).

In this study, ABA was sampled in tissues three days after the final 14<sup>th</sup> irrigation cycle in the beginning of summer (May). The lack of treatment differences in root [ABA] may have been partly due to the sampling times missing rapid changes in ABA levels that may have occurred in hours rather than days. Since half of the root systems under PRD or PRS were well watered, dry roots may not have experienced sufficient drought stress to cause treatment differences in [ABA]. In PRD-treated grapevines, ABA signals were diminished due to prolonged drying of one of side of the rootzone and  $g_s$  recovered to levels equivalent to the WW treatments (Stoll et al., 2000). The present results did not support the hypothesis of increased synthesis of ABA signals in roots in dry soil under PRD both with saline water and with good quality water.

PRS plants had higher leaf [ABA] than WW and SS plants (Table 2-3). The increased ABA in leaves of PRS plants could have originated earlier in roots, however, it also might have been attributed to locally synthesized ABA, an internal redistribution with release of stored symplastic ABA in the leaf (Soar et al., 2004; Wilkinson, 2004), and/or lower rates of ABA degradation (Beis and Pataks, 2010).

In theory, half the root system in the dry side senses soil drying and produces ABA-mediated chemical signals traveling through the xylem from the roots to shoots that reduce  $g_s$  (Davies et al., 2002; Bravo, 2005). Thus, partial stomatal closure can bring about a reduction in TR (Kang et al., 2001) and an increase in leaf WUE (Bravdo, 2005). PRD can restrict water loss and simultaneously sustain  $A_{CO_2}$  rate at the equivalent levels to WW (De Souza et al., 2003) due

to the non-linear relationship between photosynthesis and  $g_s$  (Düring et al., 1996). In this present study, the reduction in  $g_s$  (Figure 2-4A) could have been attributed to a decrease in  $\Psi_{stem}$  among all stressed plants. Numerically higher leaf [ABA] in PRS plants may have partly contributed to the lower  $g_s$ , TR (Figure 2-4B) and water consumption compared with WW plants. Since PRS plants grew less than SS plants, it is possible that [ABA] could have played a role in reducing leaf growth or regulated  $g_s$  in PRD-treated citrus seedlings.

Leaf gas exchange parameters were reduced more in PRS and SS plants than PRD probably due to accumulations of  $Cl^-$  and  $Na^+$  (Garcia-Sanchez et al., 2002) or loss of chlorophyll in salt stressed leaves (Zekri, 1991). Since  $C_i$  was significantly increased in PRS and SS plants compared to PRD and WW plants (data not shown), implying the reduction of  $A_{CO_2}$  (Figure 2-4C) was likely due to a direct biochemical reduction in photosynthesis rather than to an indirect stomatal limitation (Farquhar and Sharkey, 1982). The inhibition of  $A_{CO_2}$  under PRD may have resulted in part from partially reduced  $g_s$ , although there is no significant difference in  $g_s$  between WW and PRD plants. Although PRD and WW plants exhibited statistically similar TR rates, the larger reduction in leaf area of PRD plants than WW plants would have decreased total plant TR in PRD plants. The rates of  $A_{CO_2}$  were reduced more than those of leaf TR in leaves of all stressed plants as reflected by decreasing the rates of leaf WUE (Figure 2-5D). These results did not support the original hypothesis about WUE since leaf WUE was reduced in all stressed plants.

In conclusion, I evaluated whole plant WUE, photosynthetic WUE of leaves and other physiological responses of citrus rootstock seedlings irrigated using PRD with and without saline water. Irrigation amounts of PRD and PRS were equivalent to 64 to 72% of  $ET_c$  of well-watered plants. Although PRD-treated plants reduced growth, whole plant WUE was not affected by

PRD-treated plants relative to WW plants. PRD-treated plants reduced vegetative growth and increased RT: SH ratio. Root ABA at the end of the study was similar across treatments. PRS had highest leaf ABA and lowest growth perhaps due to the combined effects of drought and salinity stress. Higher leaf [ABA] in PRS plants may have partly contributed to the lower  $g_s$ , TR and water consumption compared with WW plants. Roots in wet soil of PRD-treated plants did not enable PRD plants to maintain water status relative to WW plants. There was no evidence for root-induced ABA regulation of stomatal behavior in PRD-treated plants. The rates of  $A_{CO_2}$  were reduced more than those of leaf TR in leaves of PRD-treated plants as reflected by decreased leaf WUE. Therefore, PRD-treated plants resulted in water savings but drought stress reduced leaf function.

Table 2-1. Average leaf dry weight (DW), stem DW, total leaf area, leaf DW per leaf area, root length, specific root length (SRL), leaf fresh weight to DW ratio (FW/DW), root FW/DW, plant water consumption, and whole plant water use efficiency (WUE) ( $n = 6 \pm \text{S.E}$ ) of *Swingle citrumelo* seedlings after 9 weeks well-water control (WW), partial root-zone drying (PRD), partial root-zone drying with saline water (PRS), and salt stress (SS) treatments. Different letters at each sampling data indicate significant differences between treatments according to Duncan's multiple range test at  $p < 0.05$ .

Trt	Leaf DW (g)	Stem DW (g)	Total leaf area (cm <sup>2</sup> )	Leaf DW/leaf area (g/m <sup>2</sup> )	Root length (cm)
WW	4.7 ± 0.28 a	6.4 ± 0.61 a	583 ± 28 a	81 ± 1.9 a	24.1 ± 2.76 a
PRD	3.0 ± 0.28 bc	4.4 ± 0.46 b	330 ± 33 bc	91 ± 2.6 a	17.8 ± 1.03 bc
PRS	2.5 ± 0.38 c	4.0 ± 0.27 b	283 ± 46 c	89 ± 2.0 a	14.1 ± 2.14 c
SS	3.8 ± 0.30 b	4.7 ± 0.69 b	417 ± 51 b	96 ± 10 a	22.1 ± 1.62 ab

Trt	SRL (cm/g)	Leaf FW/DW	Root FW/DW	Plant water consumption (L)	Whole plant WUE (g/L)
WW	4.3 ± 0.22 a	3.0 ± 0.05 a	3.6 ± 0.13 a	3.98 ± 0.14 a	4.0 ± 0.14 ab
PRD	3.3 ± 0.31 b	2.8 ± 0.12 a	2.5 ± 0.10 b	2.87 ± 0.14 b	4.5 ± 0.11 a
PRS	3.0 ± 0.47 b	3.1 ± 0.17 a	2.2 ± 0.27 b	2.55 ± 0.16 b	4.3 ± 0.21 ab
SS	3.5 ± 0.19 ab	2.9 ± 0.16 a	3.1 ± 0.10 a	3.78 ± 0.11 a	3.9 ± 0.25 b

Table 2-2. Average Nitrogen (N) concentrations (% DW in  $N_m$  and  $\text{mg cm}^{-2}$  in  $N_{la}$ ) ( $n = 6 \pm$  S.E.) in leaves, stems, and roots of *Swingle citrumelo* seedlings after 9 weeks well-water control (WW), partial root-zone drying (PRD), partial root-zone drying with saline water (PRS), and salt stress (SS) treatments. Different letters within each column indicate significant differences between treatments according to Duncan's multiple range test at  $p < 0.05$ .

Trt	Concentration			
	Leaf $N_m$ (%)	Stem $N_m$ (%)	Root $N_m$ (%)	Leaf $N_{la}$ ( $\text{mg/cm}^2$ )
WW	$3.1 \pm 0.04$ b	$1.3 \pm 0.04$ b	$2.3 \pm 0.03$ a	$0.25 \pm 0.03$ b
PRD	$3.4 \pm 0.09$ a	$1.8 \pm 0.08$ a	$2.6 \pm 0.15$ a	$0.31 \pm 0.15$ a
PRS	$3.0 \pm 0.08$ b	$1.5 \pm 0.08$ ab	$2.6 \pm 0.14$ a	$0.27 \pm 0.14$ ab
SS	$2.7 \pm 0.06$ c	$1.4 \pm 0.13$ b	$2.3 \pm 0.06$ a	$0.27 \pm 0.06$ ab

Table 2-3. Average abscisic acid (ABA) contents in leaves and roots ( $n = 6 \pm \text{S.E.}$ ) of *Swingle citrumelo* seedlings after 9 weeks of well-water control (WW), partial root-zone drying (PRD), partial root-zone drying with saline water (PRS), and salt stress (SS) treatments. Different letters within each tissue indicate significant differences between treatments according to Duncan's multiple range test at  $p < 0.05$ .

Trt	Leaf ABA (nmolABA/g DW)	Root ABA (nmolABA/g DW)
WW	13.3 $\pm$ 4.2 b	0.36 $\pm$ 0.09 a
PRD	17.8 $\pm$ 3.6 ab	0.69 $\pm$ 0.24 a
PRS	29.3 $\pm$ 8.5 a	0.87 $\pm$ 0.39 a
SS	10.4 $\pm$ 2.1 b	0.39 $\pm$ 0.12 a

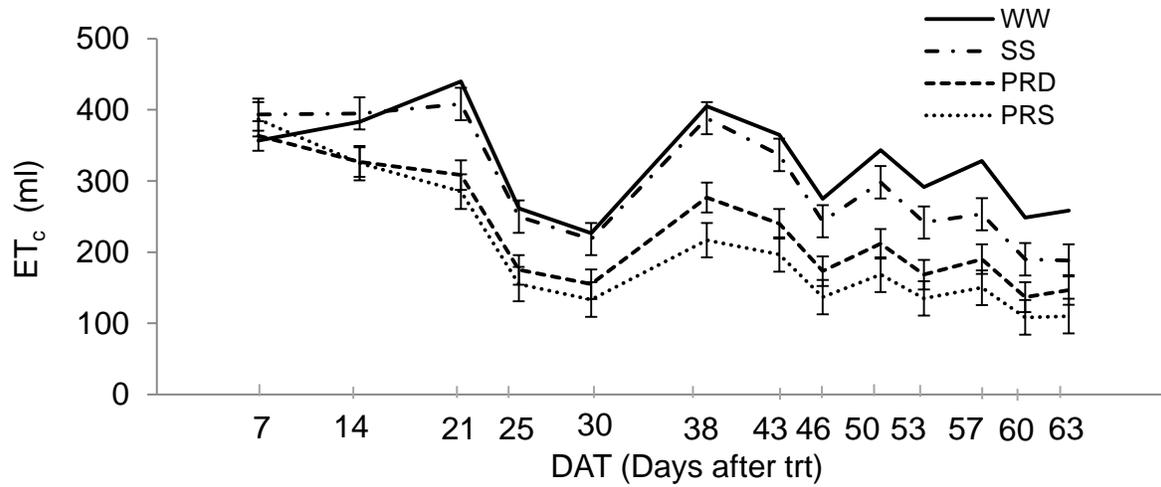


Figure 2-1. Effects of well-water control (WW), partial root-zone drying (PRD), partial root-zone drying with saline water (PRS) and salt stress (SS) treatments on average ( $n = 6 \pm \text{S.E.}$ ) evapotranspiration ( $\text{ET}_c$ ) of *Swingle citrumelo* seedlings.

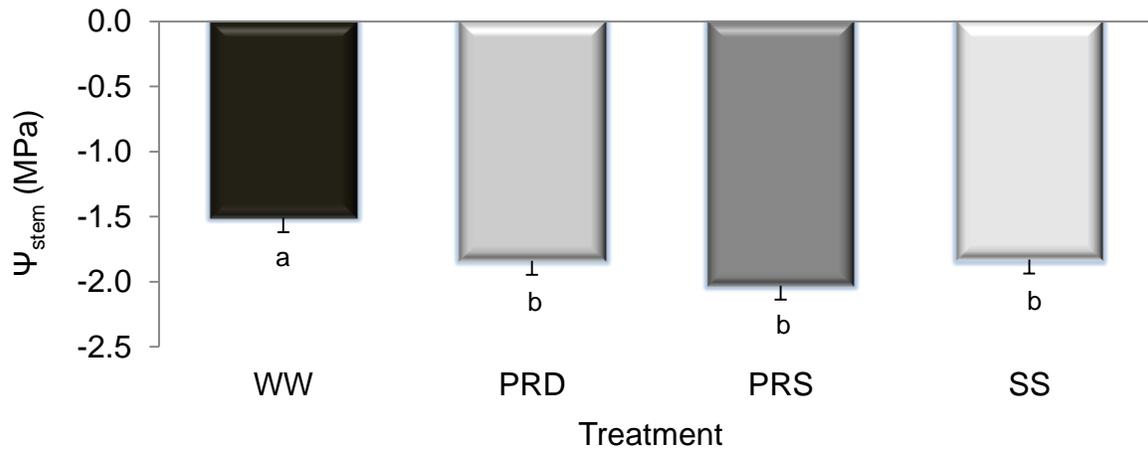


Figure 2-2. Effects of well-water control (WW), partial root-zone drying (PRD), partial root-zone drying with saline water (PRS) and salt stress (SS) treatments on average ( $n = 4 \pm \text{S.E.}$ ) midday stem water potential ( $\Psi_{\text{stem}}$ ) of *Swingle citrumelo* seedlings at the end of the experimental period (66 DAT). Different letters within each tissue indicate significant differences between treatments according to Duncan's multiple range test at  $p < 0.05$ .

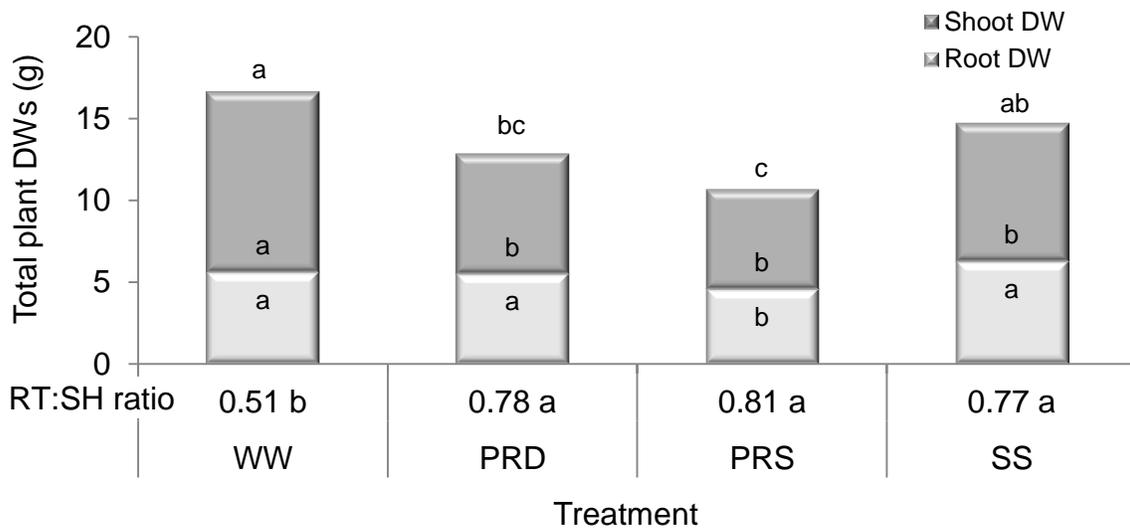


Figure 2-3. Effects of well-water control (WW), partial root-zone drying (PRD), partial root-zone drying with saline water (PRS) and salt stress (SS) treatments on average ( $n = 6 \pm \text{S.E.}$ ) shoot and root dry weights (DWs) and root to shoot (RT: SH) ratio of *Swingle citrumelo* seedlings at the end of the experimental period (66 DAT). Different letters within each tissue indicate significant differences between treatments according to Duncan's multiple range test at  $p < 0.05$ .

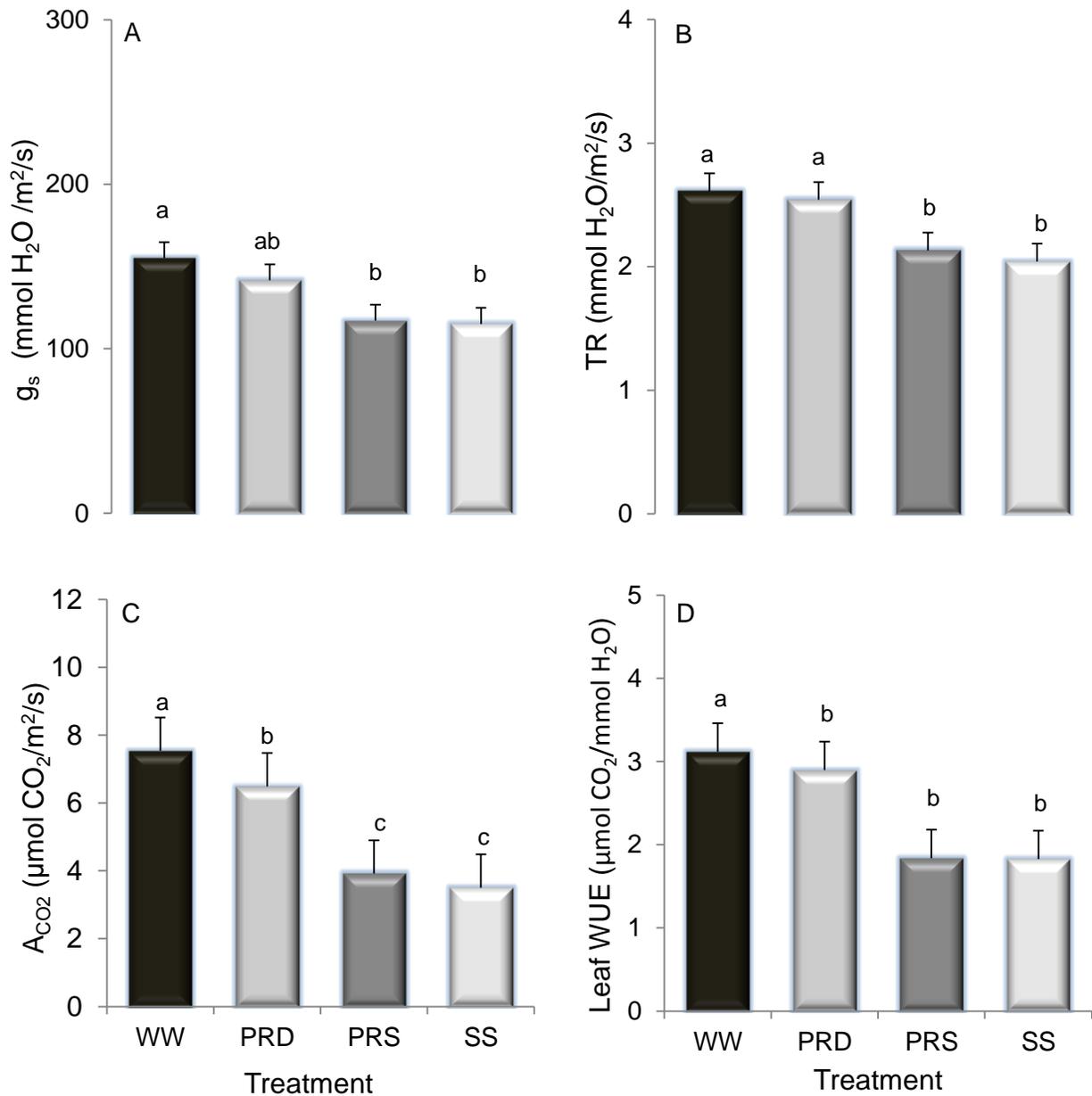


Figure 2-4. Effects of well-water control (WW), partial root-zone drying (PRD), partial root-zone drying with saline water (PRS), and salt stress (SS) treatments ( $n = 4 \pm \text{S.E.}$ ) on: A) stomatal conductance ( $g_s$ ), B) leaf transpiration (TR), C)  $\text{CO}_2$  assimilation ( $A_{\text{CO}_2}$ ) and D) leaf water use efficiency (WUE) of *Swingle citrumelo* seedlings averaged over five sample days. Different letters within each panel indicate significant differences between treatments according to Duncan's multiple range test at  $p < 0.05$ .

### CHAPTER 3 CONCLUSIONS

We evaluated whole plant water use efficiency (WUE), photosynthetic WUE of leaves and other physiological responses of citrus rootstock seedlings irrigated using partial rootzone drying (PRD) with saline water or with good quality water in a greenhouse.

Results confirmed PRD with good quality and with saline water can save irrigation water, restrict vegetative growth while leaf nitrogen (N) was not limiting regardless of treatment. Roots in wet soil of PRD-treated plants were not able to maintain a favorable plant water status relative to WW plants. Root-induced abscisic acid (ABA) regulation of stomatal behavior in PRD-treated plants was not observed in this study. However, higher leaf ABA concentrations in PRD with saline water may have partly contributed to the lower stomatal conductance ( $g_s$ ), transpiration (TR), and water consumption compared with well-watered (WW) plants. Whole plant WUE of PRD-treated plant was similar to WW plants. Rates of net CO<sub>2</sub> assimilation ( $A_{CO_2}$ ) were reduced more than those of leaf TR in PRD-treated plants resulting in decreased leaf WUE. Therefore, PRD with saline water or with good quality water resulted in water savings during spring to early summer but drought stress reduced leaf function.

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## BIOGRAPHICAL SKETCH

Ayako Kusakabe was born in Japan in 1974. She completed her Bachelor of Arts in international relations at the Tokyo International University in March 1998, and Bachelor of Science in soil and water science at the University of Arizona in December 2001, followed by a Master of Science in soil, water, and environmental sciences at the University of Arizona in August 2005. In March, 2011, she successfully defended her thesis and was awarded the M.S. in horticultural science at the University of Florida in May 2011.