An experimental system for analysis of the dynamic sap-flow characteristics in young trees: results of a beech tree

Kathy Steppe[A,B] *and Raoul Lemeur*[A]

[A]Laboratory of Plant Ecology, Ghent University, Coupure Links 653, B-9000 Gent, Belgium.
[B]Corresponding author; email: kathy.steppe@UGent.be

Abstract. This paper describes an experimental system designed for analysis of the dynamic water flow through young trees, complemented with test results of a 2-year-old beech tree (*Fagus sylvatica* L.). The system allows automatic and simultaneous measurements of a complex set of plant physiological processes at leaf, branch, stem and root levels [transpiration (E), sap flow (F) and diameter fluctuations (Δd)], in combination with the micrometeorological variables that control these processes [soil and air temperature (T_s and T_a), vapour pressure deficit of the air (D) and photosynthetically active radiation (PAR)]. A 2-d experimental period was used to study the whole-tree water transport dynamics of the young beech tree. Good correspondence between E of the leaves and F in the supporting branch was found. An increased time lag between the F measurements along the hydraulic pathway down towards the root system was observed, indicating the non-steady-state nature of the water flow. The daytime Δd of stem and branch revealed the depletion and the replenishment of internal water reserves. The daily amount of water withdrawn from internal storage was 5% of the total daily transpiration. A good linear relationship was found between the rate of change in internal water storage and the rate of change in stem diameter, having no time lag. We conclude that the data obtained with this system will allow experimental assessment of hydraulic properties in young trees and facilitate calibration of models for non-steady-state conditions of water flow in young trees.

Keywords: diameter fluctuation, sap flow, time lag, transpiration, water storage.

Introduction

The flow of water through plants in the 'soil–plant–atmosphere continuum' (SPAC) is controlled both by the vapour pressure deficit of the atmosphere and by the availability of the soil water. The equation describing the water flow through the SPAC is generally expressed as an Ohm's law analogue, which assumes that the flux density is proportional to a driving force and inversely proportional to a flow resistance (e.g. Rieger and Motisi 1990; Brisson *et al*. 1993; Lhomme 1998; Ruggiero *et al*. 1999). This Ohm's law analogue is, however, an oversimplification because it assumes a steady-state flow and hence a constant flow resistance. As such, many 'older' SPAC models regard the plants as simple, rigid tubes through which steady-state water flows occur. This is not true in reality as recently shown in studies dealing with the dynamic water transport through individual trees (Perämäki *et al*. 2001; Zweifel and Häsler 2001; Steppe *et al*. 2002). In addition to their function as a pathway for water transport, tree roots and tree stems also act as important water storage compartments (Meinzer *et al*. 2001). Because of internal water storage, leaf transpiration can start several minutes to hours earlier than water flow in the stem (e.g. Schulze *et al*. 1985). Obviously, the larger the tree, the greater the storage capacity will be, and the longer the time lag between the onset of transpiration at the crown level and the start of sap flow at the base of the tree (Goldstein *et al*. 1998). Moreover, the marked diurnal variations in stem radius also demonstrate the existence of stored water in internal reserves, which are depleted in the morning and replenished in the afternoon (Tatarinov and Cermak 1999). For these reasons, 'newer' models acknowledge the non-steady-state flow conditions of water in trees (e.g. Perämäki *et al*. 2001; Zweifel *et al*. 2001), but these models, too, are in need of calibration and validation data. However, relevant experimental information is often dispersed in literature. To fully understand the role of stem water storage in the regulation of the water flux through stems, branches and leaves of trees, and to

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characterise the effects of tree physiology during the non-steady-state flow of water, one needs an experimental system that allows measurements of the diurnal stem diameter variation in connection with other processes, like transpiration and sap flow (Goldstein *et al.* 1998; Tatarinov and Cermak 1999).

The purpose of this paper is to describe such an experimental system allowing automatic and simultaneous measurements of a complex set of plant physiological processes involved in water flow through the whole tree in the SPAC, that is, transpiration rate, sap-flow rate at root, stem and branch levels, and diameter fluctuations at stem and branch levels. Complementary measurements include micrometeorological variables that control these processes (i.e. soil and air temperature, vapour pressure deficit of the air, and photosynthetically active radiation). This experimental system allows the analysis of the dynamic plant–water relations of young trees subjected to step-changes of both atmospheric and edaphic factors. The data obtained in this way will clarify several aspects of non-steady-state flow in terms of water storage capacity and the time shifts between water uptake by the root system and water loss by leaf transpiration. Moreover, this paper aims to

complement the technical description of the experimental system with an analysis of how the system might be used to improve the understanding of water transport in trees. Therefore, test results of a young beech tree are discussed in terms of its water transport dynamics, and internal water storage.

Materials and methods

Plant material

A batch of two-year-old beech trees (*Fagus sylvatica* L.) was chosen as model system for measurement of dynamic water flow. The trees were approximately 1.3 m high and their stem diameter at the soil surface in the range 11–13 mm. All trees were grown outdoors and, at the beginning of February when freezing ends, one beech tree was selected for transplantation in a container of 0.4 m diameter and 0.4 m height, filled with a silt loam soil. Young trees were preferred over herbaceous plants because of the advantage of installing thermal sap-flow sensors on woody branches and stems, whereby overheating problems were avoided.

Tree chamber

The experimental system constructed for simultaneous control of both the atmospheric and the soil compartment around a young tree is schematically presented in Fig. 1. The system was composed of a tree chamber with dimensions $1.0 \times 0.6 \times 0.6$ m (height × width × length), which contained the above-ground parts of the young tree (see item #1
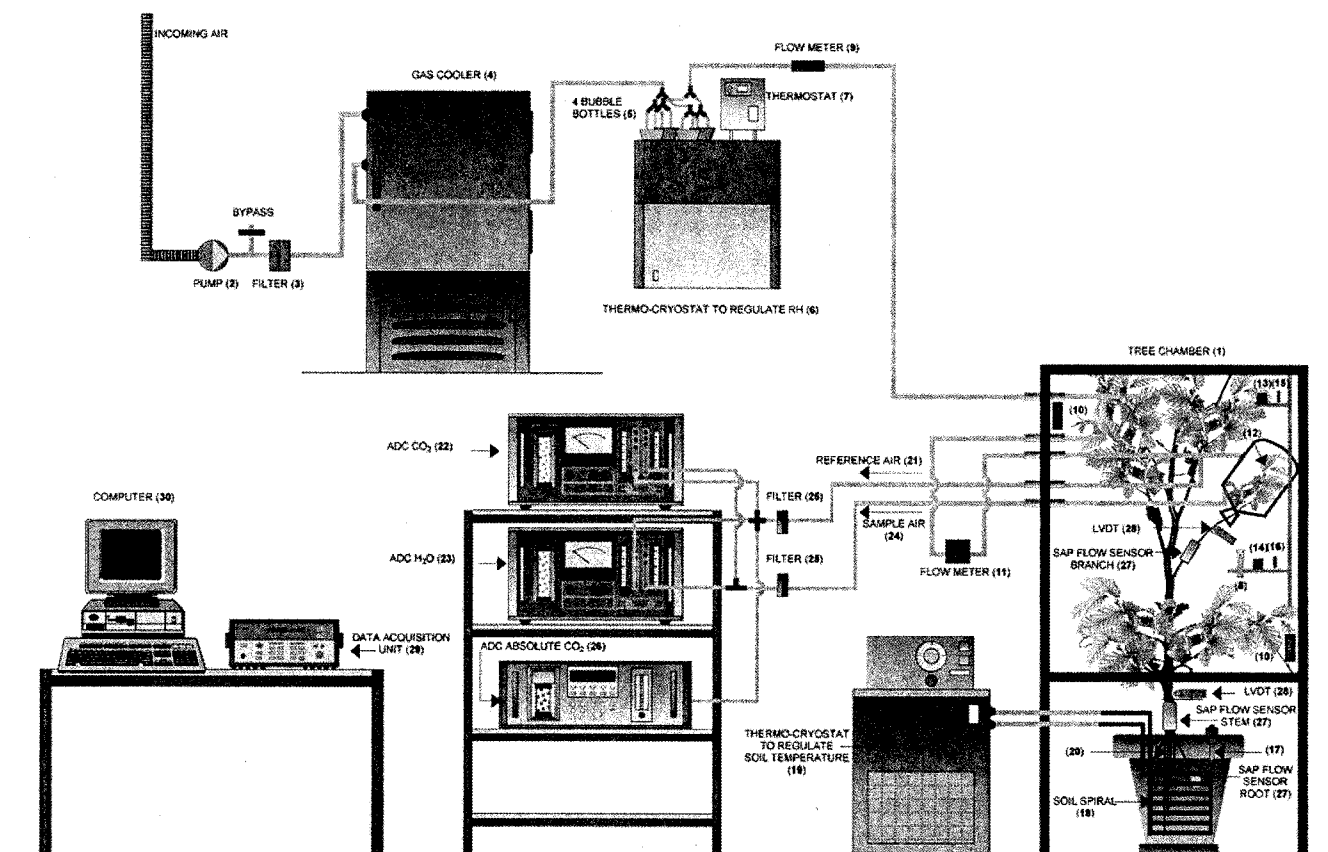

Fig. 1.   Schematic representation of the experimental system for analysis of the dynamic sap-flow characteristics in young trees. Numbers listed in the drawing refer to the sensors and equipment described in the text and in Table 1. Information about the microclimate variables and the physiological processes measured in the tree chamber is given in Table 1.

in Fig. 1). The chamber was made of transparent Plexiglas with a thickness of 2 mm. A wooden frame supported both the tree chamber and the soil container. The experimental system was placed inside a larger growth room of 2 × 1.5 × 2 m (height × width × length), which allowed the overall control of the radiation and the air temperature regime. All other control and measurement devices shown in Fig. 1 were placed outside the growth room in order to minimise disturbances of the microclimate in the tree chamber and soil container (such as extra heat production).

Microclimate of the tree chamber

Atmospheric compartment

To influence the vapour pressure deficit of the air (*D*), control of both the temperature and the humidity of the air around the transpiring leaves was required.

Humidity control in the atmospheric compartment was established by pumping outside air (#2; pump Type N 035.1.2 AN.18, KNF Verder, Aartselaar, Belgium) through a paper filter (#3; diameter = 14 cm, Hartmann and Braun AG, Frankfurt, Germany) into a gas cooler (#4; Type CG/G 73–4, Hartmann and Braun AG) operating at 4°C. This dry air was re-humidified with bubbling bottles (#5), which were kept at the chosen dew point temperature by submersion into the water bath (#6) of the thermo-cryostat (#7; UKT 2, Edmund Bühler/Otto GmbH, Hechingen, Germany). This dew point, together with the air temperature of the growth room, determined the relative humidity (humidity sensor #8), and hence the *D* imposed on the leaves inside the atmospheric compartment. A flow meter (#9; Streamline Flow Meter HFM-60, Teledyne Hastings Instruments, Hampton, VA) regulated the air volume entering the tree chamber (#1), and two fans (#10; Multifan type 4312R) assured good mixing. A fraction of this incoming air (flow meter #11) was guided to a branch bag (#12) for measurement of the transpiration rate.

The remaining factors of the microclimate, such as photosynthetically active radiation (PAR) and air temperature, were governed by the set points of the growth room. The PAR photon flux density inside the tree chamber was determined with a quantum sensor positioned above the branch bag (#13) and one below (#14); while air temperatures at these levels were recorded with thermocouples (#15 and #16, respectively).

Technical information on the sensors used in the experimental system is given in Table 1, together with the sensor accuracy specified by the manufacturer. In case of the vapour pressure deficit (*D*) and the photosynthesis (P$_n$) and transpiration (*E*) rates, the accuracy was calculated according to the theory of error propagation (Burrough and McDonnell 1998).

Soil compartment

Water supply to the root system depends on abiotic characteristics such as soil temperature and soil water availability. It is difficult to control and/or influence the soil water status (e.g. the soil water potential) dynamically. This might be done by applying nutrient solutions containing varying concentrations of osmotic active substances such as polyethylene glycol (Ranjbarfordoei *et al.* 2002). In the current experimental system water availability was allowed to change in a passive way, according to the need for replenishing the water lost by transpiration. The passive change of the soil water status was observed with a miniature type electronic tensiometer (#17).

More control could be exerted on soil temperature by means of water circulated in a copper spiral (#18). The spiral was placed parallel with the vertical container wall and was connected to a thermo-cryostat (#19; WK9 DS, Colora Messtechnik GmbH, Lorch, Germany), which circulated a mixture of 40% glycol in water at a chosen temperature. A vertical soil temperature profile was measured with thermocouples (#20) positioned at depths of 5, 15, 25 and 35 cm below the soil

surface. Horizontal temperature gradients were verified at the 15 cm depth with one thermocouple placed near the main central root and another one close to the spiral. The effects of soil temperature variation are, however, not reported here. This will be done in a later phase of the study when the influence of soil temperature on soil water transport and root uptake will be analysed.

Physiological measurements

The physiological responses of the young tree to the imposed changes in microclimate were deduced from several processes such as photosynthesis rate, transpiration rate, sap-flow rates at stem, branch and root levels and the diameter fluctuations at stem and branch levels. Each process was analysed by specific components and/or sensors installed in the atmospheric and soil compartments of the tree chamber (see Fig. 1).

Photosynthesis and transpiration rate

A polypropylene branch bag (#12) contained a secondary branch of the young tree in such a way that, for the measurements reported here, a total leaf area of 155 cm^2 was enclosed, the total whole-tree leaf area being 3965 cm^2. A part of the air at the inlet of the branch bag flowed back as reference air (#21) to the differential infrared gas analysers (CO$_2$ IRGA #22 and H$_2$O IRGA #23). The remaining part of the air flow was allowed to traverse the branch bag where the CO$_2$ and water vapour exchange could take place between the air and the leaves. The air at the outlet of the branch bag flowed back as sample air (#24) for the two IRGAs. Both the sample and the reference air were passed through paper filters (#25) in order to collect dust particles contained in the air flows. The photosynthesis and transpiration rates were calculated from the measured concentration differences between reference and sample air, the flow rate at the inlet of the branch bag (flow meter #11) and the leaf area. The expression for the calculation of transpiration rate is:

$$E = \frac{1}{A} \times (f \times \Delta\rho) \times 1000 \qquad (1)$$

where *E* is the transpiration rate per unit leaf area (mg m^{-2} s^{-1}), *A* is the leaf area enclosed in the branch bag (m^2); *f* is the flow rate at the inlet (m^3 s^{-1}) and Δρ the water vapour density difference between inlet and outlet (g m^{-3}).

A similar equation could be used to calculate the photosynthesis rate. The photosynthesis measurements are, however, not reported in this paper. They will be used when aspects of water use efficiency are studied at a later phase. At that time it will also be necessary to know the absolute CO$_2$ concentration of the air (absolute IRGA #26; ADC 7000, ADC BioScientific Ltd, Herts, UK).

Sap-flow rate

In addition to the diffusive water vapour flow by transpiration, the liquid water flow in the xylem vessels of the young tree (the sap-flow rates at root, stem and branch levels) was measured as well. Dynagage sap-flow sensors (#27) based on the heat-balance principle were used for this purpose. The sensors were installed at branch, stem and root levels, the diameters of these locations being 3.25, 11.65 and 2.7 mm. Sensor installation was performed according to the guidelines mentioned in the operation manual (van Bavel and van Bavel 1990). The sap-flow sensor at branch level was installed on the same branch that was selected for enclosure in the branch bag. For the sap-flow sensor at root level, care was taken to choose a root that could be excavated carefully. After installation of the sap-flow sensor on the upper part of the root, it was placed in the original position and covered with soil. Initially, the sap-flow rates were calculated according to the guidelines described in the operation manual (van Bavel and van Bavel 1990). However, the sap-flow estimates suffered from early morning

spikes owing to the neglect of heat storage. Thus, the measured dataset had to be corrected by including a heat storage term, as reported by Grime *et al.* (1995). For this correction the stem temperature, as well as estimates of stem and branch heat capacity (C_{st} and C_{br}) and gauge radial conductance (K_r), were needed. Diurnal courses of stem temperature were measured during the next growing season with a small thermocouple attached to the stem, and the sap-flow sensor installed in such a way that the thermocouple was located at the centre of the heater. To estimate C_{st}, C_{br} and K_r, zero sap flow conditions were required (Grime *et al.* 1995), and therefore, they were estimated at the beginning of the next growing season, before the young tree had leaves (no transpiration losses = zero sap flow). The values calculated for C_{st} (20 J °C^{-1}) and C_{br} (1.8 J °C^{-1}) were then used in combination with the dataset of stem temperature to estimate the heat storage term for correction of the measured dataset presented here.

In order to allow a meaningful comparison between the data from the sap-flow sensors installed at different positions, the measured sap
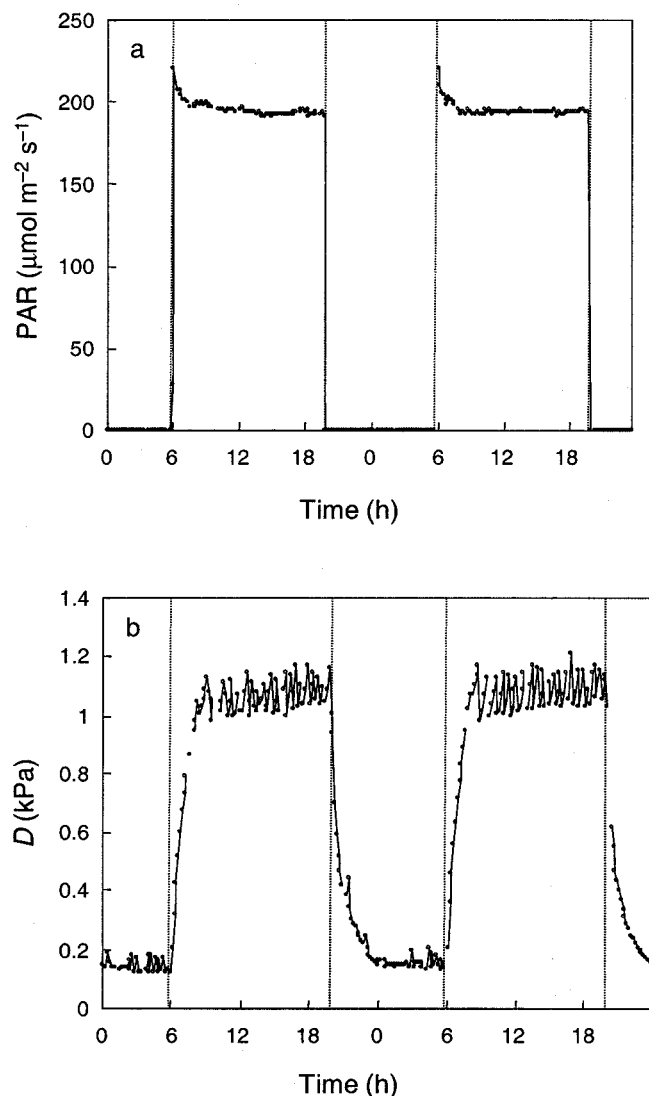
flow was normalised. In case of the sap flows at stem and branch levels, the measured values were divided by the total leaf area above the sensor. This yielded the flow rate of the sap per unit leaf area (F_{LA} in mg of water per s and per m^2 of leaf area). For the case of the sap flow at root level, the flow rate was divided by the root cross section at the sensor position (F_{RA} in g of water per s and per m^2 of cross section).

Diameter fluctuation

Small changes of stem and branch diameter were detected in this study by using linear variable displacement transducers (LVDT). Two LVDTs (#28) were installed for this purpose, one on the branch carrying the branch bag and one on the stem at ground level. The LVDT sensors were connected to an LC bridge that translated the displacements into a linearised mV signal. The LVDTs were supported by custom-made holders, made from stainless steel. A control run was carried out by installing the LVDTs on an aluminium rod of 12 mm diameter, showing that no temperature correction was required for the support.

Data acquisition

All signals from sensors and devices were logged at 10-s intervals using a data acquisition system (#29; HP 34970A, Hewlett Packard, Diegem, Belgium). A program was written (Hewlett-Packard VEE) to enable sensor measurements to be monitored continuously on the PC screen (#30). All sensor signals were averaged over 10-min periods, and the averages were stored on the hard disk for the whole period of measurements. The scan rate and averaging procedure of the data acquisition system can be adapted if fast-response information is needed during a short time period. A complete cycle of measurements took 10 d for the young tree. As the daily fluctuations did not change during the complete cycle, the data discussed in this paper refer to a 2-d sample only.

Results and discussion

Characteristics of the microclimate

Figure 2 presents a 2-d sample of the daily fluctuation of the microclimate imposed upon the young beech tree enclosed in the tree chamber. The microclimate is characterised by the photon flux density of photosynthetically active radiation (PAR) and the vapour pressure deficit of the air (D).

The daytime period was selected to last from 6 am–8 pm, which corresponds with late spring conditions in Belgium. The mean PAR during the daytime was approximately 193 μmol m^{-2} s^{-1} at the top of the beech tree. The initial increase in radiation observed at the beginning of each day was from an increased lamp output when the fluorescent lamps ('TL'D 80, Philips Lighting NV, Brussels, Belgium) warmed up.

The vapour pressure deficit of the air in the atmospheric compartment increased during the daytime to an average of approximately 1.08 kPa. This corresponded with a relative humidity of 56% at an air temperature of 20.8°C. In the

Fig. 2. A 2-d sample of the varying microclimate in the atmospheric compartment of the tree chamber enclosing a young beech tree. (*a*) PAR at the tree top level, (*b*) vapour pressure deficit of the air (*D*). The vertical dotted lines correspond to the beginning and the end of each day.

Fig. 3. A 2-d sample of the physiological responses of the young beech tree enclosed in the tree chamber. (*a*) Transpiration rate (*E*), (*b*) sap-flow rates (F_{LA}) at branch and (*c*) stem levels per unit leaf area and (*d*) sap-flow rate (F_{RA}) per unit area of root cross section. The vertical dotted lines correspond to the beginning and the end of each day.
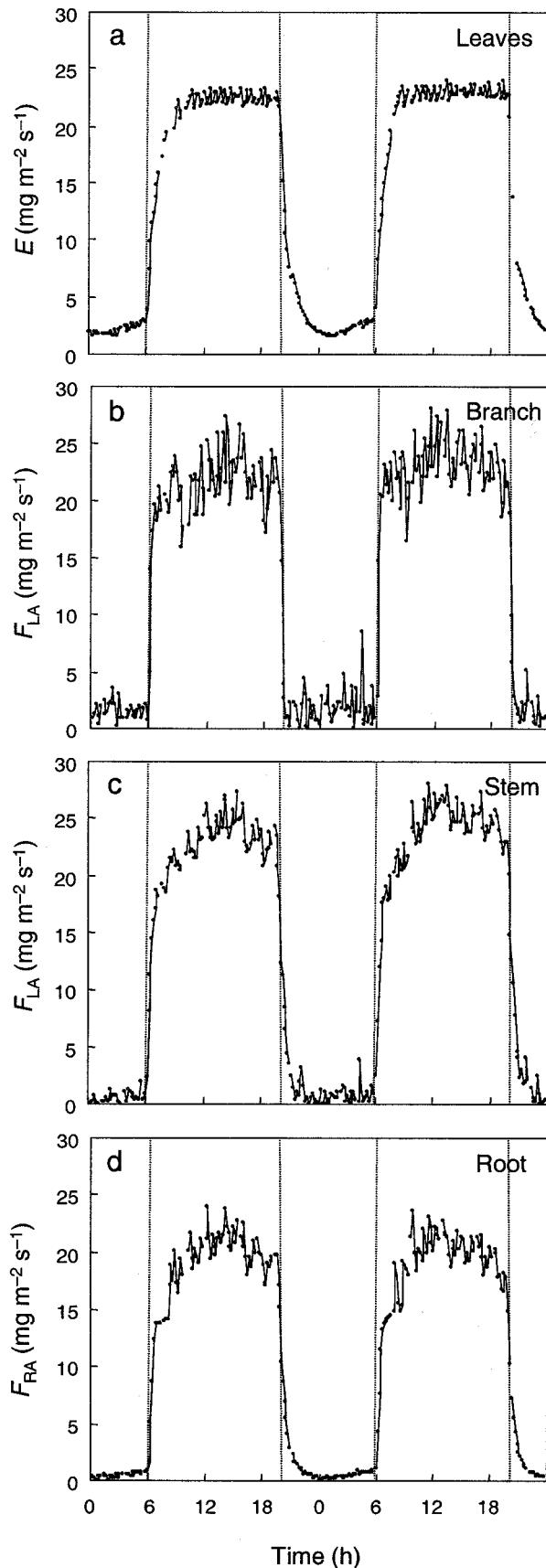
experiment the increase of D in the morning was faster than the decrease in the evening. This could be explained by the difference in heating capacity (electrical heating by the lamps) relative to the cooling capacity (water cooling) of the air conditioning unit in the growth room. The fluctuating course of D during the daytime period resulted from the hysteresis of the temperature sensor used by this unit. The hysteresis of the temperature sensor introduced a fluctuation of approximately 0.4°C around the selected air temperature set point of 22°C. During the night time the water cooling unit lowered the air temperature to 14°C as there was no electrical heating by the lamps.

The soil water content was kept near saturation during the reference period so that the young tree did not experience any drought stress. The tensiometer in the soil container indicated that the soil water potential varied between −1 and −7 kPa.

The soil temperature was not controlled, and fluctuated freely depending on the microclimate imposed in the atmospheric compartment.

Physiological responses

The responses of the young tree to the daily fluctuations in the atmospheric microclimate are shown in Fig. 3.

Transpiration rate

Figure 3*a* shows the transpiration rates per unit leaf area (E) for the beech tree measured in the branch bag. The average transpiration rate during the daytime period was 23 mg m^{-2} s^{-1}. However, some care was necessary when the E measurements were evaluated in absolute terms. The error analysis revealed that the instrumental error on E was relatively large (see the accuracy of ± 15% given in Table 1).

The water loss by transpiration during the night contributed 11% to the total daily transpiration. The night time transpiration rates indicated that the stomata of the leaves remained partly open during the night and/or that the transpiration occurred through the leaf cuticle. Epidermal impressions, obtained by the microrelief method (nail polish replicas), confirmed that a small part of the stomata remained partly opened during the night. Night time transpiration can only proceed when D around the leaves remains high (Green *et al.* 1989). In the present study the night time D remained higher than 0.12 kPa.

Sap-flow rates at branch, stem and root levels

Figures 3*b–d* show the sap-flow rates at branch, stem and root levels for the beech tree. A good correspondence between the transpiration rate of the leaves in the branch bag (E) and the sap-flow rate in the supporting branch [F_{LA}(branch)] was found when looking at absolute values (R^2=0.9; Fig. 4*a*). However, the higher scatter in F_{LA}(branch) compared with E during day and night is striking, and is probably due to a reduced precision of the

heat balance technique caused by the low sap-flow rate through the branch (see also below). When looking at daytime dynamics, Fig. 4a clearly shows a hysteresis between E and F_{LA}(branch), indicating that E lagged behind F_{LA}(branch) at the beginning and the end of the day. This is a technical artefact that can be attributed to the response time of the 'branch bag–H_2O IRGA' system compared with the sap-flow sensor. Indeed, before the concentration difference between reference and sample air could be measured with the 'branch bag–IRGA' system, both air flows had to pass through the connecting tubes inducing a significant time lag. Nevertheless, the good agreement between E and F_{LA} in terms of absolute values revealed that the sap-flow rate of a branch, measured with the heat-balance sensor, was a good indicator for the transpiration rate of the leaves positioned on that branch. Moreover, if the total leaf area of the tree is known, sap-flow rates can be scaled up, to estimate the whole-tree water consumption (Steinberg *et al.* 1990). The total transpiration rate of the tree [see F(leaves) in Fig. 5b] was calculated by upscaling F_{LA}(branch).

The good agreement found between the sap-flow rate of the whole stem [F_{LA}(stem)] and the branch [F_{LA}(branch)] ($R^2=0.95$; Fig. 4b) confirmed that the sap flow measured at a branch of the tree indeed can be used to estimate the whole-tree water consumption. The counter-clockwise hysteresis found between F_{LA}(stem) and F_{LA}(branch) indicates that for the young tree a time lag existed between stem sap flow and branch sap flow. These time lags are quantified below.

Comparison of F_{LA} measured at branch level (Fig. 3b) with the flow rate at stem level (Fig. 3c) showed that the latter displayed much less scatter. It seemed that the heat balance sensors at stem level produced more accurate results than at branch level. This might be related to the fact that, with heat balance measurements performed at stem level, a relatively larger part of heat was transported by the stem sap flow (convective heat transport) in comparison with the radial and vertical heat conduction occurring in the woody

tissues of the stem. At branch level, sap flow was less and, hence, the convective heat transport was smaller with respect to heat conduction. As such, the precision of the heat balance method was reduced.

The diurnal course of root water uptake measured with the sap-flow sensor on the beech root (F_{RA}; Fig. 3d) showed a good agreement with branch and stem sap flow (F_{LA}; Figs 3b, c).

The sap-flow responses measured at branch, stem and root level showed a time lag with respect to microclimate. The response of F_{LA}(branch) was faster than F_{LA}(stem), which in turn was faster than F_{RA}. In order to quantify the time lag differences between branch and stem sap-flow responses, the time constants were calculated from the daytime courses of sap flow. By definition, a time constant of a dynamic process indicates the time period needed to reach 63% of a step change. Therefore, the time was calculated between the onset of illumination and the time when the sap flow reached 63% of the average sap flow value obtained between 8 am–3 pm. The time constants at branch, stem and root level for the young beech tree (Figs 3b–d) were 20, 40 and 50 min, respectively. The data confirmed the well known fact that increased hydraulic resistance in the xylem vessels from leaves to roots leads to increasing time lags between the variation of the driving variable (e.g. transpiration rate) and the flow response in the upward direction of the pathway. These time lags could be interpreted in terms of storage capacity, as the time constant of a dynamic water flow is equal to the product of a hydraulic resistance with a storage capacity.

Several authors (e.g. Schulze *et al.* 1985; Steinberg *et al.* 1990; Goldstein *et al.* 1998; Perämäki *et al.* 2001; Zweifel *et al.* 2001) have also reported time lags between transpiration and sap flow varying from minutes to several hours. As this is true for large trees, it is often assumed that in small trees the flow rate of water through the stem responds immediately to changes in leaf canopy transpiration

Table 1. Physical and physiological sensors used in the experimental system presented in Fig. 1

Symbol, units, accuracy, sensor type, manufacturer and indication number of the sensors are given

Measured variable	Symbol	Units	Accuracy	Sensor type	Manufacturer	Indication no.
Photosynthetically active radiation	PAR	μmol m^{-2} s^{-1}	$\pm$ 10 μmol m^{-2} s^{-1}	Li-190S	Li-COR, Lincoln, NE	#13, #14
Air temperature	T_a	°C	$\pm$ 0.1°C	Thermocouple T	Omega, Amstelveen, Netherlands	#15, #16
Soil temperature	T_s	°C	$\pm$ 0.1°C	Thermocouple T	Omega	#20
Relative humidity	RH	%	$\pm$ 2%	RH sensor, HIH-3605-A	Honeywell, Brussels, Belgium	#8
Vapour pressure deficit	D	kPa	$\pm$ 4%	Calculated from T_a and RH		
Soil water potential	Ψ_s	MPa	$\pm$ 0.0005 MPa	Tensiometer, SWT5	Delta-T devices, Cambridge, UK	#17
Transpiration rate	E	mg m^{-2} s^{-1}	$\pm$ 15%	IRGA, ADC Model 225 Mk3	ADC BioScientific Ltd, Herts, UK	#23
Photosynthesis rate	P_n	μmol m^{-2} s^{-1}	$\pm$ 15%	IRGA, ADC Model 225 Mk3	ADC BioScientific Ltd, Herts, UK	#22
Sap-flow rate	F	mg s^{-1}	$\pm$ 10%	Dynagage SGA3-WS, SGA5-WS and SGA10-WS	Dynamax Inc., Houston, TX	#27
Diameter fluctuation	Δd	μm	$\pm$ 1 μm	LVDT, LBB, 375-PA-100 and transducer bridge 8C-35	Schaevitz, Hampton, VA	#28

(Wullschleger *et al.* 1998). This study clearly showed that even in a young tree considerable time lags can be observed. To calculate the time constants and time lags more precisely, a smaller time step for the measurements and data acquisition would be necessary.

Diameter fluctuations and internal water storage

The daily diameter fluctuation of branches and stems contains both an irreversible component due to growth and a reversible one due to changes of the water balance (Irvine and Grace 1997). This is also shown in Fig. 5*a* where the

evolution of branch and stem diameter is represented during two consecutive days. The more pronounced fluctuation at stem level in Fig. 5*a* compared with branch level was partly due to the larger diameter of the stem. A growth curve was found when the diameter maxima displayed at the end of the night time period were interconnected. The slope of these curve represented the growth rate of the branch and the stem and, after normalisation by the branch or the stem diameter, a relative growth rate (s^{-1}) was found. The time integration of the relative growth rate over 1 d for the stem and the branch was then 0.4 and 0.14%, respectively. The
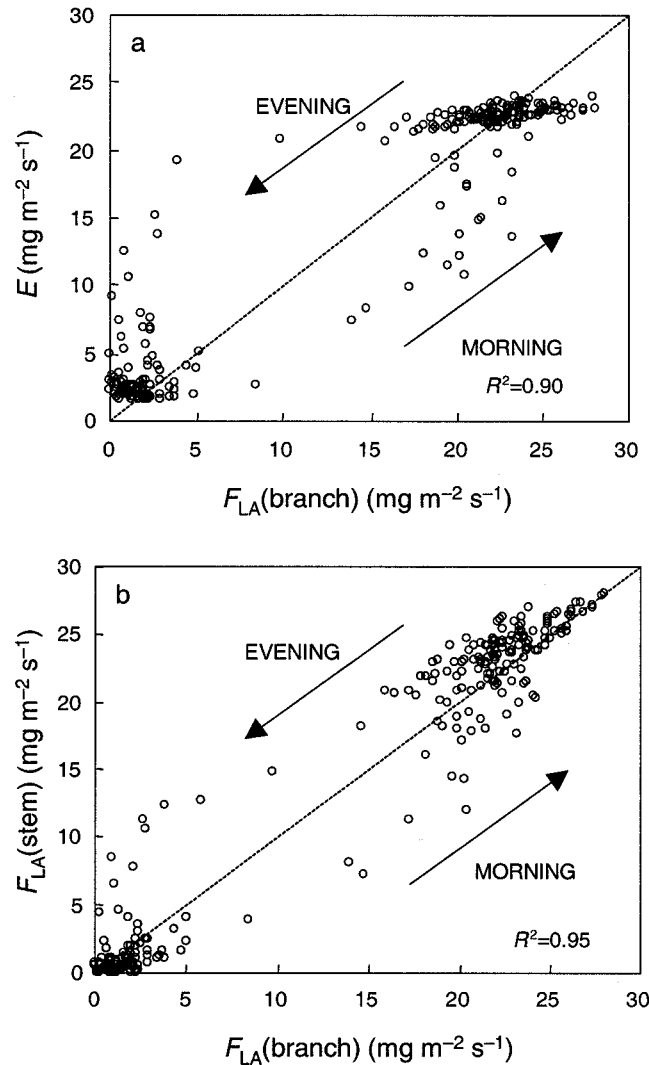

Fig. 4. (*a*) Linear relationship between the transpiration rate (*E*), measured with the branch bag, and the sap-flow rate at branch level [F_{LA}(branch)], measured with the Dynagage sap-flow sensor, for the young beech tree. (*b*) Linear relationship between the sap-flow rate at stem level [F_{LA}(stem)] and the sap-flow rate at branch level [F_{LA}(branch)] both measured with Dynagage sap-flow sensors for the young beech tree. The dashed lines represent the 1:1 relationship. The coefficients of determination (R^2) are also given. Arrows show the hysteresis phenomena.
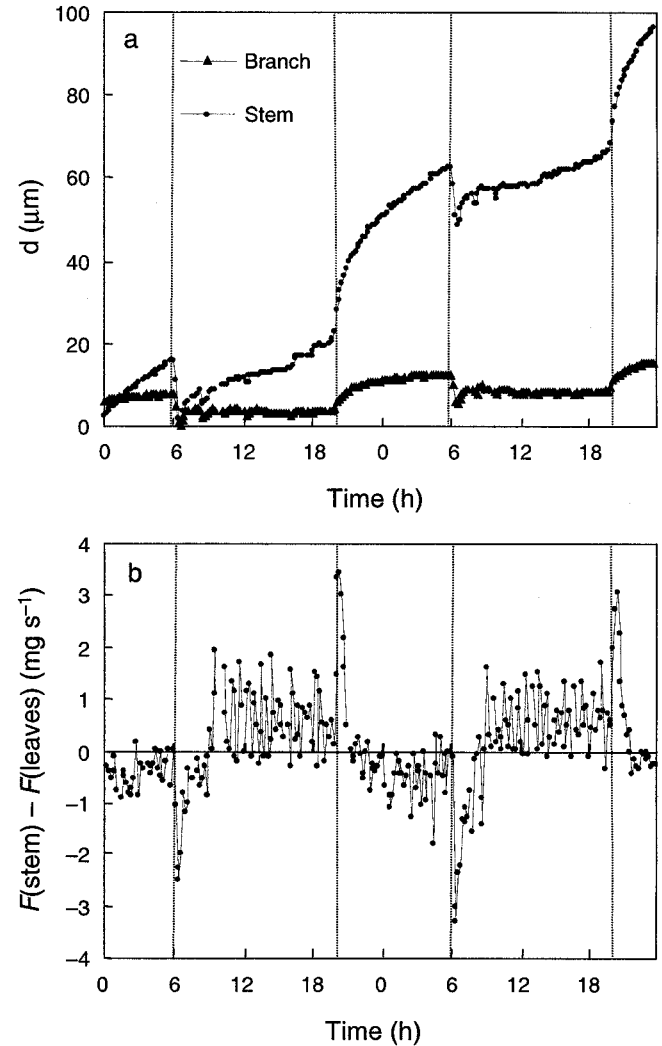
Fig. 5. (*a*) A 2-d sample of the diameter fluctuations (*d*) at branch and stem levels of the young beech tree. At the beginning of the measurements the diameters had been reset to zero. (*b*) Depletion and replenishment (negative and positive ordinates, respectively) of the total water storage in the young tree. The change of the water storage in the above-ground tree part is determined from the difference between the total stem sap-flow [*F*(stem)] and the total water loss by transpiration [upscaled transpiration rate estimated from branch sap flow towards the leaves in the branch bag, *F*(leaves)]. The vertical dotted lines correspond to the beginning and the end of each day.

time-integrated relative growth rate at branch level was less pronounced than at stem level, because the stem responds to photosynthesis carried out by the total leaf area of the young tree.

The daytime fluctuations imposed on the normalised growth signals of both the branch and the stem were more or less the same, and revealed the depletion and replenishment of the internal water reservoirs. At the onset of leaf transpiration a sharp decline of stem and branch diameter occurred.

An attempt was made to link the diameter fluctuations with the variation in internal water storage mathematically. Water storage was therefore estimated by comparing the stem sap flow of the tree [F(stem) in mg H_2O s^{-1}, as measured with sensor #27 below] with the total sap flow towards the transpiring leaf surfaces [F(leaves)]. The F_{LA}(branch) values (measured with sensor #27 at the intake of the branch bag] were therefore scaled up to tree level by multiplying with the total leaf area. Preference was given for a scaling procedure using F_{LA}(branch) instead of the direct measurement of transpiration rate (E); the reason being the different response time of the H_2O IRGA system compared with the sap-flow sensor (see above). The difference F(stem) – F(leaves) is represented in Fig. 5b for the two reference days. It can be seen that the processes of water depletion (negative ordinates) and replenishment (positive ordinates) were shown in a realistic way. The early morning consumption of stored water and the subsequent refill during the evening were displayed by the morning dip and the evening peak.

The total diurnal stem water storage capacity of the beech tree was estimated by summing the absolute values of the
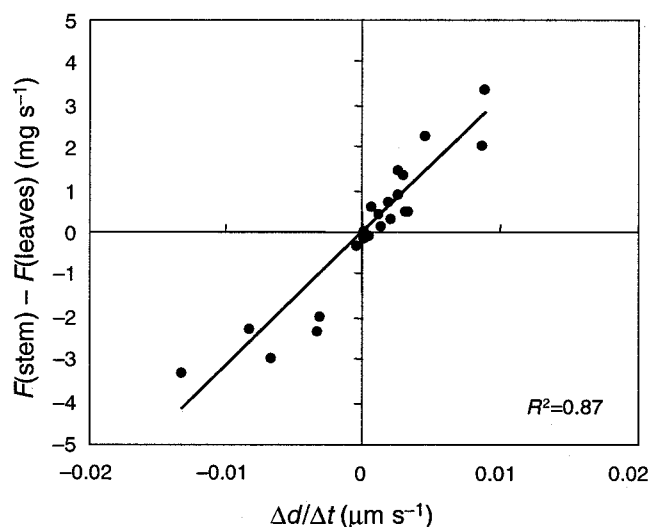

Fig. 6. Detailed analysis of the change in water storage [F(stem) – F(leaves)] in comparison with the rate of change in stem diameter ($\Delta d / \Delta t$). Data points correspond with the morning dip around 6 am (i.e. diameter shrinkage and stored water depletion) and the evening peak around 8 pm (i.e. diameter swelling and water replenishment) shown in Fig. 5b. The coefficient of determination (R^2) is also given.

negative 10-min differences |F(stem) – F(leaves)|, multiplied by the time step (10 min). The daily amount of water withdrawn from storage and subsequently replaced was 22 g d^{-1}. This meant a contribution of 5% to the total daily transpiration. Goldstein *et al.* (1998) reported similar contributions to the total daily water loss of 9–15%. This was observed in five tropical forest canopies for trees with a stem diameter ranging between 0.20–1.02 m. Our measurements showed that, although the absolute amount of water withdrawn from the internal reserves was limited in the young tree, the contribution to the total daily water loss was comparable with the values estimated for other tree species obtained in natural conditions (Schulze *et al.* 1985; Tyree and Yang 1990; Goldstein *et al.* 1998).

One could also focus on the data obtained during the morning dips (data points around 6 am) and the evening peaks (data points around 8 pm) by comparing the storage term F(stem) – F(leaves) with the rate of change in stem diameter ($\Delta d / \Delta t$). Figure 6 shows that for the non-steady-state conditions of the morning dip and the evening peak, a good linear relationship existed between the rate of change in internal water storage and the rate of change in stem diameter, without any time lag. Furthermore, the slope of the straight line, i.e. 315 mg μm^{-1}, revealed the amount of internal water the tree could mobilise per μm of stem diameter fluctuation. Simonneau *et al.* (1993) reported a similar relationship between the rate of change in stem diameter and the rate of water accumulation or loss from the above-ground plant reservoirs of a 5-year old peach tree, also having no important time lag (approximately 10 min). Génard *et al.* (2001) explained that such a slight time lag observed between the rate of change in the water storage compartment and the stem diameter could be due to the high radial hydraulic conductivity between storage compartments and the conducting xylem tissue. Zweifel *et al.* (2000) showed that changes in the stem radius of a Norway spruce [*Picea abies* (L.) Karst.] were linearly correlated with changes in the water content of the bark, and they proved that the bark was an important water storage location as long as the xylem water potential remained above –2.3 MPa. Moreover, Zweifel *et al.* (2001) stated that the rate of change in bark water content (= proportional to the measured stem radius) equalled the difference between the water flowing into and out of the stem segment. This linear relationship is also shown in Fig. 6. And although the observations of Sevanto *et al.* (2002) did not support the importance of phloem and bark as storage compartments in the stem of a Scots pine (*Pinus sylvestris* L.), the linear relationship found in Fig. 6 strongly suggested that the bark of the young beech tree did serve as a water storage compartment, being hydraulically connected to the water in the xylem with a low radial resistance. This finding is also supported by the study of Herzog *et al.* (1995) for Norway spruce. These authors, as well as others, reported that the change in stem diameter

resulted from the depletion and refilling of its extensible tissues.

Conclusions

The experimental system described in this paper is suitable for the analysis of the dynamic plant–water relations of young trees subjected to variable micrometeorological conditions. The 2-d sample of data demonstrated clearly the physiological responses during non-steady-state flow conditions in case of a young beech tree. This was done in terms of time shifts between water loss by leaf transpiration and water uptake by the root system, and also by establishing a relationship between the rate of change in internal water storage and the rate of change in stem diameter. This experimental system, tested and evaluated during this measurement cycle, is now ready for further use in studies on dynamic plant–water relations of young trees subjected to step-changes of both atmospheric and soil factors. The data obtained with this experimental system will also allow experimental assessment of hydraulic properties in young trees and facilitate calibration of dynamic flow and storage models for non-steady-state conditions of water flow in young trees.

In the future this experimental system can be improved by adding *in situ* thermocouple psychrometers installed on leaves (leaf water potential), as well as acoustic emission sensors for recording cavitation events. This might be of particular interest when studying young tree species under water stress conditions. In addition to whole-stem diameter variations, diameter fluctuations of the xylem (without bark) can also be very useful in analysing the water flow dynamics within the tree, as it provides information about water tension inside the stem.

Acknowledgments

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