

Stress Induced Water Content Variations in Mango Stem by Time Domain Reflectometry

A. Nadler,* Eran Raveh, Uri Yermiyahu, and S. Green

ABSTRACT

Close, direct, and accurate monitoring of the plant water status may serve as a practical (irrigation scheduling) and a research (climate–environmental induced physiologic changes) tool. Methods for high-frequency capacitance measurement (e.g., time domain reflectometry [TDR]) possess the potential for high resolution dielectric measurements with minimal dependence on properties of the measured matrix. The objective of this study is to test the accuracy, response time, and sensitivity of the TDR methodology in measuring changes in water status in a mango (*Mangifera indica* L., Cultivar ‘Kent’) tree stem exposed to several perturbations concerning water, salinity, fruit load, and radiation. Under several induced stress conditions, stem and root zone water content (θ) and electrical conductivity (σ) were simultaneously measured. Our study is distinct in its detailed and frequent measurements of stem water content (θ_{stem}) using short (29–70 mm) TDR probes in trees growing in a detached medium. We have found that θ_{stem} response to root zone applied salinity and water stress were negative and positive, respectively. Stem electrical conductivity (σ_{stem}) was primarily dependent on θ_{stem} and only negligibly on stem cells salinity. The θ_{stem} response time to water application was ~4 h. Two practical outcomes of our study were: (1) Because the salt content of the tree cells only slightly affected σ_{stem} , stem resistivity measurements could be used to represent dielectric changes, and (2) quite short probes could be used to include young trees of slim tree branches.

EFFICIENT IRRIGATION may be achieved by monitoring several water availability indicators: remotely related evaporation pan, closely related soil moisture distribution, or the directly linked plant water status. The latter helps overcome the doubtful relevance of plant water availability to evaporation pan characteristics such as, indirectness or low accuracy, or the spatially variable, texture-dependent water retention. Plants water status may be measured in the roots, leaves, or the stem. In the roots, a random selection may not confidentially reflect the complete root system. Also, being installed below the soil surface, more expensive water sealed sensors will be required. Several techniques, manual and automatic, can measure leaf water status: From the most reliable leaf water potential (LWP, labor intensive and unautomatable) to the turgor dependent (Phytomonitoring sensors, Phytech Technologies, Yad Merdechai, Israel) leaf width gauge clips (which requires changing leaves every 2 to 4 d). Previous

studies, based on direct stem sampling or indirect measurement of θ_{stem} (e.g., reduction in stem diameter or leaf water pressure, Ψ (Naor et al., 1995), have found that water stressed tree stems may give up to half their stored waters to the leaves (Waring and Running, 1978) before being back recharged by the roots.

Measuring θ_{stem} by TDR assumes that soil water shortage induces significant changes in the water content of tree stems. Values of $\Delta\theta_{\text{stem}}$ ranging approximately from 0.10 to 0.40 L L⁻¹ have been reported by Reynolds (1965), Waring et al. (1979), and Schill et al. (1996). Several techniques (e.g., coring and gravimetric analysis, γ -probe, and TDR) have been used in the past to monitor θ_{stem} changes over periods of days, months, and up to a year. Gibbs (1930, 1958) was one of the first to show that stems of many tree species can undergo substantial seasonal variations in θ_{stem} . Reynolds (1965) showed that water storage in the sapwood of large Douglas firs could make a significant contribution to daily transpiration under periods of high evaporative demand. Waring and Running (1978) calculated that an equivalent of about 22 mm of water, the same amount as a medium rainstorm, is stored in the sapwood of old-growth Douglas fir trees. Additional information regarding maple, pine, birch, oak, and gum under different growing and climate conditions can be found in Waring et al. (1979), Jackson et al. (1995), Clark and Gibbs (1957), and Wullschlegel et al. (1996). Recently, xylem tissue ϵ and its relations with xylem sap flux density were measured by McDonald et al. (2002) with specific equipment they have built (McDonald et al., 1999). Agreement with gravimetric analysis of excised stem segments was good. Most of the data discussed above are related to nonirrigated trees and time intervals between measurements were large. The above experimental evidence shows that TDR is capable of measuring θ_{stem} (at different time scales) in a range of tree water status.

Diurnal changes in θ_{stem} of 0.08 L L⁻¹ were found in irrigated apple trees (Brough et al., 1986) and in un-irrigated pines (*Pinus contorta*) using γ -probes. Edwards and Jarvis (1983) also reported a large drop in θ_{stem} at different radial depths in the xylem of Sitka spruce trees (at 1 and 4 m above ground), as measured by γ attenuation and gravimetric methods. Short-term (diurnal) and long-term (seasonal) changes in θ_{stem} by using TDR in natural groves of aspen, pinion, cottonwood, and ponderosa, Constantz and Murphy (1990) found absolute values of θ_{stem} between 0.20 and 0.70 L L⁻¹, with an annual change in moisture content between 15 to

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Abbreviations: DOY, day of year; DW, distilled water; EM, electromagnetic; TDR, time domain reflectometry; θ , volumetric water content; θ_{stem} , stem water content; θ_{perlite} , perlite water content; σ , electrical conductivity; σ_a , bulk electrical conductivity; σ_{stem} , stem electrical conductivity; σ_{perlite} , perlite electrical conductivity; ϵ , dielectric constant.

70% depending on tree species, as well as soil and atmospheric conditions. Absolute values of θ_{stem} as measured by TDR were in good correlation with gravimetric measurements of θ_{stem} as determined by weight loss (Holbrook and Sinclair, 1992).

Consequently, the major objective of the study was to present the competence of monitoring θ_{stem} and stem resistivity changes by the TDR technology, its accuracy, reaction time, and dimensions. Two other secondary objectives were to relate the experimental findings to the plants physiological changes and suggest guidelines for future improvements.

MATERIALS AND METHODS

Experimental Design

In August 2003, two 15-yr-old mango trees (grafted on 13–1 rootstock; Gazit and Kadman 1980) were purchased, any soil traces were washed away from the root system, and the trees were planted inside a 70-L container filled with perlite (Fig. 1). The perlite water retention was independently tested by packing it into a 10-L pot, saturating with tap water, draining, and weighing in the laboratory. During this stage two 200-mm long TDR probes, made from three rods of 3-mm diam. stainless steel at 50-mm spacing, were diagonally installed inside each container, in the lower third (Probes 7 and 9 in Containers 1 and 2, respectively) and middle third of the containers (Probes 8, and 10, in Containers 1 and 2, respectively). After 6 wk of tree recovery, when new leaves appeared, 2.9-mm holes were drilled in the stem, through a metal leader, and the trees were installed with six TDR probes, made from three rods of 3-mm diam. stainless steel at 50-mm spacing, with rods

plane parallel to the stem's long axis as follows: In Tree 1: two 70-mm long probes 0.35 and 0.50 m above the grafting and stretching the full stem diameter (1 and 2). Two additional probes, 48 (Probe 17) and 29 mm long (Probe 18), were installed 0.4 and 0.6 m higher. Two 70-mm long probes were installed in Tree 2 similarly to Tree 1 (3 and 4). Before probe installation the geometric factors, relating R to σ , were determined in the laboratory for all the probes. A 4.0-m coaxial cable (RG58U) connected each of the probes, to a multiplexer (TR-200, coaxial multiplexer 16 inputs 1 output, Dynamics Inc. Houston, TX) and a 0.9-m cable to the cable tester (Tektronix 1502B, Beaverton, OR). The TDR probes cables (50 Ω RG58) were wrapped by aluminum foil to reduce temperature changes. The TDR trace was recorded every 30 min and the probes' rods apparent length (l_a) was automatically calculated by identification of the probe's rods beginning and end-point (= reflection) by software written by S. Green (used by permission, available to everybody on request). In February 2004, four temperature sensors (Hobo thermometers and Dataloggers, Onset Computers, Bourbe, MA) were installed, one in each stem and container. The automatic irrigation system consisted of a controller, pump, two 200-L containers, and four drippers (2 L h⁻¹) per tree. Throughout the days of year (DOY) 120 to 256 the trees were irrigated for 45 min, five times a day (0800, 1000, 1200, 1400, and 1600 h) totaling to 30 L d⁻¹. Tap water ($\sigma_a = \sim 1$ dS m⁻¹) was spiked with 55 mL of a liquid fertilizer per 200 L of tap water. The liquid fertilizer was 'Shefer 3' (Fertilizers and Chemical Materials Ltd, Haifa, Israel), which contained 7:1.2:5.8 of N, P, K, and the amounts of Fe, Mn, Zn, Cu, and Mo) raising the $\sigma_{\text{input solution}}$ to 1.5 dS m⁻¹. Unless otherwise reported water and nutrition elements were in excess of trees' needs and tree canopy more than tripled. Salinity of drainage waters was determined every 2 to 3 d by collecting 10 L and analyzing a representative sample. According to previous experience regarding the effect of stem tissues curing on the TDR measurement (Wullschleger et al., 1996; Nadler et al., 2003), we have enabled 200 d to reduce installation curing effect for stable θ_{stem} values before reporting continuous measurements. Trees containers were placed over scales (Electronic scales, PM150, UWE, Sebastopol, CA), which were used mainly for measuring tree water use.

Designed treatments were as follows:

Salinity.

Three gradual salinizing–leaching cycles of the root zone by increasing the salinity of the irrigation water were accomplished by NaCl addition to the tap waters: On midnight of DOY 138 and until DOY 151 (σ_{input} was 4.2 and maximal σ_{drainage} was 5.2 dS m⁻¹) and then reduced back to 3.0 dS m⁻¹ on DOY 151 and 1.8 on DOY 153. On DOY 186 to 208 (maximal σ_{drainage} : 13.1 dS m⁻¹, in six stages; four raised the salinity: 3.7 dS m⁻¹ (DOY 186–188), 6.4 dS m⁻¹ (DOY 189–193), 7.9 dS m⁻¹ (DOY 194–197), and 10.2 dS m⁻¹ (DOY 198–201), and two leaching stages, 6.7 dS m⁻¹ (DOY 202–207), and 1.8 dS m⁻¹ (DOY 208–211). During a third salinization cycle, taking place between DOY 275 to 288 (maximal σ_{drainage} : 11.1 dS m⁻¹), daily variations in stem diameter, as well as sap chloride concentration, were monitored. Stem diameter was measured 10 cm above the grafting area, using Linear Voltage Displacement Transducer (LVDT) dendrometers (Model DF-2.5 Manufacturer: Solartron Metrology, Bognor Regis, West Sussex, UK) connected to the datalogger. Sap chloride analysis was conducted as described by Raveh and Levy (2005). Mature stem segments of about 1.3 cm in diameter (characterized by about three xylem rings) and 12 cm in length were collected at 0930 h from the trees and bark was peeled. The remaining



Fig. 1. Two mango trees in 70-L perlite containers installed with time domain reflectometry (TDR) probes (indicated by the arrows).

core of xylem was centrifuged for 25 min at $1912 \times g$ (419 rad s^{-1}) and 0 to 2°C , using a RC-5 Sorval centrifuge equipped with standard SS-34 head (inducing a stem water column pressure of 1.45 MPa). Chloride was analyzed from the extracted sap using a Corning chloride meter.

Water withholding.

For 1 (DOY 164) and 2 d (DOY 170–171), irrigation was stopped and was later followed by 3 d in which tree water-use was replenished by distilled water (DW) according to weighing (drainage was prevented). Manual irrigation was intentionally applied on DOY 180 to 183 and on DOY 160, 180, and 212 due to a faulty pump.

Radiation prevention.

The trees were covered for 24 h (from 1600 h of DOY 158) by a combination of a double layer of black net (Polisak Ltd., Kibbutz Nir-Yitzchak, Israel) and a white 2-mm cloth blocking more than 95% of the photosynthetic active radiation.

Yield removal.

Fruits were removed in two phases: On DOY 228, one third of the yield was removed and a week later, on DOY 235, the rest.

TECHNIQUES

Time Domain Reflectometry Methodology

An electromagnetic (EM) pulse composed of a wide range of frequencies (10 kHz to 1.4 GHz) is formed in the transmitter. Most commonly used is the Tektronix 1502 cable tester (Beaverton, OR). A very short-time (200 ps) pulse is launched, through a coaxial cable, into a wave guide (= TDR probe). The propagation velocity of the EM pulse generated by the TDR is $V_p = l/t$ where l is the distance (m) and t is the time (s). V_p is also related to the dielectric constant (ϵ) through $V_p = c/\epsilon^{0.5}$ where c is the speed of light. Using $\epsilon = (ct/l)^2$ and rearranging, results in $\epsilon = (l_a/l)^2$ where l_a is the measured (apparent) distance from the beginning to the end of the wave guide and l is the real (physical) length. A widely used equation relating ϵ to θ was given by Topp et al. (1980):

$$\theta = 5.3 \times 10^{-2} + 2.92 \times 10^{-2} \epsilon - 5.5 \times 10^{-4} \epsilon^2 + 4.3 \times 10^{-6} \epsilon^3 \quad [1]$$

The dielectric constant of water ($\epsilon_w \approx 80$) is larger than that of stem tissues or perlite ($\epsilon_{\text{OM}} = 2$ to 6 , $\epsilon_{\text{min}} = 3$ to 3.5), or air ($\epsilon_{\text{air}} = 1$). Assuming a negligible contribution to capacitance by mass addition due to stem growth, any changes in measured capacitance may be attributed to stem moisture changes.

When the EM pulse travels through a wave-guide embedded in any medium, the amplitude of the waves is attenuated due to dielectric losses and the electrical conductance of the medium (Cassel et al., 1994). Thus the TDR probe embedded in the medium (e.g., stem, perlite, fruit, soil) can be viewed as a lumped circuit element with impedance Z_L at the end of a low-loss waveguide (Nadler et al., 1991). Due to pulse energy attenuation along the probe rods, measurement sensitivity may decrease for rods shorter than some threshold value (typically around

40 to 70 mm, depending on probe geometry, and medium's uniformity, electrical conductivity, and moisture content). The relative permittivity obtained by the high-frequency ($> 0.5 \text{ GHz}$) TDR has a known or small temperature (T) dependence and is almost free of the medium conductivity effects (mainly ions from salts and clays). Additionally, capacitance type methods, similarly to the TDR, are (i) easily automateable, (ii) flexible in adopting many probes designs (Robinson et al., 2003), and (iii) readily collecting an unlimited number of undisturbed sampling once the probes are installed in target medium (soil, stem). The load impedance (R_L) can be measured after all multiple reflections have faded away and the pulse alternating current voltage became a direct current. At very low frequencies Z_L equals the load resistance (R_L of the TDR probe embedded in the medium), hence:

$$R_L = Z_0[(1 + \rho_{\infty})/(1 - \rho_{\infty})] \quad [2]$$

The reciprocal of R_L equals the direct current conductance and can be converted to bulk EC (σ_a) by applying the geometric cell constant K_c of the TDR probe in:

$$\sigma_a = K_c \times f_T/R_L \quad [3]$$

where f_T is the temperature factor. K_c is determined from measurement of R_L in solutions of known σ_a . All reported σ values were adjusted to 25°C according to Eq. [3] where $f_{T25} = [1 - (T_i - 25) \times 0.02]$ and T_i is the i_{th} measurement temperature (U.S. Salinity Laboratory Staff, 1954).

Values and meaning of measured stem σ_a will depend on whether it was obtained in intact or injured tissues. The passage of an electric current in a solution such as found in plant tissues, is by the movement of ions. Disturbance or injury of stem tissue releases electrolytes into the intercellular spaces causing a local, short time, increase in ions concentration that, in absence of insulation membranes effect, have higher σ_a than intact stems (Nadler, 2004). The present study reports only long-term, stable, intact stem σ_a values.

Wullschleger et al. (1996) produced an empirical relationship converting TDR measurements of ϵ into θ values for four different tree species (red maple, white oak, chestnut oak, and black gum) that were in good match with Constantz and Murphy (1990) calibration. The combined data were fitted to a second-order quadratic equation

$$\theta = -0.251 + 4.66 \times 10^{-2} \epsilon - 4.93 \times 10^{-4} \epsilon^2 \quad [4]$$

Similar calibration equations were obtained by Green and Nadler (unpublished data, 2000) from kiln dry wood blocks that, after saturation with water under vacuum, were equilibrated at different pressures on a standard soil pressure plate. The gradually drying blocks were then weighed and ϵ was determined by TDR in the moist wood after each drying stage. Under the experiment salinity levels, and according to Robinson et al.'s (2003) recent review, the maximal salinity effect on θ_{stem} is $< 0.01 \text{ L L}^{-1}$. The reported accuracy of θ_{perlite} and $\sigma_{a, \text{perlite}}$ applies to the stem θ and σ_a too. It is emphasized that the precision, namely the uncertainty of the true value is

not as important as the accuracy namely the repeatability of the values. The θ values derived from the measured ϵ have not been corrected for T effect on ϵ because the mutually compensating interaction among θ – σ_a – T is negligible (Pepin et al., 1995; Irvine and Grace, 1997).

No T dependence ($R^2 = -0.003$) for the originally measured or the T corrected stem σ_a was found and it will not be further discussed. All the findings and conclusions relate to mango trees under our experimental conditions. Measurement accuracy was evaluated from: (1) Our experimental observations obtained under non-stressing water conditions, and (2) According to specifications of the measuring device (1502 Cable Tester, Tektronix, OR), assuming screen resolution power of ± 1 pixel out of 122 (half the available, because the trace does not fill up the screen most efficiently) for a typical range of l_a values = 200–280 mm (Table 1). The measurement uncertainty may increase due to the unquantifiable effects of factors like nonuniformity of stem shrinking and swelling or trace analysis. A wide natural variability is reported for water content distributions in stems of oaks and redwoods ($\Delta\theta = 0.08$ up to 0.26 L L^{-1} , Constantz and Murphy, 1990), in red maple and white oak (Wullschlegel et al., 1996), and in pines (Irvine and Grace, 1997). It means that (i) averaging probes' output, even from the same stem, should be done cautiously, and (ii) for irrigation scheduling purposes precise results do not have an advantage over accurate ones.

Methodically, results presentation and discussion will be based on responses of θ and σ_a levels in perlite and stem, to the application of the abovementioned treatments, with respect to direction, intensity, and time, including due consideration to prevailing stress conditions. In Fig. 3 through 8 the time (ordinate axis) scale is numbered as DOY = ***.3 which, being equivalent to ~0800 h is relevant to start of stem activity.

RESULTS

Perlite

Plotting the perlite water content (θ_{perlite}) and its bulk electrical conductivity (σ_{perlite}) vs. time (Fig. 2A and 2B) verified that the TDR measurements reflected the ap-

Table 1. The θ and σ measurement ranges, scatter, and calculated experimental errors, under optimal water conditions during DOY 135 to 138.

| Observed values ranges and calculated experimental error | | | |
|--|---------------------------------------|-------------------------|---|
| Calculation according to | Parameter | Average parameter value | Std error (% of full exp. range) ($\times 10^3$) \ddagger |
| Perlite measurements (200 mm long probe rods) | | | |
| Experimental observation | σ , $\text{dS m}^{-1}\ddagger$ | 1.00 | 0.6 (0.06%) |
| Cable tester specifications | θ , L L^{-1} | 0.30 | 0.7 (0.23%) |
| | σ , dS m^{-1} | 1.10 | 0.6 (0.05%) |
| | θ , L L^{-1} | 0.33 | 0.6 (0.18%) |
| STEM measurements (48 and 29 mm long probe rods) | | | |
| Stem Exp observed (48 mm) | σ , dS m^{-1} | 1.00 | 8.0 (0.8%) |
| | θ , L L^{-1} | 0.565 | 2.2 (0.4%) |
| Stem Exp observed (29 mm) | σ , dS m^{-1} | 1.00 | 10.0 (1%) |
| | θ , L L^{-1} | 0.550 | 3.0 (0.5%) |

\ddagger According to $280 \Omega \pm 1 \Omega$ and separation power of ± 1 pixel.

\ddagger The standard deviation values have already been multiplied by 10^3 .

plied treatments: Throughout the study period (April to September 2004, DOY 120–256) θ_{perlite} fluctuated between 0.35 to 0.38 except when water supply was limited deliberately (DOY 164–174 and DOY 182–184) or accidentally (DOY 160, 180–181 and 212–213), and also when pores water salinity (σ_{drainage}) was above a threshold value of 5 dS m^{-1} (DOY 144–156 and 194–211). The independently tested pot of perlite capacity produced a similar (0.347 L L^{-1}) value.

The daily θ_{perlite} amplitudes of the deeper probes (7 and 9) were narrow (± 0.005 to $\pm 0.035 \text{ m m}^{-3}$) relative to the wider amplitudes (up to 0.05 L L^{-1}) of the shallow ones (8 and 10), indicating that the deeper part of the container was buffered and slower to drain. Total available water amount in the perlite was $\approx 20 \text{ l}$, about 2 to 3 d of average tree consumption. The amplitude of daily irrigation events (Fig. 2F) was smaller for deeper probes than that of the shallow ones (Probes 8 and 10). A demonstration of the θ_{perlite} and σ_{perlite} response to a change in irrigation rate and salinity can be seen in Fig. 2F: Exceptionally, in addition to the five daily irrigation events by fertilizers-spiked tap water, the trees were irrigated with a saline solution of 4.2 dS m^{-1} from midnight until 0700 h. On 0800 h the routine irrigation schedule was restored, still with the higher salinity solution. The sharp increase in θ_{perlite} and σ_{perlite} starting 0.5 h after midnight is clearly seen (Fig. 2F, dashed vertical line). While the shallow probe (8) reached its balanced irrigation–drainage water status immediately (DOY = 138) and has maintained it during the following days, the deeper probe indicated a θ_{perlite} increase after DOY = 138 intensive leaching.

Measured σ_{perlite} (Fig. 2B), differed from the θ_{perlite} mainly regarding its response to salinity of the irrigation water, by linearly responding to the irrigation water salinity increase for the full 1.7 to 13.2 dS m^{-1} scale, rather than nonlinearly and, only above a σ_{drainage} threshold value, as was the case in θ_{perlite} . The five irrigation events can also be observed in the σ_{perlite} cycles (Fig. 2F) that almost perfectly match the θ_{perlite} cycles. Reaction time of the TDR probes was determined only by the arbitrary measurement interval (in the current study, 30 min.) as expected according to the perlite extremely high hydraulic conductivity (HC). It should be stressed again that, although the same measuring device (Tektronix 1502 cable tester) measured both parameters, they are technically and methodically independent.

Stem

Time domain reflectometry-measured θ_{stem} response to withholding and renewal of irrigation during DOY = 164 to 174 (Fig. 2C) matched the induced water stress and its relieving. However, θ_{stem} response to the salinity increase was surprising. We could estimate the number of passing pore volumes by comparing tree daily water consumption to θ_{stem} of a cross-section. For a 70-mm stem diameter, a 40-mm sample width (assuming 20 mm above and below the rods plane), and assuming $\theta_{\text{stem}} \approx 50\%$, one pore volume is about 0.08 L . If the tree average water consumption was 4 to 8 L d^{-1} , 50 or more

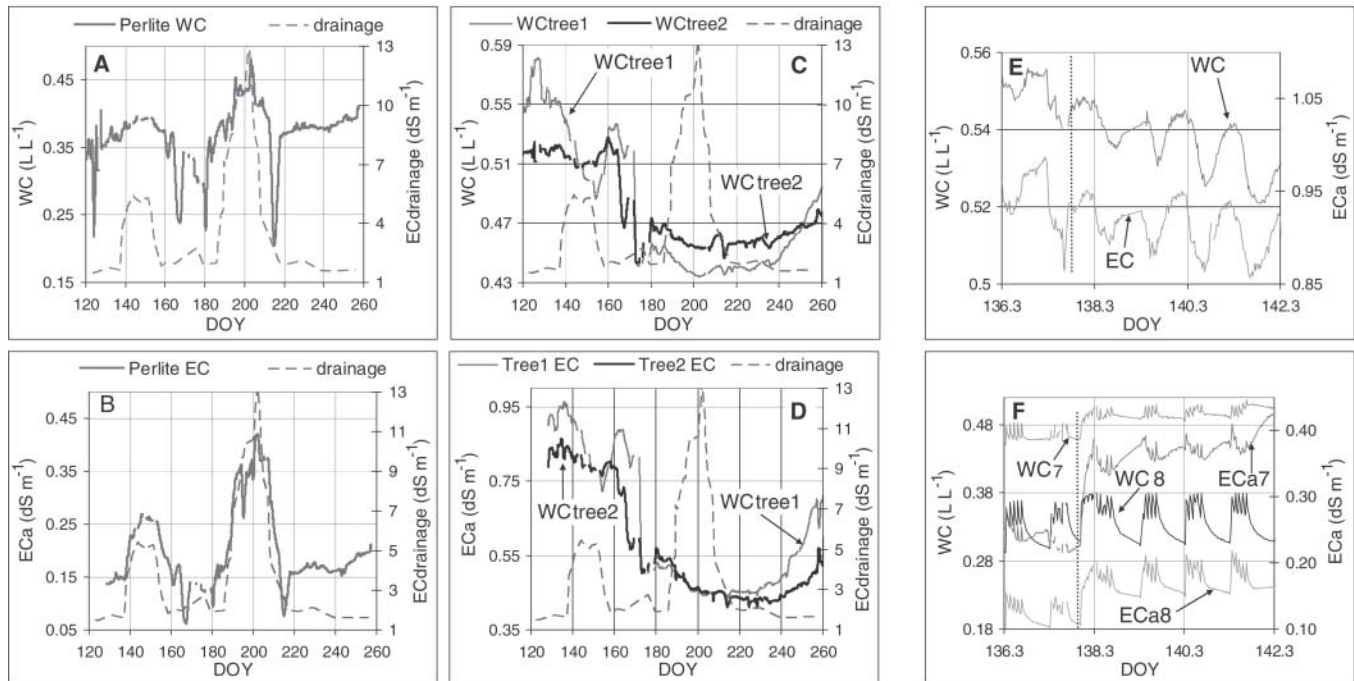


Fig. 2. (A) Averaged perlite water content (θ_{perlite}) and (B) perlite electrical conductivity (σ_{perlite}), (C) averaged stem water content (θ_{stem}) and (D) stem electrical conductivity (σ_{stem}), of the two trees during experimental period (DOY 120–260), and σ of draining solution. Also shown are (E) θ_{stem} and σ_{stem} (Tree 1), and (F) the daily five-peak θ_{perlite} and σ_{perlite} of the deep (7) and the shallow (8) perlite probes. Dotted line indicates midnight salinization start.

pore volumes flowed through the stem in an average day. Still, in the period DOY = 138 to 158 σ_{stem} changes were opposite to the salinization and leaching processes. The θ_{stem} dropped once the salinity have increased (DOY = 186) and slightly recovered when salinity was leached out (DOY = 208). It can be seen in the time-expanded scale plot (Fig. 2E) that σ_{stem} and θ_{stem} monotonously decreased during the following 3 d in spite of doubling the salinity of the irrigation water.

Induced Water Stress

During Optimal Water Content Conditions

A water stress was induced on the trees by stopping irrigation for 1 d (DOY = 164) and 2 d (DOY = 170–171, Fig. 3). Each event was followed by 3 d in which DW were replenished (according to the container's total weight, thus avoiding any salt addition) to compensate for tree water use. After 1 d without irrigation (Fig. 3A) the drop in θ_{stem} (ranging 0.465–0.545 L L⁻¹, average = 0.505 L L⁻¹) was limited to 0.015 L L⁻¹ during that day (probably due to stem water reserves) but more than doubled to 0.04 L L⁻¹ on the next day (DOY = 165) due to dwindling of stored water. Establishing back the routine irrigation schedule (167–169) recovered θ_{stem} to 60% of its initial value. Comparing daily θ_{stem} amplitudes averaged from Probes 1 and 2 ($\theta_{1-2, \text{stem}}$) before and after DOY = 164 showed amplitudes have increased, indicating that some stored water had supplied part of the tree water needs.

Stopping irrigation for 2 d lowered θ_{stem} (ranging 0.488 to 0.530 L L⁻¹, average = 0.507 L L⁻¹) by 0.065 L L⁻¹

well below the 1 d stop, and adding DW to replace water consumed by the tree could not do much. Comparing daily θ_{stem} amplitudes before and after DOY 171 and 172 showed smaller amplitudes, indicating lack of stored water that might have been used to supply the tree water needs.

The σ_{stem} values of all four probes followed θ_{stem} values during this period (Fig. 3B, 3D, and 3F) indicating that σ_{stem} was strongly dependent on θ_{stem} . Reducing irrigation rate could have affected σ_{stem} (determined by the product of volume \times concentration) in two contradicting directions: Salinity increase and θ decrease. The latter parameter was the more effective and can be seen in σ_{stem} decline against expectation (Fig. 3B, 3D, and 3F). It can be deduced that the difference in θ_{stem} between plentiful and shortage was a few percentages of water content.

A clear demonstration of the θ_{stem} measurement reaction time was presented on DOY 214 after a 2-d weekend of no irrigation. Around 0800 h (DOY = 214.33), the operator became aware of the severe water shortage and reacted by an urgent, manual application of a pail of water. Around 1400 h (DOY = 214.58), still not able to replace the faulty pump, he repeated the water addition. In both cases these wetting actions were detected by the 70-mm long TDR probes within 4 h (Fig. 4). Also present in these figures are the same events as recorded by the shorter stem probes, 48 (17) and 29 (18) mm, respectively. Although, as expected, the noise/signal ratio increased with decrease in probe length, the θ change was still detectable with the short probe and its performance could be enhanced by improving probe design and impedance matching.

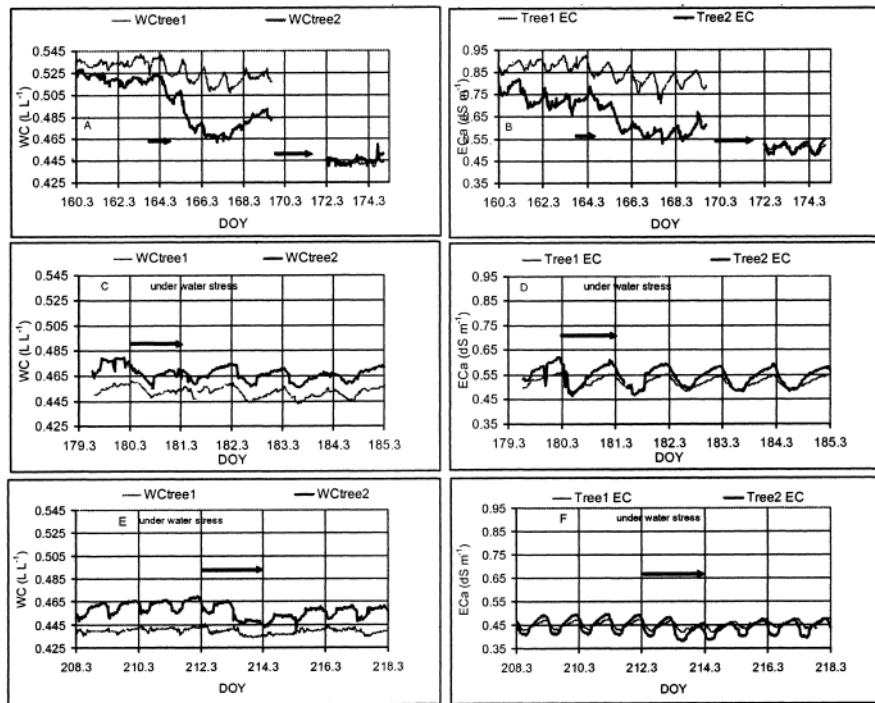


Fig. 3. Irrigation withholding tests: (A, B) Averaged θ_{stem} and σ_{stem} during a 1- and 2-d irrigation withholding under optimal irrigation conditions (DOY 160–175,.) and 1- (DOY = 179–185, C, D) and (E, F) 2-d irrigation withholding (DOY 208–218) under water stress conditions. Horizontal arrows indicate timing and length of water withholding.

Induced Salinity

On midnight of DOY 138, the salinity of the irrigation solution was doubled from 2 to 4.2 dS m⁻¹ ($\sigma_{drainage}$ = 5.2 dS m⁻¹) and was gradually reduced back to 3 on DOY 151 and 1.8 on DOY 153 (Fig. 5A, 5C, and 5E). In response to the increased salinity of the irrigation water, θ_{stem} decreased monotonously (Fig. 5) and reversed its direction only after DOY 153 salt leaching. Although the salinity of the irrigation water was doubled, σ_{stem} decreased (Fig. 5E), again indicating the stronger effect of θ in the product $\sigma \times \theta$. During the second salinization cycle on DOY 186, 189, 194, and 198 salinity of irrigation water was increased to 3.8, 5.0, 8.0, and 10.2 dS m⁻¹,

respectively, finally resulting in $\sigma_{drainage}$ = 13.2 dS m⁻¹ and lowered back to 6.7 and 1.9 dS m⁻¹ on DOY 202 and 208 (Fig. 5B, 5D, and 5F). Again, $\sigma_{perlite}$ and $\theta_{perlite}$ values reported precisely those events (Fig. 5B). Similarly to the previous salinization the σ_{stem} and θ_{stem} values showed a mirror image of the perlite (Fig. 5D, 5F).

Exposing the trees to salt stress at a third cycle (data not shown) was accompanied by monitoring daily variation in stem diameter and stem xylem sap chloride content. Expectedly, the salt stress reduced the daily stem diameter growth rate from 0.020 to 0.005 mm d⁻¹, and increased the sap chloride concentration (from baseline level of 4.5 to 14 mM chloride).

Radiation Prevention and Fruit Removal

Covering the trees for 24 h (from 1600 h of DOY 158) caused the only situation where σ_{stem} did not follow θ_{stem} (Fig. 6); until DOY 159 θ_{stem} morning values, occasionally after a short delay, decreased. During DOY 159 it had sharply increased, probably due to surplus water supply over reduced leaves demand. The excessive water not used by the tree was reflected in a slight increase (0.003 L L⁻¹) in the shallow perlite Probes (8 and 10) and in an even more pronounced increase in θ of the deep perlite Probes (7 and 9). In 3 d the routine θ_{stem} pattern was fully restored (Fig. 6A).

Around DOY 227 to 237, θ_{stem} was at its lowest levels (0.435 L L⁻¹) probably due to the consequent stressing treatments and the heavy fruit load. On DOY 228, one third of the yield (~10 Kg) was removed and a week later, on DOY 235, the rest (~20 Kg). After a delay of 2 to 3 d, θ_{stem} started a steep increase (Fig. 7A,

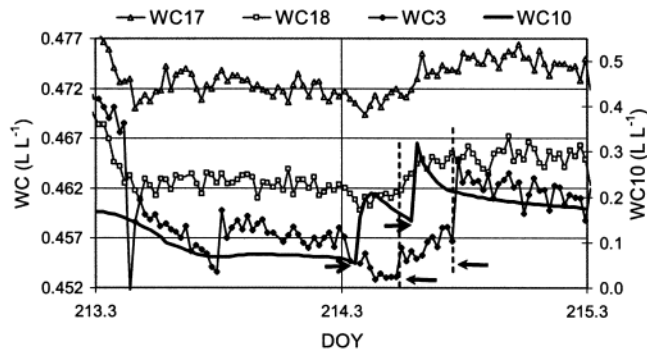


Fig. 4. Two consecutive examples of a 4-h reaction time between following a manual water addition: A sharp increase in $\theta_{perlite}$ (Probe 10, 200 mm) and θ_{stem} by probes: 3, 17, and 18 (70-, 48-, and 29-mm long rods, respectively), during DOY 214. The right facing arrows are pointing to the timing of the manual water additions (214.33 and 214.58) and the left arrows face the dotted vertical lines indicating the start of stem probes response.

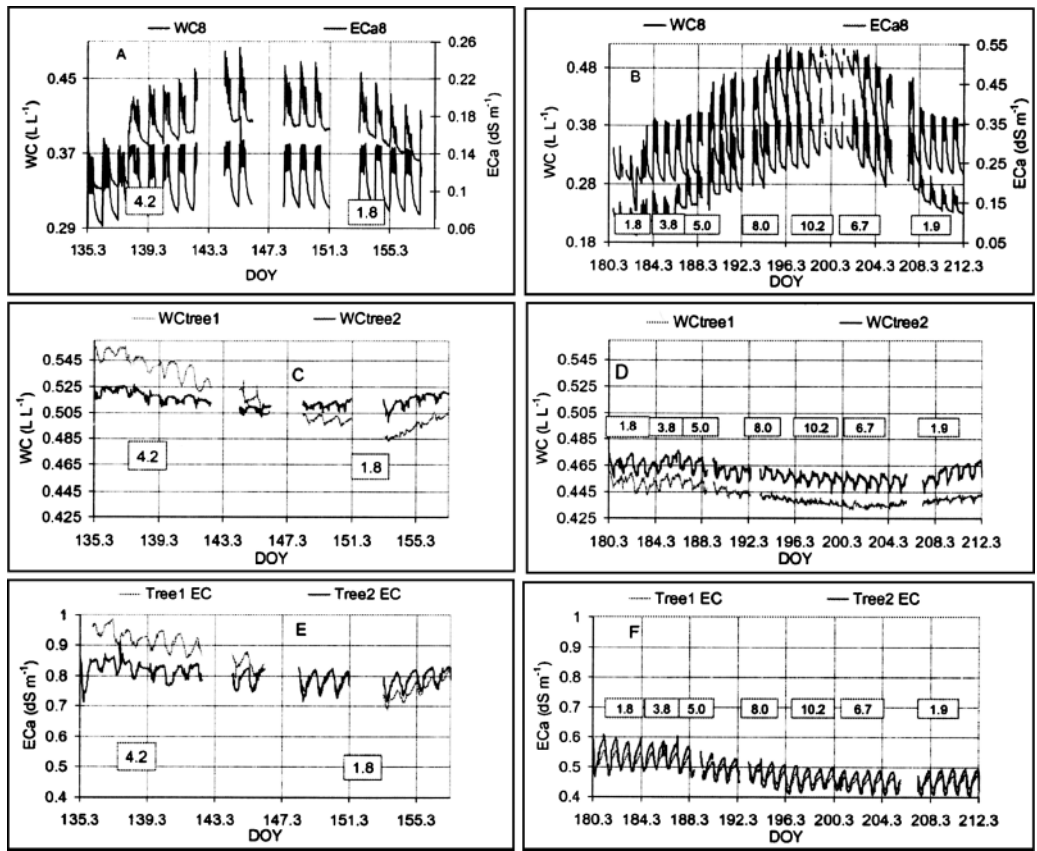


Fig. 5. Salinity tests: (A and B) Tree 1 perlite shallow probe (#8, 200 mm) θ_{perlite} and σ_{perlite} and (C and E) Trees 1 and 2 (70 mm) averaged θ_{stem} and σ_{stem} during a salinizing-leaching cycle (maximal $\sigma_{\text{drainage}} = 5.2 \text{ dS m}^{-1}$, DOY 136–157), and (D and F) during a second salinizing-leaching cycle (maximal $\sigma_{\text{drainage}} = 13.2 \text{ dS m}^{-1}$, DOY 180–212). Boxed values indicate timing and $\sigma_{\text{irrigation}}$.

with σ_{stem} following, Fig. 7B). It took nine more days (DOY 244) for the θ_{perlite} (followed by σ_{perlite} , Fig. 7C) to increase.

DISCUSSION

The perlite θ and σ_a measurements reliably reflected the applied treatments and increased our confidence in

the experimental results. Intensity of θ_{stem} response is proportional to the intensity of the induced stresses; the effect is negative and proportionally increasing from a moderate water stress, to low and then high salinity to severe water stress, and finally the largest change was caused by relieving the tree of the fruit load.

However, a puzzling situation rises from the effect of increasing salinity of irrigation water on σ_{stem} and θ_{stem} ;

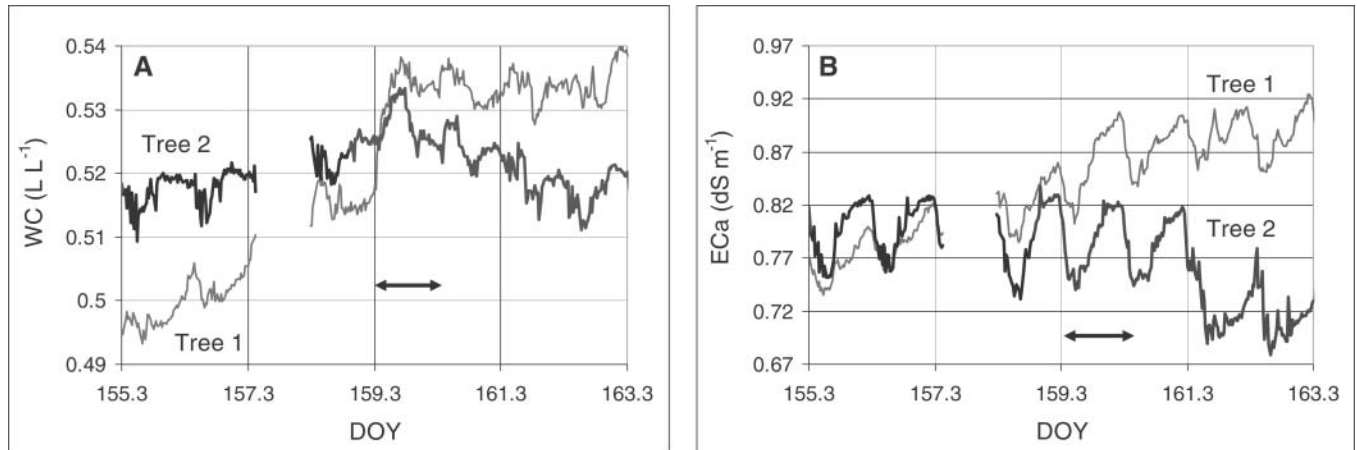


Fig. 6. (A and B) Radiation prevention test on DOY 159: Trees 1 and 2 averaged θ_{stem} and σ_{stem} (70 mm) during DOY 156 to 163. Horizontal arrows indicate timing and length of radiation prevention period.

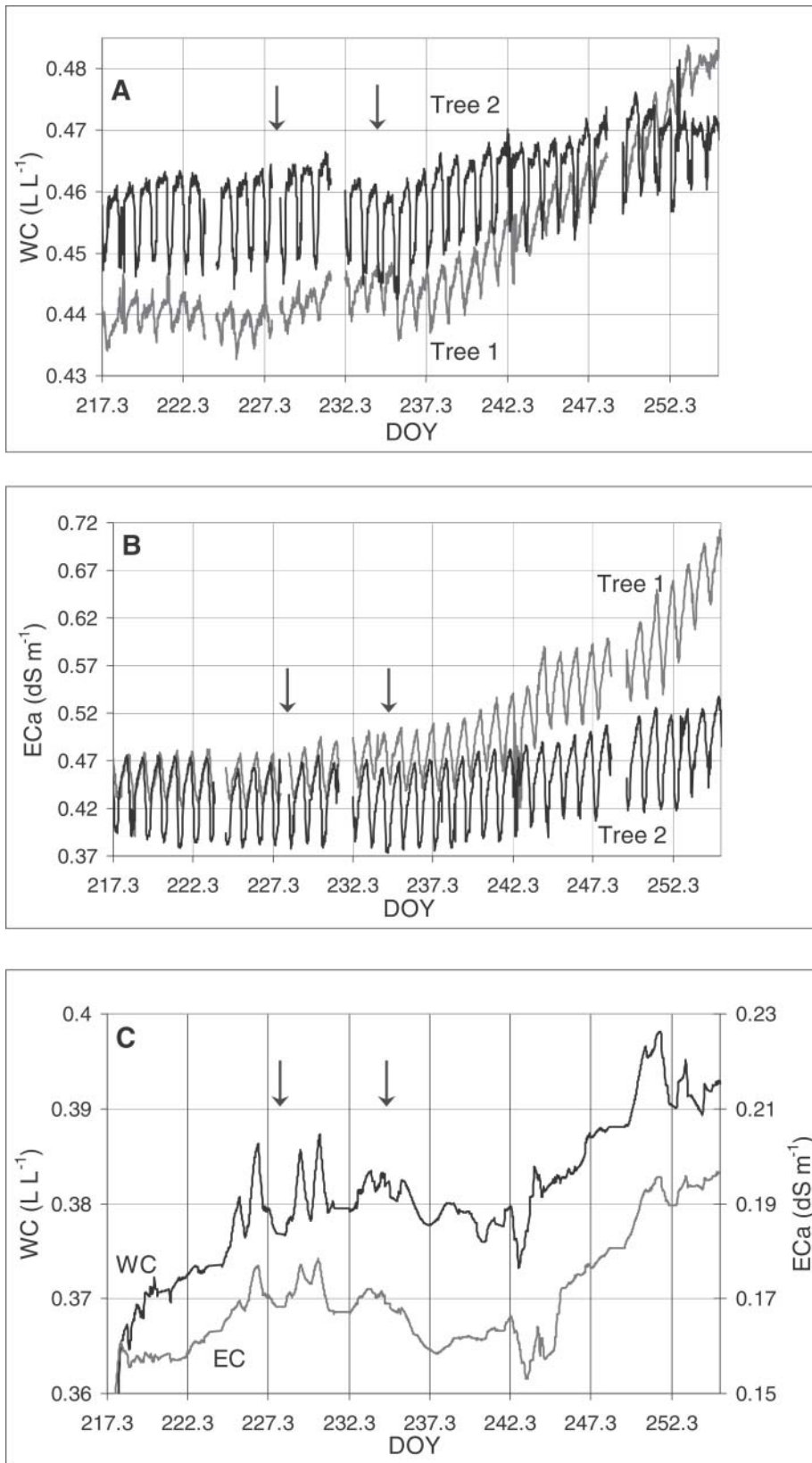


Fig. 7. (A and B) Fruit removal test: Trees 1 and 2 averaged θ_{stem} and σ_{stem} (50 mm) during DOY 217 to 254, and (C) averaged $\theta_{perlite}$ and $\sigma_{perlite}$ of both trees (200 mm). Boxed text indicates fruit removal timing and amount: 1/3 and 2/3 of total yield on DOY 228 and 235, respectively.

Table 2. R^2_{adj} by stepwise analysis between electrical conductivity (σ) of the stem or perlite probes and θ , throughout the experiment period.

| Dependent Parameter σ , by extrapolation | θ | σ , TDR measured | $\sigma_{drainage}$ | Temperature | Time, DOY |
|---|----------|-------------------------|---------------------|-------------|-----------|
| | | <u>Stem</u> | | | |
| Probe 1 (σ_{stem1}) | 0.963 | 0.890 | 0.042 | 0.045 | 0.090 |
| Probe 2 (σ_{stem2}) | 0.977 | 0.928 | 0.045 | 0.035 | 0.045 |
| | | <u>Perlite</u> | | | |
| Average of Tree 1 ($\sigma_{perlite\ 7-8}$) | 0.740 | 0.949 | 0.795 | 0.026 | 0.026 |
| Average of Tree 2 ($\sigma_{perlite\ 9-10}$) | 0.507 | 0.969 | 0.727 | 0.032 | 0.002 |

on one hand no increase in stem σ_a was detected and on the other hand θ_{stem} decreased when $\sigma_{irrigation}$ passed a certain threshold. We suggest that the decrease in θ_{stem} reflects the lower water availability due to increased salinity. It should also shift the input-output (= irrigation-tree water use) balance of the perlite toward wetter conditions, which we have actually detected.

Still we are confused, why doesn't the permittivity and resistivity, measured by the cable tester through stem installed probes, behave as 'expected' according to the theoretical dependence of σ_a on the product $C \times \theta$ (product of concentration by sampled volume), similarly to the $\sigma_{perlite}$ or σ_{soil} (Moore, 1965). Why were not the repeated increases in xylem-sap chloride concentration detected by the probes? The most probable reason is the cation exchange capacity of the cellulose cell walls, which is probably 0.3 to 0.6 $M\ L^{-1}$ (M. Tyree, personal communication, 2005). This way most σ in the stem will be through transport of cations in the cell wall. There will also be σ through living cells in the xylem (ray tissue and xylem parenchyma). The xylem lumens probably may account for only 10 to 20% of the cross-section. All the other cellulose might be 10 to 20%. The lumens of the living cells will also have about 0.5 to 1 $M\ L^{-1}$ salts. But the σ of the living cells will be reduced by the membrane conductance, which is in series with the lumens of the living cells. Thus, adding up all woody tissue σ_a sources, it will probably swamp the NaCl salinity. This explains the significantly smaller sensitivity of σ_{stem} to salinity of the irrigation water. This argument is further supported by the unusual increase in σ_{stem} due to preventing exposure of leaves to radiation of fruit removal.

Testing the dependence of σ of stem and perlite on $\sigma_{drainage}$, time, and θ , σ , and T , it was statistically found (Table 2) that σ_{stem} is determined mostly by θ ($R^2_{adj} = 0.97$) but is almost insensitive to $\sigma_{drainage}$ ($R^2_{adj} = 0.044$), while the σ of all the perlite probes is affected mostly by $\sigma_{drainage}$ ($R^2_{adj} = 0.76$) and less by $\theta_{perlite}$ ($R^2_{adj} = 0.61$).

Accepting this explanation, considering the daily perfect match between σ_{stem} and θ_{stem} cycles, the negative relations between σ_{stem} and the root zone salinizing-leaching stages, we conclude that there is an extreme difference between θ_{stem} and $\sigma_{stem\ pores}$ effect on bulk σ_{stem} where the later is negligible, at least within certain salinity limits and for several species. If this is true and limits are known we could represent θ_{stem} dielectric

changes by resistivity measurements. Considering the wider availability of resistivity measurements and lower meters prices, resistivity-based irrigation scheduling devices should be less expensive by at least two orders of magnitudes.

Additional useful technical aspects of our study relate to the reaction time and the feasibility of reducing probes' dimensions.

It goes without saying that our study does not imply that farmers are going to use the expensive and quite complicated TDR equipment. The long-term intention is that, once the capacitance based technology for sensitive and reliable detection of stem water content changes is established, simpler and less expensive devices will be developed by the industry. Beyond the achievement of a better monitoring of irrigation, the permittivity and resistivity measurements enable observation of the small changes in σ and θ of the plant's different parts as a research aid.

Practical Observations

Daily σ_{stem} and θ_{stem} cycles as a water stress indicator (Fig. 3A–B, 5C, and 8): Averaging θ_{stem} obtained by separate probes is not recommended because it may mask sharp, individual changes. Additionally to absolute θ_{stem} values, also the daily θ_{stem} amplitude may reveal a stress. A close look at both trees θ_{stem} shows that a cycle of 0.03 $L\ L^{-1}$ under optimal water conditions may drop to 0.002 to 0.008 $L\ L^{-1}$ under water shortage (Fig. 3A) or under saline solution (Fig. 5C). A typical behavior of three formats of θ_{stem} daily patterns measured by Probe 1 are shown in Fig. 8: The first (DOY 139–146) water stored in the stem are initially able to recover during the night to its previous morning level but gradually the morning depletion is larger than the afternoon recovery, in the second pattern the morning and afternoon parts are small and similar, and only after alleviation of the stress (DOY 151) is the trend reversed and the morning drop is smaller than the afternoon recovery. Another proof to water stem serving as a temporary storage for the leaves is indicated by the delay in response to irrigation withholding: While the drop in θ and σ in the

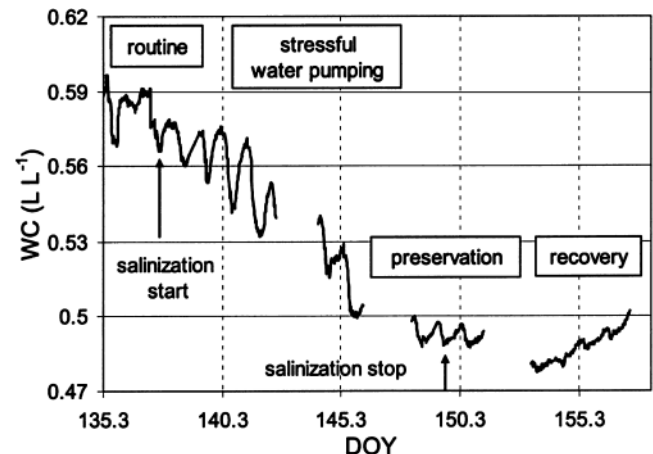


Fig. 8. Three patterns of θ_{stem} daily cycles (Probe 1, 70 mm) during a salinization-leaching process.

perlite is immediate and sharp there is a clear delay of at least 1 d, before θ and σ stem started to drop (e.g., on Days 160, 164, 180, and 211 Fig. 3 and 6).

Design of Miniature Probes

Improved results can be obtained from short probes if the design will maximize the impedance match. Future work will try to optimize this potential with due care to balance between minimizing damage to tree tissues and impedance mismatch, to maximizing sampled volume, and improving accuracy. The present study's conclusions are based mainly on integrating properties measured across the tree diameter. Probes penetrating only a fraction of the stem may be used to test how different layers of the tree cross-section may be differently affected by water stresses. Contributions of the deeper stem sections may be obtained by installing probes extending to the desired depth, which are coated by an insulating material up to the point from where measurement is planned.

Water Threshold Availability

We suggest the simultaneous measurement of stem leaves water potential and σ and θ in stem and root zone medium as a direct tool for determining threshold water content and salinity values under salinity stresses (Fig. 2A and 2B) for different species. The need for a separate determination for each species is evident from the widely reported stem variability but is quite convincing from Myers et al. (1998) findings regarding *eucalyptus grandis* and *pinus radiata* response to salinity and water deficits. These two species responded to salinity increase oppositely or at least differently regarding the following parameters: Predawn water potential, reduction in rate of leaf and stem growth, mean leaf area, and mean foliar concentration of Na^+ and Cl^- .

ϵ - θ Universal Calibration

Similar to the situation in soils (Topp et al., 1980), the existence of a universal ϵ - θ calibration will not be surprising: Concluding from the good match between ϵ - θ calibration of several species (see Background), it may be expected that the same equation could be used for several species.

Side-Product

Perlite σ_a - σ_{pores} calibration can be established from the perlite probes TDR and drainage data collected during the experiment. It may be useful for green houses growers who use perlite as their root zone medium.

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