

1 **Efficiency of Tree-Based Water Status Indicators at the Onset of Water Deficit in**
2 **Citrus**

3
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12
13 **Abstract:** This experiment evaluates the potential of using parameters based on tree trunk fluctuations for
14 detecting water deficit in citrus trees under two different water saving-irrigation strategies: sustained deficit
15 irrigation and partial root-zone drying. Three irrigation treatments were applied: 1) Control: trees were irrigated
16 with 100% of their evapotranspirative needs (ET_c); 2) 60 sustained deficit irrigation (SDI): 60% ET_c ; and 3)
17 partial root-zone drying (PRD): 100% ET_c needs, applied to only one-half of root zone. Maximum daily
18 shrinkage (MDS), trunk growth rate (TGR), and MDS ratio (ratio between MDS of stressed trees and control
19 trees) were determined. Day-to-day MDS values varied largely and could not be used to determine tree water
20 deficit. TGR did not show significant differences among treatments at this level of stress. Nevertheless, the MDS
21 ratio was a reliable indicator to measure tree water status, and it was more sensitive for detecting water deficit at
22 the onset of a water deficit in trees under SDI than in trees under PRD.

23
24 **Additional key words:** dendrometer, maximum daily shrinkage, partial root-zone drying, stem water potential,
25 sustained deficit irrigation, trunk diameter

Introduction

Irrigation strategies that reduce water applications and increase water use efficiency without affecting growth or yield [deficit irrigation (DI) strategies] are becoming important in fruit tree crops in semiarid areas (Carr, 2012; Ruíz-Sánchez et al., 2010). Deficit irrigation strategies can be applied in different forms such as regulated deficit irrigation (RDI; which applies water restrictions during certain phenological periods), sustained deficit irrigation (SDI; which delivers a uniform and reduced amount of irrigation water throughout the season), or partial root-zone drying (PRD; which keeps half of the root zone well-irrigated while the other half is allowed to dry).

The continuous control of the tree water status during DI is crucial for preventing reductions in growth and yield (Johnson and Handley, 2000). In the last decade, several articles in citrus and other fruit tree species showed that tree-based water status indicators such as the maximum daily shrinkage (MDS) of the trunk (i.e. the difference between the maximum diameter of the trunk in the early hours of the morning and the minimum diameter in the early evening), the ratio between the MDS of a stressed tree and the MDS of a well-irrigated tree (also known as signal strength or MDS ratio), and trunk growth rate (TGR; defined as the difference between the maximum trunk diameter in a day and the previous day) can become useful tools when studying the response of trees to water deficit (De Swaef et al., 2009; Ortuño et al., 2010). For instance, the MDS ratio has been successfully used in citrus as an indicator of the water status of the trees because it reduces the day-to-day variability of the solely measurement of MDS, which largely depends on environmental conditions such as evaporative demand or daily global radiations (García-Orellana et al., 2007; Ortuño et al., 2009; Velez et al., 2007). Nevertheless, the efficiency of these tree-based water status indicators to determine tree water use has never been tested under PRD irrigation, because all studies so far have been done under RDI or SDI conditions. Likewise, little emphasis has been put on studying their reliability and effectiveness at the onset of water deficit, which is especially interesting to identify water stress in fruit trees growing in semiarid areas since they are usually subjected to large variations (day/night) in temperature and relative humidity. Thus, the goal of this work was to evaluate MDS, MDS ratio, and TGR in a species, whose performance under DI has been scarcely studied, grapefruit trees in a semiarid area during the first weeks of application of SDI and PRD.

Materials and Methods

Experimental design and tree growth conditions

This experiment was conducted between October and November 2012 at the Texas A&M University-Kingsville Citrus Center, in Weslaco, Texas. Similar-sized two-year-old potted grapefruit trees (*Citrus paradisi* Macf. 'Rio Red') grafted on sour orange (*Citrus aurantium* L.) were grown in a greenhouse with open sides. During the experiment, maximum temperatures ranged from 21 to 40°C, minimum temperatures were between

60 12 and 25°C, and relative humidity values ranged between 19 and 94%. Before irrigation treatments started, and
61 since one of the treatments (PRD) required water application on only one-half of the root zone, root systems of
62 all trees were split in two halves up to the soil line and allowed to become established in adjacent 2.4 L pots that
63 were taped together (Melgar et al., 2010); this was done to assess the response of trees to the different amounts
64 of water applied and not to the different root system distribution in the pots. All pots were filled with a
65 commercial soil potting mixture (Metro-Mix 300, Sun Gro, Bellevue, WA, USA) with an average bulk density
66 of 232 kg·m⁻³, containing vermiculite, composted pine bark, Sphagnum peat moss, coarse perlite, bark ash,
67 starter nutrient charge and slow release nitrogen, and Dolomitic limestone. All trees were daily irrigated to field
68 capacity during one week so that trees recovered from the stress of splitting the roots and transplanting before
69 the application of the different treatments. The experimental design was a completely randomized design, with
70 three irrigation treatments (Fig. 1) and six replicates per treatment: 1) Control treatment, trees received 100% of
71 their evapotranspirative needs (ET_c), 50% on each side; 2) 60 SDI: trees received 60% ET_c, 30% on each side;
72 and 3) PRD: 100% ET_c was applied to only one-half of root zone. ET_c was calculated gravimetrically every
73 week by determining weight loss of each replication and trees were irrigated daily during six weeks.

74 *Soil moisture and tree measurements*

75 Soil volumetric water content (VWC) was measured every 10 days by time domain reflectometry using a soil
76 moisture probe FieldScout TDR 100 with 12 cm rods (Spectrum Technologies, Plainfield, IL, USA). The probe
77 was calibrated for this type of soil by plotting a calibration curve to determine the VWC before the experiment
78 started.

79 Trunk diameter variations were measured using dendrometers (Model DEX20, Dynamax, Houston, TX,
80 USA). Trunk diameter variations started being measured two weeks after the beginning of the experiment and
81 were collected hourly until the end of the experiment by a GPI data logger (Dynamax). Maximum daily
82 shrinkage, MDS ratio, and TGR were calculated daily in two trees per treatment until the end of the experiment.
83 Most authors in similar papers have used similar number of replications [for instance, one (De Swaef et al.,
84 2009) or three sensors (Han et al., 2012) per treatment] due to the elevated cost of the sensors and their high
85 accuracy and stability. Furthermore, the sensitivity of MDS and TGR to detect water deficit was calculated as
86 the average signal intensity to noise ratio (Goldhamer and Fereres, 2001); the signal intensity was defined as the
87 value of the variable (MDS or TGR) for the water stressed treatment divided by value of the variable for the
88 control treatment, and the noise was defined as the average coefficient of variation.

89 Stomatal conductance (g_s) and chlorophyll fluorescence (efficiency of photosystem II, F_v/F_m) were measured
90 once per week, as described in Romero-Conde et al. (2014). Midday stem water potential (SWP) was measured
91 every 10 days in one leaf per tree using a Scholander-type pressure chamber (PMS Instrument Company,
92 Albany, OR, USA; Scholander et al., 1965). At the end of the experiment, leaf fresh weight (FW), and leaf, stem,

93 and root dry weights (DW) were recorded. Leaf water content (θ_{leaf}) was calculated as $100 \times (\text{FW}-\text{DW})/\text{FW}$.
94 Root length was measured using a scanner STD4800 and the WinRhizo basic software (all from Regent
95 Instruments Inc., Quebec, Canada). Specific root length was calculated as the ratio between root length and root
96 DW. Shoot to root ratio (S/R) was calculated as the sum of leaf and stem DW divided by root DW.

97 *Statistical analysis*

98 The MDS ratio for 60 SDI and PRD were compared using a t-test ($\alpha = 0.05$, two tailed). All the other data
99 were subjected to analysis using a one-way variance (ANOVA; SPSS statistical package; SPSS, Chicago, IL,
100 USA) using Turkey's test for mean separations ($p \leq 0.05$) when a significant F-test was observed.

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Results

103 Accumulated water amounts applied to the 60 SDI (4.9 L) and PRD (5.2 L) trees by the end of the
104 experiment were 36% and 33% lower than the amount applied to control trees (7.7 L), respectively (Fig. 2). Soil
105 water content was similar in the control ($32.2 \text{ m}^3 \cdot \text{m}^{-3}$) and 60 SDI ($34.1 \text{ m}^3 \cdot \text{m}^{-3}$) treatments but the PRD
106 treatment had significantly lower soil water content ($24.2 \text{ m}^3 \cdot \text{m}^{-3}$) than the control and 60 SDI at the end of the
107 experiment [soil water content value was the average between wet ($34.9 \text{ m}^3 \cdot \text{m}^{-3}$) and dry ($13.6 \text{ m}^3 \cdot \text{m}^{-3}$) sides].

108 Day-to-day MDS values varied largely and no significant differences were observed between treatments (Fig.
109 3). The mean value for control trees was 0.16 mm, whereas 60 SDI and PRD trees had mean MDS of 0.11 and
110 0.12 mm, respectively. Nevertheless, there was a clear trend of MDS ratio in trees under the 60 SDI being
111 smaller than in PRD during the last weeks of the experiment, and significant lower values of the MDS ratio of
112 the 60 SDI trees were observed during week five (Table 1). On the other hand, the TGR was similar among all
113 treatments in each week and did not detect any significant difference in any of the weeks (Table 2). Furthermore,
114 the MDS had higher sensitivity to water deficit than the TGR (Table 3). Stem water potential, θ_{leaf} , g_s , and F_v/F_m ,
115 were not affected by deficit irrigation treatments (Table 4). All tree growth parameters were similar in control,
116 the 60 SDI and PRD trees (Table 5).

117

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Discussion

119 This study focus on assessing the effectiveness of MDS, MDS ratio, and TGR to monitor tree water deficit
120 during the first six weeks, because it is in these initial weeks when changes in tree water status normally occur.
121 Both the 60 SDI and PRD treatments were effective in reducing accumulated ET_c and water application volumes
122 since the first weeks of the study, and trees of both treatments received similar amounts of water by the end of
123 the experiment.

124 The absolute MDS value showed large day-to-day variations as a consequence of the variable environmental
125 conditions (temperature and relative humidity) and could not be used to assess water deficit. There was only one

126 date where significant differences were found for the 60 SDI treatment (second day of week four) but, based on
127 the performance of the other treatments that day, this value can be identified as an outlier. Similar variability has
128 been reported by other authors in citrus (Velez et al., 2007), and also tree-to-tree variability has been reported to
129 be higher for MDS than to SWP (Goldhamer and Fereres, 2001; Nahor and Cohen, 2003). On the other hand, the
130 MDS ratio of 60 SDI trees was smaller than the MDS ratio of PRD trees. This means that the MDS of 60 SDI
131 trees were more different from the MDS of control trees than the PRD trees were, since these PRD trees had a
132 MDS ratio close to unity. Maintaining the MDS ratio around the unity is successfully used for scheduling full
133 irrigation in citrus trees, avoiding the absence of soil water deficit (García-Orellana et al., 2007; Ortuño et al.,
134 2009; Velez et al., 2007).

135 Trunk diameter fluctuations are also known to respond sooner to water deficit than other tree water status
136 indicators such as SWP or sap flow (Goldhamer et al., 1999). Trees in the 60 SDI and PRD treatments had SWP
137 values similar to control trees. In our study, SWP values are typical of non-stressed trees or trees with a very
138 mild stress without consequences in growth (Ballester, 2013). There have been several studies in fruit tree
139 species on the effectiveness of using parameters based on trunk diameter fluctuations to detect water deficit, and
140 the different results of these studies respond mostly to the species and to the degree of soil water deficit (for a
141 review, see Ortuño et al., 2010). In that sense, the lack of a severe stress condition in the 60 SDI trees (the only
142 treatment that received less water than their tree evapotranspirative needs) was a consequence of the frequency
143 of water application in this treatment, which was the same as in control trees. Regarding TGR, all treatments
144 showed similar values for each week. Unaffected TGR could have also been a consequence of the capability of
145 trees to maintain a normal xylem sap flow rate during mild water stress (De Swaef et al., 2009). Furthermore,
146 TGR also had a lower sensitivity (signal intensity / noise ratio) to water deficit (0.68) than MDS (1.95). This low
147 sensitivity value for TGR is due to the high coefficient of variance observed in this measurement, which was a
148 consequence of the low growth rate recorded. In this regard, Ortuño et al. (2004) reported that MDS was a better
149 indicator than other parameters derived from trunk diameter fluctuations when trunk growth was very low.

150 Soil water content did not dramatically decrease in the SDI treatment with respect to the control, and tree
151 growth (measured through the leaf, stem, root, total plant DW, and S/R) as well as other physiological
152 parameters such as θ_{leaf} , g_s , and F_v/F_m , were comparable to the observed in control trees because of the mild
153 deficit. Stomatal regulation influences sap flow reduction (Jones, 1998) but the mild stress applied in this
154 experiment did not cause reductions in g_s .

155 Based on these results, the effectiveness of the MDS ratio to detect water deficit was higher in the trees under
156 60 SDI than in trees under the PRD, and thus, the MDS ratio could be an effective tool to detect differences at
157 the onset of a water deficit before any other physiological or growth parameters measured. In conclusion,
158 continuous recording of MDS and the use of the MDS ratio offer a promising possibility for its use in automatic

159 irrigation scheduling under DI even during the onset of water deficit, when trees under SDI showed more
160 sensitivity to this measurement than trees under PRD.

161

162 **Acknowledgements:** The authors thank the Consejo Superior de Investigaciones Científicas (CSIC) for funding
163 the stay of S. Elsayed-Farag at the Texas A&M University-Kingsville Citrus Center and her JAE-predoc
164 fellowship and Ayako Kusakabe, research associate at the Citrus Center, for her technical support.

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209

210 **Table 1.** Effects of the irrigation treatments on mean (n = 2) MDS and MDS ratio during the
 211 experiment.

Treatment	Week		
	4	5	6
MDS Control	0.18 a	0.13 a	0.12 a
MDS 60 SDI	0.12 a	0.07 b	0.07 b
MDS PRD	0.15 a	0.11 a	0.09 ab
MDS ratio			
MDS ₆₀ /MDS _{control}	0.67 a ^z	0.54 b	0.58 a
MDS _{PRD} /MDS _{control}	0.83 a	0.85 a	0.75 a

212 ^zWithin each column, different letters indicate significant differences at $p \leq 0.05$.

213

214 **Table 2.** Effects of the irrigation treatments on mean (n = 2) TGR (mm) during weeks four, five, and
 215 six of the experiment.

Treatment	Week		
	4	5	6
Control	0.14 a ^z	0.04 a	0.04 a
60 SDI	0.18 a	0.02 a	0.04 a
PRD	0.08 a	0.02 a	0.05 a

216 ^zWithin each column, similar letters indicate no significant differences at $p \leq 0.05$.

217

218 **Table 3.** Sensitivity to water deficit (signal intensity/noise ratio) of MDS and TGR.

Water status indicator	Signal	Noise (CV ^z)	Sensitivity
MDS	0.72	0.37	1.95
TGR	0.90	1.33	0.68

219 ^zCoefficient of variation.

220

221 **Table 4.** Effect of the irrigation treatments on mean (n = 6) stem water potential (SWP), leaf water
 222 content (θ_{leaf}), stomatal conductance (g_s), and maximum quantum efficiency of photosystem II (F_v/F_m ,
 223 dimensionless).

Treatment	SWP (MPa)	θ_{leaf} ($\text{m}^3 \cdot \text{m}^{-3}$)	g_s ($\text{mol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$)	F_v/F_m
Control	-1.1 a ^z	66.7 a	0.30 a	0.77 a
60 SDI	-1.2 a	67.0 a	0.30 a	0.78 a
PRD	-1.0 a	66.7 a	0.23 a	0.78 a

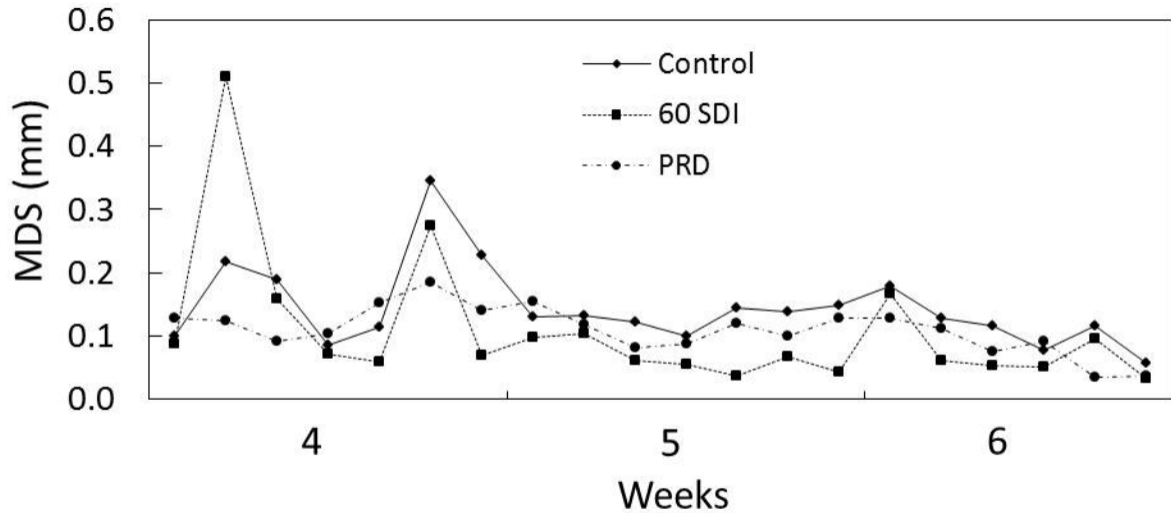
224 ^zWithin each column, same letters indicate no significant differences at $p \leq 0.05$.

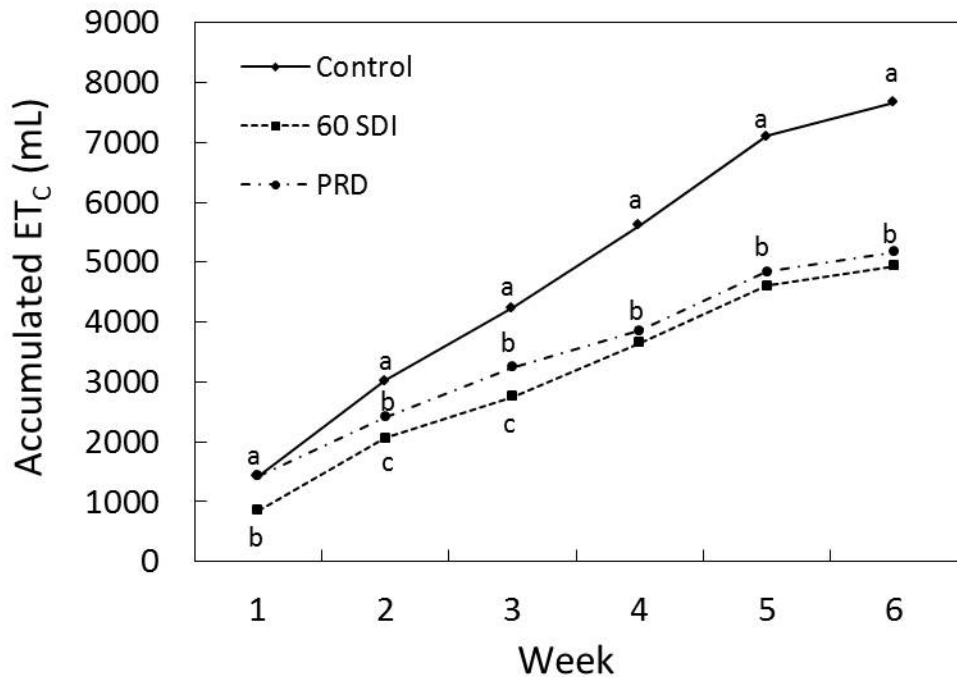
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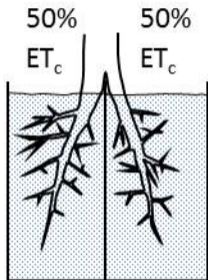
226 **Table 5.** Effects of the irrigation treatments on mean (n = 6) leaf, stem and root dry weight (DW), total
 227 plant dry weight (TPDW), root length, specific root length (SRL), and shoot to root ratio (S/R,
 228 dimensionless).

Treatment	Leaf DW (g)	Stem DW (g)	Root DW (g)	TPDW (g)	Root length (cm)	SRL ($\text{mm} \cdot \text{mg}^{-1}$)	S/R (shoot/root)
Control	6.6 a ^z	11.7 a	19.9 a	38.2 a	1515.8 a	0.81 a	0.93 a
60 SDI	4.1 a	8.8 a	16.8 a	29.7 a	1397.2 a	0.83 a	0.78 a
PRD	4.3 a	10.0 a	19.2 a	33.5 a	1302.5 a	0.65 a	0.74 a

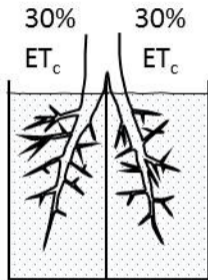
229 ^zWithin each column, same letters indicate no significant differences at $p \leq 0.05$.



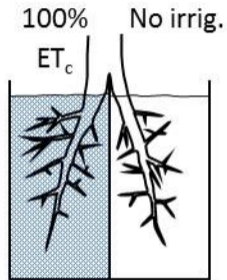




Control



60 SDI



PRD