Compensation heat-pulse measurements of sap flow for estimating transpiration in young lemon trees

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Abstract

Potted two-year-old lemon trees [*Citrus limon* (L.) Burm. f.], cv. Verna grafted on sour orange (*C. aurantium* L.) rootstock, growing in greenhouse, were subjected to drought for 33 d. Control plants were daily irrigated at field capacity. Values of sap flow (SF) were compared with transpiration (E) rates measured gravimetrically. The results underlined the robustness and high sensitivity of the compensation heat-pulse technique for estimating transpiration on a wide range of SF. Good direct correlations between E and SF rates on an instantaneous and daily basis were obtained in both treatments. On a daily basis, a common calibration curve can be used for both irrigation treatments. On an instantaneous basis, changes in SF were matches by similar changes in E in both treatments, although the relationships between these parameters presented different intercepts in each treatment. Sap flow rates were influenced by weather conditions in trees growing in non-limiting soil water conditions. This makes it possible to evaluate the significance of any sap flow measurement in relation to the reference value calculated for the vapour pressure deficit at the time the measurement was taken.

Additional key words: *Citrus aurantium* rootstock, *Citrus limon*, water stress.

Introduction

The compensation heat-pulse technique has proved to be a reliable technique for the non-destructive and continuous measurement of sap flow in woody plants (Green and Clothier 1988, 1995). Also, sap flow measurements have been proposed as a convenient way of estimating the water used by plants (Moreno *et al.* 1996, Fernández *et al*. 1997, 2001), but some limitations have been observed in the case of water stressed trees (Alarcón *et al*. 2000).

Heat-pulse velocity, measured with temperature sensors inserted downstream and upstream of a linear heater, has been related to the rate of sap flow using calibration factors derived by Swanson and Whitfield (1981). However, these theoretical calibration factors have not been widely tested (Fernández *et al*. 1997) and further tests are required.

The Verna lemon tree is a Spanish cultivar widely cultivated near the south-eastern Mediterranean coast of Spain, where the aridity of the climate and the scarcity of alternative water resources have led to the developments of new irrigation scheduling techniques to optimize water use in commercial citriculture.

The plant water status may be useful for irrigation scheduling because it has the great advantage of being

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Abbreviations: E - transpiration rate, Q - total sap flux; SF - sap flow rate, VPD - vapour pressure deficit, Ψ_p - predawn leaf pressure potential, Ψ_{pd} - predawn leaf water potential; Ψ_s - predawn leaf osmotic potential.

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directly linked to crop productivity (Goldhamer *et al*. 2003, Remorini and Massai 2003). In this sense, the measurements of both transpiration rate and water use by plants are important tasks not only for research in plant-water relations but also for irrigation management (Ferrara and Flore 2003).

For these reasons, the aim of this paper was to determine the possibilities of using compensation heat-pulse measurements of sap flow for estimating instantaneous and daily transpiration in young lemon trees under non-limiting soil water conditions and under water stress conditions. Also, in order to contribute to the interpretation of measurements of sap flow, reference values reflecting the sap flow rate response of trees under non-limiting water supply to weather conditions were obtained.

Materials and methods

Plants and culture conditions: The experiment was carried out on 2-year-old lemon trees (*Citrus limon* (L.) Burm. f.) cv. Verna on sour orange (*C. aurantium* L.) rootstock, about 1.5 m high, 2.5 cm trunk diameter, and total leaf area of about 1.3 m^2, growing in a plastic greenhouse, provided with a cooling system, in 35 dm^3 plastic pots filled with a mixture of clay loam soil and peat, containing 4 % organic matter. The soil water content at field capacity and permanent wilting point was 25 and 9 % (v/v), respectively. Plants were irrigated daily, using drip irrigation with one emitter per plant, each delivering 2 dm^3 h^{-1}, in order to maintain the soil water content at around field capacity.

On 7 June 2001, 8 lemon plants of uniform appearance were submitted to two different treatments: a control treatment (T0), in which plants were irrigated daily as indicated, and a deficit irrigation treatment (T1), in which plants were irrigated daily using 25 % of the water applied to T0 plants for a period of 33 d. Air temperature inside the greenhouse ranged between 26 and 34 ºC during the daytime and fell to 17 - 23 ºC at night. The vapour pressure deficit (VPD) reached a daytime maximum from 1.34 kPa (3rd day) to 3.51 kPa (22nd day). Photosynthetic photon flux density (PPFD) reached a daytime peak value from 1153 µmol m^{-2} s^{-1} (3rd day) to 1320 µmol m^{-2} s^{-1} (2nd day).

Measurements: The volumetric soil water content was estimated in each pot throughout the experiment using time domain reflectometry (TDR) (Topp and Davis 1985) with a pair of TDR probes installed at a depth of 200 mm midway between the trunk and pot rim.

Predawn leaf water potential (Ψ_{pd}) was measured several times during the experimental period in one mature, sun exposed leaf per plant, using a pressure chamber, following the recommendations of Turner (1988) to prevent leaf water loss during measurements. Leaves were selected at random from the middle third of the shoots. 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* (Logan, USA) vapour pressure osmometer. Predawn leaf pressure potential (Ψ_p) was derived as the difference between leaf osmotic and water potentials.

The daily course of transpiration rate (E) was measured in all plants placing the pots on top of weighing balances (capacity 150 kg and resolution of 5 g, *Scaltec Instruments model SSH91*, Heiligenstant, Germany). All the pots were covered with plastic film to avoid evaporation from the soil and to ensure that any water loss depended solely on plant transpiration. The measurements were taken every half an hour using a Campbell data logger (*Model CR10*, *Campbell Scientific Ltd.*, Logan, USA).

Sap flow rate (SF) was measured using the compensation heat-pulse technique (Swanson and Whitfield 1981) throughout the experimental period, using *MITRA-1* instrumentation (Polytechnic University, Cartagena, Spain). One set of heat-pulse probes was located on each tree. Each set consisted of a heater needle and two temperature probes of 1.8 mm diameter and 12.5 mm length, installed in parallel holes drilled radially in the trunks. Each heat-pulse probe had four thermocouple sensors (at 3, 6, 9 and 11 mm depth) to monitor the sap velocity profile over a radial depth. Sap velocity was measured following the procedure of Green and Clothier (1988) and taking into account the Swanson correction factors for a wound size of 2.4 mm (Swanson and Withfield 1981).

Once the heat-pulse velocity (V) was determined, the next step was to relate it to the actual sap flow. In our analysis, the sap flow density (J) was related to V using the equation developed by Edwards and Warwick (1984): $J = (0.505\ F_m + F_l)\ V$, where F_m and F_l are the volume fractions of wood and water, respectively. These were calculated at the end of the experiment by taking 5 mm diameter wood samples from all trees with a special auger and measuring the fresh mass, mass of displaced water by immersion in a vessel of distilled water on a balance, and oven dried mass. The factor 0.505 is related to the thermal properties of the woody matrix, and is assumed to be constant within and between species. This equation provides an estimate of the values of J at any point in the conducting sapwood. It is widely recognized that sap flux density is not uniform throughout the sapwood, for which reason our probes measured J at four radial depths. The volumetric measurement of total sap flux (Q) was obtained by the integration of these point estimates over the sapwood conducting area, which was determined at the end of the experiment by cutting the trunks and immersing them in a safranin solution overnight and measuring the radius of sapwood at the point where the heat pulse sensors were installed. The temperature signals and the corresponding heat-pulse velocities were recorded at 30 min intervals using the compensation heat-pulse instrumentation controlled by a data logger (*CR10X*, *Campbell Scientific Ltd.*).

Design of the experiment was completely randomized with four replications. One plant per replicate was used. Data were statistically analyzed using two-way analysis of variance (*ANOVA*). Linear regression differences were analyzed according to the covariance analysis.

Results

Control (T0) trees grown at soil water content around field capacity, had nearly constant Ψ_{pd} and Ψ_p of around -0.6 and 1.5 MPa, respectively, throughout the whole experiment (data not shown). However, soil moisture decreased in T1 plants from the outset of the deficit irrigation period, reaching minimum values of around 15 %, on day 7 onwards. Also, Ψ_{pd} and Ψ_p decreased to around -1.2 MPa and 1.15 MPa, respectively, on day 20 onwards (data not shown).

Table 1. Slopes, intercepts and correlation coefficients for first order relationships between instantaneous gravimetric transpiration [mmol s^{-1} tree^{-1}] and instantaneous sap flow [mmol s^{-1} tree^{-1}] in control (T0) and stressed (T1) trees on days 5, 20 and 33 of the experimental period (*n* indicates the number of mean data used for each regression). Values within a column for each treatment followed by the same letter are not different at P = 0.05 by Student *t*-test. *** - indicates significance at $P < 0.001$.

Treatment	Day	*n*	Slope	Y-axis intercept	Correlation coefficient
T0	5	48	0.96a	0.00a	0.97***
	20	48	0.87a	0.03a	0.99***
	33	48	0.92a	0.10a	0.97***
T1	5	48	0.98a	-0.06a	0.97***
	20	48	0.88a	-0.03a	0.88***
	33	48	0.77a	-0.02a	0.71***

In order to test if instantaneous SF could be used to evaluate instantaneous E, data corresponding to these parameters from three days were selected, one at the beginning (day 5), one in the middle (day 20) and the other at the end (day 33) of the experimental period. There were good correlations between instantaneous E and instantaneous SF for control (T0) and stressed (T1) trees (Table 1). The covariance analysis indicated the absence of significant differences between the slopes and the intercepts of the relationships obtained on days 5, 20 and 33 of the experiment for each treatment (Table 1).

Therefore, the data of E and SF for each treatment as a whole were correlated (Fig. 1), and the results indicated good correlations and relationships close to 1:1. The covariance analysis indicated that intercepts for both relationships were statistically different, meaning that it is impossible to represent the relationship between E and SF by one solely line.

In T1 plants, the daily courses of E and SF showed some differences (Fig. 2). At midday, E values were higher than SF values, whereas in the early morning and in the afternoon SF values were higher than E values. In the early morning these differences between both parameters gradually decreased while in the afternoon they gradually increased.

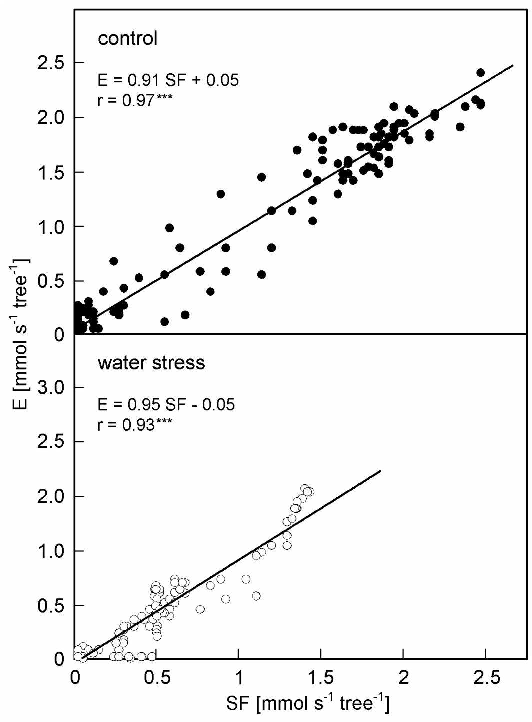

Fig. 1. Relationships for control (*closed circles*) and water stress (*open circles*) trees between instantaneous transpiration (E) and instantaneous sap flow (SF) on days 5, 20 and 33 of the experimental period. Each data point is the mean of four pair of measurements.

Although T1 trees presented lower daily SF and E rates than T0 trees, according to the covariance analysis, the relationships between both parameters in T0 and T1 plants were similar (Fig. 3). This means that an overall relationship could be used to estimate daily E on the basis of daily SF measurements (Fig. 3).

Daily SF of lemon trees growing under non-limiting soil water conditions (T0) and mean daily air vapour pressure deficit (VPD) correlated (Fig. 4), indicating that increases in the evaporative demand of the atmosphere induce sap flow increases.

Discussion

The fact that soil moisture values for T0 plants remained at around field capacity indicates that irrigation practises in this treatment were adequate. This aspect was confirmed by the fact that Ψ_{pd} values, which depend mainly on soil moisture conditions (Katerji *et al*. 1988), were high and constant. Moreover, the fact that Ψ_p values in T1 plants clearly remained above the zero suggests that the water stress developed can be considered as moderate (Ruiz-Sánchez *et al.* 1997).

The fact that the relationships between instantaneous E values and instantaneous SF values in both treatments presented different intercept values (Fig. 1) could be attributed to differences in the daily course of E and SF in T1 plants, since SF measurements in water stress conditions occasionally underestimated or overestimated E (Fig. 2).

The overestimation of E by SF measurements in the early morning and in the afternoon could be due to the rehydration of the tree organs with a part of the water transported along the stem (Alarcón *et al.* 2000). In contrast, the underestimation of E at midday reflected a depletion of the water capacitance of the tissues due to the high evaporative demand of the atmosphere and the reduced water supply to the plant from the soil (Smith and Allen 1996, Alarcón *et al*. 2000).

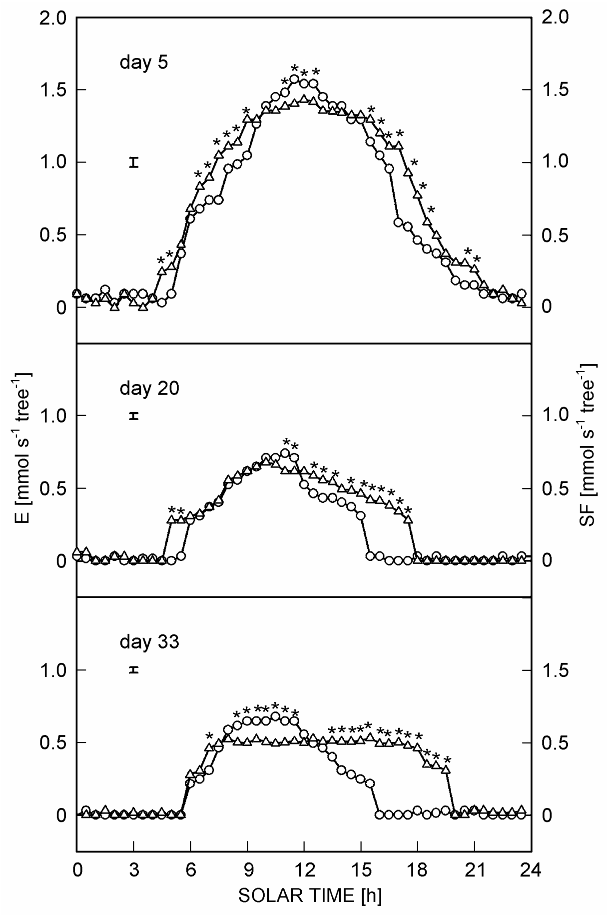

Fig. 2. Daily course of instantaneous transpiration (E, *open circles*) and instantaneous sap flow (SF, *open triangles*) in T1 plants on day 5, 20 and 33 of the experimental period. Each data point is the mean of four measurements. *Vertical bar* is twice the overall mean SE. *Asterisks* indicate statistically significant differences ($LSD_{0.05}$).

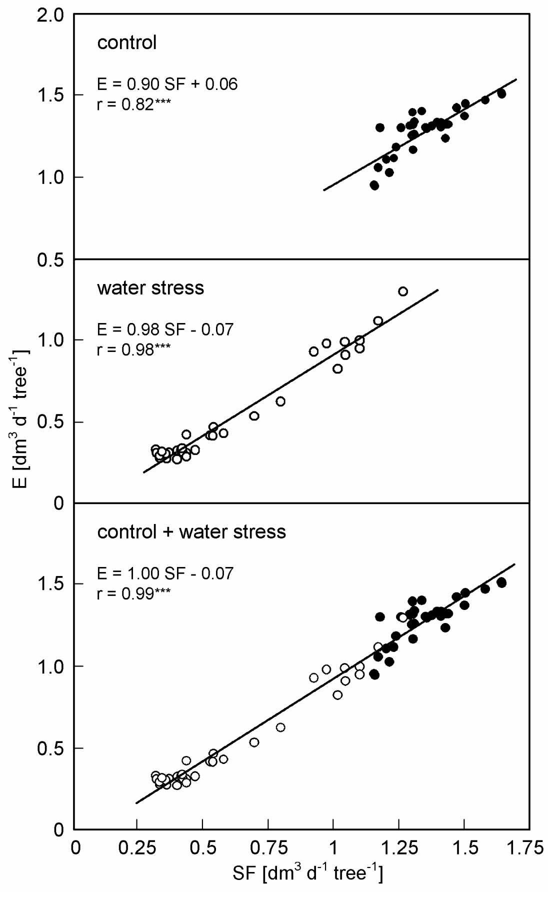

Fig. 3. Relationships for T0 (*closed circles*), T1 (*open circles*) and T0+T1 trees between daily transpiration (E) and daily sap flow (SF) during the experimental period. Each data point is the mean of four pair of measurements.

Moreover, in plants under non-steady-state water flux conditions (T1 plants) a daily compensation of these deviations between instantaneous SF and instantaneous E may explain why the relationship between daily values of both parameters was similar to that obtained in control plants (Fig. 3). In this sense, Barret *et al*. (1995) indicated that more accurate estimates of water use by plants using SF measurements were obtained when longer period of time were considered.

The good correlations obtained in both treatments between E and SF using instantaneous values, in which night time values were very low and close to zero (Fig. 1) pointed to the robustness and the high sensitivity of the compensation heat-pulse technique employed for estimating E in young lemon trees. In contrast, Becker (1998) indicated that measurements of low SF during the night are not reliable, and Barret *et al*. (1995) showed that accurate measurements of SF were obtained only above a minimum threshold sap velocity, which appeared to be determined by probe construction and xylem anatomy.

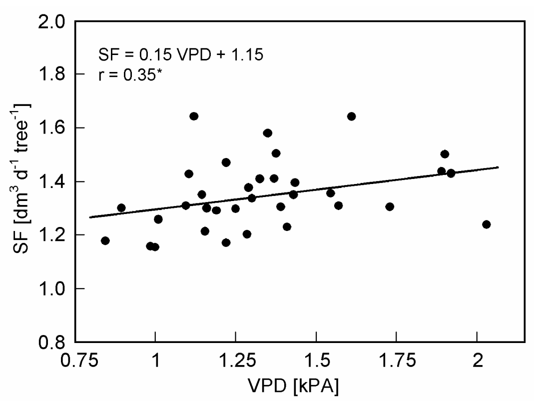

Fig. 4. Relationship for trees under non-limiting soil moisture conditions (T0) between daily sap flow (SF) and mean daily air vapour pressure deficit (VPD) during the experimental period. Each data point is the mean of four SF measurements.

In addition to the fact that E and SF rates on a daily basis correlated adequately and a common calibration curve can be used for both irrigation treatments (Fig. 3), it is important to point out that the similar slopes obtained in the relationships between instantaneous E and SF for both treatments (Fig. 1) is useful because changes in SF are associated with similar changes in E in both treatments. Čermák *et al.* (1982) and Infante *et al.* (2003) indicated that sap flow measurements are a good estimate of *Quercus* whole-tree transpiration.

The SF values were influenced by weather conditions in trees growing under non-limiting soil water conditions (Alarcón *et al*. 2000, Ortuño *et al*. 2005). The scatter of this relationship in T0 plants (Fig. 4) could be attributed to the very low variability of the environmental conditions inside the greenhouse and/or the moderate duration of the experiment. The fact that the SF and VDP of fully irrigated trees were correlated means that it is possible to develop reference relationships, and taking this into account, to evaluate the significance of any SF measurement in relation to the reference value calculated for the VPD at the time the measurement was taken.

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