

RESEARCH PAPER

# Whole-tree level water balance and its implications on stomatal oscillations in orange trees [*Citrus sinensis* (L.) Osbeck] under natural climatic conditions

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## Abstract

**Sustained cyclic oscillations in stomatal conductance, leaf water potential, and sap flow were observed in young orange trees growing under natural conditions. The oscillations had an average period of approximately 70 min. Water uptake by the roots and loss by the leaves was characterized by large time lags which led to imbalances between water supply and demand in the leaves. The bulk of the lag in response between stomatal movements and the upstream water balance resided downstream of the branch, with branch level sap flow lagging behind the stomatal conductance by approximately 20 min while the stem sap flow had a much shorter time lag of only 5 min behind the branch sap flow. This imbalance between water uptake and loss caused transient changes in internal water deficits which were closely correlated to the dynamics of the leaf water potential. The hydraulic resistance of the whole tree fluctuated throughout the day, suggesting transient changes in the efficiency of water supply to the leaves. A simple whole-tree water balance model was applied to describe the dynamics of water transport in the young orange trees, and typical values of the hydraulic parameters of the transpiration stream were estimated. In addition to the hydro-passive stomatal movements, whole-tree water balance appears to be an important factor in the generation of stomatal oscillations.**

**Key words:** *Citrus sinensis*, model, oscillations, sap flow, stomata, stem diameter variation, time lag.

## Introduction

Although stomatal oscillations were first discovered 3–4 decades ago (Lang *et al.*, 1969; Barrs, 1971; Farquhar and Cowan, 1974), they remain a subject of scientific interest because it is difficult to explain fully the mechanisms leading to the oscillations. Thus it remains difficult to develop mechanistic models to describe the water relations of plants undergoing stomatal cycling, for example, citrus trees in response to environmental variables (Upadhyaya *et al.*, 1988). This is despite the growing need for such modelling tools in the management of resources and in yield prediction, especially in capital-intensive cropping systems (Jones, 2004) such as in citrus plantations. The Navel variety of orange trees [*Citrus sinensis* (L.) Osbeck] are early maturing and are commonly grown in the warm tropical climates, while the Troyer citrange rootstock [*Citrus sinensis* × *Poncirus trifoliata* L. Raf] produces trees with less vegetative vigour and good quality fruit (Spiegel-Roy and Goldschmidt, 1996). Stomatal oscillations, which characterize the water relations of this scion–rootstock combination of orange trees, are interpreted and documented in the present report.

Recent evidence suggests that stomatal oscillations arise from responses of the stomata to leaf-level water balance involving the juxtaposition of opposing positive and negative feedback loops (Buckley, 2005; Steppe *et al.*, 2006b). The positive feedback loop has been identified with the effect of the epidermal mechanical advantage on passive stomatal hydromechanics. Loss of turgidity by the epidermal cells due to transpiration enhances stomatal opening (Shackel and Brinckmann, 1985; Franks, 2004).

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However, this ‘wrong way’ response is subsequently followed by an exponential transition to a new steady-state conductance that partially counteracts the perturbation involving guard cell movements in the negative feedback loop. It is possible that this two-phase pattern repeats itself, producing oscillations that may persist for a while before damping out, or may even occur indefinitely (Buckley, 2005).

While most studies on stomatal oscillations have focused on events at leaf level (McBurney and Costigan, 1984; Reich, 1984; Naidoo and von Willert, 1994; Zipperlen and Malcolm, 1997; Yang *et al.*, 2003), the positive feedback described above can also arise, for example, from xylem cavitation in response to increased tension, in any part of the hydraulic vascular system of the plant (Buckley, 2005). Cavitation causes a decrease in the hydraulic conductance by reducing the number of active xylem vessel elements. Water flow is impeded, causing a decrease in the water status of the leaves, hence amplifying the ‘wrong-way’ response. An important pre-condition for stomatal oscillations to be generated in this way is that cavitation repair has to occur at short intervals of the same order of magnitude as half the period of the oscillations. However, evidence for this rapid reversal remains sparse (Vogt, 2001; Bucci *et al.*, 2003; Brodribb and Holbrook, 2004).

Since the stomata are coupled hydraulically to each other and to other compartments of the transpiration stream, the role of the whole-tree level water balance in the generation of stomatal oscillations is investigated in this study. Despite the increasing availability of estimates of water storage capacity in trees, relatively little is known about the daily dynamics of discharge and recharge of stored water and their consequences for stomatal regulation of water use (Meinzer *et al.*, 2003). In this study, the dynamics of water uptake by the roots, supply to the leaves, and loss through transpiration by young orange trees are inferred from simultaneous measurements of sap flow on different organs of the trees and the stomatal conductance. Tree-level water balance is evaluated using a simple water balance model from which the hydraulic parameters of the water transport pathway are derived and related to events at leaf level. Despite the role of hydropassive mechanisms in generating stomatal oscillations, it appears that changes in the whole-tree level water balance are also important in triggering the stomatal oscillations.

## Materials and methods

### *Orchard experimental site*

Cyclic oscillations in sap flow were observed during an irrigation experiment in a commercial orchard at Mazowe Citrus Estate (MCE), Zimbabwe (17°27' S, 30°59' E, 1189 m above sea level) in November 2004. The orchard, which covers approximately 2 ha under drip irrigation, is situated on gently sloping terrain about 40 km to the north-east of Harare, Zimbabwe. The trees were 4-

year-old Navel orange trees [*Citrus sinensis* (L.) Osbeck] of the Bahianinha selection budded onto a Troyer citrange rootstock [*Citrus sinensis* × *Poncirus trifoliata* L. Raf]. The growing medium was the dark red clayey loam soil whose physical and chemical characteristics were summarized by Hussein (1982).

The microclimate was monitored using an automatic weather station located to the south-west of the orchard approximately 70 m from the edge. A CM11 pyranometer (Kipp and Zonen, Delft, The Netherlands) installed horizontally on a levelling fixture measured the solar radiation incident on the crown of the trees, while wind speed was monitored using an AL100 cup anemometer (Vector Instruments, Rhyl, UK) at 2 m height. Air temperature and humidity were monitored using an HMP35AC probe (Campbell Scientific Ltd, Shepshed, UK) inserted in a 12-plate Gill radiation shield (Vaisala Ltd, Helsinki, Finland) at ~1.5 m above the ground. Signals from all the sensors were recorded automatically at 5 s intervals, and 5 min averages were stored on a datalogger (CR23X, Campbell Scientific Ltd, Shepshed, UK).

### *Physiological measurements on orchard trees*

Branch-level sap flow rates were measured on fully exposed branches of four trees in the orchard using SGB19 heat balance sap flow sensors (Dynamax, Houston, TX, USA). The mean branch diameter on which the sap flow gauges were installed was  $18.4 \pm 0.5$  mm. Operating instructions by Baker and van Bavel (1987) were followed during sensor installation, and weather shields were also installed around each sensor. In addition, all the sensors and the exposed parts of the branches were wrapped in a double layer of aluminium foil to minimize the effects of spurious temperature gradients due to radiant heating. The sensors were connected to two dataloggers (CR23X, Campbell Scientific Ltd) with the same logging frequencies as the climatic data.

### *Potted tree experimental site*

A detailed experiment to investigate further the cyclic oscillations in sap flow observed in the orchard at MCE was set up outdoors on the flat roof of the Physics Department, University of Zimbabwe, Harare (17°79' S, 31°04' E, elevation 1450 m above sea level). Similar sets of sensors to those at MCE were used to monitor the microclimate of the potted orange trees and using the same logging frequencies.

Plant material comprised six 2-year-old orange trees of the same rootstock–scion combination as in the orchard. The trees were planted in asbestos pots, approximately 70 cm in diameter and 50 cm deep, and were 90–100 cm tall at the time of planting. Transparent plastic, approximately 200 µm thick, supported by a frame measuring 7 m × 4 m × 2 m (length × width × height), was used to shelter the trees from rain. However, all the sides of the shelter were left open to allow free circulation of air. The mean transmittance of the plastic to solar radiation, calculated as the ratio of the daily total solar radiation under the rain shelter to that outside the shelter on several clear days, was  $73 \pm 1\%$ .

Two healthy and actively growing trees were selected for the experiments and instrumented to investigate the phenomenon of stomatal cycling in detail. All six trees could not be investigated simultaneously due to limitations in the number of sensors.

### *Physiological measurements on potted trees*

Measurements of sap flow on the potted trees were made using SGA9 and SGA5 heat balance sap flow sensors (Dynamax) installed on the stem and the branch, respectively, of each of the two young model trees. The mean diameters at the stem and branch installation sections of the potted trees were 11.3 mm and 6.5 mm for the first tree and 11.6 mm and 5.4 mm for the second tree,

respectively. Care was taken to avoid the dead wood at the bud union for the installation of the stem sap flow gauges. A strip of thin plastic was wrapped around the sections where the sensors were to be installed to ensure that possible transpiration by the green parts of the young trees did not affect the signals. Weather shields and a double layer of aluminium foil were installed around the gauges. In addition, the exposed parts of the stems below the stem sap flow sensors were wound with aluminium foil to the soil surface to minimize the effects of exogenous heating on the stem, especially early in the morning and at sunset.

Stem diameter variations were monitored midway between the branch and stem sap flow gauges using two dendrometers (DEX 70 and DEX 20, Dynamax), with one dendrometer on each tree. The accuracy of the dendrometers was  $\pm 10 \mu\text{m}$ . All the sensors were connected to two dataloggers (CR23X, Campbell Scientific Ltd) programmed with a 5 s scan interval, and all signals were averaged every 5 min.

To get a clear relationship between the stomatal conductance and leaf water potential during the oscillations, the experiments were conducted on cloudless days. These conditions were not difficult to achieve as the transition period from winter to spring in Zimbabwe is characterized by cloudless conditions over many days. Consequently, the period from 5 to 7 July 2005 [Julian day of the year (DOY) 186–188] was chosen for intensive measurements of the plant variables. However, the most complete data for the simultaneous measurements of the stomatal conductance and the leaf water potential are available for 6 July 2005 (DOY 187).

Stomatal conductance was measured using the AP4 porometer (Delta-T Devices, Burwell, Cambridge, UK) on four leaves in the first tree and on one leaf in the second tree. The selected leaves were healthy, fully expanded, and exposed to solar radiation. Measurements were taken in continuous cycles throughout the day such that each leaf was sampled at least once every 5 min except during calibrations. To ensure accurate measurements, a new calibration curve was fitted every time when necessitated by a change in cup temperature ( $2^\circ\text{C}$  on average) since the instrument's calibration is strongly temperature dependent. During the porometer measurements, care was taken that the temperature difference between leaf and cup was minimal ( $<0.5^\circ\text{C}$ ). Additional leaf surface temperature measurements were taken on a representative leaf using a fine K-type thermocouple firmly fixed on the abaxial side of the leaf using a plastic paper clip. This provided an independent check of the leaf to cup temperature difference. The leaf surface temperature (data not shown) oscillated in anti-phase with the branch sap flow (see further) presumably due to the evaporative cooling dynamics.

Leaf water potential was measured on one of the selected potted trees using a thermocouple psychrometer. The measured leaves were in the vicinity of and under similar conditions to those for porometer measurements. The thermocouple psychrometer comprised four standard C-52 sample chambers (Wescor Inc., Logan, UT, USA) connected to a microvoltmeter (HR-33T, Wescor, Inc.). Chambers were kept at a constant temperature inside a white datalogger enclosure box which was well insulated and kept inside the laboratory. Leaf samples, collected with an ordinary 6 mm paper punch, were immediately wrapped in aluminium foil and put in an air-tight plastic bag and taken indoors. The samples were loaded into the sample chambers within 10 min at most of being collected. Measurements were made in the psychrometric (wet bulb depression) mode. The psychrometers were calibrated with NaCl solutions over the range 0 to  $-4.55 \text{ MPa}$ . In order to determine the dynamics of the leaf water potential, measurements were taken at between 10 min and 20 min intervals. With four C-52 sample chambers, equilibration intervals were limited to 40 min. Separate trials showed that equilibration was reached within this time interval. As a consequence, water potential measurements could not be replicated.

### Whole-tree sap flow, hydraulic resistance, and water storage

The dynamics of the stem sap flow rate ( $F$ ) as affected by changes in the transpiration rate ( $E$ ) of the whole crown due to stomatal oscillations can be described using a simple water balance model (Philips *et al.*, 1997; Steppe *et al.*, 2006a). The rate of change of the water stored in the tissues of the tree ( $W$ , kg) is related to the difference between water uptake ( $F$ ,  $\text{kg h}^{-1}$ ) and loss ( $E$ ,  $\text{kg h}^{-1}$ ) by

$$\frac{dW}{dt} = F - E \quad (1)$$

In this equation it is assumed that there is a negligible capacitance in the roots such that the stem sap flow rate ( $F$ ) equals the root water uptake. To estimate the total transpiration  $E$ , branch sap flow can be scaled up to the whole-crown level using the approach of Meinzer *et al.* (2003). In this method, the scaling factor is calculated as the ratio of the daily total sap flow at stem level to that at branch level. Multiplying the branch sap flow rate by this factor gave an estimate of the whole-crown sap flow rate. The whole-tree transpiration rate ( $E$ ) was then estimated from the whole-crown sap flow rate by imposing a 20 min time lead ahead of the whole-crown sap flow rate since the stomatal conductance led the branch sap flow by this factor (see below).

Given the fact that the stem sap flow ( $F$ ) is driven by the water potential gradient between the soil and the leaves, a typical Ohm's law analogue can be used to describe the stem sap flow according to Jones (1992)

$$F = \frac{\Psi_s - \Psi_l}{R_x} \quad (2)$$

where  $\Psi_s$  is the soil water potential measured in the root zone of the trees (MPa),  $\Psi_l$  the leaf water potential (MPa), and  $R_x$  is the hydraulic flow resistance of the whole tree ( $\text{h MPa kg}^{-1}$ ) initially assumed to be a constant for the orange trees. To describe the non-steady-state nature of the flow due to the imbalances between water uptake and loss, the capacitance ( $C$ ,  $\text{kg MPa}^{-1}$ ) of the tree, which is a measure of its ability to store water, was defined according to Jones (1992) as the ratio of the change in the tissue water content to the change in the driving water potential difference

$$C = \frac{dW}{d\Psi} \quad (3)$$

This can be simplified to

$$\Psi_l - \Psi_s = \frac{W}{C} \quad (4)$$

because the model was formulated as an electrical circuit analogue (Philips *et al.*, 1997; Steppe *et al.*, 2006a). Model-Maker Version 3 software package (Cherwell Scientific Ltd, Beaconsfield, UK) was used for the development, calibration, and validation of the model.

## Results and discussion

### Oscillations in sap flow under natural climatic conditions

A typical course of branch sap flow for the Navel on Troyer orange trees growing in the commercial orchard at MCE, Zimbabwe is shown in Fig. 1b. Although predominantly clear sky conditions prevailed (Fig. 1a) and no sudden changes in the difference between the air's actual and potential saturated value (VPD henceforth) occurred, sap flow followed cyclic oscillations. No specific disturbance in the trees' environment was needed to generate the oscillations.

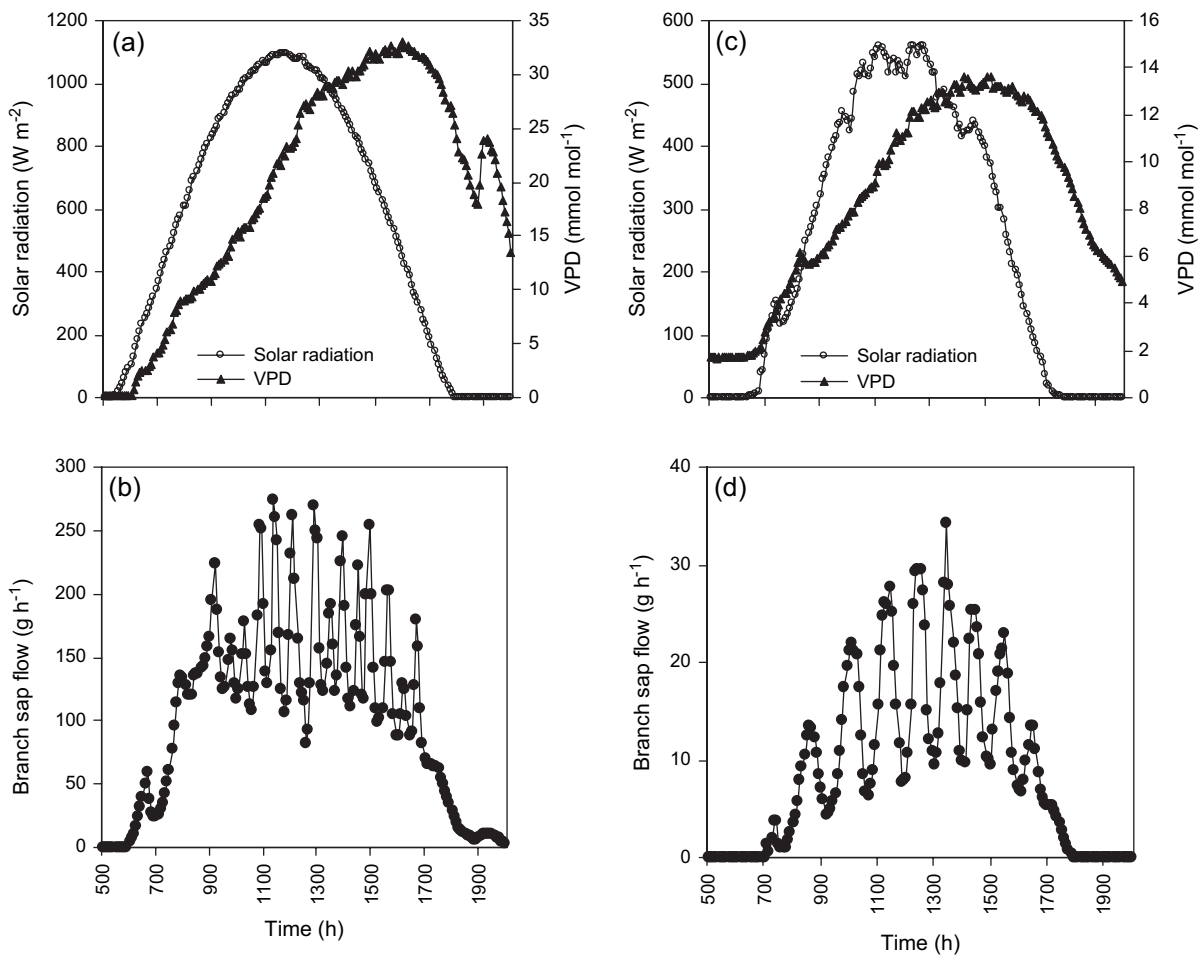
A clearer diurnal trend in sap flow was observed with the young orange trees grown in pots (Fig. 1d), illustrating that the oscillations occurred in both the field-grown and potted trees. Despite the small fluctuations and the relatively low intensities of the key driving climatic variables during the Zimbabwean winter (Fig. 1c), well-defined and sustained cyclic oscillations in sap flow were observed. Oscillations on the larger trees in the orchard had much shorter periods and were less pronounced than in the young potted trees.

#### Stomatal conductance and sap flow of potted orange trees

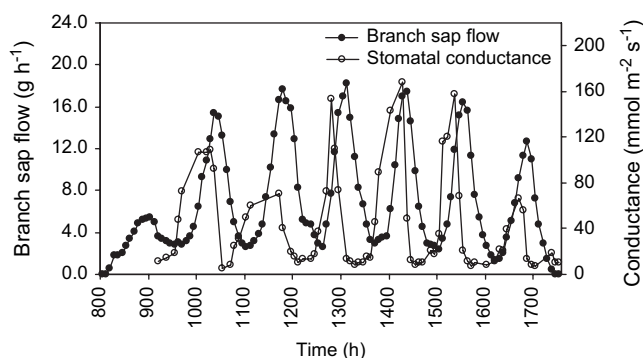
Like the sap flow, stomatal conductance followed cyclic oscillations as expected (Fig. 2). Seven clear oscillations were observed in approximately 8 h, giving a mean period of 70 min per oscillation. Since the stomatal movements and water flow through the different compartments of the transpiration stream are hydraulically coupled, the stoma-

tal conductance, branch and stem sap flows all oscillated with an identical period. Most work reported in the literature has shown oscillations with a maximum period of up to 50 min obtained mostly under controlled climatic conditions (Jarvis *et al.*, 1999; Wang *et al.*, 2001).

The stomatal conductance measured in this campaign ranged from  $5 \text{ mmol m}^{-2} \text{ s}^{-1}$  to  $175 \text{ mmol m}^{-2} \text{ s}^{-1}$  on a typical clear day. Levy and Kaufmann (1976) reported oscillations in stomatal conductance varying between  $4 \text{ mmol m}^{-2} \text{ s}^{-1}$  and  $42 \text{ mmol m}^{-2} \text{ s}^{-1}$  for 4-year-old citrus trees growing under controlled greenhouse conditions and with root systems at different temperatures. Moreover, the measured stomatal conductance in this experiment with the Navel on Troyer scion-rootstock combination was higher after sunset ( $\sim 17.30 \text{ h}$  Local Time; GMT+2 h) with a mean value of approximately  $11 \text{ mmol m}^{-2} \text{ s}^{-1}$  compared with the lowest average value of  $5 \text{ mmol m}^{-2} \text{ s}^{-1}$  reached during the day. A closer inspection of the course of the stomatal conductance in Fig. 2



**Fig. 1.** The diurnal course of the solar radiation (open circles), vapour pressure deficit of the air (filled triangles, a), and branch sap flow of a 4-year-old orange tree (b) during a typical clear day on 3 November 2004 (DOY 307) at Mazowe Citrus Estate, Zimbabwe. (c) and (d) show the same variables for a young potted orange tree under the rain shelter on 6 July 2005 (DOY 187). The cyclic oscillations in sap flow occurred under both field and laboratory conditions, and each data point on the graphs is a 5 min average.



**Fig. 2.** Comparison of the evolution of the stomatal conductance and branch sap flow in young orange trees during a typical clear day (6 July 2005; DOY 187). The stomatal conductance after sunset was higher than the minimum reached during the day due to the oscillations.

also reveals that the stomata tended to stay partially closed for significant periods of each cycle and only opened fully for shorter intervals. Direct measurements of the stomatal aperture by Kaiser and Kappen (2001) working with *Sambucus nigra* L. plants showed that during oscillations, the stomatal aperture varied between the slightly open and the closed states with a tendency for the stomatal aperture to overshoot the optimal size during opening but only for short periods. This observation explains the rapid increase in the stomatal conductance in the present study which occurred when the stomata were opening, as shown in Fig. 2.

Branch sap flow in all the trees sampled ceased shortly after sunset ( $\sim 17:30$  h) while stem sap flow occurred until 20:00 h. The continued stem sap flow occurred to replenish the internal water reserves depleted when water loss from the tree exceeded water uptake during the day. For a typical young orange tree on DOY 187, for example, total transpiration calculated as the time integral of branch sap flow scaled up to tree level from sunrise ( $\sim 06:00$  h) to sunset ( $\sim 17:30$  h) was approximately  $712.4 \text{ cm}^3$ . Of this amount, about  $103.3 \text{ cm}^3$ , i.e. approximately 14% of the total transpiration, was extracted from the internal storage reserves, while  $76.9 \text{ cm}^3$  was used to replenish the internal storage reserves during the same period. When whole-tree water loss ( $E$ ) exceeded water uptake ( $F$ ) at any instance, then the balance of water had to be withdrawn from the internal water storage reserves. The contribution of the internally stored water to transpiration was thus calculated as the time integral for all the instances when the difference between stem sap flow and transpiration was negative, and the converse was applied for the refilling of the internal storage pools. Consequently, a deficit of  $26.4 \text{ cm}^3$ , calculated as the difference between water withdrawn from the internal storage and that used to replenish the internal reserves, occurred at the end of the day. This had to be refilled after sunset through continued water uptake when transpiration had ceased. It is important to note that while discharge of

water from the internal storage occurs mainly in the morning and recharge in late afternoon on clear days for most species (Meinzer *et al.*, 2004; Steppe, 2004), more frequent recharge and discharge cycles occurred several times throughout the day for the orange trees as a result of the stomatal oscillations.

#### Dynamics of the leaf water potential

Stomata respond to environmental variables so as to maintain the water potential above a certain critical limit to avoid catastrophic declines in the water potential. For the Navel on Troyer scion–rootstock combination used in this study, the dynamics of leaf water potential were described using equation 3 in a typical whole-tree water balance model. The model calibration procedure involved systematically adjusting the values of the hydraulic parameters ( $R_x$  and  $C$ ) and the soil water potential ( $\Psi_s$ ) to minimize the squared differences between the measured and the modelled stem sap flow and the leaf water potential, weighted by the squares of the errors in the measurement of each quantity. This was achieved using the Marquardt iterative method within the Model Maker software package. Errors of 10% for the stem sap flow measurements (Baker and van Bavel, 1987) and a larger error of 20% for the leaf water potential measurements were used for the model optimization. Data collected on 6 July 2005 were used for calibrating the model.

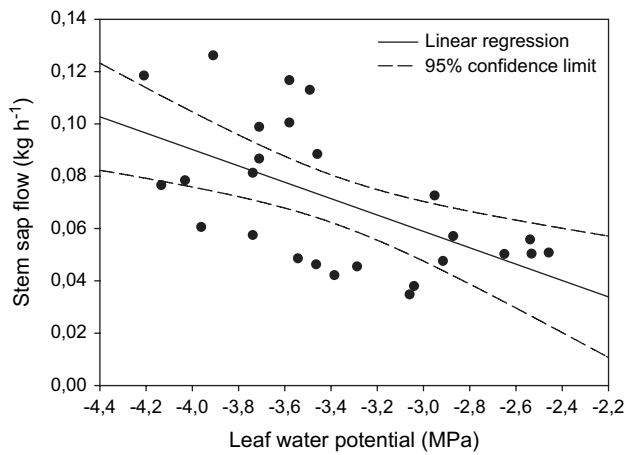
Typical values of the hydraulic parameters  $R_x$  and  $C$  that gave the best fit for the young orange trees are shown in Table 1. The soil water potential at the surface of the roots was predicted to be approximately  $-0.5 \text{ MPa}$ . In addition, the hydraulic resistance estimated by the model ( $47.9 \text{ h MPa kg}^{-1}$ ) was considerably larger than the one estimated from the inverse of the slope of the linear regression of stem sap flow against the water potential graph ( $31.0 \text{ h MPa kg}^{-1}$ ) in Fig. 3, albeit with a low coefficient of determination ( $R^2=0.36$ ). Contrary to the earlier assumption made on  $R_x$  in the model formulation above, Fig. 3 shows that the stem resistance to water transport in the young orange trees was in fact not constant throughout the day, and this is a potential source of uncertainty in the model performance. However, typical estimates of  $R_x$  and  $C$  from *in situ* measurements on intact young beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) trees (Steppe, 2004) are also shown in Table 1. These young trees were growing outdoors and were comparable in size with the orange trees investigated here. Additional estimates of the capacitance from *in situ* measurements on intact trees, larger in size than the young orange trees studied here, are in the range  $0.4\text{--}2.0 \text{ kg MPa}^{-1}$  (Meinzer *et al.*, 2004).

Figure 4a illustrates the performance of the model in predicting the leaf water potential of the young orange trees, while Fig. 4b shows the course of sap flow at the

**Table 1.** Comparison of the hydraulic parameters ( $R_x$  and  $C$ ) of three young tree species, namely citrus (*Bahianinha* Navel budded on Troyer citrange rootstock), beech, and oak trees on typical clear days

Estimates of the hydraulic parameters for beech and oak trees were obtained from Steppe (2004). The stem diameters were measured at the point of stem sap flow gauge installation.

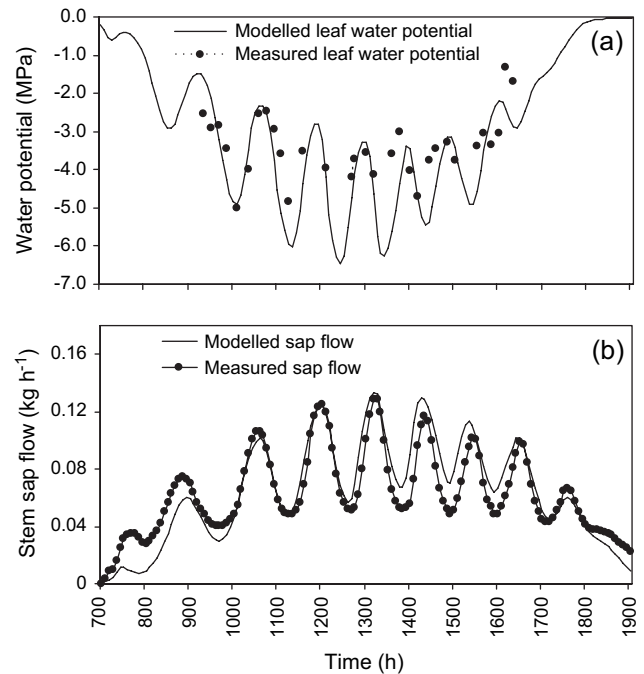
Parameter	Citrus	Beech	Oak
$R_x$ (h MPa kg <sup>-1</sup> )	48±3	39.7±0.4	17.2±0.1
$C$ (kg MPa <sup>-1</sup> )	0.28±0.02	0.0016±0.0003	0.0031±0.0007
Internal storage use (%)	14.0	2.2	3.4
Stem diameter (mm)	11.3	18.0	17.9



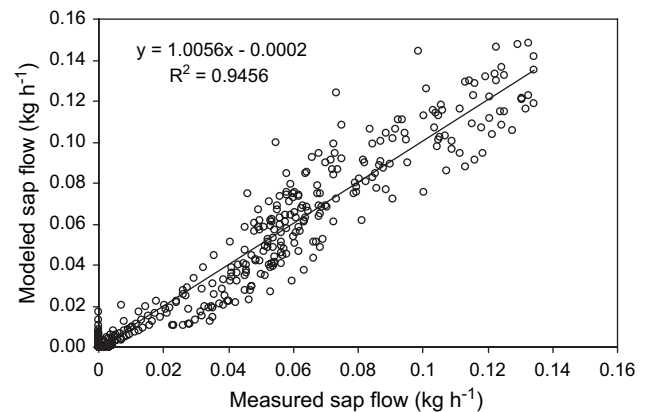
**Fig. 3.** Relationship between changes in the leaf water potential and stem level sap flow. The inverse of the slope of this graph is a measure of the hydraulic resistance of the stem, and this appeared to vary throughout the measurement period. Dotted lines indicate the 95% confidence limits.

base of the stem using the data for DOY 187. Despite the tendency by the model to predict slightly lower values than the measured ones at low water potentials, both quantities followed cyclic oscillations with an identical period to the sap flow, indicating that the oscillations in leaf water status were somewhat related to the dynamics of water transport. An improved fit of the model to the measured data could be obtained by including, for example, a variable hydraulic resistance function. Model validation was done using the data for DOY 185–186 (not used for model parameterization) as shown in Fig. 5. The model predicted the stem sap flow of the orange trees to within 10% of the true values.

It is apparent from Table 1 that the hydraulic resistance of this scion–rootstock combination of orange trees is marginally larger than that of other trees, notably 3-year-old oak and beech trees, while the capacitance is >10 times higher for the orange trees than for young beech and oak trees. Studies on different tropical trees (Goldstein *et al.*, 1998) showed that species with high water storage capacities tended to rely more on their internally stored water to sustain high transpiration rates. This was indeed true for



**Fig. 4.** (a) Comparison of the measured (filled circles) and the predicted leaf water potential (continuous line) on 6 July 2005 (DOY 187). Leaf water potential measurements were taken from 10:40 h till 17:40 h (local time, GMT+2 h). Given the long intervals between the leaf water potential measurements (10–20 min), interpolation was done with the aid of the water balance model. (b) Comparison of the predicted (continuous line) and measured (filled circles) stem sap flow of the young orange trees.



**Fig. 5.** Validation of the water balance model using sap flow data collected over a period of 2 d (DOY 185–186).

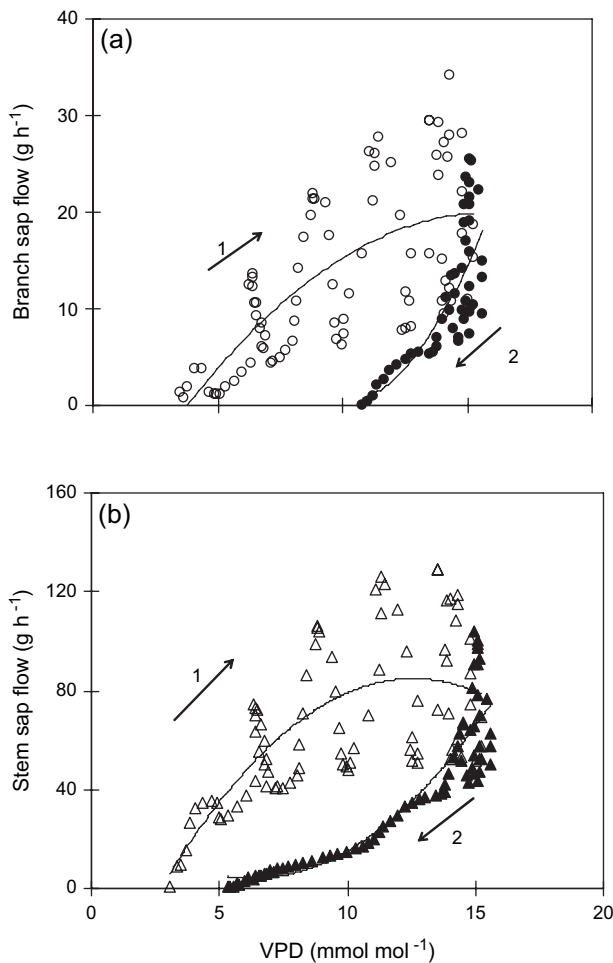
the young orange trees where the high capacitance led to a larger utilization of internally stored water during transpiration (14.0%) compared with young beech (2.2%) and oak (3.4%) on typical clear days. In addition, the humidity dependence of sap flow for the orange trees at both branch (Fig. 6a) and stem level (Fig. 6b) over a single day (DOY 187) showed a significant hysteresis effect which is a further indication of the high capacitance. In Fig. 6, the VPD was increasing (route 1) from sunrise (~06:00 h)

until after midday (14:00 h) and decreasing (route 2) from 14:05 h until sunset ( $\sim 17:30$  h). However, the hysteresis effect can also occur due to xylem cavitation (Brodribb and Holbrook, 2004; Buckley, 2005), but the occurrence of this phenomenon cannot be fully ascertained in the present study.

Despite the high capacitance, which should ensure an abundant source of water to meet the atmospheric evaporative demand, the fact that the leaf water potential of these orange trees fluctuated by as much as 2.0 MPa at regular intervals throughout a typical clear day (Fig. 4a) suggested the occurrence of periodic water deficits in the leaves, as further proven below.

#### Tree-level water balance, leaf water potential, and stem diameter variation

The dynamics of water uptake by the roots can be inferred from stem sap flow measurements, assuming there is no capacitance in the roots while the transpiration rates pro-



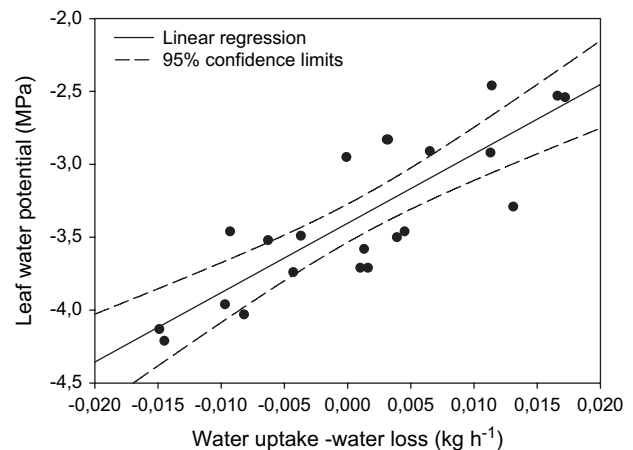
**Fig. 6.** Relationship between sap flow at branch (a) and stem level (b) with the VPD of the air during the increasing (route 1) and decreasing (route 2) VPD on DOY 187. The increasing VPD (open circles a and open triangles b) was from 06:00 h to 14:00 h, while the decreasing VPD (filled circles a and filled triangles b) was from 14:05 h to 18:00 h.

vide information on water loss from the trees. The difference in the daily courses of water uptake and loss gives the dynamics of the internal water storage of the trees. Figure 7 shows that for the young orange trees, the imbalance between water supply by the roots and demand by the leaves strongly influenced the course of the measured leaf water potential. When water loss exceeded uptake, leaf water potential dropped (more negative) while high leaf water potentials (less negative) were associated with water uptake exceeding loss.

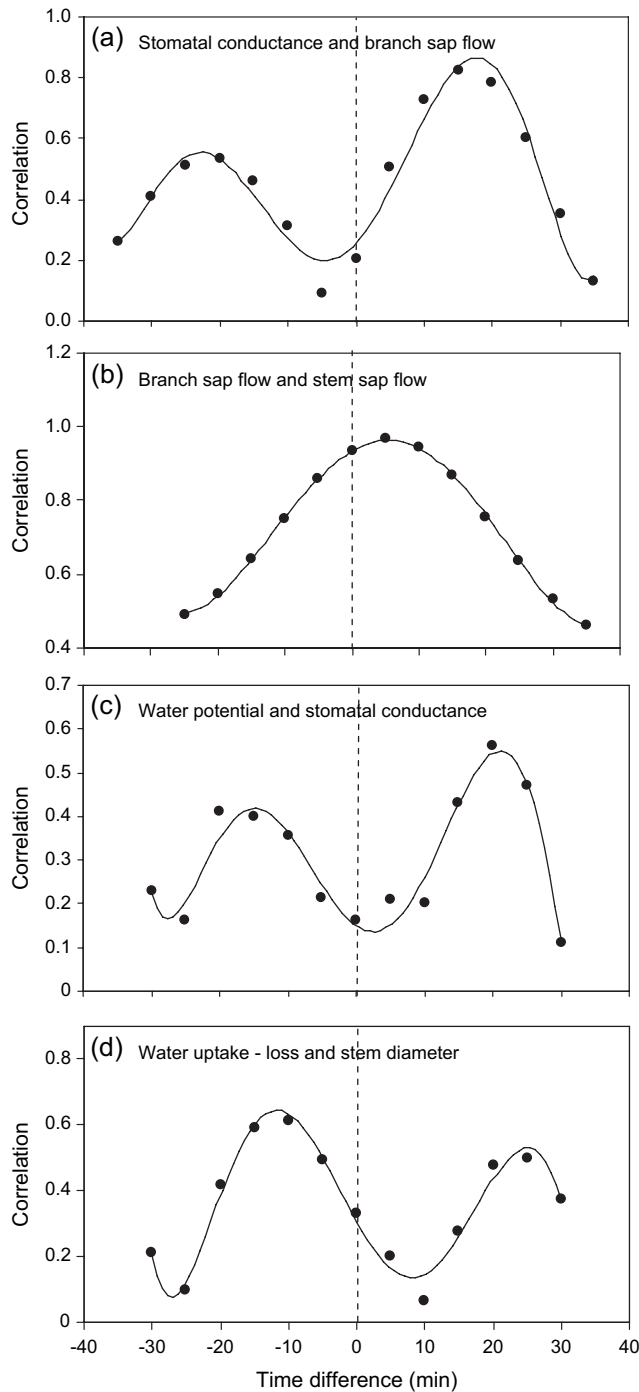
Independent evidence of the occurrence of transient changes in the internal water deficits due to changes in internal storage was shown by the evolution of the stem diameter which also followed cyclic oscillations with an identical period to the change in the internal water storage. Stem diameter variations lagged behind the changes in the internal water storage by approximately 15–20 min, as shown in Fig. 8d. According to Génard *et al.* (2001), the time lag between the rate of change in the stored water and the stem diameter could be due to a high radial hydraulic resistance between the water storage compartments in the stem and the conducting xylem tissue. For young oak and beech trees, for example, this time lag was found to be negligible (Steppe, 2004). However, for the orange trees studied here, the large time lag suggested the existence of a high radial resistance in the stem of the orange trees, thus suggesting that the high capacitance observed with these trees is probably in the leaves.

#### Time lags

Comparison of the stomatal conductance and branch sap flow as shown in Fig. 2 reveals that branch sap flow lagged behind the stomatal conductance by approximately 20 min, as clearly illustrated in Fig. 8a. The time lags were determined by cross-correlation of the two variables



**Fig. 7.** Effect of the imbalance between water uptake (stem sap flow) and water loss (branch sap flow scaled to the whole crown) on the course of leaf water potential of the young orange trees during a typical clear day. Measurements used in the graph were taken between 11:30 h and 16:30 h on DOY 187.



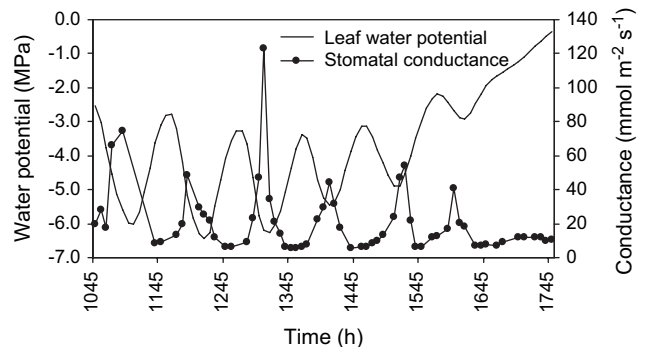
**Fig. 8.** Phase relationships of different water transport variables as influenced by the stomatal oscillations. (a) Cross-correlation between the stomatal conductance and the branch sap flow. Stomatal conductance had a phase lead of approximately 20 min compared with the branch sap flow. (b) The phase relationship between the branch and stem sap flow rates with the branch sap flow leading the stem sap flow by approximately 5 min. (c) Correlation between the stomatal conductance and the leaf water potential. Leaf water potential had a phase lead of approximately 20 min over the stomatal conductance. (d) This shows the oscillations in stem diameter lagging behind the difference between water uptake and loss by the stem by approximately 15 min. Negative values of time in the x-axis depict time lags, while positive ones show time leads. Only the larger peak correlation coefficient was considered in the analysis, and the differences in the peaks in (a), (c) and (d) were significant at  $P < 0.05$ .

by imposing systematic time shifts (Philips *et al.*, 1997). Stem sap flow best matched branch sap flow by imposing a 5 min time lag in stem sap flow, as shown in Fig. 8b. Consequently, the time lag between transpiration and water uptake by the young trees was at least 25 min, assuming no capacitance or a high resistance to water transfer at the surface of the roots. The stomatal conductance peak lagged behind the peak in the leaf water potential by approximately 20 min (Figs 8c, 9) although it is not clear whether a cause-and-effect relationship existed between these two variables.

## Conclusions

This study sought to give insights into the possible relationships between the whole-tree water balance and the phenomenon of stomatal oscillations. Although the link between the changes in the leaf water status and stomatal movements is not clearly resolved in this study, it is apparent that transient changes in the internal water deficits influenced the course of the leaf water potential and the evolution of the stem diameter. The causes of these deficits are clearly the large time lags between water uptake by the roots and loss through transpiration. Given the fact that the largest time lag occurred downstream of the branch, it is possible that there exists a high resistance between the shoots and the leaves which results in an inefficient transport of water once depleted by transpiration.

The application of the whole-tree level water balance provided useful information on the typical values of the hydraulic parameters of the transpiration stream of the young orange trees, although it could not effectively resolve events at leaf level. For instance, a leaf-level-based model is needed to differentiate between the closed loop amplification of the oscillations by hydropassive stomatal movements versus amplification arising from the lag in the water balance. The fact that the hydraulic resistance of the transpiration stream of this scion–rootstock combination of orange trees was non-constant provides



**Fig. 9.** Comparison of the diurnal course of the measured stomatal conductance and the modelled leaf water potential. This showed that water supply to the leaves influenced the size of the stomatal aperture but with a significant time lag.



further evidence that events beyond the leaf level also play an important role in the generation of the stomatal oscillations, probably through perturbations in the leaf water status. However, the causes of the variations in the hydraulic resistance are still unclear. As proposed by Buckley (2005), cavitation formation and its rapid reversal is a possible cause of the changes in the hydraulic resistance, but thorough investigations are still needed to confirm this. Recent studies conducted in intact plants using cryogenic scanning electron microscopy have shown that the number of embolized vessels can decrease during the daytime, suggesting that embolism repair can occur concurrently with transpiration (Tyree *et al.*, 1999; Bucci *et al.*, 2003).

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## References

- Baker JM, van Bavel CHM.** 1987. Measurement of mass flow of water in stems of herbaceous plants. *Plant, Cell and Environment* **10**, 777–782.
- Barrs HD.** 1971. Cyclical variations in stomatal aperture, transpiration and leaf water potential under constant environmental conditions. *Annual Review of Plant Physiology* **22**, 223–236.
- Brodribb TJ, Holbrook NM.** 2004. Diurnal depression of leaf hydraulic conductance in a tropical tree species. *Plant, Cell and Environment* **27**, 820–827.
- Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Sternberg LDASL.** 2003. Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels. *Plant, Cell and Environment* **26**, 1633–1645.
- Buckley TN.** 2005. The control of stomata by water balance. *New Phytologist* **168**, 275–292.
- Cowan IR.** 1977. Stomatal behaviour and environment. *Advances in Botanical Research* **4**, 117–228.
- Farquhar GD, Cowan IR.** 1974. Oscillations in stomatal conductance; the influence of environmental gain. *Plant Physiology* **54**, 769–772.
- Franks PJ.** 2004. Stomatal control and hydraulic conductance with special reference to tall trees. *Tree Physiology* **24**, 865–878.
- Génard M, Fishman S, Vercambre G, Huguet JG, Bussi C, Besset J, Habib R.** 2001. A biophysical analysis of stem and root diameter variations in woody plants. *Plant Physiology* **126**, 188–202.
- Goldstein G, Andrade JL, Meinzer FG, Holbrook NM, Cavelier J, Jackson P, Celis A.** 1998. Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant, Cell and Environment* **21**, 397–406.
- Hussein J.** 1982. An investigation into methods of flood irrigation of orange trees. *Zimbabwe Agricultural Journal* **79**, 73–79.
- Jarvis AJ, Young PC, Taylor CJ, Davies WJ.** 1999. An analysis of the dynamic response of stomatal conductance to a reduction in humidity over leaves of *Cedrella odorata*. *Plant, Cell and Environment* **22**, 913–924.
- Jones GH.** 1992. *Plants and the microclimate*. Cambridge: Cambridge University Press.
- Jones GH.** 2004. Irrigation scheduling: advantages and pitfalls of plant-based methods. *Journal of Experimental Botany* **55**, 2427–2436.
- Kaiser H, Kappen L.** 2001. Stomatal oscillations at small apertures: indications for a fundamental insufficiency of stomatal feedback-control inherent in stomatal turgor mechanism. *Journal of Experimental Botany* **52**, 1303–1313.
- Lang ARG, Klepper B, Malcolm JC.** 1969. Leaf water balance during oscillations of stomatal aperture. *Plant Physiology* **44**, 826–830.
- Levy Y, Kauffmann MR.** 1976. Cycling of leaf conductance in citrus exposed to natural environments and controlled environments. *Canadian Journal of Botany* **54**, 2215–2218.
- McBurney T, Costigan PA.** 1984. Rapid oscillations in plant water potential measured with a stem psychrometer. *Annals of Botany* **54**, 851–853.
- Meinzer FC, James SA, Goldstein G, Woodruff D.** 2003. Whole-tree water transport scales with sap wood capacitance in tropical forest trees. *Plant, Cell and Environment* **26**, 1147–1155.
- Meinzer FC, James SA, Goldstein G.** 2004. Dynamics of transpiration, sap flow and use of stored water in tropical forest canopy trees. *Tree Physiology* **24**, 901–909.
- Naidoo G, von Willert DJ.** 1994. Stomatal oscillations in the mangrove *Avicennia germinans*. *Functional Ecology* **8**, 651–657.
- Philips N, Nagchaudhuri A, Oren R.** 1997. Time constant for water transport in loblolly pine trees estimated from time series of evaporative demand and stem sap flow. *Trees* **11**, 412–419.
- Reich PB.** 1984. Oscillations in stomatal conductance of hybrid poplar leaves in the light and dark. *Plant Physiology* **61**, 541–548.
- Shackel KA, Brinckmann E.** 1985. *In situ* measurement of epidermal turgor, leaf water potential and gas exchange in *Tradescantia virginiana* L. *Plant Physiology* **78**, 66–70.
- Spiegel-Roy P, Goldschmidt EE.** 1996. *Biology of citrus*. Cambridge: Cambridge University Press.
- Steppe K.** 2004. *Diurnal dynamics of water flow through trees: design and validation of a mathematical flow and storage model*. PhD thesis, Ghent University, Belgium.
- Steppe K, De Pauw DJW, Lemeur R, Vanrolleghem PA.** 2006a. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. *Tree Physiology* **26**, 257–273.
- Steppe K, Dzikiti S, Lemeur R, Milford JR.** 2006b. Stomatal oscillations in orange trees under natural climatic conditions. *Annals of Botany* **92**, 831–835.
- Tyree MT, Salleo S, Nardini A, Lo Gullo MA, Mosca R.** 1999. Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? *Plant Physiology* **120**, 11–21.
- Upadhyaya SK, Rand RH, Cooke JR.** 1988. Role of stomatal oscillations on transpiration assimilation and water use efficiency of plants. *Ecological Modelling* **41**, 27–40.
- Vogt UK.** 2001. Hydraulic vulnerability, vessel refilling, and seasonal courses of stem water potential of *Sorbus aucuparia* L. and *Sambucus nigra* L. *Journal of Experimental Botany* **52**, 1527–1536.
- Wang GX, Zhang J, Liao JX, Wang JL.** 2001. Hydropassive evidence and effective factors in stomatal oscillations of *Glycyrrhiza inflata* under desert conditions. *Plant Science* **160**, 1007–1013.
- Yang HM, Zhang XY, Wang GX, Li Y, Wei XP.** 2003. Cytosolic calcium oscillation may induce stomatal oscillation in *Vicia faba*. *Plant Science* **165**, 1117–1122.
- Zipperlen SW, Malcolm CP.** 1997. Photosynthetic induction and stomatal oscillations in relation to the light environment of two dipterocarp rain forest tree species. *Journal of Ecology* **85**, 491–503.