Ann. For. Sci. 66 (2009) 501
© INRA, EDP Sciences, 2009
DOI: 10.1051/forest/2009034

Available online at:
www.afs-journal.org

Original article

Estimating sap flow from stem heat balances in *Quercus robur* L. seedlings in relation to light intensity: A comparison of two methods during the establishment phase

Magnus LÖF*, N. Torkel WELANDER

Swedish University of Agricultural Sciences, Southern Swedish Forest Research Centre, P.O. Box 49, 230 53 Alnarp, Sweden

(Received 23 October 2008; accepted 23 January 2009)

Keywords:
roots / sap flow / transpiration / water balance / water relations

Abstract

• Knowledge of whole tree seedling water fluxes is important in ecological and forestry research, especially under conditions with low transpiration, but no standard method has yet been established that provides reliable in situ measurements.
• The aims were: (1) to assess the performance of two methods for estimating sap-flows in oak seedlings following planting by correlating the data they provided with natural light intensities over a three-week period, and (2) to compare the estimates with transpiration data obtained by weighing pots.
• Estimates of sap flows obtained from data provided by constant power (Dayau-type) heat balance gauges under low light conditions (100–450 µmol m^{-2} s^{-1}) were less variable than estimates from variable power (EMS-type) heat balance gauges. The EMS-type system yielded data with little between-gauge variation, but consistently underestimated transpiration on a daily basis, a systematic error that should be corrected by other methods. The Dayau-type gauges yielded data with substantial variations, and several gauges are probably needed in research to cover these variations. Further, both systems provide rather uncertain estimates of short-time (hour) transpiration rates.
• However, provided that these considerations are taken into account, we conclude that it should be possible to use either system in various research contexts.

Mots-clés :
racines / flux de sève / transpiration / bilan hydrique / relations hydriques

Résumé – Estimation du flux de sève à partir du bilan thermique de la tige de plants de *Quercus robur* L. en relation avec l'intensité de la lumière : comparaison de deux méthodes au cours de la phase d'installation.

• La connaissance des flux d'eau de l'arbre entier est importante dans la recherche en écologie et en foresterie, en particulier dans des conditions de faible transpiration, mais jusqu'à présent, aucune méthode standard ne procure des mesures fiables in situ.
• Les objectifs étaient : (1) d'évaluer la performance de deux méthodes d'estimation des flux de sève en suivant une plantation de semis de chêne et en corrélant les données fournies par ces dernières avec les intensités de lumière naturelle sur une période de trois semaines, et (2) de comparer les estimations de transpiration avec des données obtenues par pesée des pots.
• Les estimations de flux de sève obtenues à partir des données fournies par une alimentation constante (Dayau-type) des jauges de bilan thermique sous des conditions de faible luminosité (100–450 µmol m^{-2} s^{-1}) sont moins variables que les estimations des jauges de bilan thermique à puissance variable (EMS-type). Le système EMS-type a fourni des données avec peu de variation entre les jauges, mais a toujours sous-estimé la transpiration sur une base quotidienne, une erreur systématique qui doit être corrigé par d'autres méthodes. Les jauges de type Dayau ont fourni des données avec des variations importantes, et plusieurs jauges sont probablement nécessaires pour la compréhension de ces variations. En outre, les deux systèmes fournissent des estimations plutôt incertaines des taux de transpiration pour des courtes périodes de temps (heure).
• Cependant, à condition que ces considérations soient prises en compte, nous concluons qu'il devrait être possible d'utiliser les deux systèmes, dans divers contextes de recherche.

* Corresponding author: magnus.lof@ess.slu.se

1. INTRODUCTION

There is often a near-linear relationship between water use and dry matter production in plants (de Wit, 1958). Reliable information on whole-plant transpiration rates and water use would therefore be of great interest in many ecological and silvicultural contexts. For example, it would be interesting to compare water use parameters of seedlings of important tree species in low-light conditions with competing overstory trees to improve our understanding of regeneration dynamics in near-natural systems. Moreover, it is well known that water stress often occurs in newly planted tree seedlings and may result in mortality or limited early growth, and thus economic losses in forestry (Kozlowski and Davies, 1975; Löf, 2000; Stone, 1955). Reducing such stresses or identifying tree species or provenances that have high tolerance to them would have substantial benefits. In addition, following anticipated climate changes, water is expected to become an increasingly limiting factor in many parts of the world (IPCC, 2007), and knowledge of differences in water use parameters between plant species or provenances should facilitate the development of appropriate breeding and/or management strategies. Thus, a method capable of providing continuous, reliable, non-destructive measurements of water use by whole seedlings growing in the field would be highly valuable for various purposes, but no such method has been established to date.

Several methods for measuring sap flow in small stems are, however, available. One, the constant stem heat balance method, was first applied to small herbaceous plants by Sakuratani (1981; 1984), and subsequently developed and commercialized for application to stems with diameters ranging from 2 to 125 mm (Baker and Van Bavel, 1987; Steinberg et al., 1990). Briefly, the mass flow rate of water in the stem is obtained by balancing the fluxes of constant heat into, and out of, a stem segment. This method is now widely used to study the effects of environmental factors on water flows in herbaceous stems (e.g., Baker and Van Bavel, 1987; Sakuratani, 1981; Senock and Ham, 1995) and in woody stems and roots (e.g., Coners and Leuschner, 2002; Steinberg et al., 1990; Wiltshire et al., 1995). Such measurements are reported to be generally within approximately ±10%, on a daily basis, of transpiration measurements obtained by weighing methods (Weibel and de Vos, 1994). However, large errors have been reportedly found for low and high flow rates (Gerdes et al., 1994; Shackel et al., 1992; Steppe et al., 2005). At low flow rates, the accuracy of the measurements is often poor because both the convective heat fluxes in water and temperature differences over the gauges used are small (Groot and King, 1992). To improve accuracy, several authors have proposed that the heat stored in the stem should be considered in the calculations (Dayau, 1993; Grime et al., 1995a; Valancogne and Nasr, 1993). When applying the abovementioned methods various types of loggers may be used for storing the acquired data and another advantage of using these systems is the possibility they provide for checking raw data in standard spreadsheets to verify the reliability of the results, although this is a time-consuming process.

In another method, which is technically more advanced, variable power inputs are used to maintain a constant temperature difference between a heated point and a reference point in the stem (Grime et al., 1995; Ishida et al., 1991). The advantage of this approach is that less power is supplied to the stem during periods with low sap flow rates than during periods with high rates, thereby reducing the overall power input and the likelihood of overheating causing physiological damage. The commercially available EMS-type system (T 4.2 system, EMS, Brno, Czech Republic), which includes gauges that fit stems with diameters ranging from 8 to 20 mm, is based on this method (Čermák et al., 2004). The gauges also allow stem diameter to increase during measurements, which is an additional advantage compared to other systems. Analyses based on the latter variable power approach have provided estimates of daily courses of sap flow that correspond well with transpiration measurements of *Salix* seedlings in open-top ventilated chambers (Cienciala and Lindroth, 1995). The abovementioned commercial system is user-friendly, but provides no scope for checking raw data on variables such as temperature differences.

Few comparisons have been made between methods using constant and variable power inputs (Grime et al., 1995; Kjelgaard et al., 1997), and sap-flow methods have seldom been applied for evaluating water use in seedlings during the establishment phase (Barnes, 2002; Messina and Duncan, 1993), when transpiration rates are normally low. Further, to our knowledge, no previous study has evaluated the accuracy of the EMS-type system using gravimetric data or comparing its accuracy during various light intensities with other sap flow methods. Moreover, most published evaluations of sap flows in small stems have been acquired using small numbers of sensors, and over short periods (a few days). However, thorough analysis of seedling responses to various ecological and silvicultural treatments usually requires information spanning longer periods (weeks – months) supplied by many sensors.

The aim of this study was to assess the performance of two methods for estimating sap-flows in oak seedlings following planting by correlating the data they provided with natural light intensities over a three-week period and comparing these data with transpiration data obtained by weighing pots. Sap-flow sensors of the Dayau-type use constant power inputs, the estimates they provide account for heat stored in the stem and the sensors can easily be constructed by the user, and thus produced in large numbers with relatively low overall costs. Sap-flow measurements with the EMS-type system use variable power inputs and are more expensive. It is therefore of great interest to evaluate and compare the accuracy and practical applicability of the two systems.

2. MATERIALS AND METHODS

2.1. Experimental design and plant materials

The experiments were carried out in a climate chamber (Biotron) at the Swedish University of Agricultural Sciences's Research Centre at Alnarp (55° 40' N/13° 10' E), from day of year (DOY) 115 to

DOY 179, 2006. During the experiment, the climate chamber was set to provide day/night air temperatures and relative humidities of 18 °C/12 °C and 60%/80%, respectively, with 13 h day length. However, natural light was used and due to its variability the air temperature and relative humidity varied somewhat during the daytime; increasing to > 20 °C and falling to ca. 50%, respectively, sometimes at around midday. The air temperature and relative humidity were continuously monitored (Minikin 1, EMS, Brno, Czech Republic) at one position in the chamber near foliage of seedlings and average values were recorded every 20 min. The chamber was ventilated with fresh air from outside, providing an air speed of approximately 0.3 m s^{-1}, which resulted in a change of chamber air nine times per hour.

The seedlings used in the experiment were bare-root *Quercus robur* L. (Linköping, Sweden) seedlings obtained from Tönnersjö nursery in Eldsberga (southern Sweden), lifted during DOY 114, 2006, and transported to Alnarp for transplantation in pots. The seedlings were four years old, they had been transplanted three times in the nursery and were 120–180 cm tall at the start of the experiment. In the nursery, the seedlings were pruned to approximately 50 to 75 cm.

Twenty-four seedlings were transplanted on DOY 115 into 10-L plastic pots (15.5×21 cm basal area, 31 cm high) containing between 11 000 to 13 400 g of fine-grained siliceous sand (median grain diameter, 0.44 mm), each with two 1.5 cm diameter holes in the bottom that were covered with textile nets to ensure that the sand did not leak out through them.

From DOY 115 to 143, the seedling-containing pots were irrigated twice weekly to field capacity with demineralised water. By the end of May (DOY 149), all of the seedlings had sprouted and developed a first flush of leaves. None of the seedlings had developed a second flush by the end of the experiment (DOY 179). To control aphids, the seedlings' leaves were treated with a biocide (active ingredient, liquid paraffin) twice during the experiment.

2.2. Treatments

From DOY 143 onwards, the seedlings were irrigated approximately every second or third day with a nutrient solution (Blomstra/Wallco växtnäring, 51-10-43 + micro, Cederroth International AB) diluted with demineralised water to give 1.21 mM [N] in the final solution. From DOY 157 to DOY 177 a Dayau type sap-flow gauge with constant power input (made in-house) was installed on each of eight seedlings, and a commercially available EMS-type gauge (T 4.2, EMS, Brno, Czech Republic) with variable power input (see below) was installed on each of another eight seedlings. Unfortunately, one EMS-type gauge (No. 7) was not in operation during the period DOY 171–173. The gauges were installed at heights of 30 to 61 cm above the sand surface in the pots at points where the stems were straight, their surfaces were free from knots and their diameters ranged from 13.2 to 16.5 mm (Tab. I).

2.3. Sap-flow measurements

Sap-flow was estimated using the information provided by the Dayau-type sensors (Dayau, 1993) in eight seedlings and the stem heat balance method as adapted from Sakuratani (1981) by Valancogne and Nasr (1993). This method involves measuring the heat balance components of an intact trunk wrapped in a heating band emitting a constant energy flux (Fig. 1A). The energy supplied is dissipated by conduction along the trunk and through the heated segment, and by sap-flow transport. Consequently, by measuring the energy supply and the conductive heat fluxes, it is possible to estimate the instantaneous heat flux conveyed by the sap for each seedling from the following energy balance equation:

$$Q_{sap} = E_h - (Q_{up} - Q_{down}) - Q_{lat} - Q_{sto} \quad (1)$$

where Q_{sap} is the heat flux (J s^{-1}) conveyed by the sap, E_h the energy flux (J s^{-1}) supplied by the heating wire, Q_{up} and Q_{down} are, respectively, up-stream and down-stream heat losses (J s^{-1}), Q_{lat} the lateral heat flux (J s^{-1}) and Q_{sto} the heat flux (J s^{-1}) stored in the heated segment of the trunk.

The sensors were constructed in-house, with a heating band made of flexible silicone containing a constantan heating wire on its inner surface, on top of which was a flux-meter of the same dimensions, made of silicone with six copper-constantan thermocouples along each side (Fig. 1A). The temperature difference over the heated segment was measured with two pairs of thermocouples mounted in 2-mm diameter stainless steel tubes inserted to a depth corresponding to 1/3 of the stem diameter. Water flow may vary with xylem depth in larger tree stems (Lüttschwager and Remus, 2007), but is assumed to be equal in small stems such as in this study. The first thermocouples above and below the heating band were placed at distances of 5 mm and 15 mm, respectively, and the other two were placed a further 5 mm above and below the first pair. An additional thermocouple was inserted into the stem in mid-position under the heating wire to acquire data on stored heat. Thermal insulation was provided by a 12 mm layer of insulating foam covering the whole sensor plus the section extending 5 cm above and below it. To protect the sensor from direct sunlight, it was shielded by aluminium foil. Measurements were taken at 1-min intervals using a CR10X data-logger connected to an AM416 multiplexer (Campbell, Cambridge, UK) and average values of the measurements were stored every 10 min. The gauge factors, needed for calculating Q_{lat}, were recalculated daily, assuming that sap-flow in the seedlings approached zero at 03.00 am.

Sap-flow was measured in an additional eight seedlings using commercially available EMS-type gauges (T 4.2, EMS, Brno, Czech Republic), (Čermák et al., 2004); an improved version of the gauges based on flexible external heating described by Čermák et al. (1984), Cienciala and Lindroth (1995) and Lindroth et al. (1995). In these gauges, heat is supplied by a resistance wire (Fig. 1B) and the sap flow is estimated from the heat balance of the artificially heated part of the stem, using the equation:

$$P = Q \times dT \times c_w + dT \times \lambda \quad (2)$$

where P is the heat input power (W), Q is the sap flow rate (kg s^{-1}), dT is the temperature difference at the measuring point (K), c_w is the specific heat of water (J kg^{-1} K^{-1}) and λ is the coefficient of heat losses from the measuring point (W K^{-1}). Temperature difference between heated and non-heated part of the measuring point (4 K) is kept constant by the accompanying T 4.2 data-logger unit and measured by thermo-sensors connected approximately 2 to 3 mm into the xylem (Fig. 1B), and controlled by variable power that is proportional to the amount of sap-flow. Thermal insulation was provided by accompanying foam and weather shields. The heat losses from the sensors were estimated at 03.00 am, when actual sap-flow was assumed to be zero, by daily baseline subtraction in the software used (Mini32, EMS, Brno, Czech Republic). Measurements were taken at 1-min intervals and their average values stored every 10 min.

Table I. Height and leaf area of the seedlings at the end of the experiment and positions of the sensors used for sap-flow measurements in the climate-chamber study at Alnarp, Sweden.

Sensor no.	Seedling diameter at sensor, mm	Height to sensor, cm	Seedling height, cm	Seedling leaf area, dm^2
Dayau-type system				
1	15.5	54	196	48.8
2	15.0	38	123	55.2
3	16.0	31	174	34.4
4	13.2	61	141	36.6
5	14.8	42	164	57.0
6	13.5	45	129	55.5
7	14.5	41	148	47.9
8	15.5	42	180	70.4
EMS-type system				
1	16.0	35	191	65.8
2	14.8	34	182	55.6
3	16.2	46	172	60.4
4	15.5	42	200	66.9
5	16.5	35	197	60.7
6	16.2	30	191	81.5
7	16.5	36	181	57.8
8	14.2	34	177	36.2

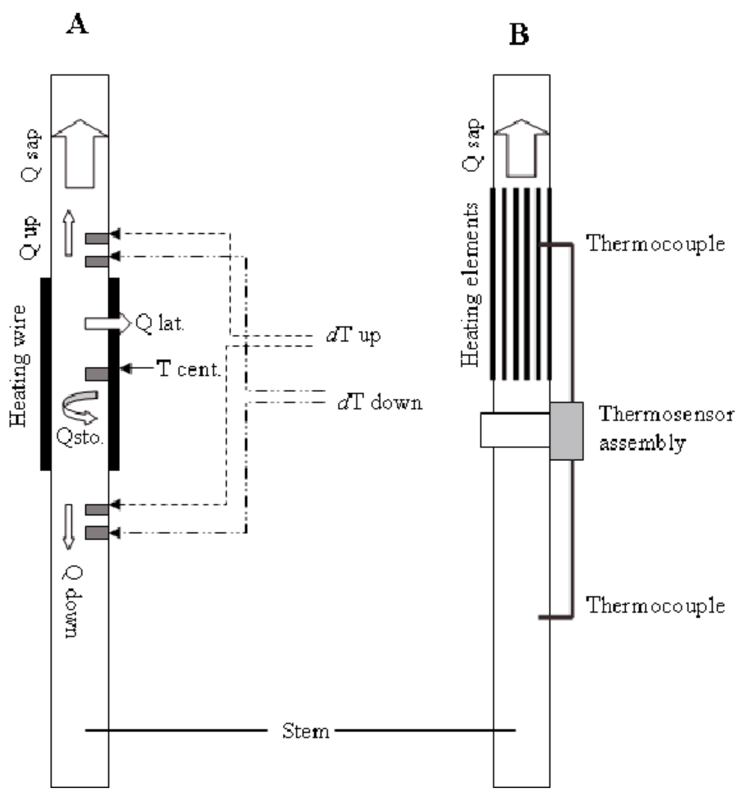

Figure 1. Schematic diagrams of the Dayau-type (A) and EMS-type (B) sap flow gauges and positions of the measured temperatures and heat fluxes (Q_{sap}, heat-flux convection by the sap; Q_{up} and Q_{down}, up-stream and down-stream conductive heat losses, respectively; Q_{lat}, lateral conductive heat flux; Q_{sto}, stored heat;dT_{up} and dT_{down}, up and down temperature differences; T_{cent}, temperature at the centre of the heated segment of the stem). All temperatures were measured using thermocouples inserted into the xylems and heating elements were wrapped around the stems in both systems.

2.4. Transpiration measurements

The transpiration rates in the 24 treatment seedlings were measured regularly by weighing the pots containing them using a B220 balance (Salter Brecknell, TE, USA), providing ± 1 gram accuracy for objects weighing up to 30 kg. During the measurement periods (DOY 161-162, 166-167, 171-172 and 172-173) each pot was weighed at 10.00 am on the first day and 10.00 am on the following day. In addition, five of the seedlings with a Dayau-type and four seedlings with a EMS-type gauge were weighed every second or third hour from 10.00 am until 20.00 pm on DOY 171 and from 10.00 a.m. until 15.00 pm on DOY 172. A thin polythene bag was cut and fastened to each pot with adhesive tape and sealed around the stem of the seedling during each measurement period to avoid soil evaporation, and between measurements the polythene bags were removed to allow aeration of the roots.

2.5. Measurements of light, seedlings and calculations

Photosynthetic photon flux density (PPFD) was measured using a LI-190SA sensor (LiCor Inc., NE, USA) in one position in the chamber above seedling foliage during the period of sap-flow measurements. The sensor was connected to a CR10X datalogger (Campbell Scientific Inc., UT, USA), which stored average values every ten minutes.

Between DOY 177 and 179, all seedlings were lifted from their pots and carefully washed in running water. The length of each seedling's new and fresh white roots (> 1 mm in diameter) was measured to the nearest cm using a ruler. We did not observe any significant amounts of new fine roots (< 1 mm). After sampling, the dry mass of leaves was determined after drying at 70 °C for 72 h. To determine the seedlings' leaf areas, a sub-sample ($n = 10$) of leaves from each seedling was photocopied and then oven-dried at 70 °C for 48 h to determine their dry masses. Leaf area was measured using a computer image system (Image access, Micro Macro Bildanalys AB, Sweden). The leaf area per seedling (LA) was calculated from the total leaf dry mass and the leaf dry mass to leaf area ratio of the sub-sample.

The acquired data were subjected to regression analysis. The period DOY 157 and 175-177 were excluded from the correlations between estimated sap flow and light intensity since gauges were mounted at different times during DOY 157 and since some seedlings experienced drought during the three last days of the experiment. Differences between transpiration measurements and sap flow measurements over various time periods were quantified by calculating the mean of the absolute relative differences (MARD):

$$\mathrm{MARD} = |100 \times \mathrm{SUM}((T_{\mathrm{sap}} - T_{\mathrm{grav}})/T_{\mathrm{grav}})/n| \qquad (3)$$

where T_{sap} is the transpiration sum measured by the sap flow sensor over the time period and T_{grav} is the transpiration sum measured by the weighing method.

3. RESULTS

The correlations between ten-minute averages of PPFD measurements during the period DOY 158-174 and ten-minute averages of sap flow estimates obtained from each of three Dayau-type gauges and three EMS-type gauges, mounted on seedlings with relatively high transpiration rates, are shown in Figure 2. In all of these cases the sap flow estimates increased non-linearly with increases in light intensity and levelled off at intensities exceeding ca 600 μmol m^{-1} s^{-1}. Generally, the Dayau-type gauges provided better, more robust correlations ($R^2 = 0.60$–0.70) with light intensity than the EMS-type gauges ($R^2 = 0.49$–0.61) when the same type of curve form was applied to data acquired for all six seedlings. Especially at low light intensities (ca 100–450 μmol m^{-1} s^{-1}), the Dayau-type gauges showed less variation compared to the EMS-type gauges. However, at higher light intensities there were substantial variations in the measurements provided by both types of gauges (Fig. 2).

Examples of daily courses of sap flow estimates for one seedling with a high transpiration rate and one with a low transpiration rate obtained from measurements by the Dayau-type and EMS-type gauges are shown in Figure 3B and 3C, respectively. Dayau-type gauges mounted on seedlings with high transpiration rates yielded sap flow curves that showed similarities to that of variations in light intensity, as illustrated by the data obtained from gauge 6 in Figure 3A and 3B. Seedlings with low transpiration rates did not show such similarities, as illustrated by the estimates obtained from gauge 3 (Figs. 3A and 3B). Considerable noise (with both positive and negative values) was observed in sap flow curves for the nights yielded by the Dayau-type sensors. The data obtained from Dayau-type gauges mounted on seedlings with low transpiration rates during the daytime were also noisy (Fig. 3B).

EMS-type gauges mounted on seedlings with high transpiration rates also yielded sap flow curves that showed similarities to variation in light intensity, as illustrated by the data from gauge 4 (Figs. 3A and 3C). However, for seedlings with low transpiration rates, the EMS-type gauges yielded extremely weak responses, as illustrated by the data from gauge 1 (Fig. 3C).

Comparisons of hourly sap flow and mean transpiration rates obtained during the daytime (DOY 171 and 172) are shown in Figure 4A and 4C. Although there were substantial deviations between the transpiration rates obtained from gravimetric measurements and sap flow rates obtained from the Dayau gauges, overall there was a strong correlation between them, as shown by the closeness of the regression line to a straight 1:1 line, the MARD value (6.2%) and the correlation coefficient ($R^2 = 0.64$) (Fig 4A). The correlation coefficient between the sap flow estimates provided by the EMS-type gauges and the transpiration data was even higher ($R^2 = 0.75$), although the sap flow values differed more, on average, from the gravimetric transpiration values (MARD, 28.7%). The results show that the EMS-type gauges had a tendency to underestimate transpiration, especially during periods with low transpiration rates, and at any given transpiration rate, across the observed range, there were significant variations in the estimates of sap flow obtained from both types of sensors.

There were weaker correspondences between estimates of accumulated sap flow obtained using both types of sensors and accumulated transpiration (DOY 171–173), including both day- and night-time values (Fig. 4B and 4D). The variations were higher ($R^2 = 0.7$) but the MARD value lower

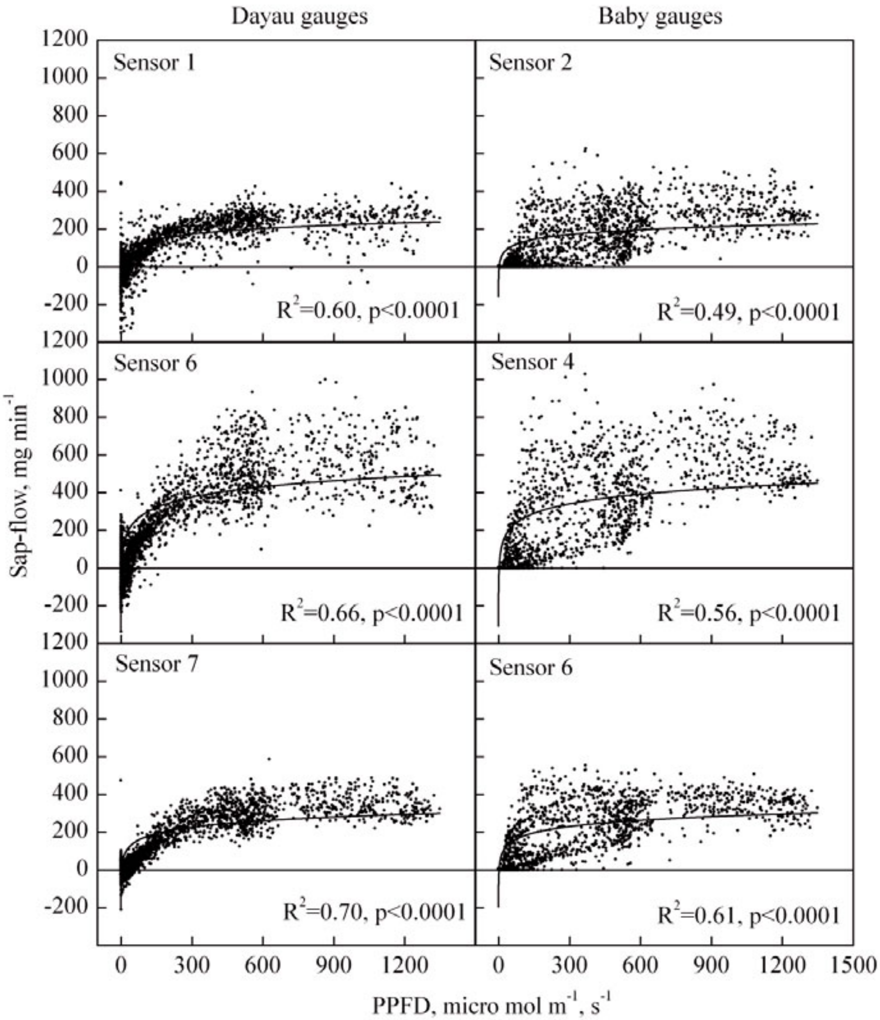

Figure 2. Sap flow (ten-min mean values) in relation to light intensity from DOY 158 to 174 for three sensors of Dayau-gauges (left boxes) and EMS-type gauges (or Baby gauges) (right boxes). No base-line subtractions were carried out for Dayau-gauges. Regression lines are according to the model: $y = \alpha + \beta \times \ln(x + 1)$.

(30.2%) for Dayau gauges than for EMS-type gauges (R^2 = 0.99, MARD = 60.2%). The sap-flow estimates obtained from EMS-type gauges showed very little variation around the regression line, but here too, the results show that these gauges had a systematic tendency to underestimate transpiration. The same trends were found when accumulated sap flow was compared to accumulated transpiration during earlier measurement periods (DOY 161-162 and 166-167) (data not shown).

In the 48-h period at the end of the experiment (DOY 171-173) there was only a very weak correlation between total seedling leaf area and transpiration sum (R^2 = 0.19; Fig. 5A), but a stronger correlation between new root length and transpiration sum (R^2 = 0.61; Fig. 5B). The transpiration rates in this period were significantly (R^2 = 0.89) higher than in the period DOY 161-162, when the light intensity sum was similar (Fig. 5C).

At the end of the experiment, we did not observe any damage to the oak stems where the heating elements had been fastened.

4. DISCUSSION

The daily estimated courses of sap flow provided by both systems tested in the experiments presented here showed similarities with daily courses of light intensity, in accordance with earlier studies (Higgs, 1994; Lindroth et al., 1995). However, there were considerable variations in the correlations between sap flow estimates and light intensity over longer periods. In seedlings with relatively high transpiration rates, sap flow estimates levelled off at light intensities higher than 600 μmol m^{-2} s^{-1} (equivalent to ca. 43% of the peak intensity, of ca. 1400 μmol m^{-2} s^{-1}) over most of the experimental

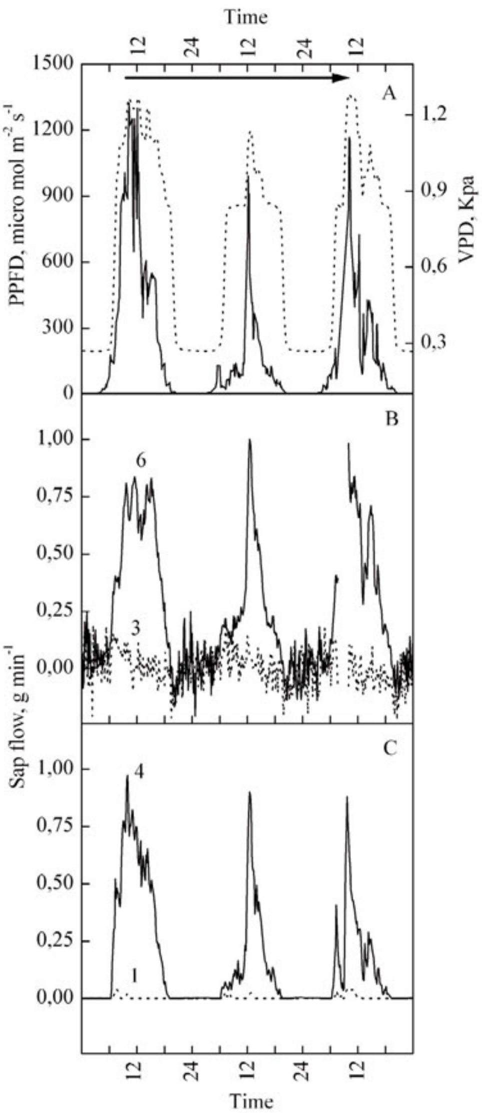

Figure 3. Influence of PPFD and VPD (A) on sap flow in two seedlings according to measurements obtained with Dayau-gauges (B) and two with EMS-type gauges (C) from DOY 171 to 173. In both B and C data for a seedling with a high transpiration rate and a seedling with a low transpiration rate are shown (seedlings 6 and 3 in B, seedlings 4 and 1 in C, respectively). The arrow in panel A indicates the period in which transpiration measurements were acquired by the gravimetrical method (used in the analyses presented in Fig. 4). The relative humidity of the air was set to 80% during night why VPD did not approach zero (A).

period, indicating, that the seedlings had probably not fully established root to soil contact. The sap flow estimates provided by the Dayau type gauges at low light intensities (ca. 100 to 450 μmol m^{-2} s^{-1}, or ca. 7 to 32% of peak irradiance) showed less variation than the EMS-type gauges. Thus, our results indicate that Dayau type gauges are a better choice than EMS-type gauges for monitoring the sap flow rates of seedlings grown in such low light environments.

We also compared estimates of sap flow provided by both systems with transpiration rates obtained from gravimetrical data. In this experiment, we examined oak seedlings with mean transpiration rates ranging from 2 to 43 g h^{-1} during the day-time and 100 to 610 g over 48-hour periods. In previous evaluations of the accuracy of sap flow measurements using stem heat balance methods the transpiration rates in the examined material have sometimes been considerably higher (e.g., Dugas et al., 1993; Grime et al., 1995; Ishida et al., 1991; Valangogne and Nasr, 1993). When comparing the variation in the same ranges of transpiration speed during day time, we have approximately the same magnitude of variation compared to other studies (Dugas et al., 1993; Grime et al., 1995a, 1995b; Kjelgaard et al., 1997; Weibel and de Vos, 1994). However, measuring sap flow in relation to gravimetrical data and using material with higher transpiration rates during measurements over 24-hour periods or longer lead to higher accumulated sap flows and consequently lower variations in regressions than those we found.

In relation to gravimetrical data, we found large variations in the responses of Dayau-type gauges during both short-term (hours) day-time measurements and measurements over 24- and 48-h periods. Thus, sap-flow estimates provided by this method for specific seedlings at specific times seem to have considerable levels of uncertainty. On the other hand, the MARD between the sap flow estimates obtained from the Dayau-type sensors and transpiration estimates obtained from gravimetric data was very low (only 6.2%) for day-time measurements, in accordance with the results of several previous investigations (e.g. Higgs, 1994; Kjelgaard et al., 1997; Sakuratani, 1981). With some exceptions (Gerdes et al., 1994; Shackel et al., 1992), most relevant previous studies have reported lower variations (Weibel and de Vos, 1994), in measurements over 24-hour periods, than we found here over 24- and 48-h periods. However, only one or a few sensors have been used in most previous evaluations of sap flows in small stems, and when larger numbers of Dayau-type sensors are used the likelihood of variation in sap-flow estimates increases, probably due to variations in the functioning of the sensors.

The accuracy of previous models of the EMS-type system has been evaluated by applying them to estimate sap flows in *Salix* trees with high rates of transpiration in open-top ventilated chambers to acquire reference data, or to compare sap flow with rates of evaporation in *Salix* stands, using the Bowen ratio method (Cienciala and Lindroth, 1995; Lindroth et al., 1995). In the present study, using a gravimetric method to obtain reference data to the EMS-type system, we found smaller variations between measurements compared to the Dayau method. Very low variations in the correlation were found when sap flows were evaluated over 24- and 48-h periods. However, the system underestimated transpiration in seedlings during periods of low flow rates (ca. 10 g per h or less), resulting in a substantial underestimation of total transpiration in all seedlings of approximately 100 g or more over 48-h periods. Kjelgaard et al. (1997) found similar trends when they compared variable power with constant power input methods. Therefore, in order to correctly evaluate water relations in seedlings during establishment in the field using the

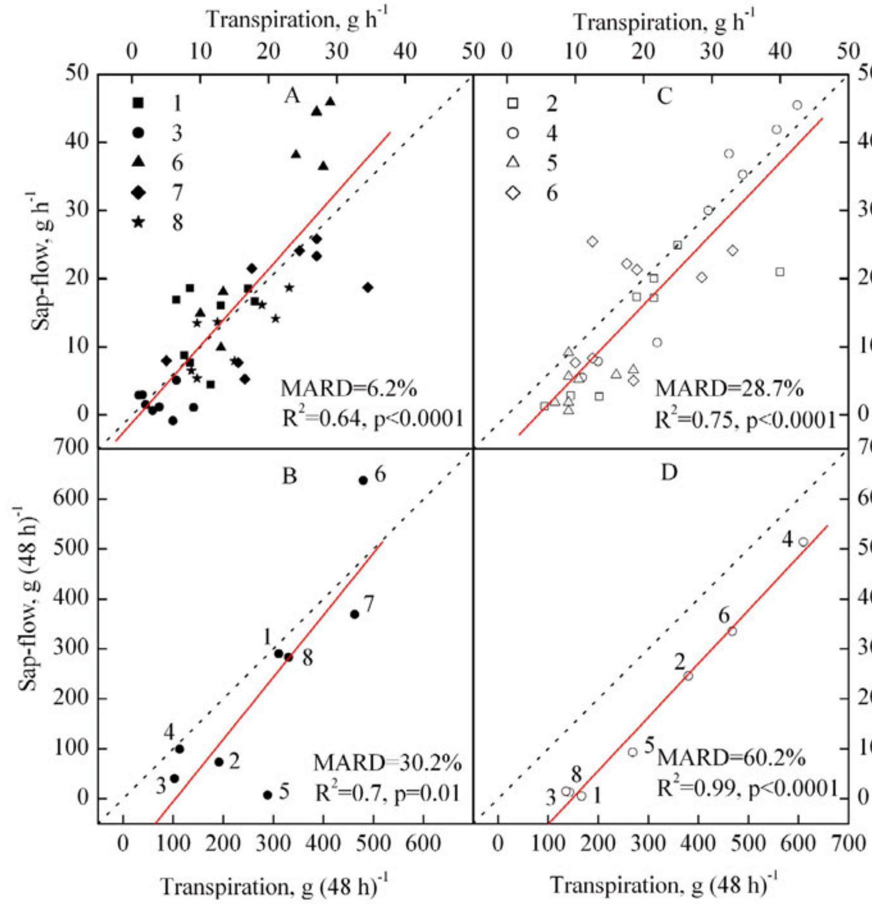

Figure 4. Correlations between hourly based sap flow and transpiration rates (A, C) and accumulated sap flow and transpiration (B, D) in seedlings measured by Dayau-gauges (A, B), and EMS-type gauges (C, D) from DOY 171 to 173. Dotted lines represent 1:1 linear correlations and solid lines are from regressions. Each symbol in A and C represents a data point for an individual seedling (five seedlings for Dayau-gauges and four for EMS-type gauges). The data displayed in B and D represent values obtained from eight and seven seedlings, respectively.

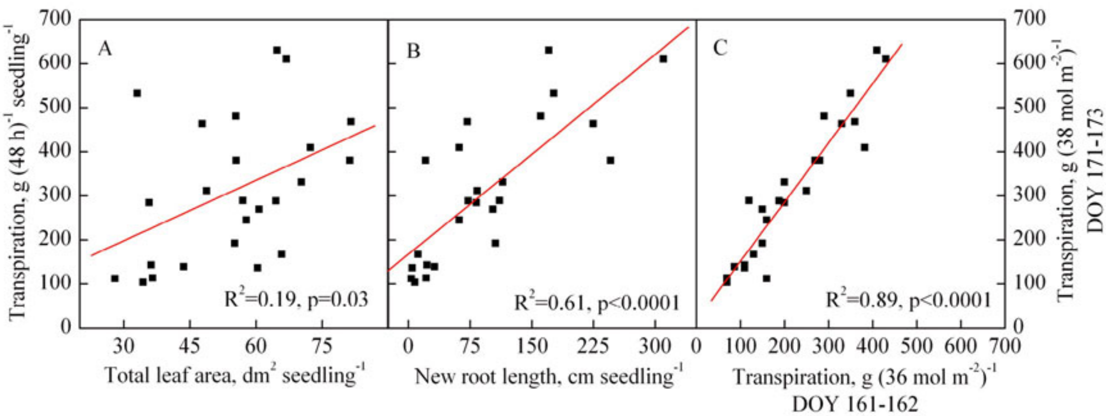

Figure 5. Correlations between accumulated transpiration from DOY 171 to 173 and seedling leaf area (A) and lengths of new roots (B). The correlation between accumulated transpiration measured in the periods from DOY 171 to 173 and DOY 161 to 162 is shown in C. In panel C the unit g per light intensity sum is used to show that the light intensity was very similar in the two periods.

EMS-type system, corrections are required for this systematic error, possibly using measurements of the weight of a reference seedling planted in a pot. Alternatively, laboratory calibration experiments might be carried out according to Coners and Leuschner (2002). A new sap flow system for small plants is under development by EMS and perhaps it will be more accurate. Furthermore, even if the variation was smaller compared to the Dayau-gauges, some variations were found between measurements, and between gauges, in the data obtained using the EMS-type system during short-term (hours) measurements in the day-time.

When comparing the two sap flow systems estimates with gravimetrical data, we found higher between-sensor and between-measurement occasion variations in the sap flow estimates obtained using the Dayau, constant input power method, in accordance with results presented by Grime et al. (1995b). This is why previous workers have drawn attention to the benefits of varying the power supply to the stem and the greater potential accuracy it provides (Grime et al., 1995a). Briefly, this should reduce the sensitivity of the gauge's response times to variations in the flow rate and maintain a higher (constant) temperature difference throughout the day, thus reducing both errors associated with the temperature differences declining to very low values, and fluctuations in stem heat storage. Heat storage is included in the Dayau method. Something that may explain why this method better estimated transpiration at low flow rates compared to the EMS-type system in line with finding by Grime and Sinclair (1999) and Steppe et al. (2005). Other factors may also have contributed to the higher variation in the sap flow estimates yielded by the Dayau method. For instance, several stainless steel tubes were inserted into the stems for the Dayau-type measurements, which may have disturbed some of the parts conducting water flows in some of the oak stems. In addition, although the difference was small, the stem positions where the Dayau-type gauges were mounted, were somewhat smaller (on average ca 1 mm) in diameter compared with seedlings where EMS-type gauges were mounted. It would probably have been an advantage to mount the different sensors to the same trees, which was not done in this study.

In the present study we did not detect any damage to the stems under the heating wires. It should therefore be possible to use either system over periods of several weeks. However, previous investigations have reported stem damage when long-term measurements (growing season) were carried out using devices similar to the Dayau-type gauges (Wiltshire et al., 1995). In this respect, the EMS-type system has a potential advantage since the gauges allow air to move along the bark surface, and stems to grow in diameter.

The choice of sap flow method for estimating transpiration during the plant establishment phase might be difficult since the transpiration rate between individual seedlings may vary considerably. This variation may be due to variation in root formation. In our study the correlation between the length of new white roots and transpiration was strong, which is in accordance with the widely recognised importance of newly established white roots for water uptake (Sands et al., 1982). However, rather unexpectedly – since plant and tree transpiration is usually closely correlated to leaf area or sunlit leaf area (e.g., Čermák et al., 2004; Ma et al., 2008; Welander and Ottosson, 2000) – there was no significant correlation between transpiration and seedling leaf area. Therefore, variations between seedlings in the rate of establishment of new white roots provide the most likely explanation for the large variations in transpiration between seedlings we observed (especially since the seedling's rates of transpiration were higher in the monitoring period DOY 171 to 173 than in DOY 161 to 162 when the light intensity sum was similar, and thus the length of their white roots probably increased during the experiment). Fine roots (< 1 mm in diameter) comprise a major component of root systems and both fine roots and suberized coarse roots may take up substantial amounts of water (Coners and Leuschner, 2005; Pierret et al., 2005). The lengths of the latter roots were not measured in the present study. However, the lengths of newly established white roots probably provides a good indication of both overall root activity and root to soil contact during seedling establishment.

5. CONCLUSIONS

Knowledge of whole tree seedling water fluxes is important in various ecological and silvicultural contexts, but no standard method has yet been established that provides reliable results in the field. The results of this study, using seedlings with stem diameters ranging from 13.2 to 16.5 mm in different phases of establishment, have several practical implications for the use of sap flow sensors to obtain estimates of transpiration in seedlings. Firstly, estimates of sap flow provided by the EMS-type system under low light conditions varied more than estimates obtained using the Dayau type gauges, thus the latter appear to be a better choice in such situations. Secondly, the EMS-type system consistently underestimated transpiration for 24-h or longer periods. This systematic error needs to be corrected, for example using gravimetric measurements of reference seedlings, laboratory calibration experiments or sap-flow estimates from Dayau-gauges, which yielded better results at low flow rates. Thirdly, when measurements were carried out over 24-hour or longer periods, the constant power, Dayau method yielded higher between-gauge variations in sap flow estimates than the variable power, EMS-type system. To overcome this problem, a possible option would be to use several gauges and rely on mean values, instead of individual values, for sap-flow estimates. The precise number of gauges to use is, however, difficult to decide. Finally, there were variations (between-gauge and between-short term measurements from the same gauges) in the estimates of sap flow during the day-time yielded by both systems. Thus, individual values for estimates of short-term transpiration rates, using either of the two methods, are rather uncertain. Nevertheless, provided these considerations are taken into account, and that seedlings with similar sizes are used, as in this experiment, it should be possible to use either system in various research situations for estimating sap flow rates in evaluations of water relations in seedlings.

Acknowledgements: We appreciate the assistance provided by Olof Hellgren and Göran Nilsson at the BIOTRON, Swedish University of Agricultural Sciences in Alnarp. We also thank John Blackwell and Sees-editing Ltd for linguistic improvements. Financial support was received from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning and the research program Sustainable management in hardwood forests.

REFERENCES

Baker J.M. and Van Bavel C.H.M., 1987. Measurement of mass flow of water in the stems of herbaceous plants. Plant Cell Environ. 10: 777–782.

Barnes A.D., 2002. Effects of phenology, water availability, and seed source on loblolly pine biomass partitioning and transpiration. Tree Physiol. 22: 733–740.

Čermák J., Jeník J., Kučera J., and Žídek V., 1984. Xylem water flow in a crack willow tree (*Salix fragilis* L.) in relation to diurnal changes of environment. Oecologia 64: 145–151.

Čermák J., Kučera J., and Nadezhdina N., 2004. Sap flow measurements with some thermodynamic methods, flow integration within trees and scaling up from sample trees to entire forest stands. Trees 18: 529–546.

Cienciala E. and Lindroth A., 1995. Gas-exchange and sap flow measurements of *Salix viminalis* trees in short-rotation forest. Trees 9: 289–294.

Coners H. and Leuschner C., 2002. In situ water absorption by tree fine roots measured in real time using miniature sap-flow gauges. Funct. Ecol. 16: 696–703.

Coners H. and Leuschner C., 2005. In situ measurement of fine root water absorption in three temperate tree species – Temporal variability and control by soil and atmospheric factors. Basic Appl. Ecol. 6: 395–405.

Dayau S., 1993. Réalisation des capteurs pour la mesure du débit de sève dans des arbres (méthode du bilan de chaleur). Cahiers Tech. INRA 31: 3–24.

De Wit C.T., 1958. Transpiration and crop yields, Institute of Biological and Chemical Research on Field Crops and Herbage, Wageningen, The Netherlands, Verlagen Landbouwkundige Onderzoekingen 64.6, 1–88.

Dugas W.A., Wallace J.S., Allen S.J., and Roberts J.M., 1993. Heat balance, porometer, and deuterium estimates of transpiration from potted trees. Agric. For. Meteorol. 64: 47–62.

Gerdes G., Allison B.E., and Pereira L.S., 1994. Overestimation of soybean crop transpiration by sap flow measurements under field conditions in central Portugal. Irrig. Sci. 14 : 135–139.

Grime V.L., Morison J.I.L., and Simmonds L.P., 1995a. Including the heat storage term in sap flow measurements with the heat balance method. Agric. For. Meteorol. 74: 1–25.

Grime V.L., Morison J.I.L., and Simmonds L.P., 1995b. Sap flow measurements from stem heat balances: a comparison of constant with variable power methods. Agric. For. Meteorol. 74: 27–40.

Grime V.L. and Sinclair F.L., 1999. Sources of errors in stem heat balance sap flow measurements. Agric. For. Meteorol. 94: 103–121.

Groot A. and King K.M., 1992. Measurement of sap flow by the heat balance method: numerical analysis and application to coniferous seedlings. Agric. For. Meteorol. 59: 289–308.

Higgs K.H., 1994. Water stress and water use in broadleaved seedlings: Evaluation of sap flow gauges in water relation research. Asp. Appl. Biol. 38: 153–163.

IPCC. 2007. Climate change, 2007. Synthesis report, Cambridge University Press, Cambridge.

Ishida T., Gaylon S.C., and Calissendorff C., 1991. Improved heat balance method for determining sap flow rate. Agric. For. Meteorol. 56: 35–48.

Kjelgaard J.F., Stockle C.O., Black R.A., and Campbell G.S., 1997. Measuring sap flow with the heat balance approach using constant and variable heat inputs. Agric. For. Meteorol. 85: 239–250.

Kozlowski T.T. and Davies W.J., 1975. Control of water balance in transplanted trees. J. Arboric. 1: 1–10.

Lindroth A., Cermak J., Kucera J., Cienciala E., and Eckersten H., 1995. Sap flow by the heat balance method applied to small size *Salix* trees in a short-rotation forest. Biomass Bioenergy 8: 7–15.

Löf M., 2000. Establishment and growth in seedlings of *Fagus sylvatica* and *Quercus robur*: Influence of interference from herbaceous vegetation. Can. J. For. Res. 30: 855–864.

Lüttschwager D. and Remus R., 2007. Radial distribution of sap flux density in trunks of a mature beech stand. Ann. For. Sci. 64: 431–438.

Ma L., Lu P., Zhao P., Rao X.-Q., Cai X.-A., and Zeng X.-P., 2008. Diurnal, daily, seasonal and annual patterns of sap-flux-scaled transpiration from an Acacia mangium plantation in south China. Ann. For. Sci. 65: 402.

Messina M.G. and Duncan J.E., 1993. Irrigation effects on growth and water use of *Quercus virginiana* (Mill.) on a Texas lignite surface-mined site. Agric. Water Manage. 24: 265–280.

Pierret A., Moran C.J., and Doussan C., 2005. Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. New Phytol. 166: 967–980.

Sakuratani T., 1981. A heat balance method for measuring water flux in the stem of intact plants. J. Agric. Meteorol. 37: 9–17.

Sakuratani T., 1984. Improvement of the probe for measuring water flow rate in intact plants with the stem heat balance method. J. Agric. Meteorol. 40: 273-277.

Sands R., Fiscus E.L., and Reid C.P.P., 1982. Hydraulic properties of pine and bean roots with varying degrees of suberization, vascular differentiation and mycorrhizal infection. Aust. J. Plant. Physiol. 9: 559–569.

Senock R.S. and Ham J.M., 1995. Measurements of water use by prairie grasses with heat balance flow gauges. J. Range Manage. 48: 150–158.

Shackel K.A., Johnson R.S., and Medawar C.K., 1992. Substantial errors in estimates of sap flow using the heat balance technique on woody stems under field conditions. J. Am. Soc. Hortic. Sci. 117: 351–356.

Steinberg S.L., Van Bavel C.H.M., and McFarland M.J., 1990. Improved sap flow gauge for woody and herbaceous plants. Agron. J. 82: 851-854.

Steppe K., Lemeur R., and Dierick D., 2005. Unravelling the relationship between stem temperature and air temperature to correct for errors in sap-flow calculations using the stem heat balance sensors. Funct. Plant Biol. 32: 599–609.

Stone E.C., 1955. Poor survival and the physiological condition of planting stock. For. Sci. 1: 90–94.

Valancogne C. and Nasr Z., 1993. A heat balance method for measuring sap flow in small trees. In: Boghetti M., Grace J., and Raschi A. (Eds.), Water transport in plants under climatic stress, Cambridge University Press, Cambridge, pp. 166–173.

Weibel F.P. and de Vos J.A., 1994. Transpiration measurements on apple trees with an improved stem heat balance method. Plant and Soil 166: 203–219.

Welander N.T. and Ottosson B., 2000. The influence of low light, drought and fertilization on transpiration and growth in young seedlings of *Quercus robur* L. For. Ecol. Manage. 127: 139–151.

Wiltshire J.J.J., Wright C.J., Colls J.J., and Unsworth M.H., 1995. Effects of heat balance stem-flow gauges and associated silicone compound on ash trees. Agric. For. Meteorol. 73: 135–142.