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Water status indicators of lemon trees in response to flooding and recovery

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Abstract

Potted 2-year-old lemon trees [*Citrus limon* (L.) Burm. fil, cv. Verna] grafted on sour orange (*C. aurantium* L.) rootstock were subjected to flooding for 3 d. Control plants were irrigated daily to field capacity. Continuously (sap flow, trunk diameter fluctuations) and discretely (predawn and midday leaf water potential, leaf conductance) measured plant-based water status indicators were compared. The sensitivity of the maximum daily trunk shrinkage signal intensity to flooding and its behaviour during the recovery period demonstrated that this indicator is more feasible than the others for use in automatic irrigation. The responses to flooding of continuously and discretely measured plant-based water status indicators were very similar to those observed in response to drought stress indicating that it necessary to use soil water measurement automatic sensors to detect the cause of the stress. The results underlined the robustness of the compensation heat-pulse technique for estimating instantaneous and daily transpiration rates on flooding stress and recovery.

Additional key words: *Citrus limon*, leaf conductance, leaf water potential, plant water relations, sap flow, trunk diameter fluctuations.

Introduction

The main direct effect of flooding stress is to limit the availability of oxygen to the roots. Nevertheless, specific plant responses to flooding vary as a function of plant material (Nicoll and Coutts 1998), plant age, time and duration of the stress, and the floodwater characteristics (Kozlowski 1984). Moreover, in assessing the plant resistance to flooding it is also important take into consideration the ability of plants to recover from transient waterlogging of the root system (Smith *et al.* 1990).

The principal flooding symptoms are changes in the leaf water status (Nicolás *et al.* 2005), decrease in leaf gas exchange parameters (Domingo *et al.* 2002), leaf abscission and epinasty (Sánchez-Blanco *et al.* 1994), anatomical and morphological adaptations (Kawase 1981, Savé and Serrano 1986), chlorosis, necrosis and a reduction in growth (Bradford and Yang 1981, Kozlowski 1984, Ashraf and Arfan 2005).

However, studies on the behaviour of continuously recorded plant-based water status indicators (PBWSI), such as sap flow (SF) and parameters derived from trunk diameters fluctuations (TDF), under occasional flooding conditions are very scarce (Nicolás *et al.* 2005). Moreover, knowledge of the behaviour of these PBWSI is essential when their continuous and automatic registers are used for automatic irrigation scheduling (Moreno *et al.* 1996, Shackel *et al.* 1997, Goldhamer and Fereres 2004).

For these reasons the aim of this study was *1*) to assess the response of continuously recorded PBWSI to

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Abbreviations: Ψ_{md} - midday leaf water potential, Ψ_p - predawn leaf pressure potential, Ψ_{pd} - predawn leaf water potential, Ψ_s - leaf osmotic potential, DOY - day of year, E - transpiration rate, g_l - leaf conductance; MDS - maximum daily trunk shrinkage, PBWSI - plant-based water status indicators, PPFD - photosynthetic photon flux density, R_{s+p} - plant plus soil resistance to water flow, SF - sap flow, TDF - trunk diameter fluctuations, VPD - mean daily air vapour pressure deficit.

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flooding stress, *2*) to compare their sensitivity and reliability in detecting the stress compared with other, conventional discretely measured plant water status indicators, and *3*) to use the SF measurements to evaluate instantaneous and daily transpiration rates (E) of the young lemon trees during flooding and recovery periods.

Materials and methods

Plant material, experimental site and treatments: The experiment was conducted in a plastic greenhouse provided with a cooling system, using 2-year-old potted lemon plants (*Citrus limon* (L.) Burm. fil, cv. Verna) grafted on sour orange (*C. aurantium* L.) rootstock. Plant, soil and culture conditions were similar to those described by Alarcón *et al.* (2005). Plants were drip irrigated daily, using one emitter per plant, each delivering 2 dm^3 h^{-1}, in order to maintain soil water potential at around field capacity.

At midday on 13 July 2001, day of year (DOY) 194, eight lemon plants were submitted to two different treatments: control plants (T0), irrigated daily as indicated, and flooded plants (T1) for a period of 3 d. The plants were flooded by placing their pots inside larger plastic containers filled with tap water at 25 ºC, with the water level maintained 3 - 4 cm above the soil surface. For recovery purposes, the pots containing the stressed plants were removed from the water, drained and then placed in the same conditions as the control plants. Recovery was studied over a period of 15 d.

The design of the experiment was completely randomized with four replications per treatment. One plant per replicate was used. A two-ways analysis of variance was performed and means were separated by $LSD_{0.05}$ range test. Linear regression differences were analyzed according to the covariance analysis.

Measurements: The oxygen content of the soil water surrounding the roots (soil-O_2) was measured with an *Orion*, model *810*, dissolved oxygen meter (*Orion Research Inc.*, Beverly, USA). One suction probe was installed in four pots per treatment. Measurements were made in extracted soil water immediately after collecting.

Leaf water potential was measured at predawn (Ψ_{pd}) and at midday (Ψ_{md}) (12:00) in one mature, sun-exposed leaf per replicate, using a pressure chamber, following the recommendations of Hsiao (1990). After measuring Ψ_{pd}, the leaves were frozen in liquid nitrogen and the osmotic potential (Ψ_s) was measured after thawing the samples and extracting the sap, using a *Wescor 5520* vapour pressure osmometer (*Wescor*, Logan, USA). Predawn leaf pressure potential (Ψ_p) was derived as the difference between leaf osmotic and water potentials.

Leaf conductance (g_l) was measured at midday for a similar number and type of leaves as for leaf water potential, using a steady-state porometer (*LI-1600*, *LI-COR*, Lincoln, USA).

Plant plus soil resistance to water flow (R_{s+p}) was measured according to Sands and Theodorou (1978) and Savé and Serrano (1986).

Plant transpiration rate (E) was measured by placing the pots on top of weighing balances (capacity 150 kg and resolution of 5 g, *Ohaus Co.*, model *3150510*, Pine Brook, USA). All the pots and water containers were covered with plastic film to prevent evaporation and to ensure that any water loss depended solely on plant transpiration. The mass of each pot was recorded every half hour using a *CR10X* datalogger (*Campbell Scientific Ltd.*, Logan, USA).

Sap flow (SF) and micrometric trunk diameter fluctuations (TDF) were measured on four trees per treatment, using the compensation heat-pulse technique and linear variable displacement transducers (LVDT), respectively. The measurement procedures of both parameters have been described in detail by Ortuño *et al.* (2004b) and Alarcón *et al.* (2005).

Photosynthetic photon flux density (PPFD), air temperature and relative humidity were measured continuously by two *Hobo H8 Loggers* (*Onset Computer Corporation*, Bourne, USA), and vapour pressure deficit (VPD) was calculated from air temperature and relative humidity data.

Results

During the experimental period, the VPD inside the greenhouse reached a daytime maximum of between 1.37 kPa (DOY 200) and 3.68 kPa (DOY 209). PPFD reached a daytime peak value of between 818 (DOY 200) and 1254 µmol m^{-2} s^{-1} (DOY 196). Air temperature ranged between 27 and 36 ºC during the daytime and fell to 19 - 22 ºC at night.

In the control plants the content of soil-O_2 was almost constant at around 0.26 mmol dm^{-3}. In contrast, in T1 plants, soil-O_2 levels sharply decreased, reaching minimum values of only 0.08 mmol dm^{-3} at the end of the flooding period. During the recovery period, the soil-O_2 content progressively increased achieving similar values to those of the control plants 11 d after the end of the flooding period (DOY 208).

In T0 plants, Ψ_{pd}, Ψ_{md} and Ψ_p showed high and constant values throughout the experiment (Fig. 1). From the outset of the stress period, Ψ_{pd}, Ψ_{md} and Ψ_p values were reduced in T1 plants, these reductions becoming significant from DOY 196 onwards. Minimum Ψ_p values were reached on DOY 197, and on DOY 198 in the case of Ψ_{pd} and Ψ_{md}. When the plants were removed from the water Ψ_{pd}, Ψ_{md} and Ψ_p increased, reaching similar values to those of the control plants on DOY 208.

Flooding caused a progressive reduction of SF and g_l from the beginning of the stress period (Fig. 2*A*,*B*). During the recovery period, SF exhibited a moderate tendency to achieve a partial recovery and g_l values continued to decrease until DOY 212, when partial recovery was observed.

On the second day of flooding (DOY 196), the stress induced a sharp increase in maximum daily trunk shrinkage (MDS) values (3.25 times higher than those in control plants) (Fig. 2*C*). However, during the recovery period, MDS presented lower values in T1 than in T0 plants.

In order to assess the strength of the discretely and continuously recorded PBWSI, the signal intensity of each was defined as its relative value (T0/T1 or T1/T0). All indicator signals increased as a consequence of flooding, although careful analysis points to certain differences in their behaviour. SF, Ψ_{pd}, Ψ_{md}, and g_l signal intensities increased progressively as a result of flooding, whereas, MDS signal intensity strongly increased only on the second day of stress (DOY 196). Moreover, during the recovery period SF and g_l signal intensities clearly remained above unity, while Ψ_{pd}, and Ψ_{md} signal intensities gradually decreased, and MDS presented values below unity.

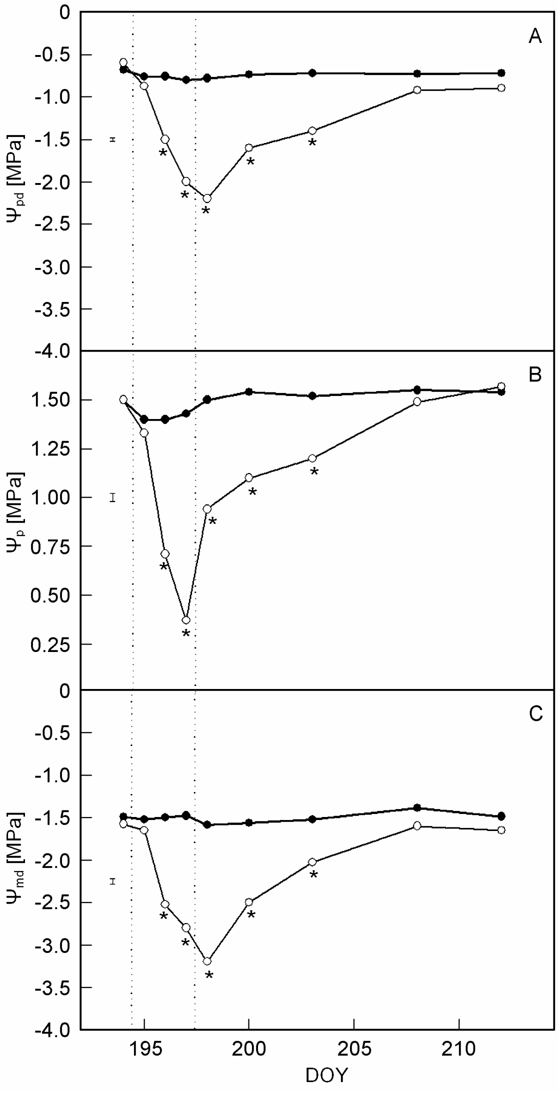

Fig. 1. Predawn (Ψ_{pd}) (*A*) and midday (Ψ_{md}) (*C*) leaf water potential, and predawn leaf pressure potential (Ψ_p) (*B*) in T0 (*closed symbols*) and T1 (*open symbols*) plants during the experimental period. Each data point is the mean of four measurements. *Vertical dotted lines* indicate the beginning and the end of the flooding period. *Vertical bar* is twice the overall mean SE. *Asterisks* indicate statistically significant differences by $LSD_{0.05}$.

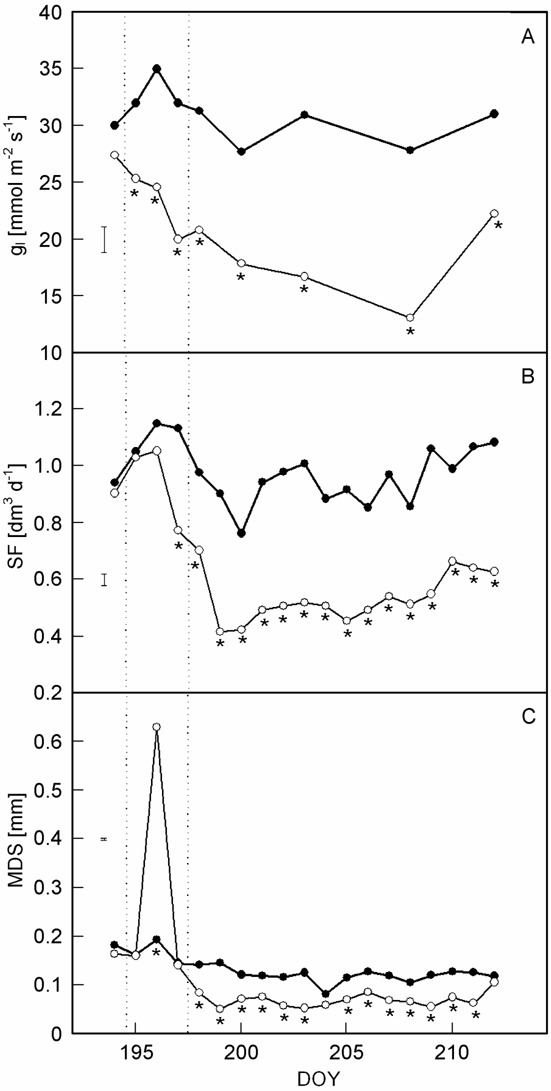

Fig. 2. Midday leaf conductance (g_l) (*A*), daily sap flow (SF) (*B*) and maximum daily trunk shrinkage (MDS) (*C*) in T0 and T1 plants during the experimental period. Each point is the mean of four measurements. Symbols as in Fig. 1.

To test whether SF rates could be used to evaluate E during flooding stress and recovery periods, instantaneous data corresponding to these parameters from both treatments during the stress (DOY 195 and 196) and recovery (DOY 198, 205 and 212) periods were selected. There were good direct correlations between instantaneous E and SF rates for T0 and T1 trees. The covariance analysis indicated that the relationships for T0 and T1 trees were not statistically different, meaning that it is possible to represent the relationship between E and SF by one solely line ($E = 1.01 \times SF + 0.01$, $r = 0.98^{***}$).

Following a similar procedure, but using daily data, we also concluded that SF could be used to evaluate E by means of a first order fit and by pooling data during the flooding and recovery periods ($E = 1.06 \times SF - 0.05$, $r = 0.99^{***}$).

Discussion

Soil inundation displaces the air present in the soil pores and, according to Kawase (1981), the rapid depletion of soil-O_2 is due to it being taken up by plant roots and soil microorganisms. The slow soil-O_2 content recovery when plants and soil were removed from water has also been observed in other woody crops (Domingo *et al.* 2002).

The fact that there was a significant decrease in the g_l on DOY 195 (Fig. 2*A*), when Ψ_{pd}, Ψ_{md}, Ψ_p, SF, MDS (Figs. 1 and 2) and R_{s+p} were similar in T0 and T1 plants, indicated that this stomatal regulation did not occur as a consequence of a water deficit in the leaf and can be related to the effect of a hormonal signal transmitted from the roots to the shoots (Zhang and Davies 1990, Else *et al.* 1996, Yordanova *et al.* 2005).

On the other hand, the fact that T1 plants showed low Ψ_{pd}, Ψ_{md}, Ψ_p, SF, and g_l values on DOY 196 and 197 (Figs. 1 and 2), and that R_{s+p} reached higher values than in T0 plants at the same time, suggests the existence of a prevailing negative hydraulic message generated by O_2-deficient roots due to the increase in R_{s+p}. As a consequence, the stomatal regulation observed at that moment could be considered the result of a leaf water deficit (Dell'Amico *et al.* 2001).

The increase in R_{s+p} caused by flooding can be attributed to the decrease in O_2 in the root zone (Jackson and Kowalewska 1983, Dell'Amico *et al.* 2001). Also, it is known that flooding reduces the ability of roots to absorb water (Holbrook and Zwieniecki 2003).

During the recovery period, Ψ_{pd}, Ψ_{md} and Ψ_p gradually recovered, although the high R_{s+p} and low SF and g_l values took longer to recover (Figs. 1 and 2). This fact suggests that during the recovery period there was no close relation between these parameters, with a prevailing behaviour similar to that described at the beginning of the flooding period. Moreover, stomatal closure induced by flooding has been demonstrated in other crops (Dell'Amico *et al.* 2001, Nicolás *et al.* 2005), and has generally been interpreted as a mechanism to restrict water loss (Bradford and Yang 1981, Jackson and Drew 1984). The reduction of SF in flooded plants during this assay confirms this idea (Fig. 2*C*).

The strong signal intensity of MDS in response to flooding and the fact that its values after the stress were clearly lower than unity emphasise the greater feasibility of using this indicator. Although Ψ_{pd} and Ψ_{md} presented high signal intensity values in response to flooding, the delay in achieving values near or below unity after flooding indicated that both indicators are less useful than MDS as indicators for use in automatic irrigation.

In any case, the fact that the responses to flooding of continuously and discretely measured PBWSI were very similar to those produced in response to drought stress (Ortuño *et al.* 2004a,b) indicated that the cause of stress cannot be diagnosed solely by the studied parameters. For this reason, we conclude that it is necessary for precise irrigation scheduling to complement plant-based water status indicator measurements with soil water measurements using automatic sensors.

The fact that very good direct correlations between E and SF rates were obtained on an instantaneous and daily basis in T0 and T1 plants and common calibration curves could be used for both treatments during the flooding and recovery periods indicated that the changes occurring in SF and E during these periods were proportional. This underlined the robustness of the compensation heat-pulse technique for estimating transpiration, not only in non-limiting soil water conditions or water stress conditions (Čermák *et al.* 1982, Infante *et al.* 2003, Alarcón *et al.* 2005), but also in situations of flooding stress.

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