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# Partial Root Zone Drying with and without Salinity Affects Water Use Efficiency of Citrus

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**A partial root zone drying (PRD) experiment was conducted in a greenhouse with and without saline water on split-root 'Swingle' citrumelo [*Citrus paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.] seedlings potted in an autoclaved fine sandy soil to evaluate leaf water use efficiency (WUE) and photosynthetic responses. Roots of all plants were split in half and the following five treatments were applied: 1) both root halves irrigated with 50% ETc each [100% ETc control or well-watered (WW)], 2) one-half irrigated with 100% ETc while the other received no water (PRD), 3) one-half irrigated with 50% ETc water plus 50 mM NaCl while the other received no water (PRS), 4) both sides irrigated with 50% ETc plus 50 mM NaCl (SS), or 5) one-half irrigated with 50% ETc and one-half irrigated with 50% ETc plus 50 mM NaCl (WS). Citrus seedlings in the PRD and PRS treatments used 33% to 42% less water than the WW controls. PRD and PRS treatments restricted shoot growth while root growth was less affected, leading to an increase in root: shoot ratio. Dry weight (DW) of PRD and PRS plants were 23% to 33% less compared to plant DW of WW controls. PRD and PRS dry sides induced higher levels of abscisic acid (ABA) in roots but not in leaves. Leaves above the PRD dry side had higher net $CO_2$ assimilation rate ($A_{CO2}$) compared to the WW controls and the PRD wet side. Leaves in both halves of PRS plants were lower $A_{CO2}$ than in WW plants. PRD resulted in water savings without loss of leaf function, but the mechanism that regulates stomatal closure and transpiration appeared to be independent of ABA concentrations in roots.**

Efficient water use has become an important issue in recent years (Tang et al., 2005). Improvement in growth per unit of water used, water use efficiency (WUE), is an ultimate goal in many agricultural systems. Shortages of available water call for optimum irrigation management in order to enhance WUE of field crops for sustainable agricultural output (Wakrim et al., 2005). One such practice is partial root zone drying (PRD), which is an 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. In addition to allowing precise control of irrigation water applications, mild water stress maintained through PRD irrigation may reduce excess shoot vigor and competition for carbohydrates (CHO) by the growing tips, and promote a shift in the CHO partitioning towards reproductive tissues. These changes in plant metabolisms may positively affect fruit yield and quality (Loveys et al., 2000). If yields are not reduced, PRD offers the potential for higher WUE of crops compared to full irrigation (Dos Santos et al., 2003; Stoll et al., 2000); although PRD irrigated crops require frequent applications of water to only part of the root zone (Dos Santos et al., 2003; Leib et al., 2004; Stoll et al., 2000).

From the viewpoint of citrus pest management, regulating vegetative growth 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 feeding by citrus leaf miner and the asian citrus psyllid (*Diaphorina citri* Kuwayama), the vector of 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 has only been successfully practiced in areas of low rainfall where soil water availability can be controlled using irrigation (Hutton, 2004). Drought stress is common in the relatively dry spring and fall seasons in FL (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. The high rainfall in Florida makes controlling soil water almost impossible during summer. There may be potential to use fixed PRD, where one-half of the root zone is consistently irrigated with water while the other receives no water, in order to impose a limited amount of drought stress during summer.

Although PRD may have the potential to improve WUE in citrus production, 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 water availability to crops due to osmotic and salt ion stress (Syvertsen et al., 1989). Any additional salt stress may induce differences in physiological responses to drought of crops under PRD irrigation. PRD-grown plants may respond differently to salinity if part of the root system receives good quality water. There is no information on PRD irrigated citrus with high quality water or under saline conditions. Therefore, the objectives of this experiment were to evaluate water use, photosynthetic WUE (amount of $CO_2$ fixed per amount of water transpired), and physiological responses of citrus rootstock seedlings irrigated using PRD with and without saline water.

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## Material and Methods

This study was conducted at the University of Florida Citrus Research and Education Center, Lake Alfred. Roots of thirty 6-month-old 'Swingle' citrumelo [*Citrus paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.] seedlings were divided into presumably equal halves with each positioned in a split-root pot, filled with autoclaved fine sandy soil. Plants were grown in a temperature-controlled greenhouse. All seedlings received identical dilute fertigation management throughout the experiment. Twenty-two days after establishing the split root system, the following five treatments were imposed: 1) both root halves irrigated with 50% ETc each [100% ETc or well-watered (WW) control], 2) one-half irrigated with 100% ETc while the other received no water (PRD), 3) one-half irrigated with 50% ETc water plus 50 mM NaCl while the other received no water (PRS), 4) both sides irrigated with 50% ETc plus 50 mM NaCl (SS), or 5) one-half irrigated with 50% ETc and one-half irrigated with 50% ETc plus 50 mM NaCl (WS). There were six replicate seedlings per treatment.

Water was supplied to the wet sides at about three to eight day irrigation intervals based on the crop evapotranspiration (ETc, measured by weight loss) to maintain contrasting soil moisture status in the root zones. In the PRD and PRS treatments, the dry halves were allowed to dry out and there was no alternating of the treatments on the root halves. Sodium chloride (NaCl) was applied in irrigation water in increments of 15 to 20 mM until the final concentration of 50 mM was reached to avoid osmotic shock.

Net assimilation of $CO_2$ ($A_{CO2}$), stomatal conductance, transpiration, intercellular $CO_2$ concentration, and photosynthetic water use efficiency (WUE = $A_{CO2}$ $E^{-1}$) were measured periodically during the experiment on single mid-stem leaves using a portable photosynthesis system (LI-6200; LI-COR Inc.) with a small cuvette. Gas exchange parameters were measured before and after irrigation events. In addition, some of the non-destructive leaf measurements were duplicated on leaves on opposite sides of the shoot to determine if the different root zone treatments differentially influenced the response of leaves directly above that root half. At final harvest (62 d after the onset of treatment, DAT), leaves, stems, and root dry weights (DW) were recorded. Abscisic acid (ABA) content of leaflet and root extracts was measured by an enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody for ABA (Liu et al., 2003). 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. Irrigation, physiological, and harvest data were subjected to analysis of variance (ANOVA) procedures (SAS Institute Inc., 2002–03).

## Results and Discussion

**Water Savings and plant biomass production.** Crop evapotranspiration (ETc) of PRD and PRS plants was consistently lower compared to the WW control plants (grown with 100% evapotranspirational loss replacement) and reasonably stable from 20 DAT until the final harvest (62 DAT). Plant water use of PRD and PRS plants was only 58% to 67% of WW plants. Application of PRD and PRS in this experiment saved 33% to 42% of irrigation water.

The mild drought stress significantly reduced total plant biomass production by 23% in PRD and 33% in PRS compared to the WW control. In both drought treatments, shoot growth was inhibited more than root growth, leading to an increased root to shoot ratio (0.78 in PRD and 0.75 in PRS) compared with the WW control. This significant reduction in shoot growth may maintain a more favorable water balance in leaves under water deficit (Taiz and Zeiger, 2006).

**Leaf and root thickness.** Plants in the PRD and PRS treatments developed higher leaf and root thicknesses (greater DW per unit leaf area and lower SRL, Kusakabe et al., 2009) compared to the WW control, which may be adaptations to increase capture of resources under unfavorable resource environments (Lambers et al., 2009) such as nutrient, light and water-limited conditions. These anatomical modifications such as an increased amount of cell-wall materials may enhance leaf and root longevity (Eissenstat and Achor, 1999) by potentially enhancing more structural defense from wilting or herbivory, increasing root diameter, enhancing plant anchorage and water flow functions (Wright and Westoby, 1999). Unlike the PRS treatment, leaves above the PRD dry side were thinner than the PRD wet side, indicating that the PRD dry side maximized the surface area for aboveground resource capture. The PRD dry side does not only invest more biomass in roots, but also makes relatively thick roots with a low SRL, compared to the PRD wet side. This indicated that the PRD dry side invested carbon for storage or physical defense in order to survive the water-limited conditions.

**Root abscisic acid concentrations.** There were no significant differences in ABA accumulation in leaves among all treatments (data not shown). ABA in roots growing in the PRD and PRS dry sides were 2.1 and 1.4 times higher, respectively, compared to the PRD and PRS wet sides. At low water potentials when ABA levels are high, ABA promotes root growth and inhibits shoot growth (Saab et al., 1990). In addition, ABA may stimulate water flow by increasing root hydraulic conductivity and by enhancing ion uptake, which can increase the water potential gradient between the soil and the roots and increase root growth.

**Leaf gas exchange.** Treatment differences did not significantly affect the rates of stomatal conductance and transpiration (data now shown), although relatively higher ABA production from the root tissues under PRD and PRS dry sides was directly related to the soil water availability in the drying side. In this experiment, the mechanism that regulates stomatal closure and transpiration appeared to be independent of ABA produced in the roots. Leaves above the PRD dry side had higher $A_{CO2}$ compared to the WW plants and the PRD wet side (Kusakabe et al., 2009). In addition, leaves above the PRD dry side had 1.4 to 1.5 times higher $A_{CO2}$ than above PRD wet side. This may have been due to higher root growth in the dry side that reflects the potential for resource acquisition resulting from the increase in ABA levels, and in turn, ABA-induced changes in membrane properties (alternations in the lipids or in the protein transporters) (Taiz and Zeiger, 2006), osmoregulation (McNaughton, 1991; Oosterhuis and Wullschleger, 1987; Taiz and Zeiger, 2006) or hydraulic lift (Dawson, 1993; Harton and Hart, 1998). However, leaves in both halves of PRS plants had lower $A_{CO2}$ than WW plants. A reduction of $A_{CO2}$ in PRS plants could be explained by an accumulation of $Cl^-$ or $Na^+$ in leaves rather than a stomatal limitation.

## Conclusions

We evaluated photosynthetic WUE and physiological responses of citrus rootstock seedlings irrigated using PRD with and without saline water. PRD and PRS of citrus seedlings conserved 33%

to 42% of irrigation water, but total plant DW was 23% to 33% less than in WW controls. PRD and PRS treatments restricted shoot growth while root growth was less affected resulting in an increase in root: shoot ratio. Roots located in the unwatered PRD and PRS dry sides produced higher levels of abscisic acid which apparently was not transported to leaves. Leaves above the PRD dry side had higher net $CO_2$ assimilation rate ($A_{CO2}$) than WW plants, while PRS had lower $A_{CO2}$ than WW plants. Higher $A_{CO2}$ occurred in leaves above the PRD dry side than above the PRD wet side. Therefore, PRD resulted in water savings without loss of leaf function.

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