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Ian Leslie Seymour

Use of sap flow measuring techniques to estimate water-use of multi-stem plants

Supervisor: Dr Paul Burgess
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Abstract

In the UK, coppice willow is a potentially high yielding energy crop which if widely planted could have significant effects of hydrology. This is one reason why researchers are interested in developing reliable techniques for estimating whole-plant water use of such trees. In recent years various sap flow measuring techniques have become commercially available. The aim of this thesis was to evaluate the reliability sap flow measurement and to consider issues related to best practice when used on multi-stemmed woody plants. The thesis starts by reviewing methodologies for estimating tree water-use with particular focus on sap flow gauges. Subsequently over three years, experiments were undertaken using a commercially available Stem Heat Balance (SHB) sap flow gauges, manufactured by Dynamax Inc, Houston, Texas, on coppice willow grown in lysimeters. Plant responses were monitored through a range of soil-water conditions from flood to drought. Over three years a methodology for deriving an accurate assessment of total plant water-use using a lysimeter water balance (LWB) was developed. Whole-plant water-use, estimated from scaled up sap flow measurements from individual stems were compared against LWB values. Both, stem basal area and leaf area were used as scalars to derive values of plant water-use.

In the final experiment, four out of eight, different sized sap flow gauges, with the appropriate scalar, gave estimates similar (±7%) to LWB values of whole-plant water-use over a period of 'unstressed' growing conditions. Variation in the accuracy of estimates was considered to be a function of a) error inherent to the SHB technique, b) error in scalar values used to derive whole-plant estimates, and c) apparently autonomous responses of individual stems to changes in soil water status. In non-water stressed conditions and where sap flow rates are high, errors from the technique were minimised by selecting 'large' stems (16-19 mm diameter). Under extreme water stress conditions, reductions in leaf area can result in errors if the estimate is based on stem-diameter. Where individual stem flow rates were perturbed by changes in soil-water conditions, selecting an 'intermediate' sized stem (in this case 10-13 mm diameter) appeared to minimise errors.

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Abbreviations and symbols

Symbol	Description	Unit
ΔS	Change in soil water content	(L)
ΔT	Change in temperature	(°C)
Δt	Signal averaging period	(s)
ΔT_o	Change in temperature at zero sap flow	(°C)
ΔT_{rad}	Change in radial temperature	(°C)
ΔT_{st}	Change in stem temperature	(°C)
θ	Volumetric water content of soil	($m^3\ m^{-3}$)
®	Registered trade mark	
^2H	Stable isotope of hydrogen (deuterium)	
^3H	Radio isotope of hydrogen (tritium)	
AWS	Automatic weather station	
AWSETv3.0	Automatic weather station ET_o software	
C	Correction Factor	
CHPV	Compensation Heat Pulse Velocity	
C_P	Specific heat of water (4.186 J g^{-1} °C^{-1})	
C_{st}	Heat capacity of stem segment	(J °C^{-1})
d	day	
D	Drainage	(L)
D1	First drying treatment phase	
D2	Second drying treatment phase	
D_2O	Deuterium oxide	
DC	Direct current	
DS	Drought-stressed treatment	
dT	Temperature difference	(°C)
DTI	Department of Trade and Industry	
dT_{min}	Threshold minimum temperature difference	(°C)
E_s	Evaporation from soil surface	(L)
ET	Evapotranspiration	
ET_o	Reference crop evapotranspiration	
F	Sap flow rate	(g s^{-1})
FC1	First field capacity treatment phase	
FC2	Second field capacity treatment phase	
g_s	Stomatal conductance	(mol H_2O m^{-2} s^{-1})
h	hour	
H_2O	Water	
ha	Hectare	(10000 m^2)
HDM	Heat Dissipation Method	
HFD	Heat Field Deformation Method	
HPV	Heat Pulse Velocity Method	
HRM	Heat Ratio method	
hrs	hours	
I	Irrigation	(L)
i	Day of lysimeter measurement	
K_{app}	'Apparent' coefficient of radial heat conductance used to determine K_{rad}	
K_{rad}	Coefficient of radial heat conductance	
$K_{rad\text{-}used}$	K_{rad} value used in sap flow calculation	
$K_{rad\text{-}best\ fit}$	K_{rad} used to give best fit to LWB	

K_{rs}	Coefficient of gauge thermal conductance	
L	litres	
LA	Leaf Area	(m^{-2})
LHP	Laser-diode heat pulse gauge	
Ltd.	Limited company	
LWB	Lysimeter water balance	
M_{i-1}	Lysimeter mass at end of preceeding day *i*	($g\ d^{-1}$)
M_i	Mass of lysimeter on day *i*	($g\ d^{-1}$)
$M_{post\ irrigation}$	Mass of lysimeter post irrigation	($g\ d^{-1}$)
$M_{pre\ irrigation}$	Mass of lysimeter pre-irrigation	($g\ d^{-1}$)
$M_{post\text{-}rain}$	Mass of lysimeter post rain	($g\ d^{-1}$)
$M_{pre\text{-}rain}$	Mass of lysimeter pre-rain	($g\ d^{-1}$)
N	North	
P	Effective precipitation	(L)
$p<0.05$	Probability at 95% confidence level	
P_{in}	Power supplied to heater	($W = J\ s^{-1}$)
$plant^{-1}$	Per plant	
PWP	Permanent Wilting Point	
Q_{dn}	Heat flow below the heater	($J\ s^{-1}$)
Q_f	Convected heat flow rate	($J\ s^{-1}$)
Q_h	Heater power	($J\ s^{-1}$)
Q_{ho}	Dissipated heater power at zero sap flow	($J\ s^{-1}$)
Q_l	Heat loss to adjacent sapwood	($J\ s^{-1}$)
Q_r	Radially conducted heat flow	($J\ s^{-1}$)
Q_{rad}	Radial heat loss	($J\ s^{-1}$)
Qs	Stem heat storage	($J\ s^{-1}$)
Q_{up}	Heat flow above the heater	($J\ s^{-1}$)
Q_v	Vertically conducted heat flow	($J\ s^{-1}$)
r^2	Coefficient of determination	
R_h	Heater resistance	(Ω)
R_s	Stemflow precipitation	(L)
R_t	Through fall precipitation	(L)
SBA	Stem basal area	cm^{-2}
SF	Scaled frequency	
SHB	Stem Heat Balance	
spp.	Species	
SRC	Short Rotation Coppice	
T	Transpiration	(mm) or ($g\ d^{-1}$)
T_i	ET derived from lysimeter mass balance	($g\ d^{-1}$)
TSHB	Trunk sector heat balance technique	
T_x	Thermocouple probe $number_{(x)}$	
VHI	Variable Heat Input	
W	West	
WW	Well-watered	
x	Horizontal chart axis	
y	Vertical chart axis	

1. INTRODUCTION

1.1. Background

The UK Government's new and renewable energy policy (Department of Trade and Industry, 2000) acknowledges that energy crops will make a significant contribution to meeting the target of 10% of energy being supplied by renewable energy sources by 2010. It is anticipated that up to 150,000 ha of energy crops will need to be established in England and Wales for this contribution to be realised (Stephens *et al.*, 2001). The implications of such policies on groundwater hydrology need to be fully assessed to ensure that any proposed planting scheme would be viable in terms of crop yield and sustainable in terms of environmental impact within a given catchment.

Short Rotation Coppice (SRC) willow (*Salix* spp.) is the principal crop currently under investigation for suitability as an energy crop. The potential of SRC willow to produce high yields of biomass with minimal agronomic inputs, in the temperate climate of the UK, make it an attractive choice as an energy crop. A number of studies have been carried out in recent years at Cranfield University Silsoe to address various issues related to growing SRC willow (Souch *et al.,* 2000; Brierley *et al.*, 2001; Martin and Stephens (2001); Stephens *el al.* 2003*;* Souch *et al.,* 2004; Bonneau, 2004). This thesis builds primarily on work done by Martin and Stephens (2001) that used lysimeters to study the growth and water-use of SRC willow grown on Oxford clay.

Sap flow gauges have gained increasing acceptance as an appropriate technique for quantifying water-use of woody perennials, capable of producing reliable data outputs, provided care is taken with installation and operation of the gauges (Grime and Sinclair 1999) Stem heat balance (SHB) sap flow gauges, developed in the 1980's (Sakuratani 1981; Baker and van Bavel 1987) and made commercially available in the 1990's (Dynamax Inc., Houston, TX, USA), have been used in a number of studies (Lindroth 1995; Hall *et al.,* 1998) aimed at quantifying the rates of transpiration and therefore, the water use requirements of SCR crops.

Physical and financial considerations impose limits on the number of gauges that can be employed and the amount of replication that can be built into many experiment designs. These limitations inevitably require assumptions to be made when results are scaled up from single stem sap flow rates, to whole plant, then to the plot and field scale. This thesis considers the assumption made by various investigators (Hall *et al.*, 1998; Allen and Grime 1995), that the relationship between sap flow, leaf area and stem basal area is consistent for all the stems of a multi-stemmed woody perennial. This is particularly pertinent for investigations involving SRC willow in which stem counts per stool regularly exceed 25 viable stems and stem diameters on an individual plant can range from 7 mm to greater than 50 mm.

1.2. Aims

The aims of the thesis are:

- To evaluate the reliability of SHB sap flow gauges as a means of estimating plant water-use.
- To recommend best practice when using SHB sap flow gauges to determine water-use of a multi-stemmed woody perennial.

1.3. Structure and approach

This MPhil thesis is divided into seven chapters and was completed on a part-time basis whilst the author was employed as a technician within the Institute of Water and Environment, Cranfield University, Silsoe. The literature review briefly outlines the range of methodologies used for quantifying water-use of woody perennials, with a more detailed review of sap flow measurement techniques. The author's own work related to sap flow was undertaken as three experiments in 2001, 2002 and 2003. These experiments are reported in chronological order. The implications of findings from these experiments are discussed in the final chapter.

2. LITERATURE REVIEW

This literature review briefly describes the reasons why researchers and practitioners wish to measure tree and crop water use. It then briefly describes the range of methods used, and concludes with a focus on plant-based measurements of transpiration.

2.1. Introduction

Hydrological modelling of the effect of woody vegetation at catchment scale and climate change studies require knowledge of canopy dynamics, in terms of the capacity to intercept precipitation and of subsequent rates of evaporation and transpiration (*ET*) for individual trees and up to forest scale (Meiresonne *et al.*, 2002). Along with the need to evaluate the impact on groundwater from planting schemes such as those associated with biomass crops, knowledge of plant water-use is of value for a range of crop and environmental management issues. For example, the ability to quantify accurately crop water requirements can aid irrigation management, leading to more efficient use of water resources as well as maximising yield potential of tree and other woody perennial crops (Li *et al.*, 2002). It may also enable improved design of tree planting to optimise productivity (Lott *et al.,* 1996; Sellami and Sifaoui, 2002). An improved understanding of plant responses to competition between individuals for resources such as water can aid decision making for various management practices such as such thinning or pruning (Forrester *et al.*, 2003), as well as the environmental impacts of deforestation (Giambelluca *et al.*, 2003).

Various methods for quantifying plant water-use have been developed. Each of these methods has their own particular advantages and disadvantages (Wullschleger *et al.*, 1998; Wilson *et al.*, 2001). Suitability for any given study will depend on a range of technical and logistical considerations. Such issues are particularly important when extrapolating results from a single plant to estimate transpiration at field or catchment scale (Wilson *et al.*, 2001). These methods include indirect estimates of water-use and direct plant water-use measurements.

2.2. Indirect estimates of plant water-use

Estimates of plant water use can be derived from the construction of water and energy balances, and these can be done for a range of spatial and temporal scales.

Water balance

Water balance methods are often used to provide an estimate of crop evapotranspiration (*ET*). Measurements of soil water content are carried out using instruments such as neutron probe, capacitance probes or gravimetric measurements. Changes in the available water within the root zone are monitored and from these measurements, together with rainfall and drainage data, a water budget can be established, from which an evapotranspiration (*ET*) value can be deduced.

$$ET = P - D - \Delta S \qquad \text{Equation 2.1}$$

Where: *P* is effective precipitation; *D* is drainage and ΔS is change in soil water content. Although this method is relatively simple to use, it is difficult to measure actual drainage losses, and therefore estimates are required to effectively close the water balance (Cuenca *et al.*, 1997). Errors may also result from water uptake below the measurable depth of the soil moisture probes. Deep rooting trees may tap these resources, resulting in a significant under-estimation of *ET* (Wilson *et al.*, 2001). Problems may also be encountered when extrapolating to a larger scale, due to the high degree of spatial variability of soil water content (Dunin, 1991).

Lysimeters

A lysimeter is a contained column of selected soil or undisturbed field soil with facilities for sampling and monitoring the movement of water and chemicals. Lysimeters use a combination of water-balance and gravimetric measurements to establish the water-use of plants grown in the lysimeter (Edwards, 1986; Daamen *et al.*, 1993). This methodology can therefore provide a useful mechanism for calibrating and validating other plant based measurements, such as porometry and sap flow. The use of lysimeters allows the growing conditions of the plant to be controlled and manipulated. Detailed studies of plant water-use can therefore be carried out for a range of growing conditions. However the physical limitation of growing plants in containers restricts this methodology to relatively small scale studies. Scaling up from results observed with container grown to field grown plants can also be problematic due to the assumptions that need to be made when comparing the lysimeter to a 'natural' system. The accuracy of measurement is dependent on the capacity to measure each of the inputs and outputs to the lysimeter including: precipitation (rain, snow and fog), canopy interception, drainage, biomass increase and losses (e.g. leaf fall). Accuracies of the order 0.06 mm m^{-2} soil surface, have been reported for studies using weighing lysimeters (Fritschen *et al.*, 1973)

Energy balance

Energy balance techniques based on the Penman-Monteith equation, Bowen ratio and Eddy Covariance can be used to estimate crop evapotranspiration. These techniques use a range of micrometeorological measurements to determine tree canopy water vapour fluxes. The spatial scale of energy balance studies are by necessity an order of magnitude greater than plant based studies and as such are best suited to field-based or forest-based estimate (Lindroth and Iritz 1993: Wilson *et al.*, 2001).

2.3. Plant water-use measurements

Plant water loss from individual plants can be measured by porometry, the use of tented and ventilated chambers, radio or stable isotope tracers, and sap flow gauges. These methods provide a means of quantifying the transpiration component of ET.

Porometry

Porometry is based on gaseous exchange measurements made from leaves or plants enclosed in a chamber in which water vapour entering the chamber is compared to that leaving it. This may be measured by the use of humidity sensors, capacitance sensors or by infra-red gas analysis (Pearcy *et al.*, 1989). Using these measurements, stomatal conductance (g_s, units: mol H_2O m^{-2} s^{-1}) can be determined from which it is possible to estimate transpiration rates.

Porometry is a labour intensive technique that provides relatively poor temporal resolution (Dugas *et al.*, 1993). Scaling up transpiration rates calculated from measurements of stomatal conductance made from individual leaves is problematic, requiring estimates for the degree of heterogeneity in terms of leaf age, boundary layer conductance, and levels of illumination within the tree crown or canopy (Ansley *et al.*, 1994). Short term studies (24 h) using porometry however, have shown estimates of transpiration that correlated well with other techniques such as sap flow (Steinberg *et al.*, 1990a; Zhang *et al.*, 1997)

Whole plant measurements of transpiration have been made by enclosing plants, even large trees, in ventilated chambers and monitoring water vapour flux. This approach has logistical limitations in terms of portability, prohibiting its use for many studies. Also, more critically, the microclimate within the chamber may not represent 'normal' growing conditions for the plant and thus interpretation of observed data is open to question (Goulden and Field, 1994).

Isotopes

The radio-active isotope of hydrogen, tritium (^3H), has been used as a tracer for estimating water-use of trees (Kline *et al.*, 1970; Waring and Roberts, 1979) but health and safety issues limit the suitability of this technique. The stable isotope of hydrogen, deuterium (^2H or D), is now generally used in preference to ^3H as it is safer to use and is subject to less regulation (Wullschleger *et al.*, 1998).

A known amount of deuterium oxide (D_2O) is injected into the tree stem and samples of transpired water are collected, from bags enclosing foliage at different positions in the crown, over a set period of time. The concentration of D_2O within the transpired water can then be analysed using mass spectrometry and a flow rate determined (Hall *et al.*, 1998). This technique only permits mean daily flow rates to be calculated; even so it has been used to validate estimates derived from techniques such as sap flow and porometry (Hall *et al.*, 1998).

2.4. Sap flow measurement techniques

Various methodologies have been devised and developed since the 1930's that use heat as a tracer to detect and quantify movement of sap within the stem of plants. Measurements are based on the thermodynamics of heat applied to a stem and the resultant conduction and convection of the heat. A key advantage of heat tracer sap flow systems over the techniques described above is that they can be easily automated enabling continuous records of plant water-use, with high time resolution (Smith and Allen 1996). Various approaches to sap flow measurement are described in Table 2.1 and are examined in more detail below.

Heat pulse velocity method

The heat pulse velocity (HPV) method for measuring sap flow was first developed by Huber and Schmidt (1937). The sap flow velocity is determined from the time taken for a pulse of heat, applied at a point in the stem, to travel a known distance along the stem. The rate of flow is determined from the velocity multiplied by the cross sectional area of the 'sap conducting' portion of the stem. One commercially available heat pulse gauge is the Compensation Heat Pulse Velocity Method (CHPV), based on three probes (Greenspan Technology Pty. Ltd., Australia.) A heater probe, consisting of a small heater element encased in a hypodermic needle, is inserted into the woody stem, as close to the major xylem vessels as possible (Figure 2.1). The second and third probes are encased thermocouple or thermistors. The second probe is inserted a known distance in the same vertical plane as the heater and is used to detect the heat downstream of the heater. The third probe is installed upstream of the heater to enable compensation for the stem's capacity to conduct heat to be made in the calculation of sap velocity. This potentially allows low flow and reverse flow measurements to be made (Smith and Allen, 1996).

Table 2.1 Summary of the key characteristics of selected sap flow methods with web site addresses of commercially available gauges.

Technique	Stem diameter	Comments
Xylem sap flow velocity		
Compensation Heat Pulse Velocity (CHPV) And Heat Ratio Method (HRM)	> 30 mm	Advantage: low sap flow rates Greenspan sap flow sensor **Http://www.advmnc.com/greenspan/sapflow.html** ITC International Pty Ltd. Http://www.ictinternational.com.au/brochures/hrm.pdf
Laser-diode heat pulse		Still under development Advantage: improved accuracy of heat application and temperature measurements; Non-invasive; Potential for miniaturization.
Xylem sap mass flow		
Heat Dissipation Method (HDM) Granier method.	> 70 mm	Similar equipment to sap flow velocity techniques but measuring the dissipation of heat applied to a point on the stem away from a reference temperature sensor, fixed upstream of the heater Two probe sets per tree are required for 75-150 mm in diameter, and four probe sets per tree for trees >150 mm in diameter. Advantages: probes are relatively simple to construct and thus less expensive than heat balance equipment. Disadvantages: similar to sap velocity methods in that variations in sap carrying tissue around the circumference of trees requires a number of probes to inserted per tree for accurate estimates of sap flow. Thermal Dissipation Probe (Dynamax) Http://www.dynamax.com/p9.htm
Heat Field Deformation Method (HFD)	> 70 mm	Variation of HFD using an array of four thermocouple probes. Advantage: improved accuracy of low and reverse flows Disadvantage: flow rate calculation is dependent on accurate positioning of probes. Wounding of stem may also influence accuracy of measurements. EMS: http://www.emsbrno.cz
Stem Heat Balance (SHB)	2-150 mm	Advantage: no calibration required Disadvantage: suitable for a limited range of stem sizes; expensive and delicate instruments. Dyangauge: http://www.dynamax.com EMS: http://www.emsbrno.cz
Trunk sector heat balance	> 150 mm	Advantage: no calibration required; suitable for large stem size Disadvantage: invasive technique. EMS: http://www.emsbrno.cz

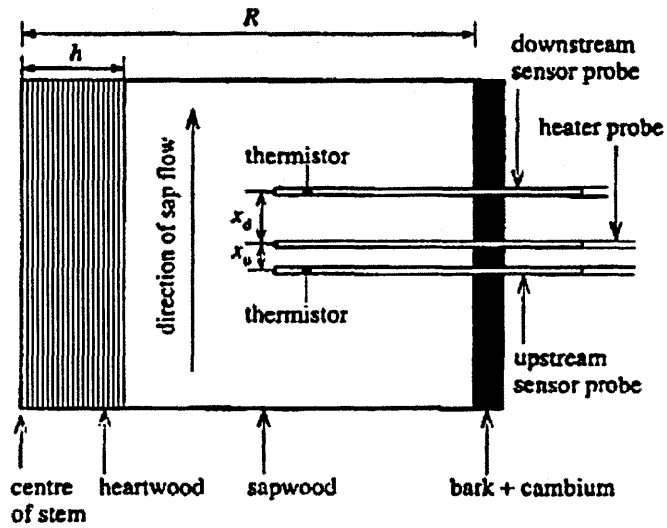


Figure 2.1 Schematic diagram of the Compensation Heat Pulse Velocity sap flow method (CHPV) installed in a stem of radius R *to outer edge of cambium layer and with heart wood radius* h. *The upstream thermistor probe is installed at a distance of* x_u *below the heater and the downstream thermistor probe is installed a distance of* x_d *above the heater (From: Smith and Allen, 1996)*

A disadvantage of this system is the invasive nature of the installation, where probes are inserted into the plant tissue. The resulting wounds may influence calculations of sap flow, requiring some form of correction (Swanson and Whitfield 1981). Accurate positioning of the heater and sensor probes relative to each other is critical as misalignment can cause significant error in the calculation of sap velocity (Smith and Allen, 1996). Also, to overcome uncertainties related to the non-homogeneity of sap carrying xylem tissue in the radial profile of the stem, empirical calibration is necessary to validate sap flow rates and a number of probes per stem may be required to improve accuracy of these calculations. A further development of the CHPV method is the commercially available Heat Ratio Method (HRM) (ITC International Pty. Ltd., Armidale, NSW 2350, Australia) which derives values of sap flow velocity from the ratio of heat detected by the thermocouple probes inserted above and below the heater probe. To overcome error associated with wounding of stem tissue and asymmetries in probe alignment, multiple temperature readings are taken between 60-100 seconds after each heat pulse (Burgess *et al.*, 2001).

Laser-diode heat pulse gauge

A recent development of the HPV method has been proposed by Bauerle *et al.* (2002). The laser-diode heat pulse gauge (LHP) uses a fibre-coupled diode laser as a heat source and infrared thermocouple temperature sensors (Figure 2.2).

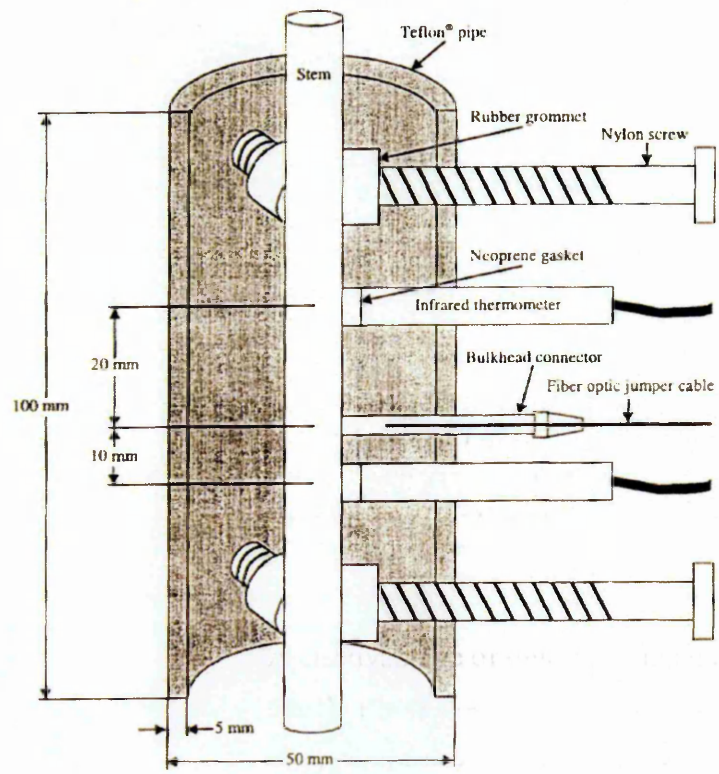


*Figure 2.2 Cross section diagram of a Laser-diode heat pulse gauge (LHP) showing the relative positions of infrared thermometers to the laser heat source (applied through fibre optic jumper cable) in teflon pipe housing (*From: Bauerle et al., *2002).*

Improved accuracy of sap flow measurements are claimed for this technique due to the increased precision in applying and measuring the heat pulses (Bauerle *et. al.*, 2002). An additional benefit is that this method is non-invasive to the plant tissue. However laser devices are currently expensive. It is also acknowledged that further development of this methodology is still required before it is adopted in field studies or used on stems of large diameter. The potential for miniaturisation of this system is highlighted for possible future development.

Heat-Dissipation Method

The heat dissipation method (HDM) was devised by Granier (1985). As with HPV, this method requires the insertion of probes into the woody plant stem (an integral heater/temperature probe and a second temperature probe) (Figure 2.3). The method differs from the HPV in two respects: firstly, the heater is powered continuously, applying constant heat to the stem; secondly, the temperature sensing probe is installed 'upstream' of the heater and measures the dissipation of heat generated by the heater. Thus, as sap flow increases, the temperature difference *(ΔT)* between the two probes decreases.

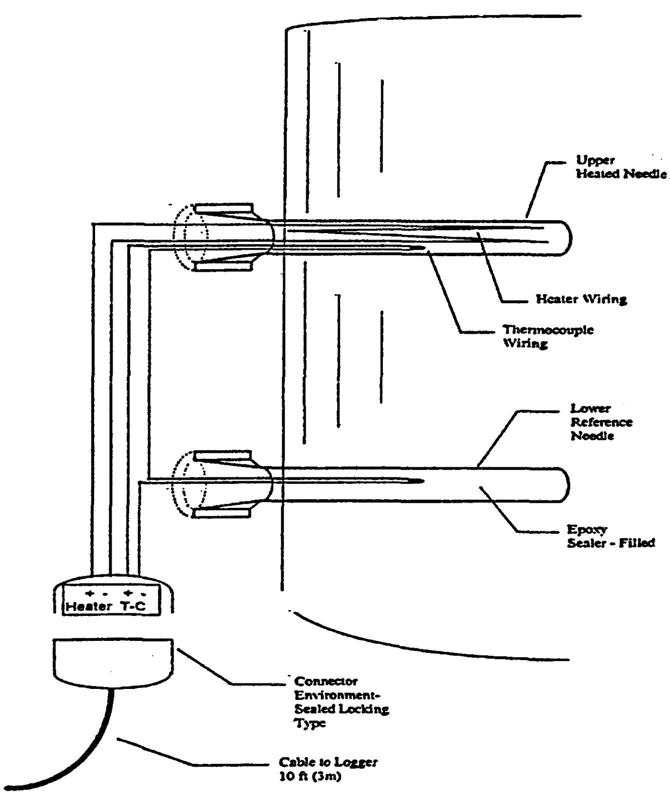


Figure 2.3 Schematic diagram of a Heat Dissipation Method (HDM) showing the wiring configuration of the integrated heater/thermocouple probe and the upstream thermocouple probe (From: Dynamax Inc,. 1997).

These gauges are of simple construction and hence relatively inexpensive. The method of installation is also relatively easy and less precision is required when inserting the probes. This is because the distance and positioning of the probes relative to each other is not critical to the calculation of sap flow rate.

In developing this method, Granier established an empirical relationship between the actual sap flow velocity *(V)* measured from sections of trunk and a dimensionless 'flow index' value *(K)* derived from measured values of ΔT. Calibration is recommended when this method is to be used on species for which no previous validation has been made (Smith and Allen, 1996). Again, in order to overcome uncertainties related to the non-homogeneity of the sapwood, a series of gauges may be required per stem. Two sets of probes are recommended for trees of trunk diameter between 125 and 200 mm and four sets per tree of trunk diameters greater then 200 mm (Dynamax Inc., 1997).

Heat field deformation method

The heat field deformation (HFD) method is a variation of the HDM and was developed by Nadezhdina and Cermák (Nadezhdina, 1992, 1999; Nadezhdina *et al.*, 1998; Nadezhdina and Cermák, 1998). It is commercially available from Environment Measuring Systems (EMS), Turisticka 5, 62000 Brno, Czech Republic (http://www.emsbrno.cz) and Dendronet (denronet@wo.cz). This system uses a set of four thermocouples, configured as two separate pairs, to measure the deformation of the heat field around a heater inserted radially into the stem. The construction of the heater and thermocouples probes is similar to those in the HDM design, using stainless steel hypodermic needles 1- 1.2 mm in diameter (Figure 2.4). The main advantage of the HDF method compared to the HDM is the increased sensitivity to very low flow rates and the ability to measure basipetal sap flow. The detection of basipetal and low night re-saturating flows could prove to be useful indicators of plant water stress (Cermák *et al.*, 2004). The disadvantages of installing the extra thermocouples are a) problems associated with the additional wounds to the stem and, b) the increased complexity of installation and error resulting from inaccurate positioning of the probes. As with the HDM, multiple gauge installations are required for studies on large trees.

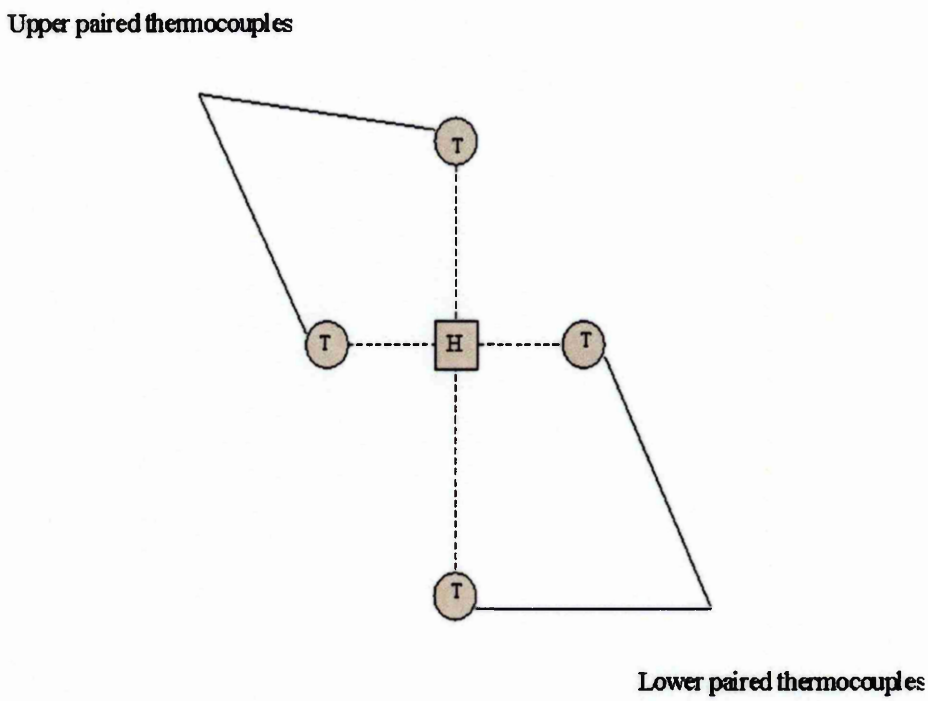


Figure 2.4 Schematic diagram of a heat field deformation sensor showing the positions of the differentially wired, upper and lower thermocouple pairs(T) relative to the heater probe (H).

Stem heat balance

Stem heat balance (SHB) sap flow gauges were developed in the 1980's (Sakuratani, 1981; Sakuratani, 1984; Baker and van Bavel, 1987; Steinberg *et al.*, 1989). They can be used on both woody and herbaceous stems and became commercially available in the 1990's (Dynamax Inc., Houston, TX, USA). These gauges are available for a range of stem sizes from 2 to 150 mm in diameter. The SHB gauges consist of an encased copper wire heater element installed around the full circumference of the stem (Figure 2.5). The heater is powered from a DC electrical supply, controlled through a voltage regulator, to give a continuous constant voltage. The voltage applied to the heater is usually within the range 1 to 10 V, dependent on stem size and sap flow rates during the period of measurement. Differentially wired copper-constantan thermocouples are used to measure the increase in temperature of the sap passing through the heated section of stem. Radial losses of heat from the inside to the outside of the gauge are also determined using differentially wired thermocouples located within the thermopile (Sakuratani, 1981). Using these measurements in a heat balance equation, the heat dissipated by convection of sap flow per unit time (Q_f) can be calculated and from this, the mass sap flow can be determined

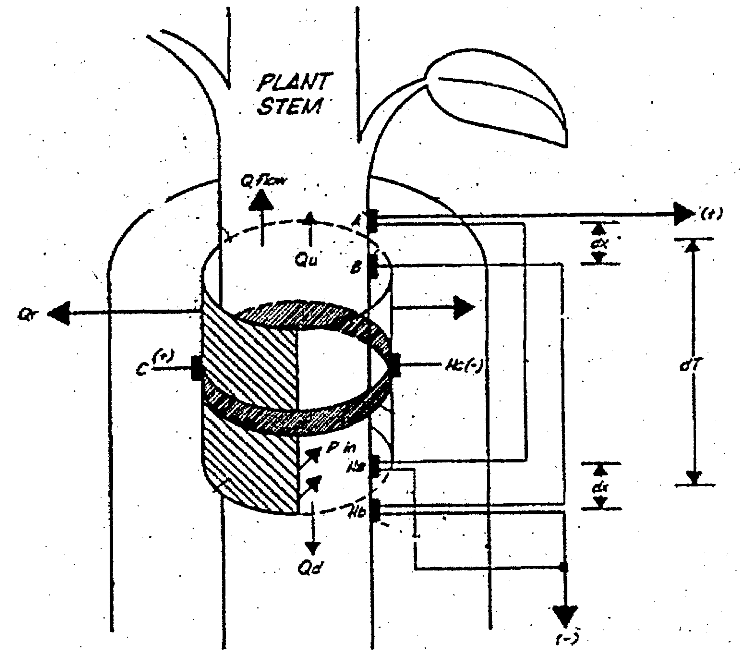


Figure 2.5 Schematic diagram of a SHB gauge showing the wiring configuration of the integrated thermopile heater and the relative positions of the differentially wired, upstream and downstream thermocouples (From: Dynamax Inc., 2005).

Heat balance calculations of sap flow

The basic heat balance equation, for a specified time period, for the stem heat balance gauge is stated as (Equation 2.2)

$$Q_h - Q_f - Q_{up} - Q_{dn} - Q_{rad} = 0 \qquad \text{Equation 2.2}$$

Where: Q_h represents the heat supplied to the stem by the gauge heater, Q_f represents the heat convected by the sap flow movements within the stem; Q_{up} and Q_{dn} represent the heat conducted up and down the stem through the stem tissue; and Q_{rad} represents the radial heat loss from the stem.

The value of Q_h (units: J s^{-1}), the heat supplied to the stem by the gauge heater, is determined by applying a preset, regulated voltage (V; units: V) to the heater element of the gauge for which the resistance (R_h; units:Ω) is known (Equation 2.3).

$$Q_h = V^2 / R_h \text{ (from Ohms Law)} \qquad \text{Equation 2.3}$$

The values of Q_{up} and Q_{dn} are calculated from the measurements taken from thermocouples sited above and below the heater element, using an approximate value for the thermal conductivity of the plant stem (Sakuratani, 1984) and the measurement of stem diameter at the point where the gauge heater is installed. The value of Q_{rad} represents the radial heat loss from the stem (Equation 2.4).

$$Q_{rad} = K_{rad}\, \Delta T_{rad} \qquad \text{Equation 2.4}$$

Where: ΔT_{rad} (Units: °C) is the temperature difference between the heater element and the insulation material of the gauge as determined by radial thermocouple measurements. K_{rad} (Units: Js^{-1} °C) is assumed to be a constant for each gauge installation and represents the thermal conductivity of the gauge materials and is calculated from the heat balance equation (Equation 2.5).

$$K_{rad} = (Q_h - Q_{up} - Q_{dn}) / \Delta T_{rad}. \qquad \text{Equation 2.5}$$

The sap flow, F (units g s^{-1}), is calculated from Q_f (Equation 2.6).

$$F = Q_f / (C_p\, \Delta T) \qquad \text{Equation 2.6}$$

Where: C_p is the specific heat of water at constant pressure (4.186 J g^{-1} $°C^{-1}$), and ΔT is the temperature increase of the sap. As reported by Delta-T Devices Ltd (1999), from work carried out by Sakuratani (1981) and Baker- Van Bavel (1987), sap is regarded as having a water content of approximately 99%.

Heat balance method using variable heat inputs

A development of the heat balance method described above, in which the heater is powered continuously by a constant voltage, is the variable heat input method (Figure 2.6). This method can be employed successfully when using a modified heat balance gauge design described by Ishida *et al.*, (1991), quoted by Kjelgaard *et al., (*1997). This design uses three differentially wired thermocouples: one placed upstream and one downstream of the heater, with the third located at the heater. The heat input for this gauge configuration can then be controlled in such a way as to maintain a constant temperature difference between the heater thermocouple and the downstream thermocouple. By regulating the heat in this manner it avoids the risk of damaging the stem tissues through over-heating during periods of low or zero sap flow rates. Also the power consumption of individual gauges is lower, which may prove significant for battery operated systems installed in remote locations (Smith and Allen, 1996). This method could potentially generate more accurate measurements of sap flow since fluctuations in heat storage of the stem is reduced (Grime et *al.*, 1995). However, Kjelgaard *et al.* (1997) reported generally better results from constantly heated gauges in a study evaluating these heat balance techniques for a range of plant species. This, coupled with the added complexity of electrical circuitry and or data-logger programming, make the variable heat input (VHI) method less attractive for most applications.

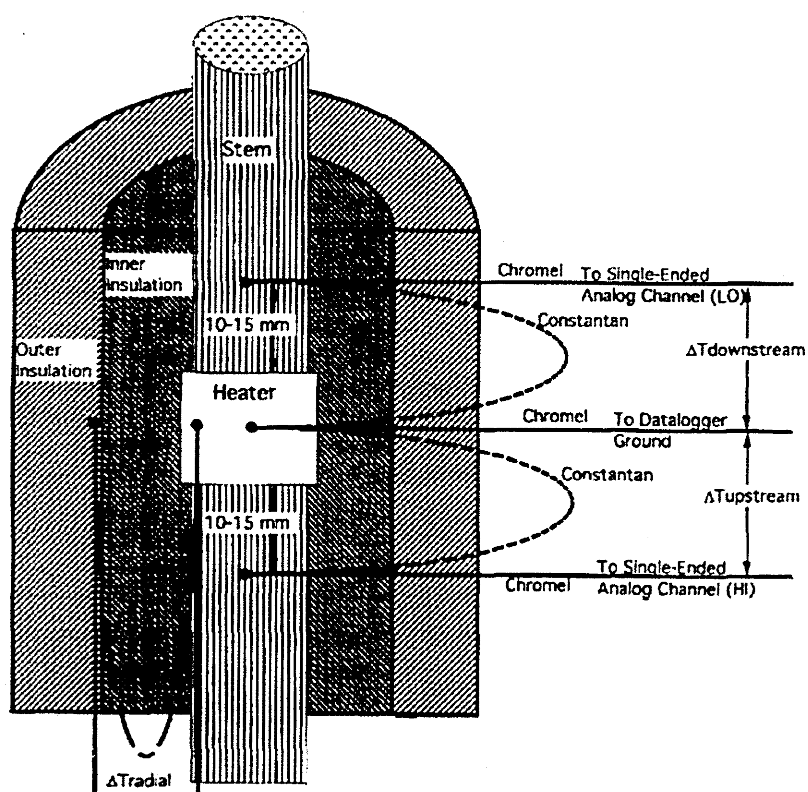


Figure 2.6 Schematic diagram of an alternative stem heat balance (SHB) gauge (Ishida et al., *1991) suitable for use with variable heat input (VHI), showing the wiring configuration of the differentially wired, upstream, central and downstream thermocouples. Also showing the positioning of the differentially wired thermocouples used to estimate radial heat losses. (From: Kjelgaard et al., 1997).*

Trunk sector heat balance

The trunk sector heat balance technique (TSHB) works on a similar principle to the SHB approach, in that sap flow rates are determined from the heat balance of heated stem tissue. However, only a sector of trunk is heated rather than the whole stem. This allows the TSHB method to be used on stems greater than 120 mm in diameter. The heater elements used in this system are inserted into the stem of the plant.

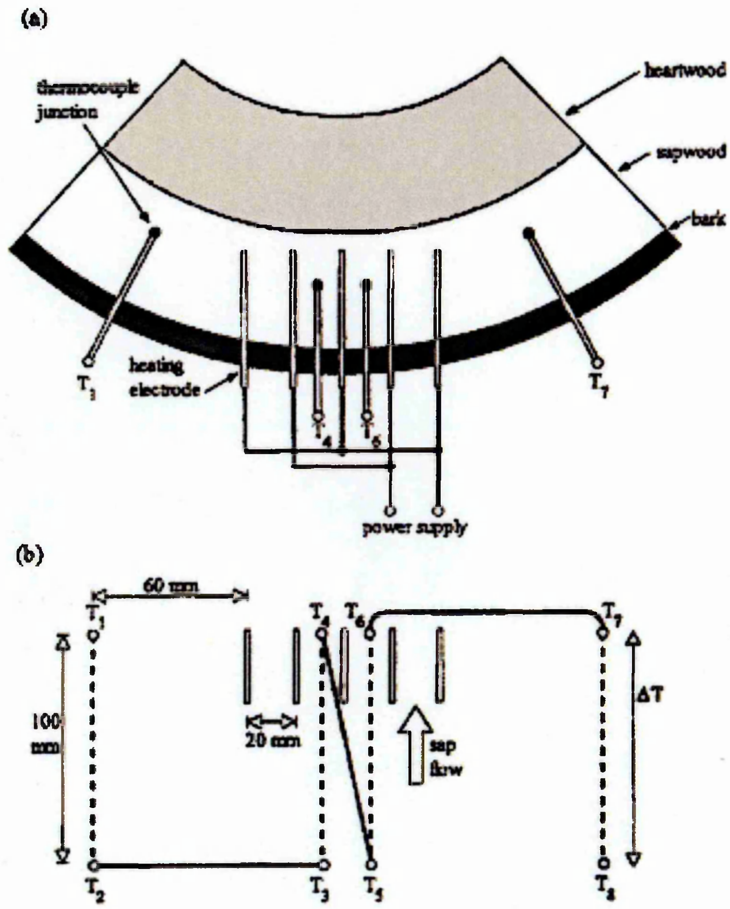


Figure 2.7 Schematic diagram of a TSHB gauge installation. a) Shows the horizontal cross section with the positioning of the five heater electrodes and the four thermocouples installed level with the top of the heater elements. b) Shows the relative position of all eight differentially wired thermocouples and the heater electrodes within the vertical plane (From: Smith and Allen, 1996).

The methodology, is summarised by Smith and Allen (1996) from work done by Cermák *et al.* (1973; 1976; 1984; 1995) They explain that five stainless steel electrode plates are installed in a parallel configuration, 20 mm apart, on the same lateral plane within a sector of stem sapwood. A series of eight differentially wired thermocouples, each housed in 1 mm diameter metal probes, are installed into the sapwood, adjacent to the heater plates. Four are positioned level with the top of the heater plates, with the inner two positioned 10 mm either side of the centre plate (3) (Figure 2.7). The other two are located a distance of 60 mm from each of the two outer heater plates (1 and 5). The second set of four probes are aligned 100 mm below the first set (in the same vertical plane) and are thus outside the heated zone. The measurement taken from these thermocouples is the net increase in temperature (ΔT) attributed to the heated sector of trunk. From ΔT the heat flow rate (Q_f) convected by sap passing through the inner segment of sapwood bounded by heater plates 2 and 3, is calculated using the following heat balance (Equation 2.7):

$$Q_h = Q_v + Q_r + Q_l + Q_f \qquad \text{Equation 2.7}$$

Where Q_h is the electrical power dissipated as heat in the trunk sector, Q_v is the vertically conducted heat, Q_r is the radially conducted heat, both internally towards the heartwood and externally through the bark and Q_l is the heat lost in to the adjacent sapwood. The value of Q_l is considered to be negligible since the heater plates 1 and 5 ensure there is no lateral thermal gradient within the heated sapwood segment. The values of Q_v and Q_r are established during periods of zero sap flow from equation 2.8:

$$Q_v + Q_r = K_{rs}\,\Delta T \qquad \text{Equation 2.8}$$

Where K_{rs} is a thermal conductance coefficient assumed to be constant for each individual gauge installation that can be derived from equation 2.9:

$$K_{rs} = Q_{ho} / \Delta T_o \qquad \text{Equation 2.9}$$

Where Q_{ho} and ΔT_o are values of Q_h and ΔT recorded when sap flow is assumed to be zero. As with the SHB method, Q_f can then be converted to a mass flow rate using the specific heat capacity of sap (assumed to be that of water). The mass flow rate for the central heated segment of the stem is then multiplied by the ratio of the width of this segment to the circumference of the stem in order to establish a mass flow rate for the whole tree. As is the case with HPV and HDM, where measurements are carried out on only a portion of the sapwood, assumptions have to be made with regard the homogeneity of the non-measured areas of sapwood. For increased confidence in estimates of sap flow for the whole plant, multiple gauge installations may be needed on a single stem (Cermák *et al.*, 1995).

2.5. Scaling-up sap flow measurements

For hydrological impact assessments to be made from water-use estimates derived from sap flow, it is necessary to extrapolate measurements taken from individual gauges up to whole plant and then to entire stand level. Various biometric scalars have been used successfully for this purpose, including: stem basal area, stem diameter at breast height, leaf area and sapwood area (Hatton *et al.*, 1995). The relationship between gauge output and scalar is normally determined by regression analysis with the resulting equation used to estimate water-use of entire stand. Choosing a scalar that is easy to survey accurately at stand level is therefore important. It is also important to ensure that trees (or stems) chosen for sap flow measurement span the full range of values for that scalar (Wullschleger *et al.* 1998).

In their consideration of issues related to scaling up water-use estimates, Wullschleger *et al.,* (1998) also commented that, as well as giving attention to the spatial variation within an area of study, it is necessary to take into account potential temporal variation. They cite work done by Hatton and Wu (1995) who investigated problems associated with temporal scaling of water-use estimates on the basis of leaf area. This work showed water-use of individual eucalypt trees was linearly related to leaf area during periods of abundant soil water (soil matric potential > -50 kPa), but that the relationship was temporally unstable, becoming non-linear in periods of water deficit when the soil matric potential was less than –200 kPa). Long term temporal variation was also demonstrated by Vertessy *et al.*, (1995) who found that age of tree affected the relationship between stem diameter and water-use. It was found, in a study of mountain ash stands of different ages, that this problem could be over come by using sapwood area as a scalar (Haydon *et al.*, 1996). In this study the sapwood area was shown to reach a peak of 10.5 m^2 ha^{-1} in 15 year-old stands, declining gradually to 2.4 m^2 ha^{-1} in 200 year-old stands, whilst mean sap flow velocities had been shown to be similar for ash trees of this age range.

A number of studies investigating water–use of SRC willow and poplar have been carried out using both leaf area and stem basal area as scalars (Hall *et al.*, 1996; 1998). Cienciala and Lindoth (1995) reported a linear correlation for both stem basal area and leaf area, with transpiration from coppiced *S. viminalis* trees.

2.6. Summary

In reviewing the various methodologies used to quantify plant water-use it is apparent that the choice of technique employed in a study will depend, not only on its aims but also on the temporal and spatial scale. The sap flow methods described have the potential for use in a wide range of investigations. Since sap flow techniques are suited to data-logger recording, outputs from individual gauges can provide high temporal resolution over prolonged time periods. Replicated experimental designs are also possible using the various sap flow techniques although; financial considerations may impose constraints on the number of gauges. Provided that estimates of water-use for individual plants are accurate, the use of appropriate scalars allows the estimation of plant water-use up to forest or plantation scale (Wullschleger *et al.*, 1998) especially were stands are uniform (Smith and Allen, 1996).

The remainder of this thesis aims to evaluate the reliability of the stem heat balance technique for estimating water-use of coppice willow, and to recommend best practice when used on a multi-stemmed plant. Stem basal area and leaf area are examined as two possible scalars to derive values of whole plant water-use. Consideration is given to the effect of temporal variation in the sap flow rates of individual stems of a multi-stemmed plant on estimates of whole plant water-use.

3. PRELIMINARY INVESTIGATION ON USE OF SAP FLOW GAUGES

This chapter describes an initial study carried out during July and August 2001 using stem heat balance (SHB) sap flow gauges on coppice willow plants grown in lysimeters.

3.1. Objectives

The objectives of this study were to: a) estimate water-use of coppice willow using SHB sap flow gauges on stems of different girths, and b) to test the consistency and reliability of the SHB technique under various growing conditions.

3.2. Methodology

Site

The study was undertaken, at Cranfield University, Silsoe, England (52° 0' N; 0° 26' W; altitude 60 m). The experiment formed an unreported part of a research project (Martin and Stephens, 2006a; 2006b) involving 24 lysimeters.

Figure 3.1 Lysimeter with coppice willow plant and Stem heat balance sap flow gauges installed on two stems (2001 experiment).

Meteorological measurements were taken using a Mini-Met (Skye Instruments Ltd., Llandrindod Wells, Powys, UK) automatic weather station (AWS) sited locally to the lysimeter on a 2 m mast. Air temperature, relative humidity, solar radiation, rainfall and wind run were all recorded hourly. Reference crop evapotranspiration (ET_o) (Allen et al., 1998; Allen, 2000) was determined from these measurements using AWSET v3.0 (14) software (Cranfield University, 2002).

Lysimeters

Four lysimeters were selected for this experiment. The lysimeters were constructed from 200 l white plastic barrels with a diameter of 0.5 m and a height of 1 m. A 12 mm gate valve was fitted 0.15 m from the bottom of the barrel to enable control of drainage water. Gravel (19 mm grade) was placed in the bottom of the barrels to a depth of 0.2 m. Soil was then placed on top of the gravel to a depth of 0.75 m to provide a growing medium for the willow.

In each lysimeter a coppiced willow plant (*Salix viminalis,* cultivar: 'Jorr') was grown. Willow cuttings of 0.25 m length were set in the centre of each lysimeter in April 1999. At the end of the first growing season the stems were cut back to leave 25 mm of stem above soil level. This was done to encourage multiple stem growth from the coppiced stool. The experimental measurements were taken in July and August 2001, thus the coppiced willow plants were in their second growing season after cut back.

Experimental treatments

The experimental treatments comprised two soil types (Oxford clay and a Cottenham series sandy loam) and two watering regimes. The dry bulk density for each soil type was 1500 kg m^{-3} and 1270 kg m^{-3} respectively.

Two irrigation treatments were imposed on each pair of lysimeters (Table 3.1). One lysimeter of each soil type was irrigated throughout the study period with the aim of preventing the willow plant becoming drought stressed. The other two lysimeters were subjected to a drought stress treatment. The irrigation regime for the non-drought stressed ('well-watered') treatment was managed with the objective of maintaining the soil moisture, in the top 10 cm horizon, at values assumed to be close to 'field capacity' for each soil type.

During July 2001, two 'drying cycles' were imposed on the drought-stressed treatments (13-17 July and 22-28 July). Prior to the start of each 'drying cycle', the soil moisture contents for the drought stressed and well-watered treatments were similar. The drought-stressed treatment was allowed to progressively dry out until the leaves of the willow showed visible signs of wilting. Water was then applied, to restore the soil moisture content to values similar to the well-watered treatment. The first drying cycle was affected at the beginning and end by rainfall but through the second drying cycle no precipitation was recorded.

Table 3.1 Start and end dates of each soil-water treatment phase imposed on the drought-stress treatment lysimeters.

Soil-water treatment phase		Start date	End date
Drought phase 1	(D1)	13 July	17 July
Re-wetting phase 1	(W1)	18 July	21 July
Drought phase 2	(D2)	22 July	28 July
Re-wetting phase 2	(W2)	29 July	3 Aug

Water balance measurements

The water inputs to the lysimeter included irrigation (*I*); rainfall penetrating the leaf canopy, termed 'throughfall' (*Rt*); rainfall intercepted by the stems of the plant and then tracked down the stem to the soil, termed 'stemflow' (*Rs*). The water losses from the lysimeter included evapotranspiration from the plant (*ET*); evaporation from the soil surface (*Es*) and drainage (*D*). The change in the water content of the soil in the lysimeter is given the term ΔS. The water balance is stated as (Equation 3.1):

$$\Delta S = I + Rt + Rs - Es - D - ET \qquad \text{Equation 3.1}$$

In 2001, the irrigation (*I*) was carried out manually. Measured volumes were applied to the individual plants as required in a single 'flood irrigated' daily dose. *Rt* was measured using a 'mini rain-gauges' installed, on the soil surface, at the base of each plant. The volume of rain captured in the gauge was recorded after every rainfall event and the measurements were scaled up to give estimates of *Rt* for the whole lysimeter. *Rs* was measured on selected stems by intercepting the flow of water down the stem using a plastic collar and diverting this flow into a bottle. The water captured was measured after each rainfall event. From these values estimates of *Rs* were made for the whole plant.

Drainage from the lysimeter drain tap was measured on a daily basis. The drainage water was collected and measured using a measuring cylinder. The tap was then closed until the next reading was taken. *Es* was assumed to be negligible due to the relatively poor linkage between the atmosphere and soil surface (lying 10 cm below the rim of the lysimeter and shaded by the plant).

Soil water status measurements

Soil water content was measured using a Diviner2000® capacitance soil moisture probe (Sentek Pty. Ltd Adelaide, South Australia). A single access tube was installed in each lysimeter allowing a 70 cm soil profile to be scanned. The Diviner2000® is configured to take measurements every 10 cm down the soil profile. The soil moisture percentage calculations were made using the soil specific calibration equations cited by Groves & Rose (2004) for:

a) sandy loam : $\theta = 0.441SF^{2.756}$ Equation 3.2

and

b) clay soil: $\theta = 0.514SF^{3.371}$ Equation 3.3.

For comparison,

c) the factory default calibration equation:

$$\theta = 0.494SF^{3.108}$$ Equation 3.4

is plotted along side the sandy loam and clay soil calibrations (Figure 3.4). Where: θ is the volumetric water content of the soil (m^3m^{-3}) and *SF* a dimensionless Scaled Frequency term determined from the recorded probe signal. The signal detected by the probe is a function of the capacitance properties of the surrounding soil media, which in turn is proportional to water content (Paltineanu and Starr, 1997; Bosch, 2004).

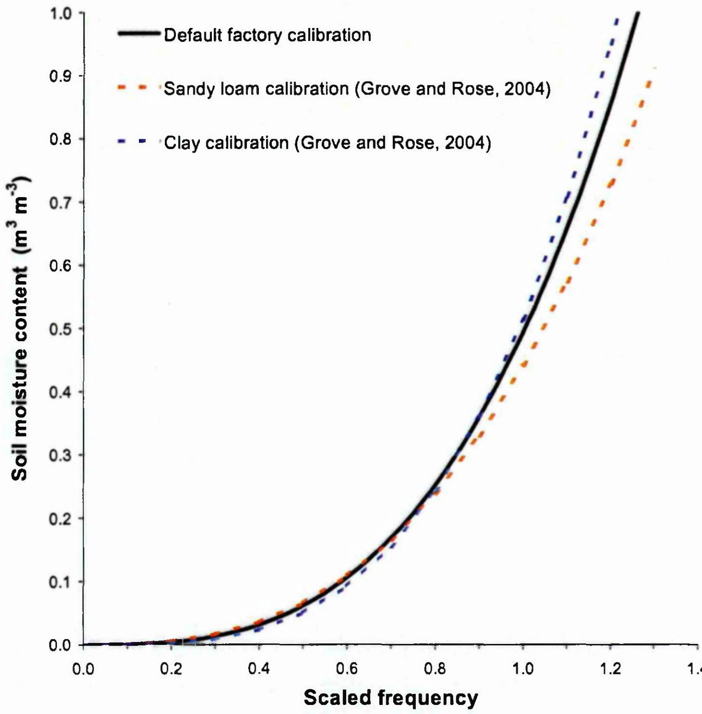


Figure 3.2 Soil specific calibration curves defined by Groves & Rose (2004) for a sandy loam ($\theta = 0.441SF^{2.756}$) and clay soil ($\theta = 0.514SF^{3.371}$) compared to the default factory calibration ($\theta = 0.494SF^{3.018}$)..used to convert the scaled frequency (SF) signal values recorded by Diviner2000® capacitance probe to volumetric soil moisture content (θ).

Sap flow measurements

On 10 July 2001 ten Dynagage sap flow gauges (Dynamax Inc., Houston, TX, USA) were fitted to selected stems on each of the four willow plants in a 'paired' configuration (Table 3.1). The stems chosen for each pair of gauges were matched as far as possible for: stem diameter, leaf area and orientation within the lysimeter (Table 3.2). Gauges were installed in accordance with the manufacturers instructions. Preparation of the stems prior to installation involved ensuring that the area of stem to be covered by the gauge was clean and smooth by rubbing lightly with a fine grade emery cloth and coating with liquid teflon supplied by the manufacturers. Silicone grease was applied to the inner surfaces of the gauge as recommended to by Dymamax, to ensure good thermal contact between the stem and the gauge heater. The insulation material supplied with each gauge was also secured according to instruction and the recommended 'Blue Tac' seal was applied to the top of the upper insulation collar. Gauge 'Shelters' described by Hall, *et al.* (1998) were not used as it was considered the stems were already 'crowded' and heavily instrumented. Instead a supplementary layer of heavy duty universal grease was applied over the top of the 'Blue Tac' seal, to provide a flexible water seal that would deflect water running down the stem away from the gauge. A DL2e Delta-T Devices Ltd. data-logger was used to record the data from the gauges. Measurements were recorded every one minute and averaged every 10 minutes.

Table 3.2 Description of treatments and sap flow gauge allocation.

Soil type	Soil-water treatment		Gauge size (nominal stem diameter)
Sandy loam	Drought stressed	(SD)	9, 10 and 19 mm
Sandy loam	Well-watered	(SW)	9, 10 and 19 mm
Oxford clay	Drought stressed	(CD)	13 and 16 mm
Oxford clay	Well-watered	(CW)	13 and 16 mm

Calculation of sap flow per stem

The outputs from the logged data were computed using the software supplied with the Dynagages to give a calculated sap flow rate (*F,* units g s^{-1}) which is derived from the flow of heat (Q_f; units: J s^{-1}) convected away from the heater by xylem water flow. Qf is calculated from the basic heat balance equation (Equation 3.3):

$$Q_h - Q_f - Q_{up} - Q_{dn} - Q_{rad} = 0 \qquad \text{Equation 3.3}$$

Where: Q_h is the heat supplied to the stem by the gauge heater, Q_f is the heat convected by the sap flow movements within the stem; Q_{up} and Q_{dn} are the heat conducted up and down the stem through the stem tissue; and Q_{rad} is the radial heat loss from the stem.

The value of Q_h (units: J s^{-1}) is determined by applying a preset, regulated voltage (V; units: v) to the heater element of the gauge for which the resistance (R_h; units: Ω) is known.

$$Q_h = V^2 / R_h \text{ (from Ohms Law)} \qquad \text{Equation 3.4}$$

Q_{up} and Q_{dn} are calculated from the measurements taken from thermocouples sited above and below the heater element, using an approximate value for the thermal conductivity of the plant stem (Sakuratani, 1984) and the measurement of stem diameter where the gauge heater is installed. The value of Q_{rad} represents the radial heat loss from the stem (Equation 3.5).

$$Q_{rad} = K_{rad}\,\Delta T_{rad} \qquad \text{Equation 3.5}$$

Where: ΔT_{rad} (units: °C) is the temperature difference between the heater element and the insulation material of the gauge as determined by radial thermocouple measurements. K_{rad} (units: J °C^{-1} s^{-1}) is assumed to be a constant for each gauge installation and represents the thermal conductivity of the gauge materials and is calculated from the heat balance (Equation 3.6):

$$K_{rad} = (Q_h - Q_{up} - Q_{dn}) / \Delta T_{rad}. \qquad \text{Equation 3.6}$$

The sap flow, F, (units: g s^{-1}) is calculated from Q_f by (Equation 3.7):

$$F = Q_f / (C_p\,\Delta T) \qquad \text{Equation 3.7}$$

Where: C_p is the specific heat of water (4.186 J g^{-1} °C^{-1}), and ΔT is the temperature increase of the sap. As reported by Delta-T Devices Ltd (1999) based on work carried out by Sakuratani (1981) and, Baker and van Bavel (1987), sap is regarded as having a constant water content of approximately 99%.

Leaf area and stem basal area

Leaf area (*LA*) was assessed on 18 July. Leaves on each stem were classified into seven 'types' $(t_1 - t_7)$ depending on their size and position on the stem (Table 3.3) Numbers of each classification 'type' per stem were counted. The length and width of a representative proportion of leaves (n=10) of each 'type' were measured on the plant and a mean value derived. Leaves were removed from adjacent 'guard row' plants and their width and length measured and then digitally scanned to assess leaf area using image analysis software (WinDIAS Delta-T Devices Ltd., Burwell, Cambridge, UK.).

Table 3.3 Leaf characteristics for each classification 'type' used to estimate leaf area per stem based on position on stem and nominal leaf length(mm).

Type	Position on stem	length(mm)
t_1	Leaves on main stem	95-120
t_2	Leaves on main stem	75-95
t_3	Leaves on main stem	55-75
t_4	Leaves on main stem	30-55
t_5	Leaves on branch off main stem	70-95
t_6	Leaves on branch off main stem	30-55
t_7	Leaves on branch off main stem	20-30

A relationship was established between leaf area and the measurements of length and width. A leaf coefficient of 0.7 was thus derived allowing total leaf area per stem *(LA)* to be estimated from the number of leaves of each of the seven leaf types (t), and the mean length *(L_t)* and mean width *(W_t)* of those leaf types (Equation 3.8)

$$LA = \sum_{t=1}^{t=7} 0.7\, L_t W_t n_t \qquad \text{Equation 3.8}$$

Stem diameters at height of gauge installation were measured on 10 July, prior to installation of the gauges, using digital vernier callipers (Table 3.4). Stem base diameters were measured and 28 July. From these measurements stem basal area *(SBA)* was calculated and these values were used as scalars to derive whole plant water-use estimates from single stem sap flow measurements (Table 3.5 and 3.6).

Table 3.4 Stem diameters of stems selected for sap flow measurement at a) point of gauge installation; b) stem base also showing leaf area at start of measurement period for each selected stem and each plant.

Treatment	Gauge size (nominal stem diameter)	Stem diameter at gauge (mm)	% of whole plant stem basal area	Leaf area stem^{-1} (cm^2)	% of whole plant leaf area
SW	19 mm	18.6	2.3	3540	3.3
SW	10 mm	10.7	1.2	752	0.7
SW	9 mm	10.7	1.0	878	0.8
SD	19 mm	18.8	2.3	3272	4.2
SD	10 mm	10.3	1.1	763	1.0
SD	9 mm	9.4	1.1	78	0.1
CW	16 mm	15.9	1.9	3551	7.8
CW	13 mm	11.6	1.3	1351	3.0
CD	16 mm	16.3	1.9	2674	7.0
CD	13 mm	12.5	1.5	1603	4.2

3.3. Results

Water balance

During the sap flow measurement period (13 July to 3 August 2001) there were two large rainfall events, on 18 July (35 mm) and on 19 July (25 mm) (Figure 3.3a). ET_o was low on these days, at 1.57 and 1.52 mm respectively, with the lowest ET_o being recorded on 2 August at 1.26 mm d^{-1}. The maximum value of ET_o was 3.85 mm d^{-1}, recorded on 29 July.

Estimates of mean daily lysimeter evapotranspiration (ET; units: kg d^{-1}) were derived from the lysimeter water balances for each soil type, for the periods: 13-20 July, 21-31 July and 1-3 August (Figure 3.3b). Throughout the monitoring period the sandy loam lysimeters gave consistently higher ET rates (ranging from 9.5 to 13.6 kg d^{-1}) than the clay (ranging from 4.4 to 7.3 kg d^{-1}). The ET rates progressively decreased over the course of the experiment for both soil types.

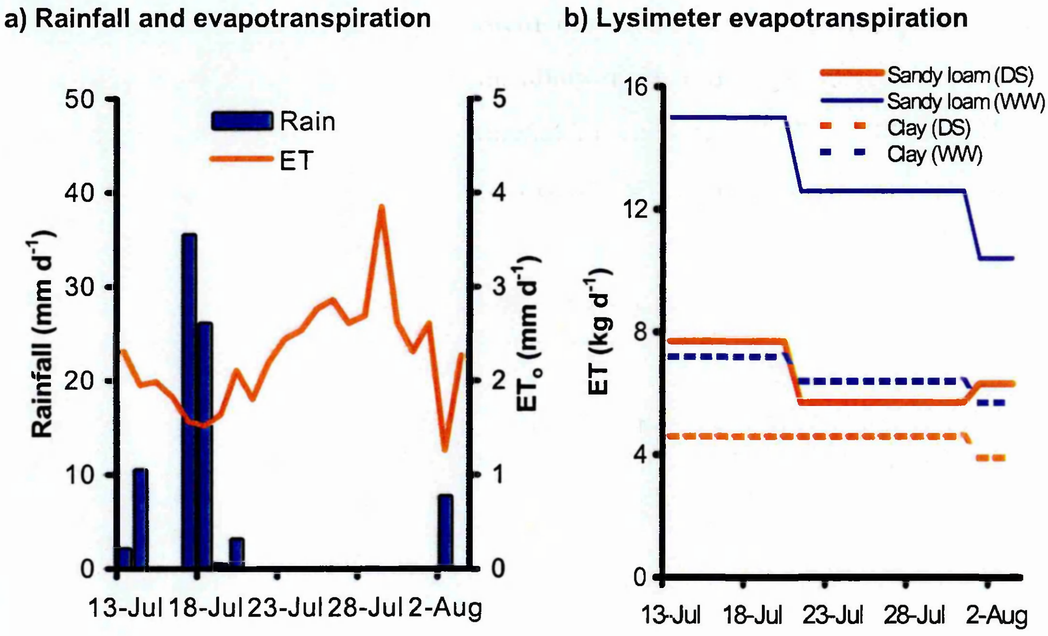


Figure 3.3 a) Daily rainfall and reference crop evapotranspiration (ET_o) b) estimated mean daily evapotranspiration derived from the lysimeter water balance for each soil type and soil-water treatment (drought-stressed (DS) and well-watered(WW)), for the three periods between 13 July and 3 August

Soil water

The volumetric water content (measured using the Diviner2000® capacitance probe) in the sandy loam drought-stressed treatment, when subjected to the drying cycles (13-17 July and 21-28 July) declined to 17.5 % in the top 10 cm. A similar pattern occurred in the clay soil drought stressed treatments with a minimum soil-water content value of approximately 30 % in the top 10 cm.

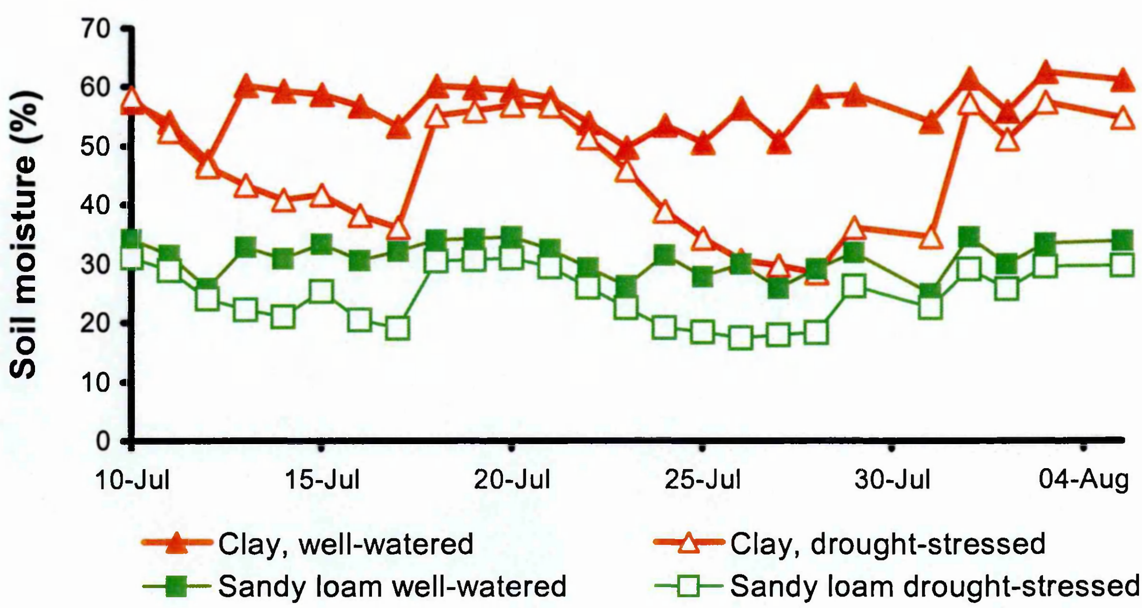


Figure 3.4 Daily volumeteric soil-water content in the top 10 cm horizon of each lysimeter from 10 July to 5 August, recorded using a Diviner2000® capacitance probe. Illustrated, is the pattern of wetting and drying imposed on the drought stressed treatments relative to the well watered treatments, for each soil type.

Sap flow measurements: sandy loam treatment

Well-watered

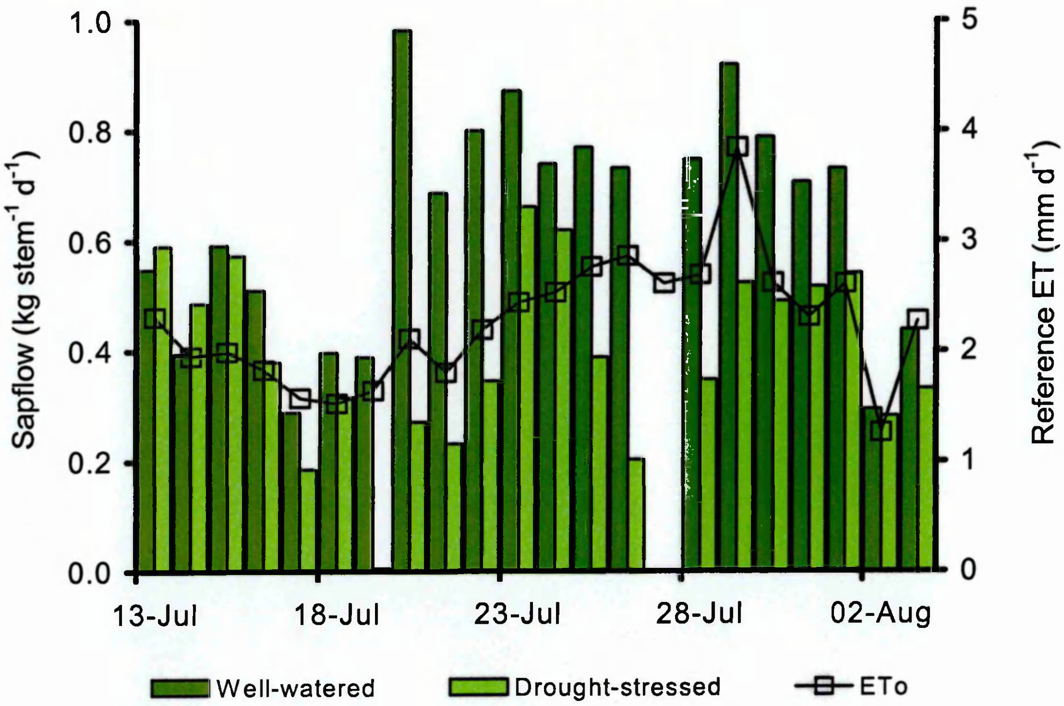
In the well-watered sandy loam treatment, the sap flow rate, measured by the 19 mm gauge, ranged from 0.3 to 0.6 kg d^{-1} between 13 and 19 July, and 0.6 to 0.9 kg d^{-1} between 20 July and 1 August (Figure 3.5a). The daily sap flow appear to follow a similar pattern to ET_o with co-efficient of determination (r^2) of 0.54. The daily sap flow for the well-watered treatment 10 mm gauge showed a similar relationship with ET_o (r^2

= 0.57) (Figure 3.5b). Mean daily sap flows recorded for the first seven days were less than 60% of those recorded subsequently up to 1 August for both gauges.

Drought-stressed

In the drought-stressed sandy loam treatment, the sap flow rate, measured using the 19 mm gauge closely matched that of the 19 mm well-watered treatment for the first three days of monitoring (Figure 3.5a). From 16 to 19 July the sap flow rates were lower, relative to well-watered treatment, with values close to zero recorded on 19 July. The drought-stressed plant then showed a gradual recovery in sap flow rate but it was still less than half that observed in the well-watered treatment from 20 to 22 July rising to 85% of the rate observed for the well-watered treatment by 24 July (Figure 3.5a). This suggests there was a time-lag between the imposition of the drying cycle treatments and plant response. The second drying cycle showed similar effects in suppressing sap flow, with rates declining from 24-26 July (missing data for 27 July). Sap flow again recovered in response to re-wetting up of the soil profile, although only returning to approximately 80% of the well-watered treatment. The 10 mm gauge also showed reduced flow rates in response to drought-stress. However the mean daily sap flow (13-15 July) of the well-watered treatment (0.09 kg d^{-1}) was 70% of that for the drought treatment (0.13 kg d^{-1}). Also in the first re-wetting phase, (19-22 July), the mean daily sap flow rate was very similar to well-watered treatment and on the 23 July was approximately 30% greater than that recorded by the 10 mm well-watered treatment gauge. For the second drying cycle there was again a reduction in sap flow followed by a partial recovery (Figure 3.5b).

a) Sandy loam 19 mm sap flow gauges



b) Sandy loam 10 mm sap flow gauges

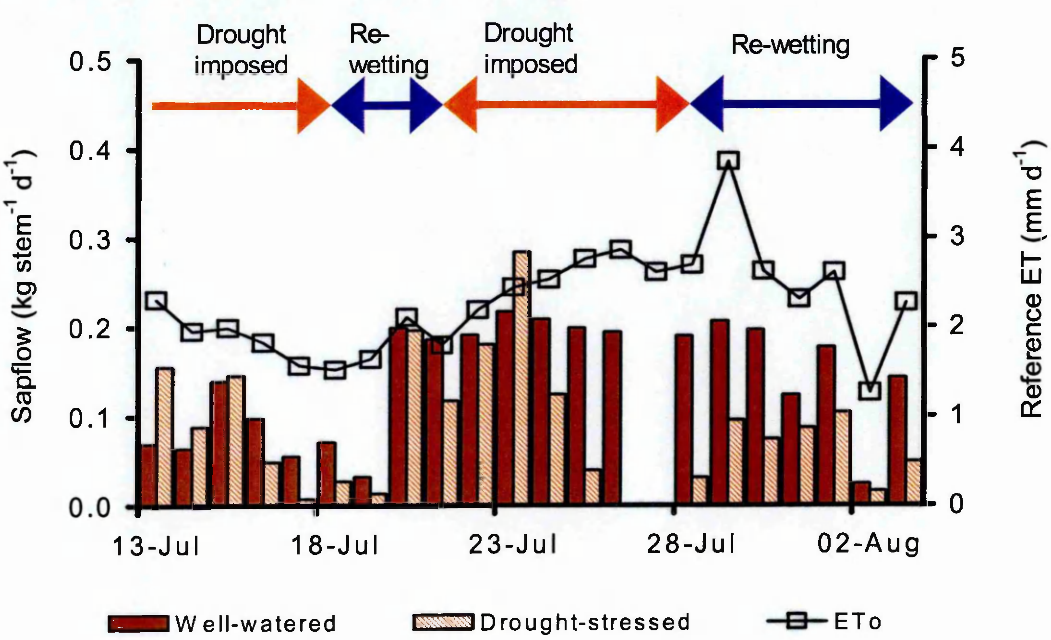


Figure 3.5 The relative responses of individual stem sap flow rates (kg stem^{-1}d^{-1}) for: a) the 19 mm sap flow gauges and b) the 10mm sap flow gauges, for the sandy loam well-watered and drought-stressed treatments, with corresponding reference ET_o (missing data for 27 July). The periods of drying and re-wetting for the drought stressed treatment are shown in figure b).

Sap flow measurements: clay treatment

Well-watered

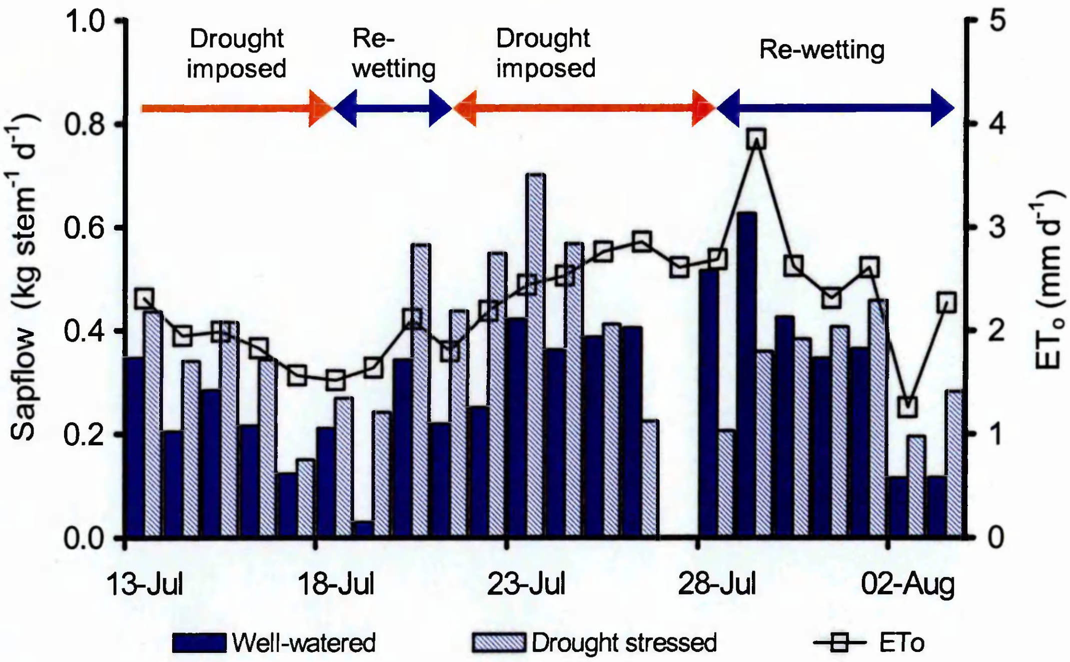
In contrast to the sandy loam treatments, sap flow rates for the well-watered clay treatment were generally lower than the drought-stressed treatment for both gauge sizes. Over the whole monitoring period the mean daily sap flow from the 16 mm gauge in the drought treatment (0.38 kg d^{-1}) was 25% greater than that of the well-watered treatment (0.30 kg d^{-1}) (Figure 3.6a). The sap flow rate, measured with the 16 mm gauge ranged from 0.03 to 0.42 kg d^{-1} between 13 and 23 July (with a mean value of 0.24 kg d^{-1}). From 24 July to 3 August the values ranged from 0.12 to 0.63 kg d^{-1} (with a mean value of 0.37 kg d^{-1}). The 13 mm gauge showed a similar pattern of daily sap flow, but with lower sap flow rates. Both 16 mm and 13 mm gauges gave values that were well correlated to ET_o ($r^2 = 0.77$ and $r^2 = 0.83$ respectively).

Drought-stressed

For the period 13 to 23 July sap flow rates measured by the 16 mm gauge tended to follow the pattern of ET_o ($r^2 = 0.76$) with no indication of negative impact resulting from the first drying cycle (Figure 3.5a). The range of sap flow rates for this period was from 0.15 to 0.7 kg d^{-1} (with a mean value of 0.4 kg d^{-1}). The second drying cycle affected the stem sap flow rates in a similar way to that observed in the sandy loam drought-stressed treatment, with a decline in daily sap flow rates followed by a recovery phase. Post-recovery, the drought-stressed sap flow rates exceeded the well-watered sap flow rates for the remaining period (31 July to 3 August) (Figure 3.5b).

The 13 mm gauge in the drought-stressed treatment failed on the 21 July due to water leaking into the gauge short circuiting the thermopile and damaging to a number of the thermocouples. This prevented the gauge being used for the remainder of the investigation period. The flow rates recorded prior to gauge failure showed a similar pattern to that of the drought stressed 16 mm gauge, but with mean daily values (0.10 kg d^{-1}) that were 500% greater than those recorded from the corresponding well-watered 13 mm gauge (0.02 kg d^{-1}) (Figure 3.6b).

a) Clay 16 mm sap flow gauges



b) Clay 13 mm sap flow gauges

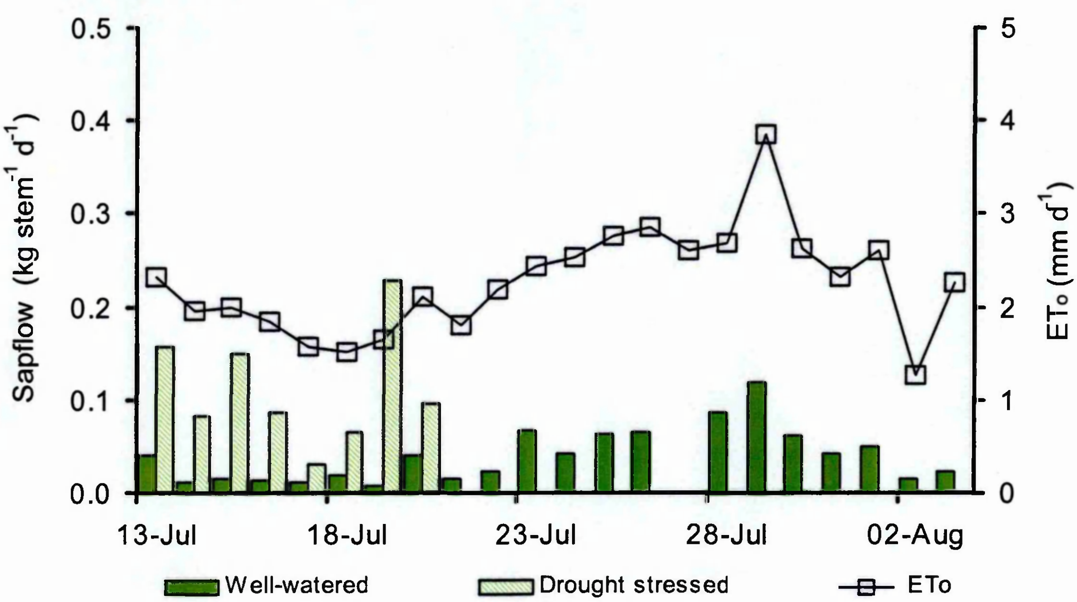


Figure 3.6 The relative responses of individual stem sap flow rates (kg stem^{-1}d^{-1}) for: a) the 16 mm sap flow gauges and b) the 13 mm sap flow gauges, for the clay well-watered and drought-stressed treatments, with corresponding reference ET_o (missing data for 27 July). The periods of drying and re-wetting for the drought stressed treatment indicated apply to both figure a) and b). Missing data for the 13 mm gauge from 21 July was due to failure of the gauge.

Stem basal area and leaf area measurements

Stem basal area calculated from stem base diameter measurements taken on 28 July ranged from 1.0 -1.2 cm^2 for the 10 mm gauge stems to 4.0 - 4.2 cm^2 for the 19 mm gauge stems. The corresponding values for the 13 mm and 16 mm gauge stems were 1.3 - 1.7 cm^2 and 2.7 cm^2 respectively. The whole plant SBA calculated for the sandy loam well-watered and drought-stressed plants were 82.0 cm^2 and 68.1 cm^2 respectively. For both clay treatment plants, the whole plant SBA was approximately 50% of the sandy loam treatment with values of 37.4 cm^2 for the well-watered and 35.3 cm^2 for the drought-stressed treatments (Table 3.3).

Leaf area was assumed to be unchanged throughout the monitoring period. The values for leaf area ranged from 0.08 m^2 for the 10 mm gauge stems to 0.33 - 0.35 m^2 for the 19 mm gauge stems. The corresponding values for the 13 mm and 16 mm gauge stems were 0.14 - 0.16 m^2 and 0.27 - 0.36 m^2 respectively (Table 3.3). The whole plant LA calculated for the sandy loam well-watered and drought-stressed plants were 10.8 m^2 and 7.8 m^2 respectively. For both clay treatment plants, the whole plant LA was less than 50% of the sandy loam treatment with values of 4.5 m^2 for the well-watered and 3.8 m^2 for the drought-stressed treatments.

Comparison of methods to estimate whole plant water use

Scaling up from the individual stem sap flow measurements to estimate water use of the whole plant was done on the basis of stem basal area and leaf area (Hall *et al.*, 1998) The sap flow measurements reported in Figures 3.5 and 3.6 were used to estimate the total water use of the plant (Table 3.4).

The total plant water-use, calculated using the sap flow rates of the sandy loam treatments, scaled up on the basis of stem basal area (129-288 kg $plant^{-1}$), was similar to that calculated from the lysimeter water balance (123-251 kg $plant^{-1}$). However for the clay treatments a greater discrepancy was observed between sap flow and the lysimeter water balance totals.

Table 3.5 Calculation of water-use per stem for the period 13 July to 3 August 2001.

Soil Treatment	Water Treatment	Gauge Diameter (mm)	Total Sap flow (kg)	Measured stem SBA (cm^2)	Measured stem LA (m^2)	Total plant SBA (cm^2)	Total plant LA (m^2)
Sandy loam	Well-watered	19	14.06	4.0	0.35	82.0	10.8
		10	3.17	1.2	0.08		
	Droughted	19	8.56	4.2	0.33	68.1	7.8
		10	1.90	1.0	0.08		
Clay	Well-watered	16	6.79	2.7	0.36	37.4	4.5
		13	0.91	1.3	0.14		
	Droughted	16	8.17	2.7	0.27	35.3	3.8
		13	0.90	1.7	0.16		

Table 3.6 Scaling up of the results from an individual stem to the whole plant based on: (a) stem basal area (SBA), (b) leaf area (LA) compared to lysimeter water balance (LWB) data for the period 13 July to 3 August 2001.

Soil Treatment	Water Treatment	Gauge diameter (mm)	Plant water-use estimates: Sap flow scalar SBA (kg)	Sap flow scalar LA (kg)	lysimeter LWB (kg)	*SBA /LWB*	*LA /LWB*
Sandy loam	Well-watered	19	288	429	251	1.15	1.71
		10	217	455		0.86	1.81
	Droughted	19	139	203	123	1.13	1.65
		10	129	193		1.05	1.57
Clay	Well-watered	16	94	84	125	0.75	0.67
		13	26	13		0.21	0.10
	Droughted	16	107	117	85	1.25	1.38
		13*	na	na		na	na

**Clay droughted 13 mm gauge data not included due to gauge failure.*

For the sandy loam treatments, using leaf area to scale up the sap flow measurements resulted in an overestimate of plant water use (193-455 kg plant^{-1}) when compared to the lysimeter water balance values (123-251 kg plant^{-1}). This was equivalent to an overestimate of 57–87%. When using leaf area to scale up the sap flow measurements from the clay treatments the 16 mm well-watered gauge under-estimated the lysimeter-predicted value by 33% and the drought-stressed over-estimated by 38%. The 13 mm well-watered gauge gave a very poor estimate of water-use, under-estimating the lysimeter water balance value by 90%.

Effects of gauge size on the estimate of plant water use

Within the sandy loam treatments, the estimated **total** plant water-use calculated from sap flow was similar for the 10 mm gauge and the 19 mm gauge. Figure 3.7a shows that there was almost a 1:1 relationship (r^2 = 0.85) between **daily estimates** of water use/unit SBA, calculated from sap flow measurements made with the 10 mm gauge and with the 19 mm gauge.

However, for the drought-stressed treatment the relationship between the scaled up values determined for the 10 mm and 19 mm gauges is poorer (r^2 = 0.39) (Figure 3.7b). There are four 'out-liers' from the regression line. These points correspond to the 'recovery phase' (20 to 23 July) that followed the first drying cycle. For these four days the estimates of water-use/unit SBA were higher for the 10 mm gauge than for the 19 mm gauge (Figure 3.8). If these points are not included in the regression analysis an r^2 of 0.8 is achieved, indicating good correlation between the two gauges for the majority of the monitoring period.

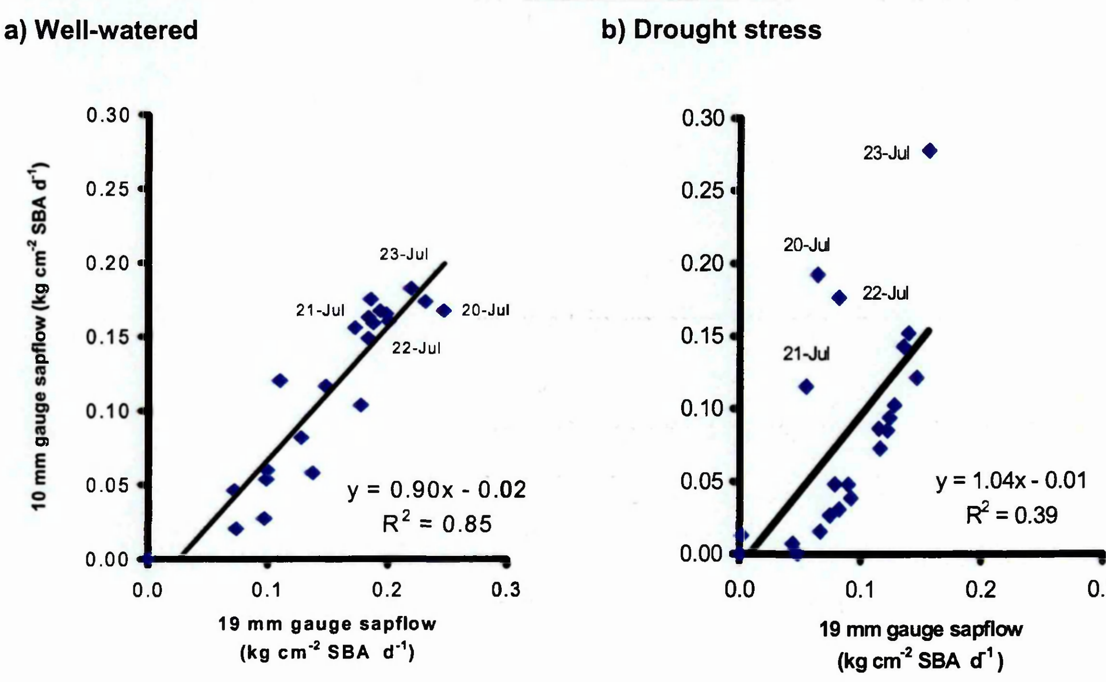
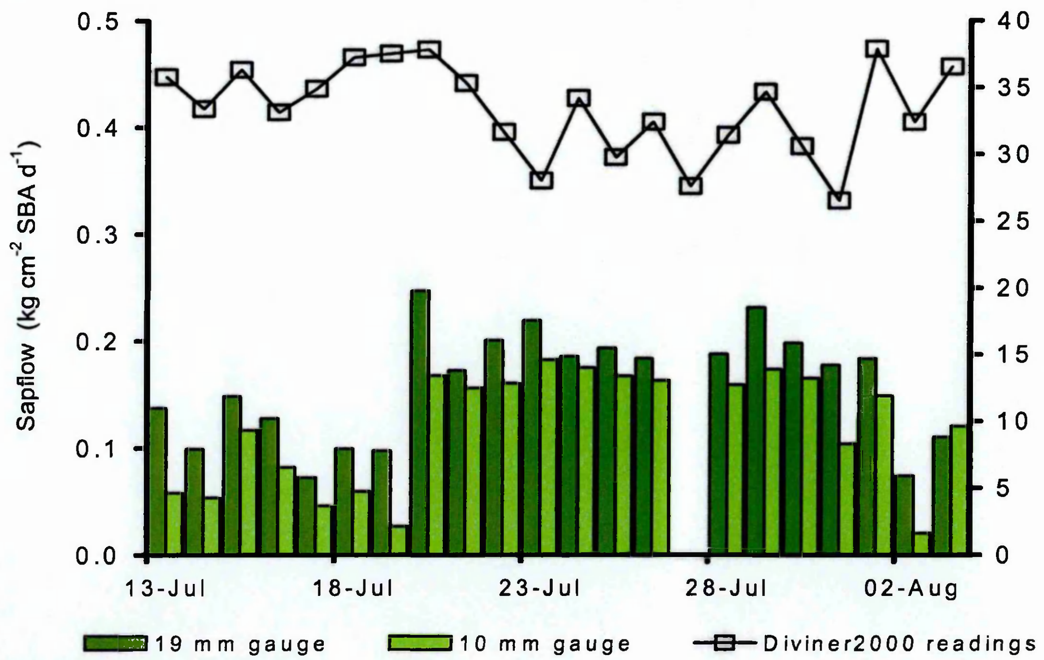


Figure 3.7 Linear regression of daily sap flow measurements (kg d^{-1} cm^{-2} stem basal area) for the 10 mm and 19mm gauges installed on the sandy loam treatment in a) well-watered and b) drought stressed conditions

a) Sandy loam well-watered



b) Sandy loam drought-stressed

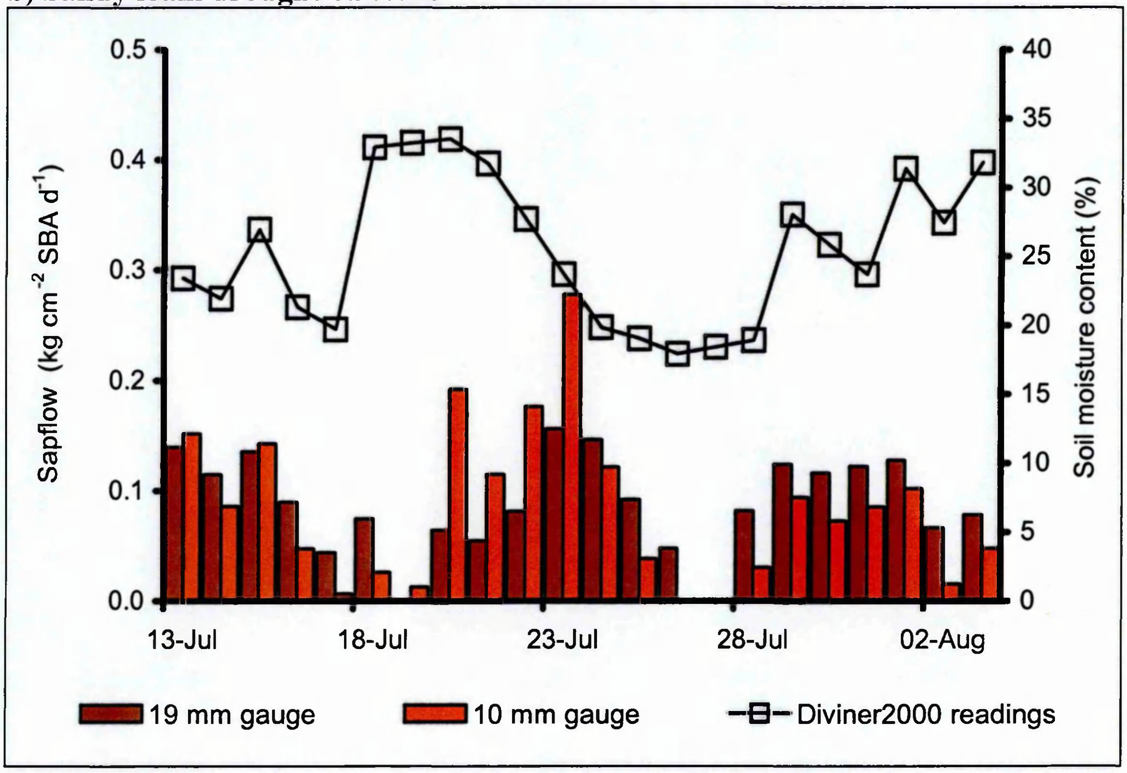


Figure 3.8 Relative daily sap flow of the gauges installed on the sandy loam: a) drought treatment and b) non-stressed treatment, expressed in kg sap flow cm^{-1} stem basal area day^{-1} between 13 July and 3 August 2001, showing change in soil moisture in the top 10 cm soil horizon. Note the differing response to drought stress of the 19 mm and 10 mm stems between 21 and 24 July.

In the clay well-watered treatment, there was some correlation between estimates of daily sap flow made with the 16 mm gauge and the 13 mm gauge ($r^2 = 0.59$) (Figure 3.9). However, the 13 mm gauge gave very low estimates of plant water-use equivalent to only 14% of those calculated using the 16 mm gauge.

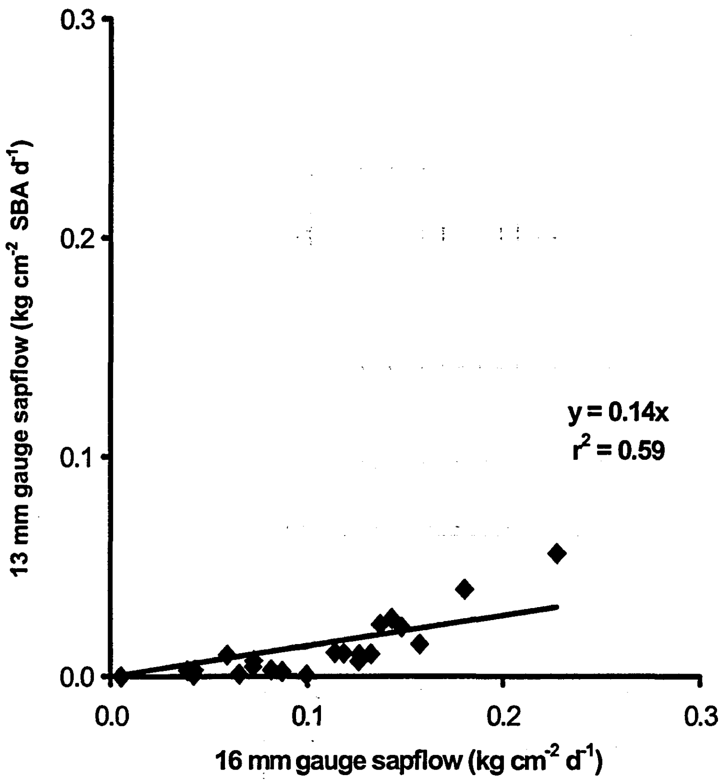


Figure 3.9 Linear regression of daily sap flow measurements per unit stem basal area (SBA) for the 13 and 16 mm gauges installed on the clay well-watered treatment for the period 13 July to 3 August 2001.

3.4. Discussion

Lysimeter water balance and reference ET values

To overcome uncertainties in calculating the volume of water stored within the soil profile, a mean daily ET was calculated for periods when it was assumed the soil water status was the same at the start and end of a period. Hence ET was calculated for three discrete periods (13-20 July, 21-31 July and 1-3 August (Figure 3.3b)). These periods were based on the soil-water status of the drought-stressed treatment returning to 'field capacity' following a drying phase (i.e. when drainage water was recorded first after a re-wetting phase). Hence, validation of the ***daily*** sap flow measurements was not possible.

For the first period, 13-20 July, estimates of daily water-use for all treatments, based on the lysimeter water balance, were higher than the second (21-31 July) and third (1-3 August) periods (Figure 3.3b). This did not correspond to sap flow estimates or ET_0 values, which both showed the highest water-use during the second period. Over-estimation in water-use for the initial period may have resulted from difficulties in estimating the actual volume of water 'funnelled' into the lysimeter along the stems (stemflow) during rainfall events recorded for this period (Figure 3.3a). The relationship established between rainfall and stemflow (R_s) was based on measurements made from number of selected stems. Although this was considered to be a reasonable predictor of R_s it was affected by both rainfall intensity and wind direction during a rainfall event (Martin, 2001). Nevertheless, the plant water-use estimates calculated for the whole period from the lysimeter water balance still provide a useful measure with which to validate the sap flow measurements.

Effects of soil type on sap flow rates

For the sandy loam treatments, stem basal area of the well-watered plant (82 cm^2) was 20% greater than that of the drought treatment plant (68 cm^2) and leaf area was 38% greater. However stem basal area and leaf area of the two 19 mm stems was similar. This could indicate that the general growing conditions in the well-watered lysimeter were more favourable to plant growth and that factors independent of the watering regime were influencing plant responses. However sap flow rates observed during the first three days of monitoring (13 –15 July) indicated that the two 19 mm were capable of achieving similar flow rates. This also appeared true for the two 10 mm stems, in that their sap flow rates were also similar to each on the 14 and 15 July (Figure 3.5) . The drying cycles imposed on the sandy loam drought treatment appeared to perturb the relationship between sap flow and ET_0 for the second drying phase (Figure 3.5). An overall reduction in stem sap flow (for both stem sizes) of 40% compared to the well-watered treatment was noted for the period 13 July to 3 August (Table 3.5).

In the clay well-watered treatment there was no shortage of available water. However, on comparing the sap flow rates with the clay drought-stressed treatment, it is observed that higher sap flow rates were recorded for the drought stressed treatment for most of the period of study. This appears to indicate that the soil conditions in the well-watered treatment where limiting to plant growth in some respect. The soil structure characteristics of the Oxford clay make it prone to anaerobic conditions which could prove to be a limiting factor for plant growth. For this soil type there appears to be only a small range of soil moisture content that is conducive to optimum growth of the willow plant (Martin, 2001).

Reliability of sap flow measurements

Generally, daily sap flow corresponded with the daily pattern of ET_0 and responded to the changes in soil-water status. Scaling up on the basis of SBA gave estimates closer to lysimeter water balance values than those derived from LA. This could have been due to the increased potential for error associated with estimating LA, compared to the relatively simple measurements of stem diameter required to calculate SBA.

The total plant water-use estimates of the sandy loam treatments, scaled up using SBA, compared well with the water balance values with differences in the range ± 5 -15% (Table 3.6). By comparison, the estimates of water-use calculated using the 13 mm gauges on the clay well-watered treatment were only 20% of the lysimeter water balance values for ET. Sap flow rates measured on this stem were very low. Difficulties associated with measuring low sap flow rates using constant power SHB gauges are described by Grime and Sinclair (1999). To overcome some of these problems the use of a 'low flow' filter (described more fully in Chapter 5) is built into the sap flow calculation. This filters out potentially erroneous values when certain 'low flow' conditions are encountered. The manufacturers default settings for the 'low flow' filter were applied to all sap flow calculations presented in this chapter. Difficulties associated with determining the K_{rad} constant (Equation 3.6) are also acknowledged by Grime and Sinclair (1999). The K_{rad} value applied to the sap flow calculations in this chapter were determined in accordance with the manufactures instructions (Dynamax inc., 1997), from a period of measurements when sap flow was assumed to be zero.

Error in determining K_{rad} and applying the 'low-flow' filter could have resulted in the under-estimation of sap flow rates reported for this stem.

Effect of gauge size on sap flow measurements

Under the conditions imposed on the plant during this study, the 10 and 19 mm stems show an apparent difference in behaviour in response to (or recovery from) drought stress (Figure 3.7b). These differences effectively 'cancelled each other out' in terms of the total water balance recorded in this study. However, it is conceivable that, over extended periods of drought-stress or fluctuating water availability, the differing responses of individual stems could induce significant error in estimates of whole plant water-use.

3.5. Conclusions and recommendations

Results presented in this chapter indicate that SHB sap flow gauges can be used successfully to estimate the water-use of multi-stemmed woody perennials. Estimates within 15% of the LWB were achieved from the plants grown in sandy loam with sap flow was scaled up on the basis of SBA. Poorer estimates (> ± 25% LWB) were reported for the plants grown in the 'heavy' Oxford clay.

Periods of low sap flow rates, induced by less favourable growing conditions, such as the plant grown on the 'heavy' well-watered (possibly 'over-watered') Oxford clay, may have contributed to the discrepancies between scaled up estimates of plant water-use from gauges installed on the same plant (Grime and Sinclair, 1999). Sensitivity of the sap flow calculation to correctly determining Krad and applying the 'low flow' filter is explored more fully in Chapter 5.

Also, as seen with sandy loam drought-stressed treatment, the stems of a multi-stemmed plant appear to behave independently in response to stress. Further investigation into the extent and duration of these apparently 'independent stem responses' is suggested in order to increase confidence in the use of SHB gauges for estimating the water-use requirements of multi-stemmed plants. This issue is examined in more detail in the following chapters.

The choice of scalar had a big effect on water-use estimates with stem basal area proving the most satisfactory in this study. Large overestimates of more than 50%, relative to lysimeter based estimates, resulted from the sandy loam treatment values scaled up on the basis of leaf area. Care is therefore needed when applying a scalar (Wullschleger *et al.* 1998).

The failure of the 13 mm gauge installed on the clay drought stressed treatment also has implications in terms of reliability of SHB gauges. The delicate nature of the gauges makes them prone to damage under field conditions which must, therefore, be an important consideration when choosing appropriate measurement techniques for any given study

4. SAP FLOW RESPONSES OF INDIVIDUAL STEMS

The overall aims of the thesis are to evaluate the reliability of stem heat balance (SHB) sap flow gauges as a means of estimating plant water-use and to determine best practice when using SHB sap flow gauges to determine water-use of a multi-stemmed woody perennial. This chapter describes the work undertaken in 2002.

4.1. Introduction

In 2002, the focus was on the sap flow responses of *individual* stems to changes in available soil water. The experiment was again based on the SRC willow grown in lysimeters (as described in Chapter 3). A single lysimeter was used and SHB sap flow gauges were installed on 10 of the 22 stems on the plant. The overall aim was to test the hypothesis that transpiration (sap flow) from the individual stems of a multi-stemmed plant is *consistently* proportional to the stem basal area (SBA) and/or leaf-area (LA) of each stem, for a broad range of growing conditions. The following objectives for this experiment were set:

1. to subject the willow plant to a wide range of soil water conditions,
2. to estimate plant water-use using a lysimeter water balance, compared to ET_o,
3. to determine the effect of changes in soil water content on the sap flow of different sized stems,
4. to compare the estimates of plant water-use derived from sap flow measurements scaled-up on the basis of SBA and LA with those from the lysimeter.

4.2. Method

Experimental site

The study was undertaken on a single willow plant (*Salix viminalis,* cultivar: 'Jorr'), grown in a lysimeter, at Cranfield University, Silsoe, England (52°0' N; 0°26' W; altitude 60 m). The experimental measurements were taken between July and August 2002. The lysimeter was placed on level ground and was positioned to avoid shading from any other plants.

Meteorological measurements

As described in chapter 3, daily reference crop ET_o values were determined using AWSET v3.0 software (Cranfield University, 2002), from meteorological measurements recorded by a locally sited automatic weather station (AWS). The range of ET_o for the period 17 July to 16 Aug was 1.6 to 4.2 mm d^{-1} with a mean value of 2.8 mm d^{-1} (Figure 4.1).

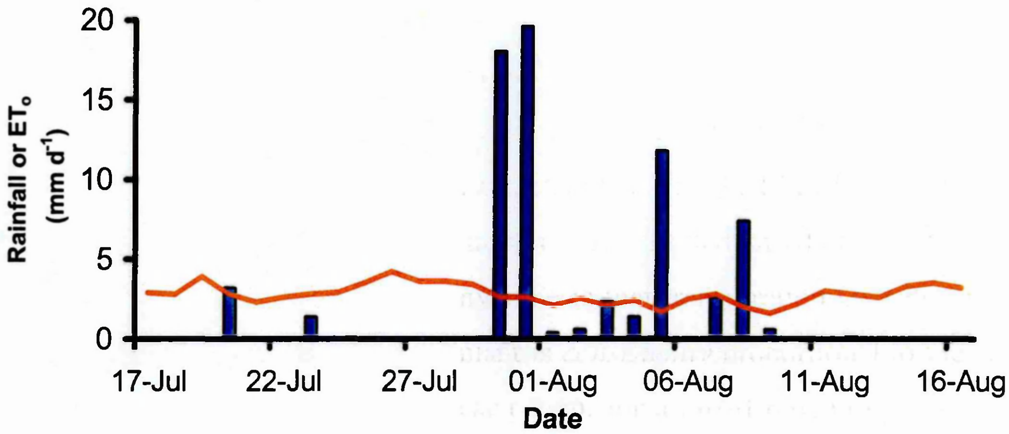


Figure 4.1 Daily reference evapotranspiration (ET_o) as calculated from data supplied from the AWS from 17 July to 16 August 2002 using AWSET v3.0 (14) software (Cranfield University, 2002) together with rainfall.

Lysimeter

The chosen lysimeter was selected from those lysimeters set up in April 1999 as part of a larger experiment (Martin and Stephens, 2001). Thus in 2002, the selected coppiced willow plant was in the third growing season after cut back. The construction of the lysimeter is described in detail in Chapter 3. The soil in the lysimeter was a Cottenham series, sandy loam soil, with a dry bulk density of 1270 kg m^{-3}.

Soil water treatments

To allow observations of plant response to be made for a range of soil water conditions, irrigation of the plant was controlled to allow four treatment phases (Table 4.1).

Table 4.1 Description of the four wetting and drying phases imposed in 2002.

Time period	Description of phase
17 to 23 July	FC1 (First field capacity)
24 July to 3 August	D1 (First drying)
4 to 9 August	FC2 (Re-wetting + second field capacity)
10 to 16 August	D2 (Second drying phase)

During the first seven days (FC1) of the measurement period, daily irrigation ensured that the soil water status was above field capacity. This was verified by the drainage from 19 to 23 July. No irrigation was applied on 23 July before the start of the first drying phase (D1). Irrigation was applied on 26 and 27 July to avoid the soil drying out too quickly. There was precipitation on 30 July (18 mm) and 31 July (19 mm). Smaller rainfall events were recorded during the following ten days with the most significant recorded on 5 August (12 mm) and 8 August (7 mm). Irrigation on the 4 August commenced the second field capacity phase (FC2). A second drying phase (D2) was started on the 10 August, and continued until 16 August (Figure 4.2).

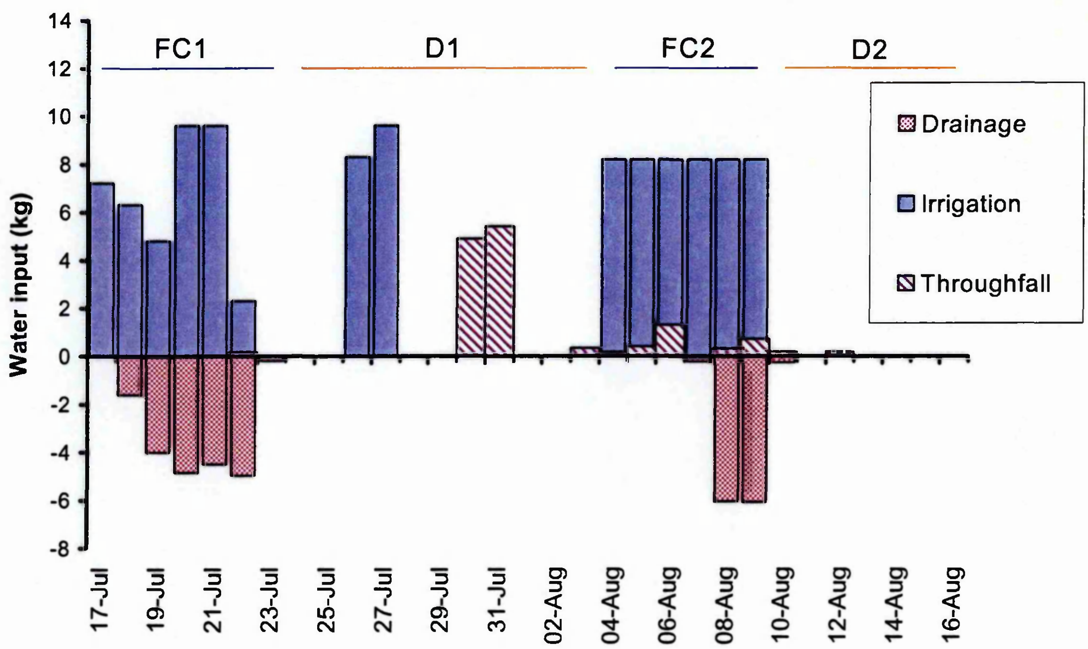


Figure 4.2 Irrigation, throughfall and drainage components of the water balance from 16 July to 16 August 2002.

Water balance measurements

As in chapter 3, the lysimeter water balance (LWB) was derived using Equation 4.1:

$$\Delta S = I + R_t + R_s - E_s - D - ET \qquad \text{Equation 4.1}$$

Where: ΔS is the change in the soil water content in the lysimeter; I is irrigation; R_t is throughfall precipitation penetrating the leaf canopy; R_s is stemflow precipitation, intercepted by the stems of the plant and then tracking down the stem to the soil; E_s is evaporation from the soil surface; D is drainage and ET is evapotranspiration from the leaves of the plant. Irrigation was automated using a timer solenoid valve between a header tank and a drip emitter on the soil surface next to the base of the plant. During periods of drought, the solenoid valve was opened for three minutes each hour, providing 0.4 kg of water per hour. An in-line flow meter monitored the irrigation input. Drainage flow was measured by connecting the gate valve installed in the lysimeter to a tipping bucket rain gauge (Skye Instruments) connected to a DL2E datalogger (Delta-T Devices Ltd., Burwell, UK). A lid on the rain gauge prevented rainfall from entering (Figure 4.3).

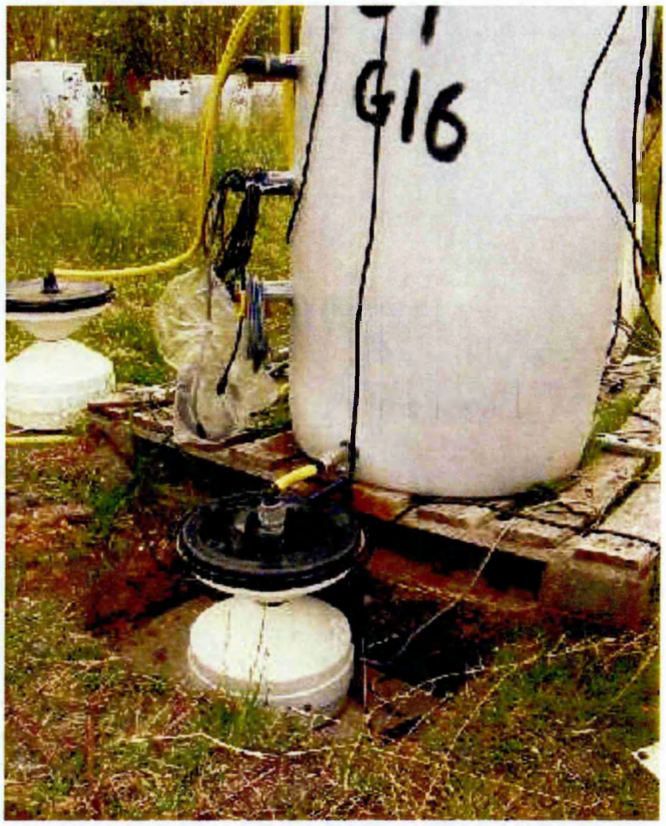


Figure 4.3 Lysimeter installation showing the arrangement of tipping bucket rain gauges (Skye Instruments) for monitoring the drainage and throughfall components of the water balance. Also shown are the three equitensiometers (Delta-T Devices Ltd) positioned down the soil profile at depths of 10, 30 and 50 cm.

Throughfall precipitation (R_t) was collected from a 110 mm diameter funnel (Figure 4.4) positioned close to the soil surface near the centre of the lysimeter. This was then measured using a tipping bucket rain gauge (Skye Instruments Ltd., Llandrindod Wells, Powys, UK), with a covering lid, connected to a DL2E datalogger (Delta-T Devices

Ltd., Burwell, UK). Due to the complications associated with quantifying stemflow precipitation (R_s), this input was minimised by diverting water tracking down the stems to cause it to fall outside the lysimeter catchment. This was achieved by use of foam collars and a length of plastic tubing attached to each stem to form a drip barrier (Figure 4.4). For reasons mentioned in the previous chapter, the evaporative loss from the soil surface of the lysimeter (E_s) was assumed to be negligible and it was not measured directly.

Figure 4.4 Dynagages installed on ten stems of the coppiced willow plant, grown in a lysimeter. Also shown is the 110 mm funnel positioned to collect throughfall precipitation and the foam collars and a length of plastic tubing attached to each stem to deflect stemflow water out of the lysimeter.

Soil water status measurements

As in the 2001 experiment, soil water content was measured using a Diviner2000® capacitance probe. A single access tube was installed in the lysimeter allowing a 70 cm soil profile to be scanned. The Diviner was configured to take measurements every 10 cm down the soil profile. The volumetric soil water content was calculated using the calibration curve for the sandy loam soil type derived by Grove and Rose (2004), reported in chapter 3.

Soil water potential was measured using three equitensiometers (Delta-T Devices Ltd , Burwell, Cambridge, UK.), positioned down the soil profile at depths of 10, 30 and 50 cm. They were installed through holes in the side of the lysimeter and sealed using a silicon sealant. The 30 cm equitensiometer was connected to a datalogger (Delta-T Devices Ltd, Burwell, Cambridge, UK.) and measurements were taken hourly. Milli-volt measurements were taken daily from the 10 and 50 cm equitensiometers using a hand-held reader. The milli-volt outputs from each equitensiometers were converted to water potentials using the calibration equations, provided by the manufacturer for each sensor (Table 4.2).

Table 4.2 Calibration equations supplied by Delta-T Devices Ltd, Burwell, Cambridge, UK for EQ2 Equitensiometers installed at three depths in the lysimeter. Where y is the soil matric potential (kPa) and x is the mV output signal from the sensor.

Depth installed	Manufacturer's identifier	Calibration equation
10 cm	EQ2 118/021	$y = -1.77.10^{-12}x^6 + 4.38.10^{-9}x^5 - 4.48.10^{-6}x^4 + 2.43.10^{-3}x^3 - 7.42.10^{-1}x^2 + 1.21.10^2x - 8.32.10^3$
30 cm	EQ2 68/20	$y = 1.46.10^{-10}x^6 - 3.27.10^{-7}x^5 + 3.00.10^{-04}x^4 - 0.145.10^{-1}x^3 + 3.857.10^{+1}x^2 - 5.311.10^3x + 2.95.10^5$
50 cm	EQ2 118/020	$y = 2.46.10^{-12}x^6 - 4.36.10^{-9}x^5 + 2.87.10^{-6}x^4 - 7.770.10^{-4}x^3 + 2.32.10^{-2}x^2 + 2.71.10^1x - 3.74.10^3$

Sap flow measurements

In total ten Dynagage sap flow gauges (Dynamax Inc., Houston, TX, USA) were fitted to ten selected stems on the willow plant. Installation was carried out according to manufactures instructions, as described in chapter 3. There were two gauges of each of the following diameters: 19, 16, 13, 10, and 9 mm. A datalogger (Delta-T Devices Ltd) recorded the milli-volt output from each gauge. Between 17 July and 16 August, readings were recorded each minute and averaged every 30 minutes. The stem heat balance calculations were carried out as described in Chapter 3, again using the manufacturers default settings for the 'low flow' filter (Delta-T Devices Ltd., 1999). The value of K_{rad} applied to the sap flow calculations were determined from a period of measurements when sap flow was assumed to be zero (i.e. between 02.00 and 03.00 hrs over consecutive days at the beginning of the monitoring period).

Stem basal area and leaf area

On 26 June 2002, stem diameter measurements were taken prior to the start of the measurement period using digital vernier callipers. These initial measurements were made at both gauge height (used in the sap flow calculation) and stem base from which stem basal area (SBA) was calculated. On 2 August and 14 October 2002, two further measurements were taken to give an indication of the rate of plant growth (Table 4.3).

Table 4.3 Diameters of stems selected for sapflow measurement, recorded between 26 June and 14 October 2002. Also showing size of gauge installed on each stem.

		Diameter at gauge (mm)	Stem base diameter (mm)		
Stem no.	gauge size	26/06/02	26/06/02	02/08/02	14/10/02
18	19 mm	18.9	22.0	20.4	21.4
24	19 mm	18.3	21.0	21.8	22.2
12	16 mm	15.8	18.7	17.5	17.9
15	16 mm	16.8	20.8	22.2	22.9
2	13 mm	13.3	15.9	16.9	18.6
13	13 mm	12.5	14.0	14.0	14.4
21	10 mm	10.4	11.8	12.7	13.0
25	10 mm	10.3	12.9	14.4	15.2
7	9 mm	8.9	11.3	10.8	10.9
16	9 mm	9.3	12.7	13.4	13.7

The morphological development of the *Salix viminalis,* cultivar: 'Jorr,' coppice willow stool is such that leaves emerge in clusters along the length of each stem (Figure 4.5). Midway through the monitoring period (26 July), each cluster length on each stem was measured. On the same day clusters with a wide range of lengths, were harvested from adjacent lysimeters with willow plants of same age and size, growing under similar conditions to the plant under observation.

Figure 4.5 Photograph showing the morphology of clusters of leaves along the length of Jorr (Salix viminalis) stem.

The leaves from these clusters were digitally scanned to assess leaf area using image analysis software (WinDIAS Delta-T Devices Ltd., Burwell, Cambridge, UK.). A relationship was established between the length and the leaf area of the clusters (r^2 = 0.75; Figure 4.6), which provided a relatively quick method of estimating leaf area.

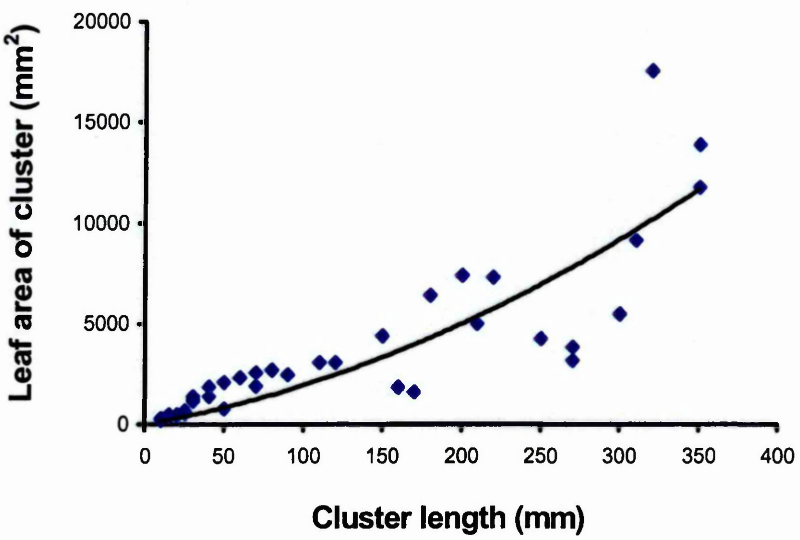


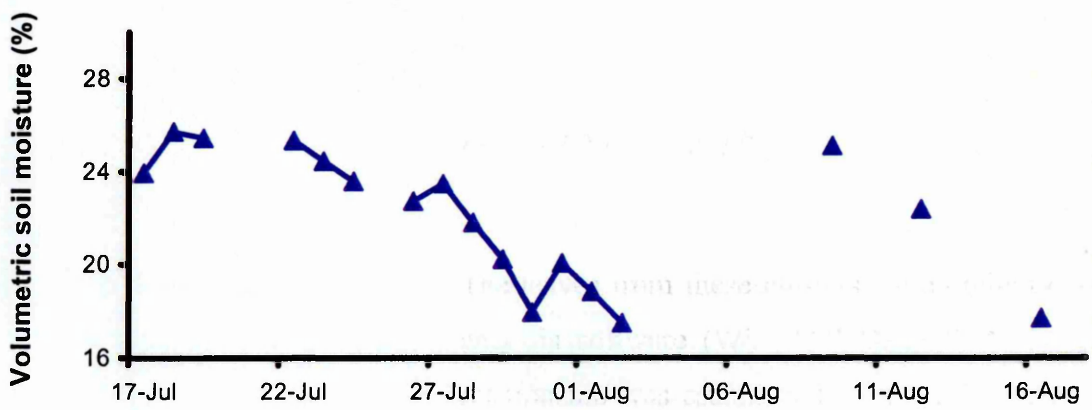
Figure 4.6 Relationship between leaf area and cluster length (Area = 0.0566 Length^2 + 13.591 Length; r^2 = 0.743).

4.3. Results

Soil water status

The volumetric soil water content in the lysimeter was controlled to impose four treatment phases (Table 4.1). It decreased from a mean value of approximately 24.5% between 17 and 23 July (FC1) to approximately 17.5% on 3 August (end of the D1). Following re-wetting, the soil water content increased to about 25% on 9 August (FC2), before declining to 17.7% on 16 August (end of D2).

a) Soil water content



b) Soil water potential

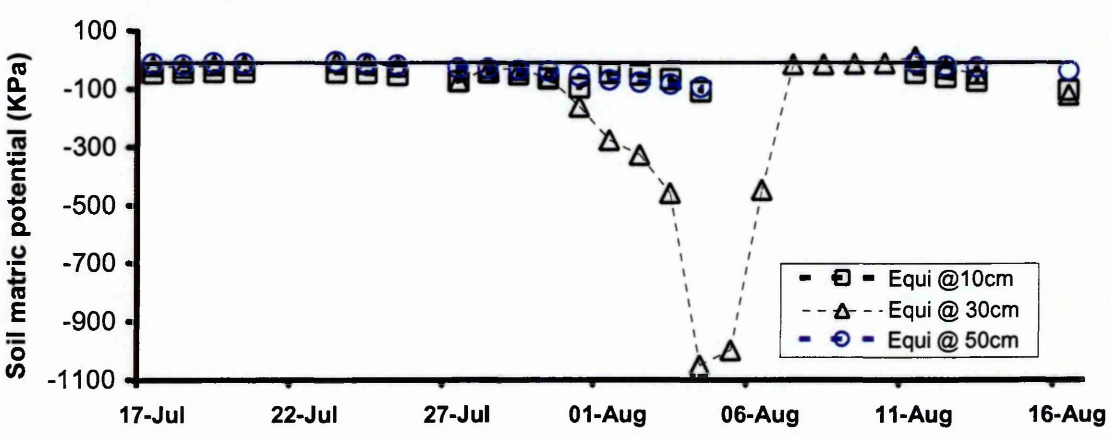


Figure 4.7 Soil moisture as determined by the Diviner2000 for the 70 cm profile of the lysimeter and b) soil matric potential as measured at depths of 10, 30 and 50 cm using delta-T equitensiometers.

The soil matric potential, at 30 cm depth remained high until 28 July, declined to a minimum (about –1000 kPa) on 4 August before increasing when the soil was re-wetted. The D2 phase had less effect on soil matric potential at 30 cm depth, with the minimum value less than –300 kPa (Figure 4.7).

The soil matric potential at 10 and 50 cm however, showed little response to the drying cycles (D1 and D2). Values for the 10 cm horizon ranged from about –40 to –110 kPa and the lowest matric potential values for the 50 cm horizon only reached –90 kPa (Figure 4.7 b).

The soil water content recorded by the Diviner 2000 was plotted for points representing each of the wetting and drying phases. An extra data set, collected in January 2003, when it was assumed that the lysimeter was close to saturation and when the willow plant would not be extracting water, was also included (Figure 4.8). It can be observed from this figure that the sequence of wetting and drying affected the soil profile in a consistent manner. The measurements at 30 and 40 cm appear to be significantly drier than the other horizons for each stage of wetting and drying. This could be attributed to some variation in soil conditions in the immediate vicinity of the diviner access tube, such as a large stone or air pocket.

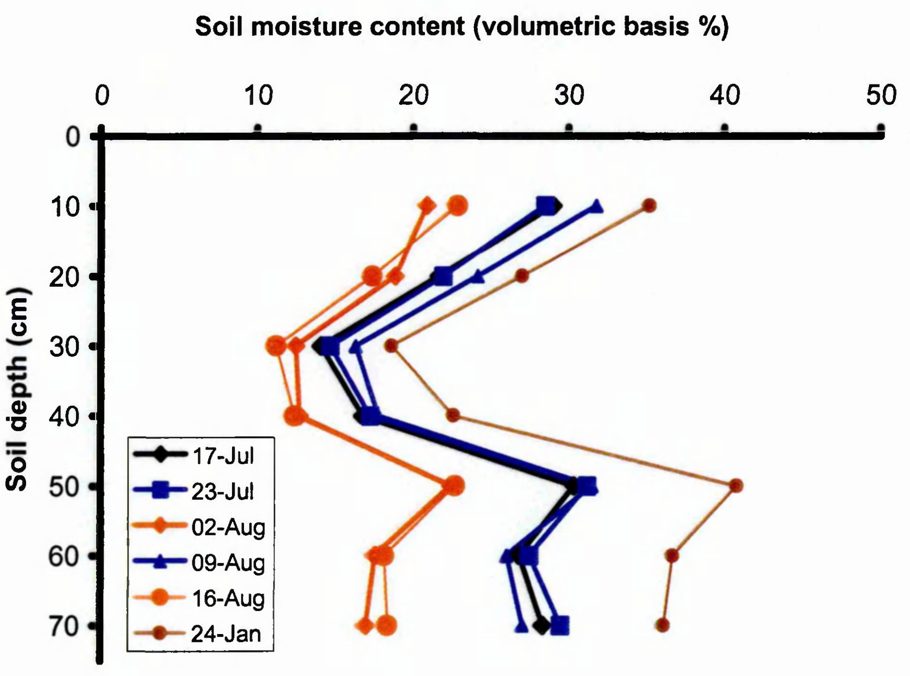


Figure 4.8 Soil moisture content as measured by the Diviner2000 probe for each 10 cm horizon of the 70 cm soil profile for key dates in the wetting and drying cycles.

The soil water contents for the 10, 30, and 50 cm horizons, recorded by the Diviner2000 moisture probe, were plotted against the corresponding equitensiometer readings for each of the dates represented above. Also for comparision a soil moisture release curve for the sandy loam soil used in the lysimeter (Martin, 2001), together with a standard soil moisture release curve for a 'typical' sandy soil are included in the same figure (Figure 4.9). The water content and potential values for the 10 and 50 cm horizons correspond well with the soil moisture release curve of the 'lysimeter' sandy loam. The values for the 30 cm horizon however lie well below this curve, relating more closely to the soil moisture release curve of the 'typical' sandy soil.

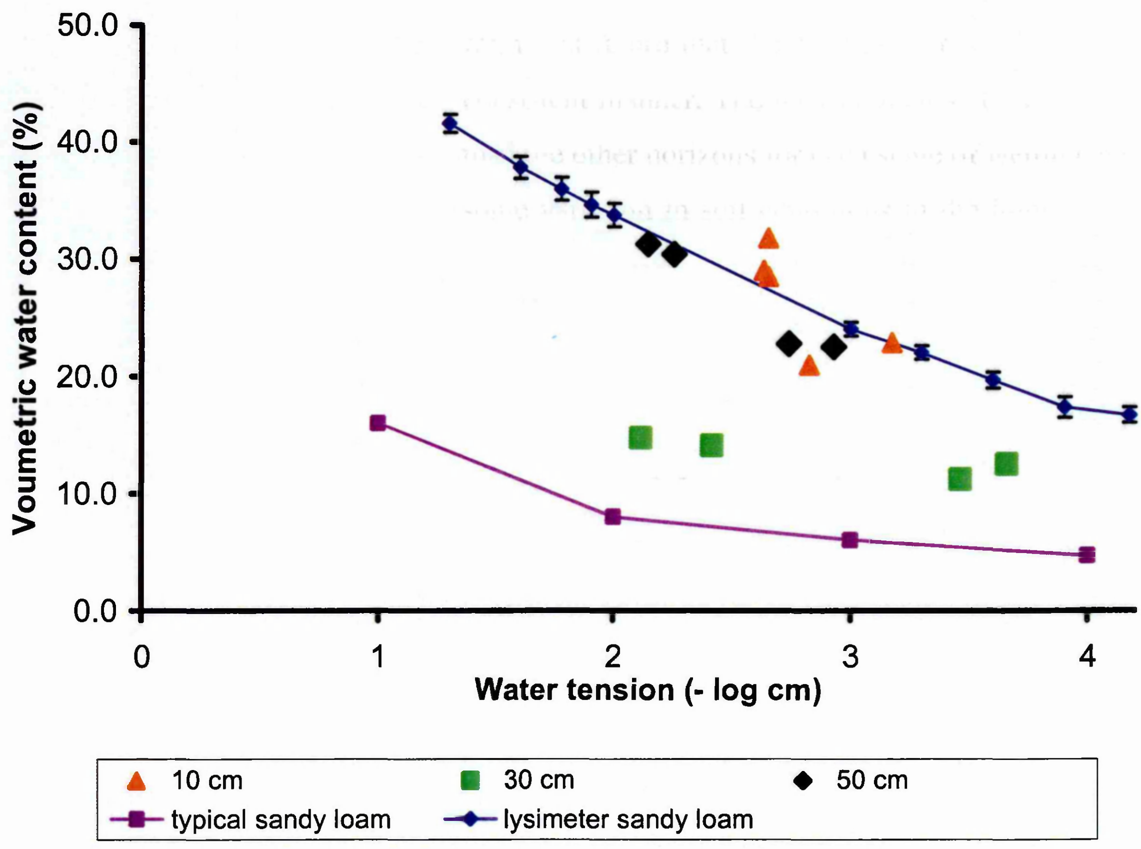


Figure 4.9 Soil moisture release curves for the Cottenham series sandy loam soil used in the lysimeter and a 'typical' sandy soil, together with measured values of soil moisture and water tension, recorded for the 10, 30 and 50 cm horizons of the lysimeter.

Calculation of lysimeter soil water balance

The measurements of water inputs, outputs and changes in soil-water content (based on Diviner2000 measurements) were used to estimate plant water-use for each soil-water treatment phase (Table 4.4). The daily water-use was estimated to be 2.4 -3.2 kg for the FC1, FC2 and D2 periods, and 4.5 kg for the D1 period.

Table 4.4 Water balance totals for each of the soil-water treatment phases of the study period, showing calculated plant water-use totals.

Phase	Time period	Initial soil moisture (kg)	Water input (kg)	Water output (kg)	Final soil moisture (kg)	Water use (kg)	Water use ($kg\ d^{-1}$)
FC 1	17-23 July	50.6	40	20.2	51.8	18.5	2.7
D 1	24 July-3 Aug	51.8	30.1	0	31.8	50.1	4.5
FC 2	4-9 Aug	31.8	50.1	12.7	50.3	18.9	3.2
D 2	10-16 Aug	50.3	1.6	0	35.2	16.7	2.4
Total	17 July- 16Aug		121.8	32.8		104.2	3.3

Sap flow rates of individual stems

The daily totals of sap flow for each stem were calculated and plotted alongside daily ET_o values from 17 July to 16 August 2002 (Figure 4.10). The gauge installed on stem 2 (13 mm) failed during the monitoring period and therefore was not included in the analysis.

Daily sap flow rates per stem generally increased with increasing stem size. The greatest sap flow rates were recorded by the two 19 mm gauges and one 16 mm gauge (stem 15). The main exception to this order was the 16 mm gauge on stem 12 which gave relatively low values, similar to the 9 and 10 mm gauges (Table 4.3). This ranking of sap flow rates with stem size is also illustrated in Figure 4.11a.

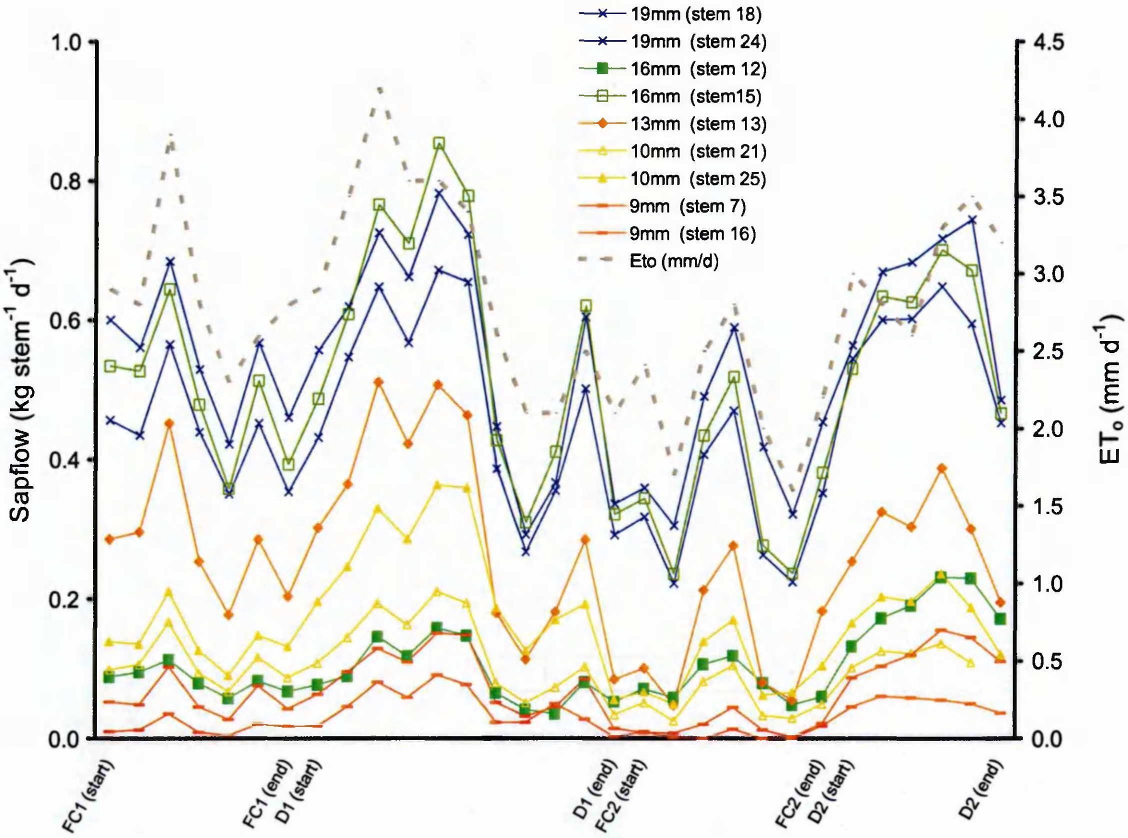


Figure 4.10 Daily sap flow calculated for each stem heat balance (SHB) sap flow gauge (kg stem^{-1} d^{-1}), together with ET_o for monitoring period 17 July and 16 August 2002. The start and end of the two field capacity phases (FC1, FC2) and the two drought phases (D1, D2) are indicated.

Table 4.5 Mean daily sap flow (g stem^{-1}d^{-1}), for each gauge, for each of the soil-water treatment phases and for the whole monitoring period.

Stem diameter	Soil-water treatment phase FC 1	D 1	FC 2	D 2	Whole period
19 mm (18)	436	484	376	492	454
19 mm (24)	547	556	490	552	540
16 mm (12)	84	92	91	161	105
16 mm (15)	493	572	405	518	510
13 mm (13)	279	310	159	252	261
10 mm (21)	105	123	62	94	101
10 mm (25)	140	228	110	159	170
9 mm (7)	16	45	7	44	31
9 mm (16)	57	84	20	103	70

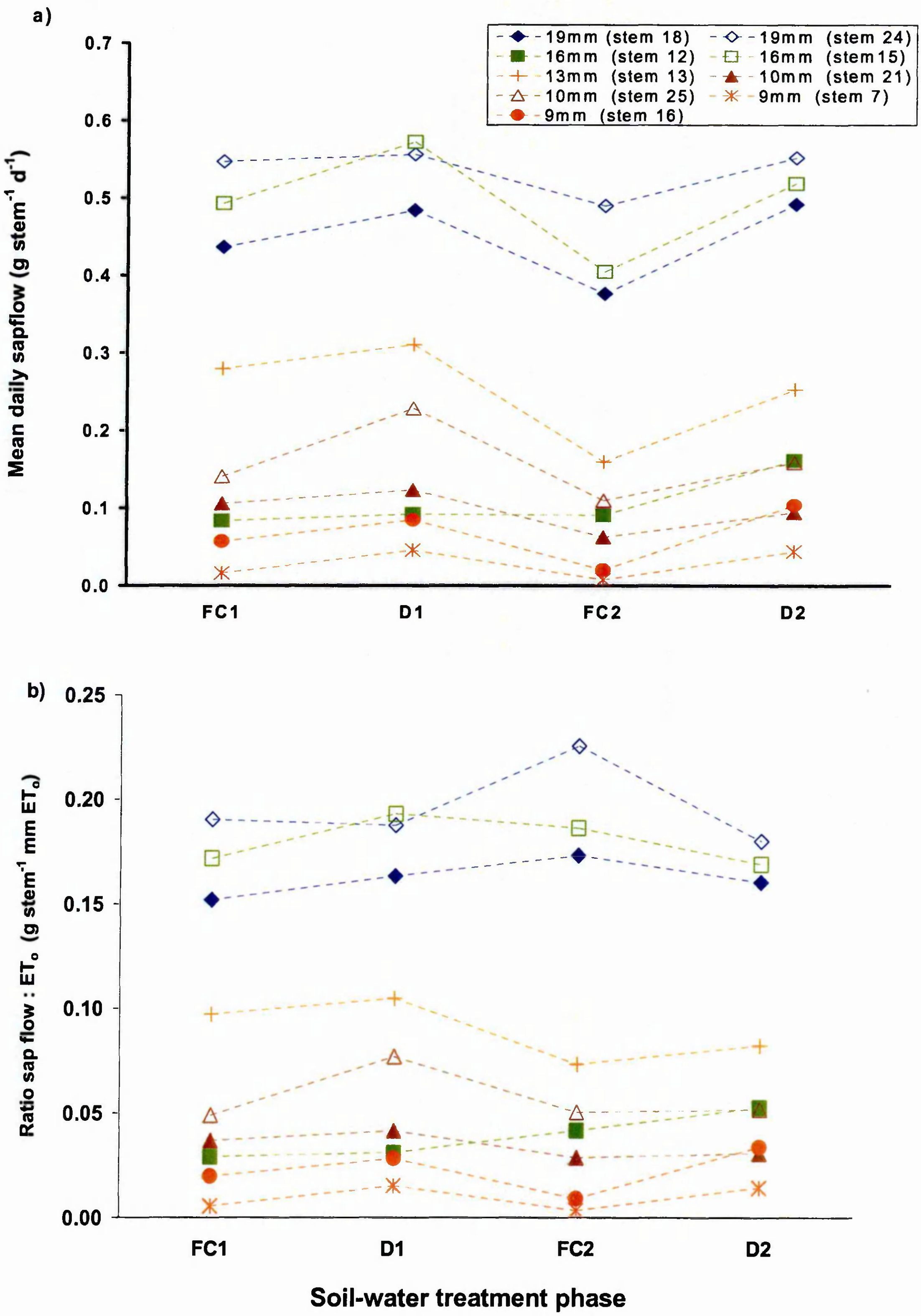


Figure 4.11 Sap flow rates recorded using stem heat balance (SHB) sap flow gauges for each stem size are shown as: a) Mean daily sap flow (g stem^{-1}d^{-1}) and b) Mean daily sap flow adjusted for ET_o (g stem^{-1}mm$^{-1}ET_o$). These means indicate the relative changes in flow rate for each stem, for each soil-water treatment phase. (FC1, FC2 are the two field capacity phases and D1, D2 are the two drought phases.)

A linear regression of the daily sap flow rate of each stem and ET_o for the first two phases (FC1 and D1) gave coefficients of determination (r^2) from 0.61 to 0.91 (Table 4.4). For the FC2 phase the coefficients of determination for each stem remained between 0.66 and 0.91, apart from stem 7 for which $r^2 = 0.27$. In the final phase (D2), the coefficient of determination (r^2) was below 0.4 for all stems.

Table 4.6 Values of the coefficient of determination (r^2) for each stem for linear regressions of sap flow against ET_o for each soil-water treatment phase and for the whole monitoring period 17 July to 16 August 2002.

	Soil-water treatment phase				
Stem gauge and stem number	FC1 r^2	D1 r^2	FC2 r^2	D2 r^2	Whole period r^2
19 mm (18)	0.73	0.82	0.91	0.05	0.75
19 mm (24)	0.69	0.81	0.74	0.20	0.75
16 mm (12)	0.73	0.81	0.74	0.34	0.40
16 mm (15)	0.68	0.77	0.91	0.20	0.78
13 mm (13)	0.84	0.89	0.79	0.06	0.86
10 mm (21)	0.81	0.86	0.86	0.02	0.83
10 mm (25)	0.88	0.77	0.69	0.03	0.69
9 mm (7)	0.71	0.61	0.27	0.23	0.51
9 mm (16)	0.68	0.76	0.66	0.39	0.67

The mean sap flow rates (g stem^{-1} d^{-1}) calculated for each soil-water treatment phase were adjusted, to take into account changes in ET_o, by dividing mean daily sap flow by the mean daily ET_o (mm d^{-1}) for each phase. This is given the term 'transpiration ratio' by Hall *et al.,* (1996; 1998) (Figure 4.11b). An analysis of variance (ANOVA) (StatSoft, Inc, 2005) of mean daily sap flow rates adjusted for ET_o (g cm^{-2} SBA: mm ET_o) showed significant differences ($p<0.05$) between soil-water treatment phases for each stem, except stem 24 (19 mm).

Stem basal area

The plant had 22 stems, which ranged in diameter from 5 to 22 mm (Figure 4.12). The stems selected for sap flow measurements (9-19 mm) provided a good representation of the range and distribution of stems on the plant.

The measurements of stem diameter taken on the 26 June, 2 August and 14 October 2002 indicated an overall increase of stem basal area. A small mean increase in stem diameter of 0.98 mm occurred over 110 days (Table 4.5). Therefore, to simplify the scaling up sap flow measurements made from the individual stems, to derive an estimate of water-use for the whole plant, it was assumed there was no change in stem diameter for the monitoring period (17 July - 16 August). The stem diameters recorded on 2 August 2002 were the values used to calculate stem basal area (SBA).

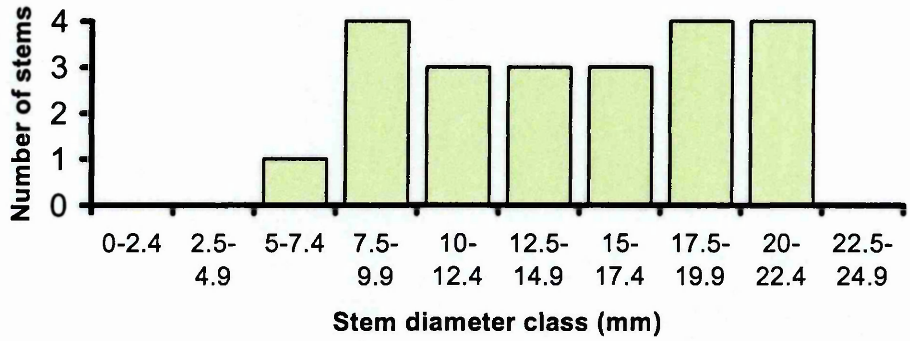


Figure 4.12 Distribution of numbers of stems, for the range of stem diameters, on the coppiced willow plant chosen for this study.

Table 4.7 Mean stem basal diameter measurements for all 22 stems of the plant under study for the period 26 June 2002 to 14 Oct 2002

Date	Mean stem diameter (mm)	Standard error
26-Jun-02	14.7	±1.00
2-Aug-02	15.0	±1.03
14-Oct-02	15.6	±1.06

Leaf area calculations

The leaf area estimates were based on the measurements of cluster length. Leaf areas per stem ranged from 43 cm^2 to 1702 cm^2 with a mean of 555 cm^2. The mean ratio of leaf area: stem base area was approximately 285:1 (Figure 4.13). However, Stem 13 (13 mm) had a LA:SBA ratio of approximately 600:1.

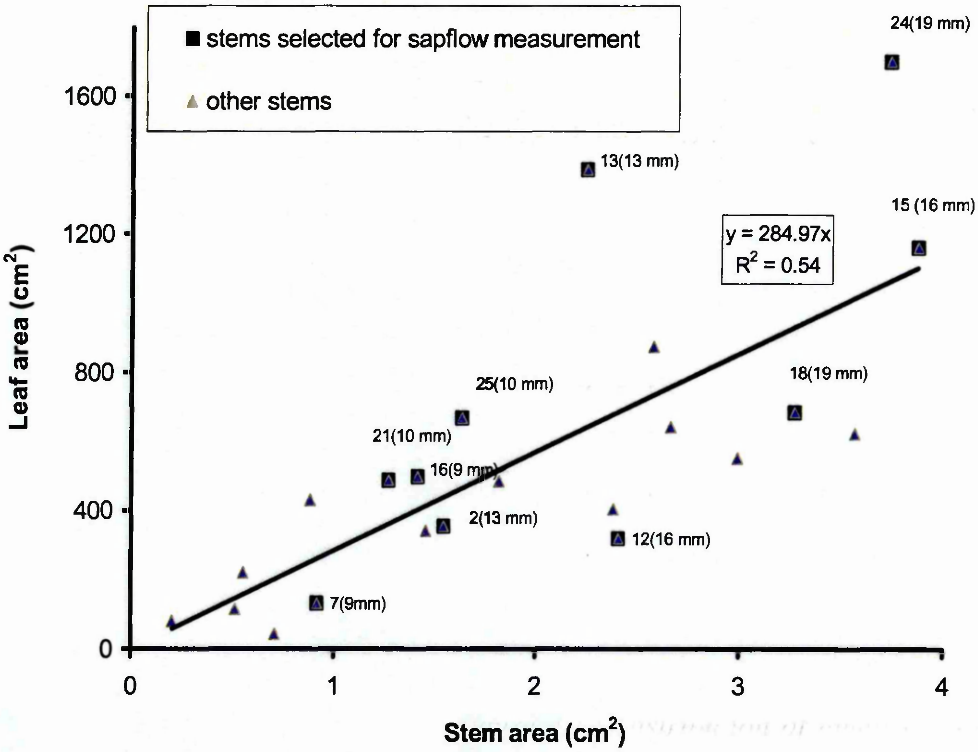


Figure 4.13 Relationship between leaf area and stem basal area of all stems on the plant under observation depicting individual stems selected for sap flow measurements and the size of gauge installed.

Sap flow per unit stem basal area and per unit leaf area

The daily values of sap flow (g stem^{-1}) in Figure 4.10 and the stem diameter measurements taken on 2 August (cm^2 stem^{-1}) were used to calculate the daily sap flow rate per stem per unit SBA (g cm^{-2}). Similarly daily sap flow rates for each stem per unit LA (g cm^{-2} LA) for each phase were calculated from the daily sap flow rates and leaf area estimates for each stem (Figures 4.14a and 4.14b). A wide range of mean sap flow rates were observed between individual stems and between each soil-water treatment phase. An analysis of variance (ANOVA) (StatSoft, Inc, 2005) of both SBA and LA adjusted values.showed significant ($p<0.05$) differences in sap flow rates for each stem between soil-water treatment phases. Daily values per unit SBA ranged from 8 g cm^{-2} SBA recorded for stem 7 (9 mm gauge) during the FC2 phase to 150 g cm^{-2} SBA measured by the stem 24 (19 mm gauge) during the D1 phase. Daily values per unit LA ranged from 0.04 g cm^{-2} LA recorded during the FC2 phase by stem 16 (9 mm gauge) to 0.72 g cm^{-2} LA for stem 24 during the D2 phase.

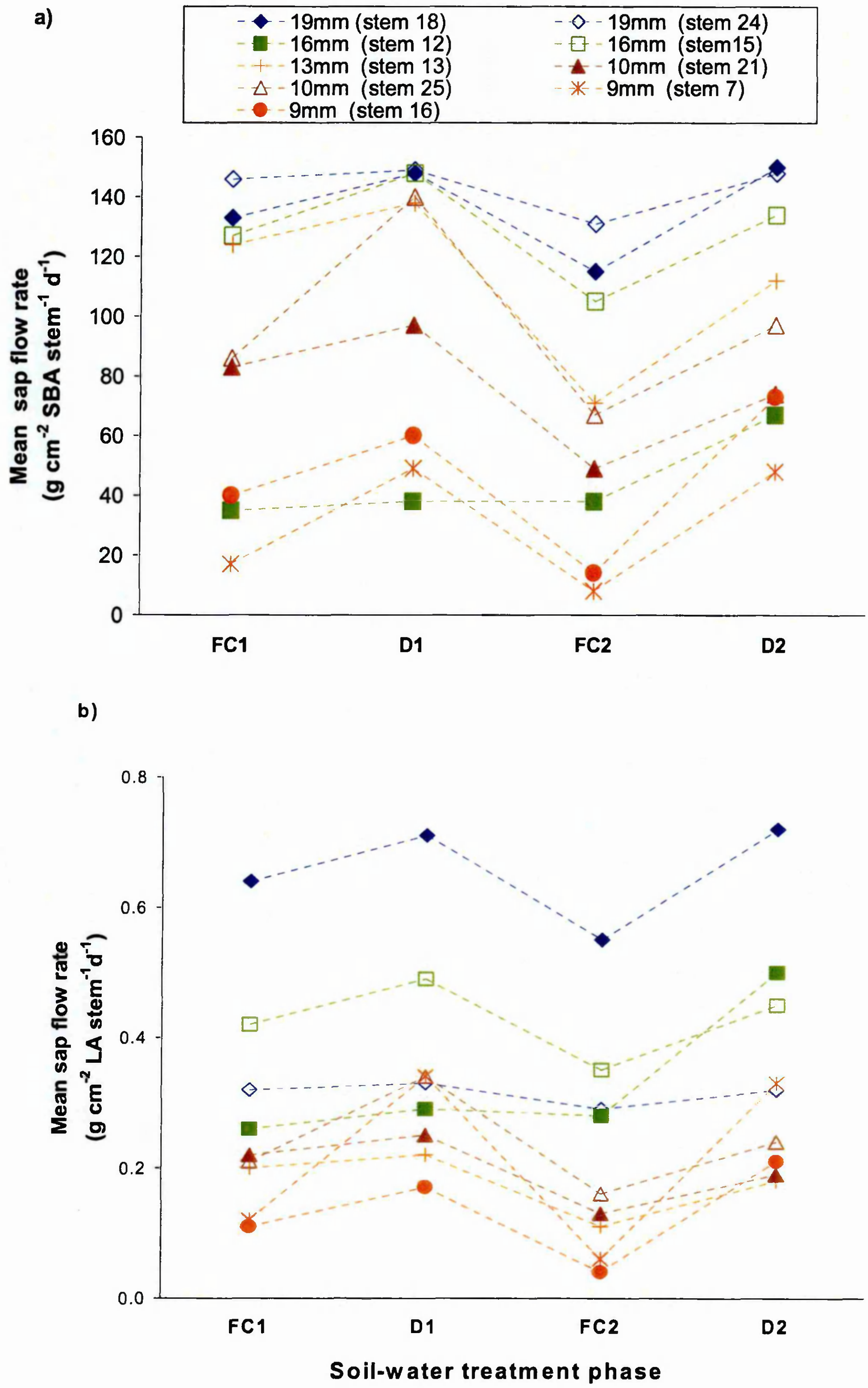


Figure 4.14 a) *Mean daily sap flow (g cm^{-2} SBA stem^{-1}d^{-1}) recorded using SHB sap flow gauges of different sizes for each soil-water treatment phase. b) Mean daily sap flow (g cm^{-2} LA stem^{-1}d^{-1}) recorded using SHB sap flow gauges of different sizes for each soil-water treatment phase.*

Stems were ranked according to the mean daily sap flow rates per unit SBA and LA and homogenous groupings were determined using analysis of variance (ANOVA) post-hoc Fisher LSD test (StatSoft, Inc, 2005) for stems with similar sap flow rates for each phase (Table 4.6a.). It is noted from the ranking of SBA adjusted sap flow that the stems are ranked in order of stem diameter for each of the soil-water treatment phases. The exception to this order was Stem 12 (16 mm) which ranked similar to the 9 mm gauged stems.

Table 4.8 Stems ranked in ascending order of mean daily sap flow rates a) per unit SBA (g cm^{-2} SBA $stem^{-1}d^{-1}$) and b) per unit LA (g cm^{-2} LA $stem^{-1}d^{-1}$) for each soil water treatment phase. Also showing homogenous grouping based on analysis of variance (ANOVA) post-hoc Fisher LSD test (StatSoft, Inc, 2005).

Stem diameter and number	Phase FC1	D1	FC2	D2
a) Stem basal area				
9 mm (stem 7)	17 a	49 a	7 a	56 a
16 mm (stem 12)	35 a	38 a	32 ab	78 ab
9 mm (stem 16)	40 a	60 ab	12 a	85 b
10 mm (stem 21)	83 b	97 b	42 bc	87 b
10 mm (stem 25)	86 b	140 c	58 bc	114 c
13 mm (stem 13)	124 c	138 c	61 c	131 c
16 mm (stem 15)	127 c	148 c	90 d	156 d
19 mm (stem 18)	133 c	148 c	99 d	176 d
19 mm (stem 24)	146 c	149 c	112 d	172 d
b) Leaf area				
13 mm (stem 13)	0.20 a	0.22 a	0.10 a	0.21 a
9 mm (stem 16)	0.23 a	0.34 ab	0.07 a	0.48 b
9 mm (stem 7)	0.24 a	0.69 c	0.10 a	0.77 c
10 mm (stem 25)	0.42 b	0.68 c	0.28 b	0.55 b
10 mm (stem 21)	0.43 b	0.50 bc	0.22 ab	0.44 b
16 mm (stem 12)	0.52 bc	0.58 bc	0.49 c	1.16 d
19 mm (stem 24)	0.64 c	0.66 c	0.49 c	0.75 c
16 mm (stem15)	0.84 d	0.99 d	0.59 c	1.03 d
19 mm (stem 18)	1.27 e	1.41 e	0.94 d	1.65 e

By contrast, when ranking according to LA adjusted values the pattern is less clear (Table 4.6b.). Stem 13 (13 mm) ranks in the lowest grouping for all phases. With the exception of stem 13, all other stems are ranked in ascending order of gauge size for the two field capacity phases. However, for the D1 phase, the mean sap flow per unit LA of stem 12 (16 mm) and stem 24 (19 mm) was similar to the two 10 mm gauge stems and stem 7 (9 mm). In the D2 phase Stem 7 gave significantly higher values than the other 9, 10 and 13 mm stems with a mean sap flow rate corresponding to that of the 19 mm gauged Stem 24.

Estimates of whole plant water-use

Estimates of water use for the whole plant were made by scaling up the calculated totals of sap flow per unit SBA and unit LA for each soil-water treatment phase and for the whole period of monitoring (17 July –16 August). These estimates were then compared to the estimates of plant water-use calculated from the lysimeter water balance (LWB) (Figures 4.15 and 4.16).

On the basis of stem basal area, estimates of plant water-use for the whole period, generated from the smallest (9 mm) stems were lower than values derived from the lysimeter. By contrast the largest (19 mm) stems produced the largest overestimates relative to lysimeter values (Figure 4.15) Estimates closest to the LWB were derived from the intermediate sized stems with the 10 mm stems producing the best estimates. The underestimate generated by Stem 12 (16 mm) appears to be an anomaly with regards the general pattern of larger stems tending to overestimate and smaller stems tending to underestimate LWB values (Figure 4.15). This pattern is observed with the estimates based on leaf area with the four largest stems producing overestimates and the five smaller stems underestimating LWB values. The 19 mm Stem 18 values scaled up on the basis of leaf area showed the greatest deviation from the LWB, whereas improved estimates were generated for Stems 7, 12, 15, 24 and 25 (Figure 4.15).

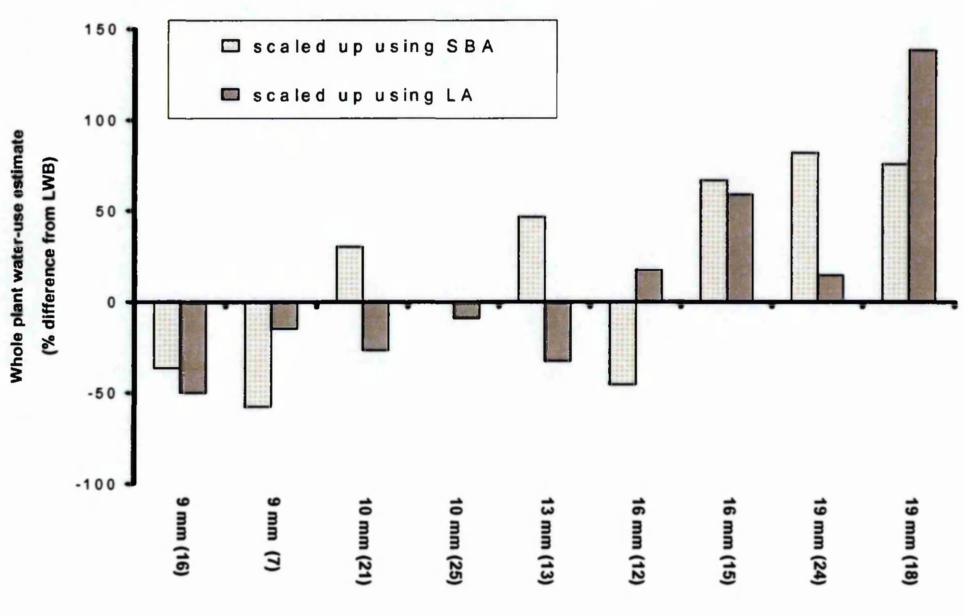
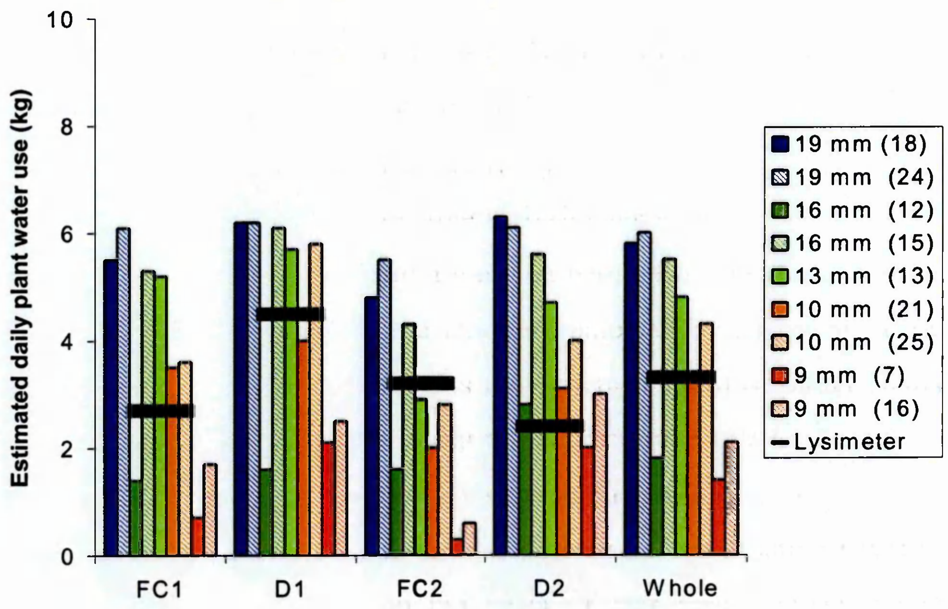


Figure 4.15 Estimates of plant water-use for the period 17 July - 16 August 2002 based on sap flow measurements from individual stems scaled up on the basis of SBA and LA, expressed as % difference of LWB estimates of water-use.

For each of the soil-water treatment phases, estimates derived on the basis of both stem-basal-area and leaf area showed levels of variation relative to LWB values that are not easily predicted (Figure 4.16).

a) Based on stem basal area



b) Based on leaf area

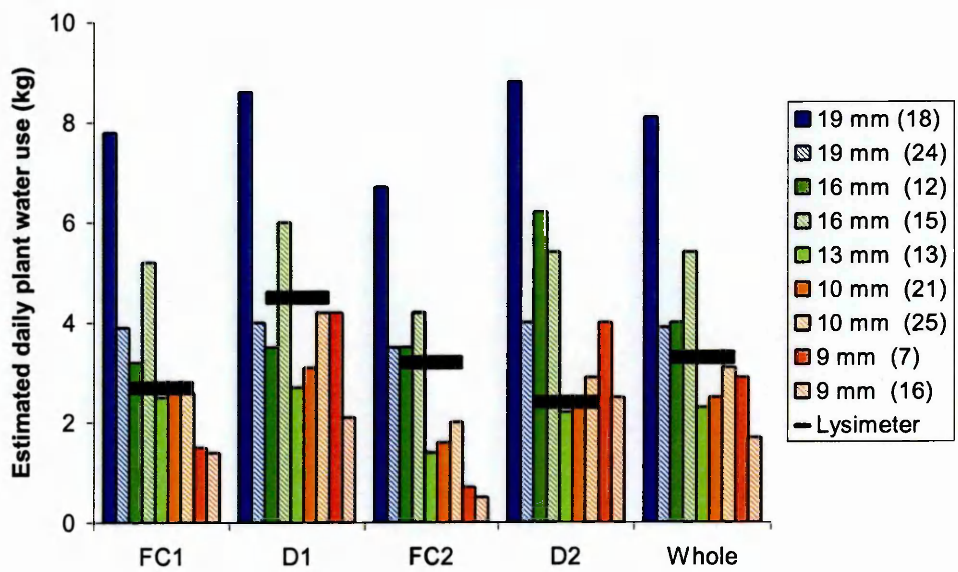


Figure 4.16 Estimates of daily plant water use (kg plant^{-1} d^{-1}), for each gauge, scaled up on the basis of (a) unit stem basal area and (b) unit leaf area together with the estimate of daily plant water use based on lysimeter water balance (LWB).

4.4. Discussion

This section discusses each of the five objectives stated at the beginning of the chapter. Recommendations are then made in the conclusion

Soil-water treatment phases

The first objective was to subject the plant to a wide range of soil-water conditions, from non-limiting to drought approaching permanent wilting point (PWP). The soil water contents indicate that a pattern of wetting and drying was implemented. The low values of soil water content recorded at 30 and 40 cm horizons could be attributed to some variation in soil conditions in the immediate vicinity of the diviner access tube, such as a large stone or air pocket. The equitensiometer results indicated that during the drying phases, the soil matric potential for the majority of the plant root zone was in the upper end of the available water range with values between -10 and –200 kPa. The equitensiometer at the 30 cm horizon however, did measure tensions tending towards permanent wilting point (-1500 kPa).

Lysimeter water balance

The objective was that the lysimeter would provide an accurate measure of plant water-use that could be used to validate the sap flow measurements. However certain limitations of the water balance methodology are evident. Firstly the stemflow and evaporation components of the water balance were assumed to be zero. The stemflow component was assumed to be zero because an attempt was made to divert all water funnelled down the stems, out of the system. Martin and Stephens, (2002) have shown that large volumes of water can track down stems of willow depending on the rainfall intensity. A failure to divert all of this water would have resulted in underestimates of plant water-use. Secondly the mass of water stored in the soil at the start and end of each treatment phase was uncertain, since they were based primarily on the Diviner2000® readings. As previously mentioned potential errors in the 30 and 40 cm horizon readings could influence the lysimeter calculations.

The values of daily plant water-use calculated from the lysimeter were of the same range as reference crop evapotranspiration assuming a ground cover equivalent of 1 m diameter for the canopy of the willow plant (Table 4.9). However whereas the lysimeter estimate of water-use was high in the D1 period, the calculated ET_o rate was similar to the preceding period. This anomaly casts doubt on the integrity of the lysimeter water balance estimates for individual phases. The difficulties in quantifying the soil water capacity at the start and end of each phase may have contributed to error in the LWB estimates for each phase. Also error in quantifying the 'throughfall' precipitation entering the lysimeter (which was highest during the D1 phase) could have resulted in an overestimate of water-use for this phase.

Table 4.9 Estimates of daily plant water use kg plant^{-1} d^{-1} based on lysimeter water balance (LWB) and daily reference evapotranspiration (ET_o)

Soil-water Treatment phase	17-22 July FC 1	23 July-3 Aug D 1	4-9 Aug FC 2	10-16 Aug D 2	Whole period
Lysimeter	2.7	4.5	3.2	2.4	3.3
ET_o assumed over 1 m^2	2.9	3.0	2.2	3.1	2.8

Effect of soil water content on sap flow

The strong correlation between sap flow and ET_o during the first three soil-water treatment phases suggests that the prevailing weather had a dominant effect on daily plant water use. Similar observations were made by Hall *et al.* (1998) in a study using SHB sap flow gauges on field grown coppiced willow. Their study noted that the ratio between the sap flow rate (*T*) and reference evapotranspiration rate (T / ET_o) remained between 1 and 2.5 and showed less variation than T (the measured sap flow rate) through a period of increasing soil water deficit, indicating that transpiration was controlled primarily by the evaporative demand. However Hall *et al.* (1998) did record a transpiration ratio of less than 1 for a period of more severe drought. The poor correlation recorded for all stems for the D2 phase indicates that during this phase the soil water status had a greater influence on plant water-use than the prevailing meteorological conditions.

The ranking of the sap flow of individual stems tended to be correlated with stem size, with the large stems showing the greater sap flow rates. In general the stems showing the greatest sap flow rates under conditions of minimal drought stress were the same stems that showed the greatest sap flow rate under drought stress conditions (Figure 4.16). However there is some evidence that individual stems showed different responses to the imposed changes in soil moisture. This is most apparent from the LA adjusted values of stem 7 (9 mm gauge) which had the lowest sap flow when the soil was wet, and the third or fourth highest sap flow when the soil was dry (Figure 4.16b).

Scaling-up to estimate plant water-use

Scaling up from single stem measurements to the whole plant on the basis of stem basal area led to estimates of daily plant water-use across the whole period ranging from 1.4 to 6.0 kg d^{-1}, compared to a lysimeter estimate of 3.3 kg d^{-1} (Figure 4.16a) The estimate given by the 10 mm gauge on stem 21 (3.3 kg d^{-1}) matched that of the lysimeter.

Errors in estimates of water-use based on scaled up sap flow rates can be attributed to the method for i) calculating sap flow and ii) scaling up. Sources of error in SHB sap flow methodology are documented by Sakuratani (1981), Baker and Neiber (1989), Senock and Ham (1993), Zhang *et al*, (1997) and Grime and Sinclair, (1999). Sakuratani (1981) reported sap flow estimate error within ±10% of gravimetric measurements of water–use of soy bean and sunflower. By contrast, in a study evaluating the use of SHB gauges against lysimeter values for various plant species, Kjelgaard *et al.* (1997) reported relative absolute errors ranging from 6% to over 66%. Hatton *et al.* (1995), in a study investigating the relative performance of several scaling up techniques (including LA and SBA) for estimating the transpiration of a <u>stand</u> of poplar box (*Eucalyptus populnea)* also concluded that the main source of error was from the estimate of individual <u>tree</u> water-use rather than scaling up processes. As stated in the previous chapter, the potentially large errors that can occur during periods of low sap flow rates should be mitigated to some extent by the manufacturers default settings for the 'low flow' filter (Grime and Sinclair, 1999). These were applied to all sap flow calculations presented in this chapter; however the low flow errors are explored in more detail in Chapter 5.

Compared to the estimate of water use for the whole plant derived from the lysimeter, the sap flow, expressed on a per leaf area or per stem basal area basis for an individual stem, tended to be less for small than for large stems (Figure 4.16). Grime and Sinclair (1999) cite research by Groot and King (1992), Shackel *et al.* (1992), Senock and Ham (1993), Weibel and de Vos (1994), Grime *et al.* (1995) and Zhang *et al.* (1997) on the effect of heat 'stored' in the stem tissue on the heat balance calculation.

As reported by Grime *et al.* (1995), the value of the heat energy stored in the stem (Q_s; units: J s^{-1}) can be derived by measuring the change in absolute temperature (as opposed to the differential temperature gradient) of the heated segment of stem. This requires an extra thermocouple to be installed on the stem, at the centre of the heated segment. Q_s is defined as (Equation 4.2):

$$Qs = C_{st}\,(\Delta T_{st}/\Delta t) \qquad \text{Equation 4.2}$$

Where C_{st} is the heat capacity of the stem segment (J K^{-1}); ΔT_{st} is the change in stem temperature during each signal averaging period, Δt. The revised heat balance equation is thus given as (Equation 4.3):

$$C_{st}\,(\Delta T_{st}/\Delta t) = Q_h - Q_{up} - Q_{dn} - Q_{rad} - Q_f \qquad \text{Equation 4.3}$$

Where for a given time period: Q_h is the heat supplied to the stem by the gauge heater, Q_f is the heat convected by the sap flow movements within the stem; Q_{up} and Q_{dn} are the heat conducted up and down the stem through the stem tissue; and Q_{rad} is the radial heat loss from the stem. By including the heat storage term Q_s it is not necessary to assume steady state conditions (i.e. that Q_s is always zero) required for the heat balance calculations used in this study.

Grime *et al.* (1995) report that the inclusion of a stem heat storage term (Q_s) can give improved temporal resolution of instantaneous values of sap flow and improved estimates of cumulative daily sap flow values, especially when sap flow rates are low. It was also found that as stem size increased, inclusion of Q_s became increasingly important. Hence the increasing stem water-use per unit leaf area with increased stem size observed in this study could be due to changes in Q_s. The recommendations made by Grime and Sinclair, (1999) for the inclusion of Q_s however, refer to stem sizes greater than 25 mm. They imply that the maximum expected error for smaller stems, if Q_s is not included in the sap flow calculation, would be approximately 10-15%. The variation in the values reported here is greater than could be explained by Q_s alone and thus, is likely to be due to a combination of factors.

The method of scaling up (either on stem basal area or leaf area) can give substantial differences in the estimate of plant water use. The estimates based on stem basal area ranged from 1.4 to 6.0 kg d^{-1} (–58% to 82% of the lysimeter value), whereas estimates based on leaf area ranged from 1.7 to 8.1 kg d^{-1} (–50% to 138%). The relatively poor correlation (r^2 = 0.54) between SBA and LA of individual stems (Figure 4.13) is a reason for this variation.

As mentioned in Chapter 2, work done by Hatton and Wu (1995) showed water-use of individual eucalypt trees was linearly related to leaf area during periods of abundant soil water, but that the relationship was temporally unstable, becoming non-linear in periods of water deficit. There is some evidence that water-use estimates based on leaf area reported in this chapter were affected by changes in soil water content (Figure 4.16b).

4.5. Conclusions and recommendations

1. Although a range of soil-water contents were imposed, more extreme drought stress should be tested in a future experiment.
2. Refinements are needed to the lysimeter water balance calculations to allow better validation of sap flow measurements. The next experiment should incorporate load cell measurements of the lysimeter, to provide gravimetric data on changes in soil water status.
3. The method employed in this experiment resulted in wide variations in estimated stem water use on a stem-basal area or leaf area basis. Hence using the method cited, it is unwise to estimate the plant water use of a multi-stemmed plant from sap flow measurements of a single stem.
4. Compared to estimates from a lysimeter water balance, there was a tendency for water-use, expressed on a stem-basal area or a leaf area basis, to be underestimated for small stems (9 to 10 mm) and overestimated for large stem (16-19 mm). Using a combination of stem sizes would appear to moderate the final values obtained.

5. VALIDATION OF SAP FLOW MEASUREMENTS

The overall aims of the thesis are to evaluate the reliability of SHB sap flow gauges to estimate water use of a multi-stem plant and to determine best practice when scaling up sap flow measurements taken from stems of a multi-stemmed plant. This chapter describes the experimental programme undertaken in 2003.

5.1. Introduction

Building on the work presented in Chapters 3 and 4, a further study was carried out in 2003. The experiment in 2003 was essentially a re-run of the lysimeter-based experiment in 2002, but included a load cell to determine changes in the mass of the lysimeter. The objective of this was to provide a more reliable validation of the sap flow measurements. A second objective was to increase the range of soil water treatments to examine more fully the hypothesis, stated in the previous chapter, that the transpiration from individual stems, of a multi-stemmed plant, is consistently proportional to stem basal area (SBA) and leaf area (LA).

5.2. Method

Experimental site

This study was again carried out on a single willow plant (*Salix viminalis,* cultivar: 'Jorr'), grown in a lysimeter, at Cranfield University, Silsoe, England (52°0' N; 0°26' W; altitude 60 m). The experimental measurements were taken between July and September 2003. The lysimeter was sited on level ground and was positioned to avoid shading from any other plants.

Meteorological measurements were again taken using a Skye Instruments Mini-Met automatic weather station (AWS) sited close to the lysimeters on a 2 m mast. Air temperature, relative humidity, solar radiation, rainfall and wind run were recorded every 20 minutes and reference crop (grass) evapotranspiration (ET_o) was determined from these measurements using AWSET v3.0 (14) software (Cranfield University, 2002).

Lysimeter and water balance measurements

The lysimeter was the same type used in the two previous experiments. A willow plant established in April 1999 in a sandy loam soil with approximate bulk density of 1270 kg m^{-3} (Martin, 2001) was used. The load cell was supplied by Griffith Elder & Co. Ltd. (Bury St Edmunds, Suffolk, UK.). This was connected to a PC and DATAMAID® software was used to convert the digital signal from the load cell to a mass (units: kg). The load cell range and resolution was 0-500 kg ± 20 g. The lysimeter mass was recorded every 20 mins as the mean value of ten successive readings taken immediately prior to the recorded measurement.

The daily evapo-transpiration from the willow plant (*ET*; units: g d^{-1}) was determined from Equation 5.1:

$$ET = (M_{i-1} - M_i) + I_i + R_i - Es_i - D_i \qquad \text{Equation 5.1}$$

Where M_{i-1} is the mass of the lysimeter at the end of the preceding day, $M_{t=i}$ is the mass of the lysimeter at the end of day i, I_i is the irrigation; R_i is the rainfall; Es_i is the evaporation from the soil, and D_i is the drainage (all on day i and expressed in units of g d^{-1}).

The irrigation, up to 10 kg d^{-1}, was applied manually as a single dose. The amount of irrigation was calculated from the mass of the lysimeter measured by the load cell immediately prior ($M_{pre\text{-}irrigation}$) and immediately post irrigation ($M_{post\text{-}irrigation}$) (Figure 5.1) (Equation 5.2).

$$I = M_{\text{post irrigation}} - M_{\text{pre irrigation}} \qquad \text{Equation 5.2}$$

In a similar way, the value for net rainfall (*R*) was determined from changes in the mass of the lysimeter linked to rainfall events recorded by the meteorological station. Assuming that $M_{pre\text{-}rain}$ and $M_{post\text{-}rain}$ is the mass immediately before and immediately after a rainfall event respectively, the rainfall was defined (Equation 5.3) as:

$$R = M_{\text{post-rain}} - M_{\text{pre-rain}} \qquad \text{Equation 5.3}$$

This negated the need to divert stem-flow water out of the system, as attempted in the previous experiment.

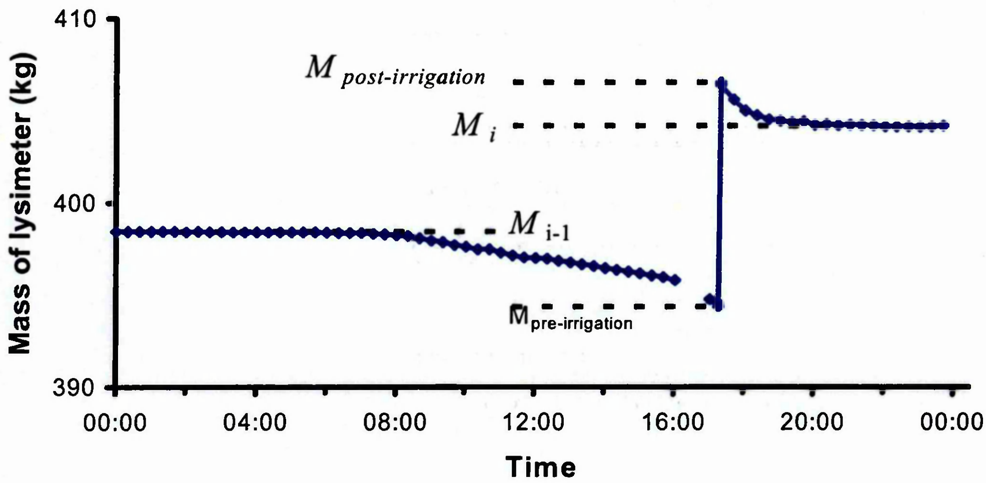


Figure 5.1 Daily change in the mass of the lysimeter, showing the effect of an irrigation application.

The quantity of drainage (*D*), measured using a measuring cylinder, was recorded when the lysimeter gate valve was open. For most of the period of this experiment the gate valve tap was kept shut. A 50 mm layer of 19 mm graded gravel was placed on the soil surface to act as a barrier between the soil surface and the atmosphere to reduce the potential for evaporative losses from the soil. Thus the value of the soil evaporation (*Es*), was assumed to be zero on all days. Equation 5.3 can therefore be re-written to calculate *ET* on the basis of measurements of mass recorded using the lysimeter load-cell:

$$T_i = M_{i-1} - M_i + M_{\text{post irrigation}} - M_{\text{pre irrigation}} + M_{\text{post-rain}} - M_{\text{pre-rain}} - D \qquad \text{Equation 5.4}$$

Soil water status measurements

Soil water content was again measured using a Diviner2000 capacitance probe (Syntec) at 10 cm intervals along a single 60 cm access tube installed vertically in the lysimeter. The soil moisture percentage calculations were made using the standard calibration data provided with the Diviner2000 software.

Three equitensiometers (Delta-T Devices Ltd) were installed as in previous experiment, in the soil profile at depths of 10, 30 and 50 cm. All three equitensiometers were connected to a datalogger and measurements were taken every 20 minutes. The milli-volt outputs of the equitensiometers were converted to kPa using the manufacturer's calibration equation as reported in chapter 4 (Table 4.2).

Three theta probes (Delta-T Devices Ltd) were also installed at depths of 10, 30 and 50 cm in a similar manner to the equitensiometers. These were connected to the same datalogger and measurements were taken every 20 minutes. The milli-volt outputs were converted to values of soil moisture using the instrument calibration equations supplied by Delta-T Devices Ltd for a 'generalised mineral soil type'(Table 5.2)

Table 5.1 Calibration equations supplied by Delta-T Devices Ltd, Burwell, Cambridge, UK for Theta probes installed at three depths in the lysimeter. Where y is the volumetric soil moisturecontent (m^3m^{-3}) and x is the mV output signal from the sensor.

Depth installed	Model number	Calibration equation
10 cm , 30 cm and 50cm	ML2x-UM-1.21	$y= 4.68.10^{-17}x^6 -1.49.10^{-13}x^5 +1.84.10^{-10}x^4 -1.09.10^{-7}x^3 + 3.15.10^{-5}x^2 -3.60.10^{-3}x + 1.41.10^{-1}$

Sap flow measurements

The willow plant chosen for the study had 22 stems ranging from 5.2 mm to 22 mm in diameter. Nine Dynagage sap flow gauges (Dynamax Inc., Houston, TX, USA) were fitted to nine selected stems on the willow plant. The gauges were again installed in accordance with the manufactures instructions, as described in chapter 3. Sap flow measurements commenced on 17 July. There were two gauges of 19, 16, 10, and 9 mm and a single 13 mm gauge. A datalogger (Delta-T Devices Ltd) was used to record the milli-volt output from each gauge. Between July and September readings were recorded each minute and averaged every 20 minutes. Calculations of sap flow were based on the stem heat balance method described in the previous chapter. The non-selected stems were removed for the plant on 30 July to leave the nine stems to which gauges had been fitted.

5.3. Results

Meteorological measurements

For the period 15 July to 29 September 2003, the value of ET_o ranged from 0.5 to 4.1 mm d^{-1} with a mean value of 2.2 mm d^{-1} (Figure 5.2). The weather conditions from 15 July to 1 August was characterised by warm sunny days interspersed by periods of high rainfall intensity. Between 18.00 hrs 16 July and 23.00 hrs 17 July, 77 mm of rain was recorded; between 03.00 hrs and 23.00 hrs on 25 July, 95 mm was recorded; between 16.00 hrs 29 July and 23.00 hrs 30 July, 58 mm was recorded and 92 mm was recorded on 1 August. The rain was virtually continuous for each of these rainfall events with rain recorded in each hour for the duration of each event. A period of drier weather followed through August. Two major rainfall events were recorded in September: 54 mm was recorded between 10 September (02.00 hrs) and 11 September (23.00 hrs), and 76 mm was recorded on 22 September (13.00-23.00 hrs).

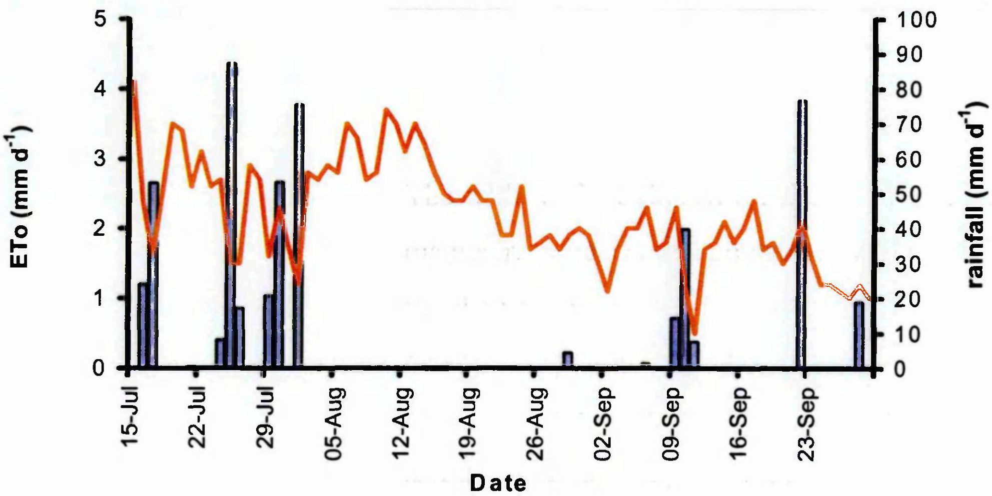


Figure 5.2 Daily reference evapotranspiration values as calculated from data supplied from the AWS from 15 July to 29 September 2003 using AWSET v3.0(14) software (Cranfield University, 2002) together with daily rainfall (mm).

Measurement of water inputs and outputs

The measurement period started on 15 July 2003 (Figure 5.3). The sequence of wetting and drying (Table 5.1) commenced with a period during which the lysimeter was maintained at 'field capacity'. Drainage water was recorded on 18, 21 and 24 July. The 'field capacity' phase was continued until 3 August. The drainage tap was closed on 24

July hence no subsequent drainage was recorded. From 3 to 8 August there were no water inputs to the lysimeter as the plant was subjected to the first drying phase.

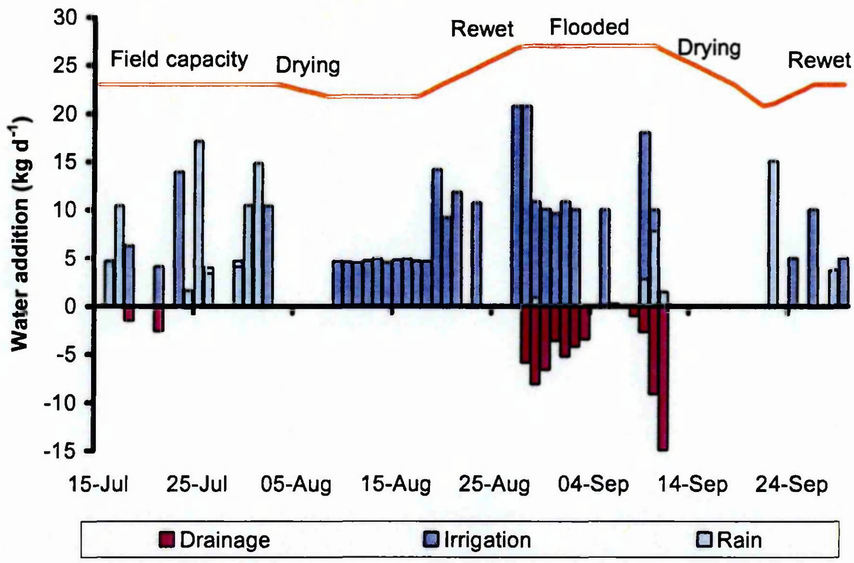


Figure 5.3 Irrigation, rainfall and drainage components of the lysimeter water balance from 15 July to 29 September 2003.

Table 5.2 Description of the seven wetting and drying phases

Time period	Description of phase
18 July to 3 August	Field capacity
4 August to 8 August	First drying
9 August to 17 August	First dry
18 August to 27 August	First re-wetting
28 August to 10 September	Flooded
11 September to 21 September	Second drying
22 September to 29 September	Second re-wetting

Irrigation was applied on 9 August when the willow plant showed the first signs of wilting. Irrigation was then applied late afternoon, daily, at a rate of approximately 5 kg d^{-1}, from 9 to 18 August. The objective of this was to maintain the mean water content close to permanent wilting point but with sufficient water to keep the plant alive. This level of irrigation was based on observations of plant response, allowing visible signs of wilting to occur through the day but alleviating this late afternoon by irrigation. On 19 August the soil was re-wetted and maintained close to field capacity until 27 August. From 27 August to 10 September, water was applied to **saturate** the soil, allowing surface ponding of water. The second drying phase occurred between 11 and 22

September, followed a second wetting stage with rainfall on 22 and 28 September and irrigation on 24, 26 and 29 September.

The lysimeter mass was measured daily using the load cell. It started at 397 kg on 18 July, reached a maximum of 427 kg on 29 August (during the flood treatment), and declined to 373 kg by the end of the second drying period (Figure 5.4).

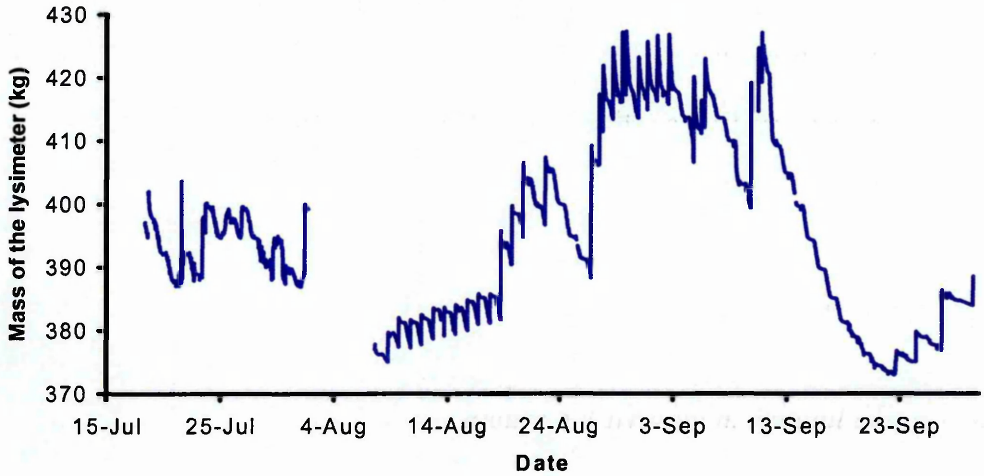


Figure 5.4 Daily weight change in the lysimeter for the period 18 July to 28 September 2003.

Soil water content measurement

The mean soil moisture content of the lysimeter was measured using both the Diviner and theta probes. The moisture content measured by the Diviner2000 probe ranged from a maximum of 47% during the saturated phase down to a minimum of 20% during drought phase (Figure 5.5a). During the period 15 July to 3 August, when the soil was assumed to be at field capacity, the mean moisture content was 33%. The soil moisture values for the 10, 30 and 50 cm soil horizons showed that the sequence of wetting and drying had an effect throughout the whole soil profile (Figure 5.5b). The greatest daily fluctuations in soil moisture content were observed in the 10 cm horizon. The 10 cm horizon also showed the greatest range of values (15.8 - 46.8% compared to 21.3 - 49.3% at 50 cm). The moisture content at 30 cm matched more closely that of the 10 cm horizon (y= 1.03x; r^2 0.8; where 30 cm is x) than the 50 cm (y= 1.27x; r^2 0.75; where 30 cm is x) horizon. However, the magnitude of the daily changes and the range of values recorded at this depth were less (17.3 % - 44.1%) than at 10 cm. For the majority of the period, the soil remained wettest at the 50 cm depth.

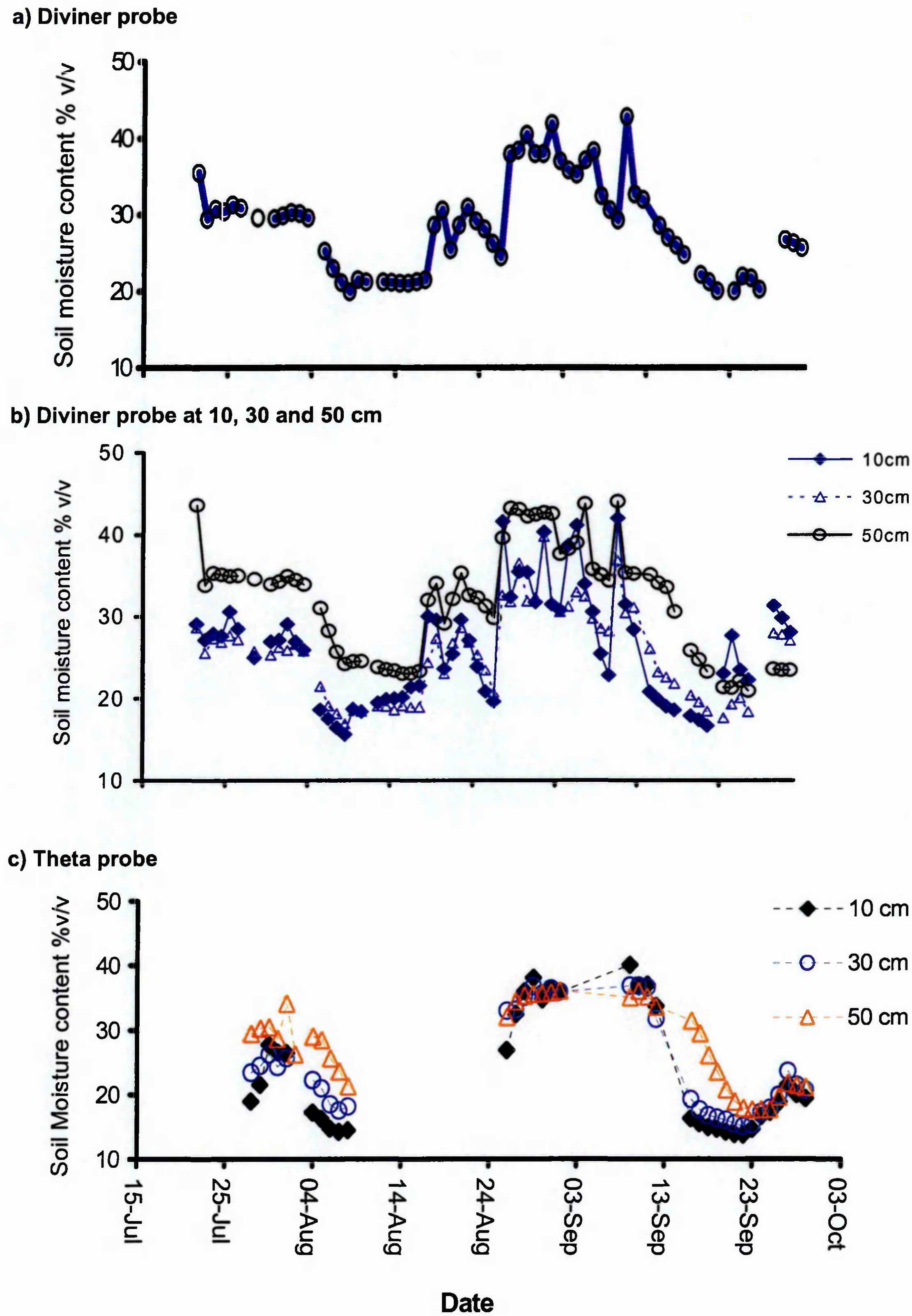


Figure 5.5 Daily values of soil moisture content (% v/v) recorded by the Diviner 2000 probe for a) the whole lysimeter soil profile and (b) three depths (10, 30 and 50 cm)and c) by the theta probe in the lysimeter soil profile during the period 15 July to 29 September 2003.

The daily means of the Theta probes measurements follows the same pattern of wetting and drying indicated by the Diviner, for the three depths; 10, 30 and 50 cm. (Figure 5.5c). The minimum values recorded for the first drying phase were 16, 17 and 24% water content at 10, 30 and 50 cm respectively. For the second drying phase minimum water contents at the same depths were 17, 18 and 22%, indicating increase drying at depth, potentially resulting in more severe drought conditions for this phase. Mean values of 32-48% soil moisture were recorded during the saturated phase.

The soil moisture measurements recorded by the Diviner 2000 were also plotted for key stages of the measuring period, representing each of the wetting and drying phases. As in 2002, the sequence of wetting and drying affected the whole soil profile (Figure 5.6). As with the 2002 readings, the values at 30 cm appear to be consistently drier than the 40 and 50 cm horizons for each stage of wetting and drying.

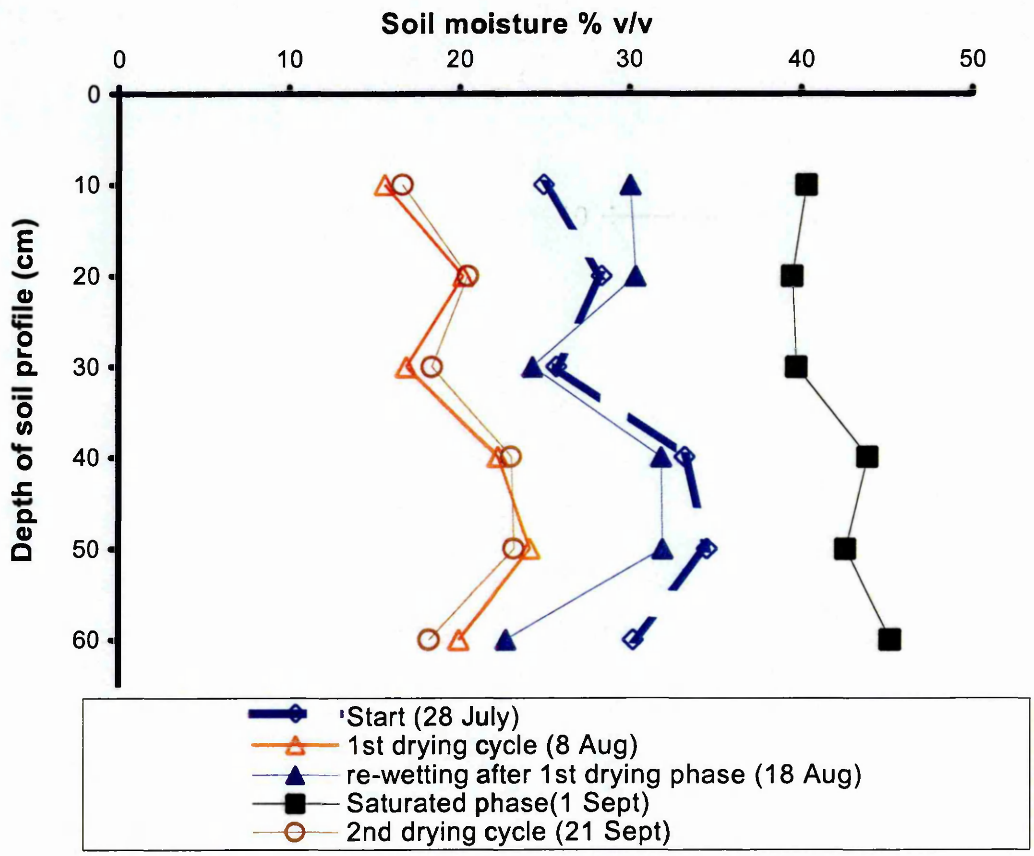


Figure 5.6 Soil moisture content as measured by the Diviner2000 probe for each 10 cm horizon of the 60 cm soil profile for key points in the wetting and drying cycles.

Soil water potential

The soil matric potential measured by the equitensiometers was consistent across the range of depths and closely matched the soil moisture measurements (Figure 5.8). The second drying phase was more severe than the first, in that tension at 10 and 50 cm exceeded permanent wilting point (PWP). The deeper soil horizons dried out more slowly than the 10 cm horizon. This drought and the flooding regime covered a wider range of soil water conditions than in 2002.

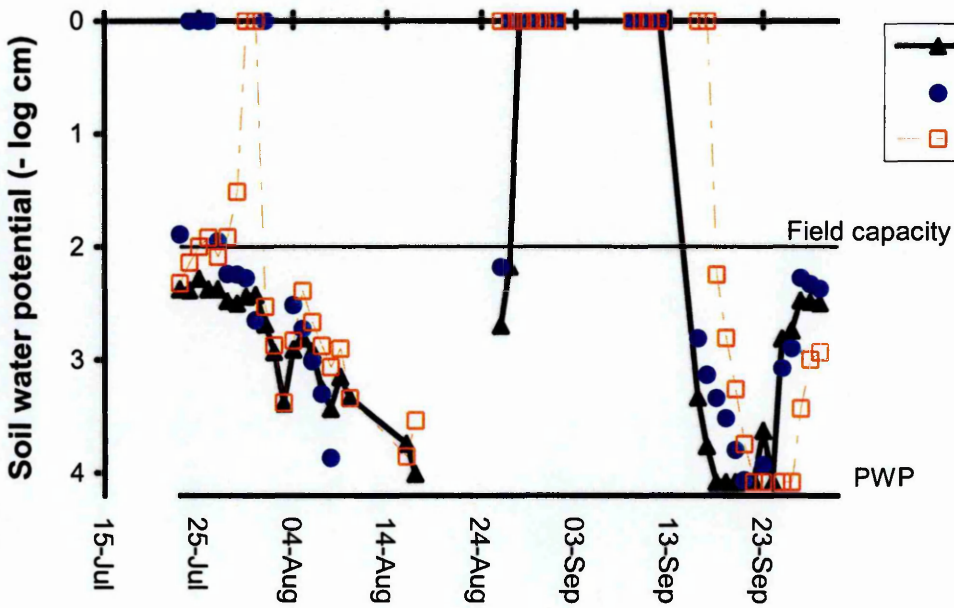


Figure 5.7 Daily mean values of soil matric potential as recorded using equitensiometers at depths of 10, 30 and 50 cm.

Estimates of plant water-use

Lysimeter: daily water-use by the willow was estimated from the lysimeter water balance (LWB) (Figure 5.9b). The estimates of plant water-use ranged from 0 to 8 kg d^{-1}. Problems with the PC resulted in no data from 2 to 7 August inclusive.

Sap flow: the estimates of tree water use using sap flow, ranged from 4 kg d^{-1} on 4 August to 0.32 kg d^{-1} on 22 September (Figure 5.9c). Equipment failure resulted in three periods during which no data were collected (11-16 August; 21-26 August, and 2-9 September). In addition on 11 August, the 19 mm sap flow gauge on stem 4 failed and could no longer be used for the remainder of the monitoring period.

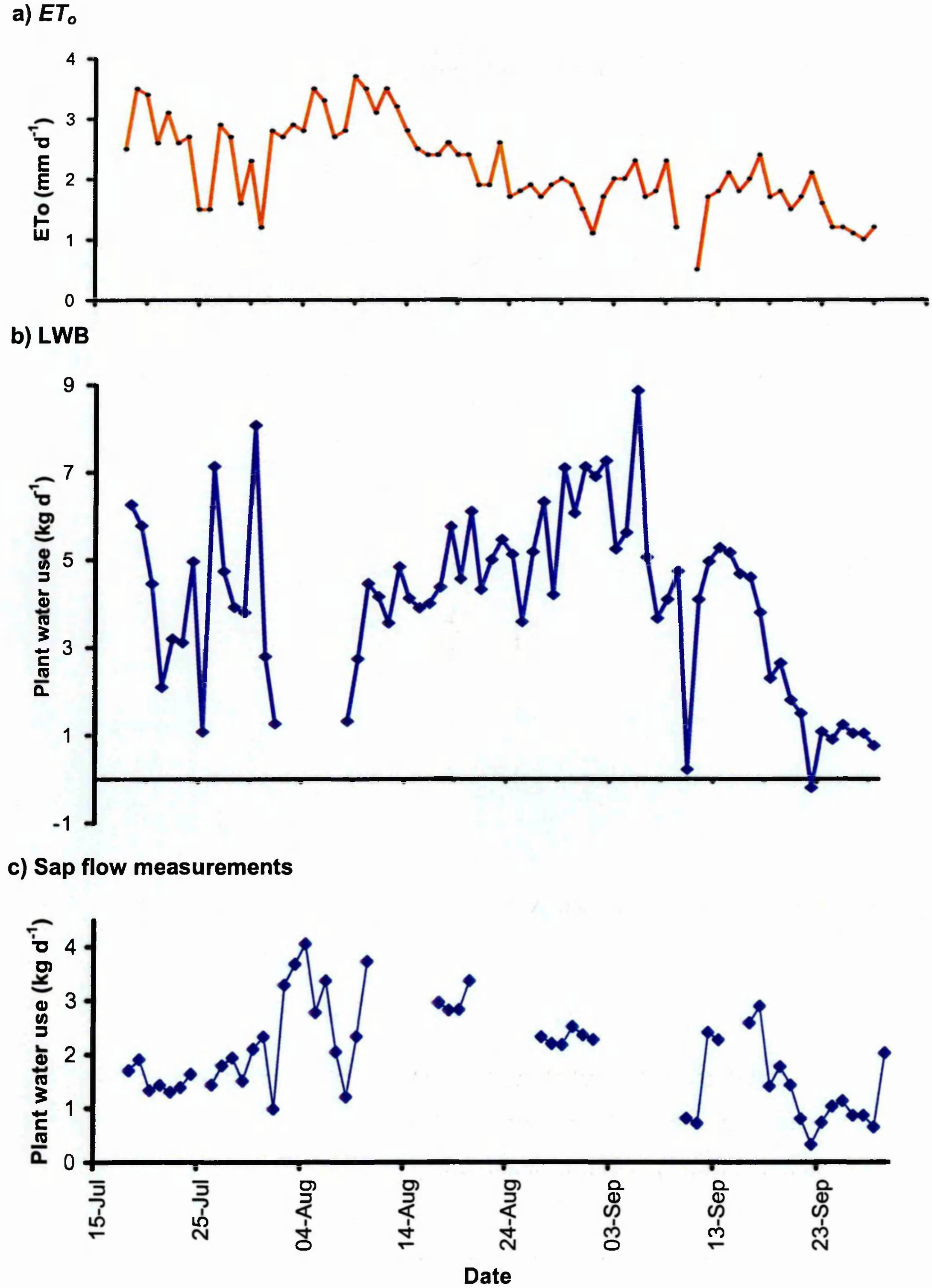


Figure 5.8 a) Reference-crop evapotranspiration ET_o b) Estimates of daily plant water-use derived from lysimeter water balance and c) initial estimates of daily plant water-use derived from the sum of sap flow measurements of all stems, for the period 17 July to 29 September 2003.

Comparison of sap flow and load-cell estimates of water use

A linear regression comparing the lysimeter and sap flow measurements of plant water-use showed that the sap flow measurements tended to result in lower estimates of water-use (y = 0.49x, r^2 = 0.48; Figure 5.9). The relationship before 30 July corresponds to a period when there were 22 stems on the plant. Stems to which SHB gauges were not fitted were excised on the 30 July. During the flooding phase of the measurement period (28 August to 10 September), problems were encountered in ensuring that the lysimeter container was fully sealed. This probably resulted in drainage losses that were not fully accounted for in the water balance calculations. These difficulties were most acute between 27 and 31 August inclusive. By removing these values (Figure 5.9) the correlation coefficient (r^2) between the plant-water-use calculated from sap flow measurements with that from the lysimeter water balance was 0.75. The gradient was 0.6, indicating that the sap flow calculations generally predicted lower water use than the lysimeter.

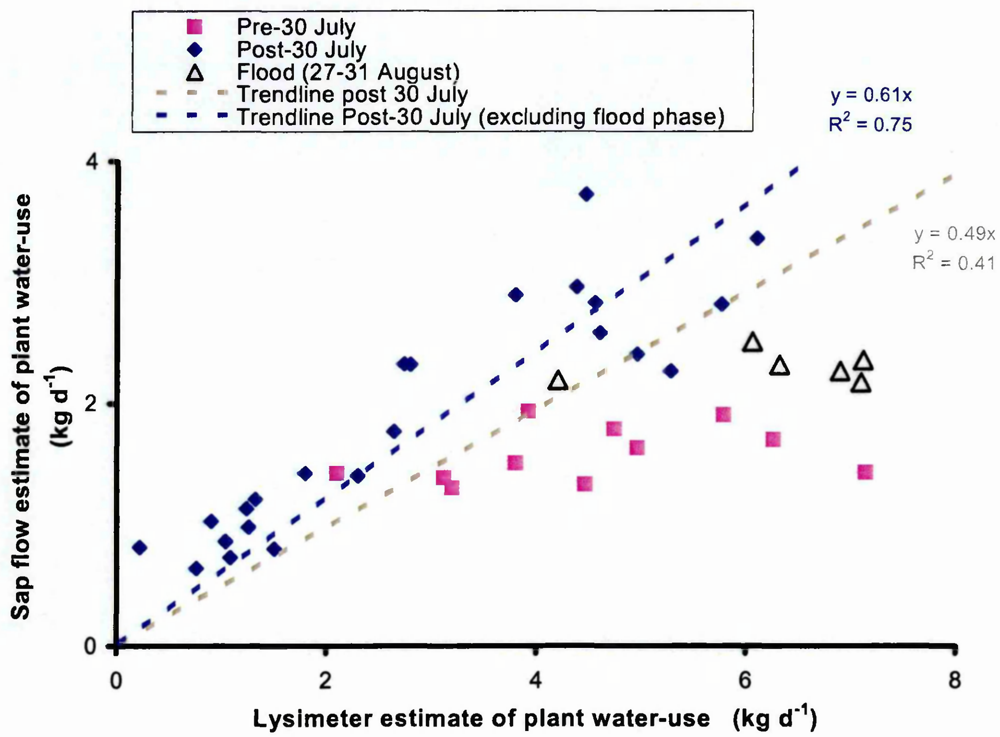


Figure 5.9 Plant water-use estimates derived from SHB gauges against those calculated from the lysimeter for the period 17-30 July (pre-pruning of stems not selected for sap flow measurements) and 31 July to 29 September 2003 post pruning. Also highlighting values recorded through the flood phase.

The daily records were further analysed in order to identify possible sources of error that could explain this under-estimation in the sap flow calculations. A typical example of sap flow and load cell data for one day is plotted in Figure 5.10. This shows that for the period from midnight to midday, the water use predicted by the sap flow matches that recorded by the lysimeter load cell. However from midday to 19.00 hrs the sap flow rate appears to slow relative to that recorded by the load cell. After 19.00 hrs, the sap flow appears to be zero whereas the load-cell continues to records mass changes through to midnight.

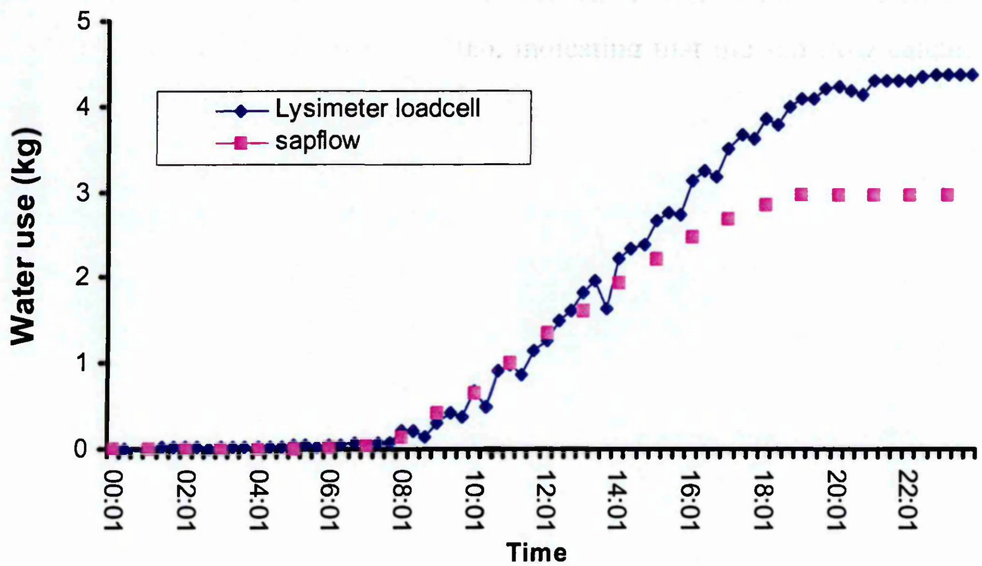


Figure 5.10 The cumulative weight change recorded by the lysimeter load cell and estimates of daily water-use calculated from sap flow measurements, for the 17 August 2003

Modification of K_{app}

As stated in Chapter 2, the calculated value for sap flow, for a specific period of time, is based on the Q_f value (the heat convected by sap flow in the stem) in the heat balance equation (Equation 5.5).

$$Q_f = Q_h - Q_{up} - Q_{dn} - Q_{rad} \qquad \text{Equation 5.5}$$

Where Q_h is the heat supplied to the stem by the gauge heater; Q_{up} and Q_{dn} represent the measured values of the heat conducted up and down the stem respectively through the stem tissue respectively; and Q_{rad} is the radial heat loss from the stem.
The value of Q_{rad} is the product of two components (Equation 5.6)

$$Q_{rad} = K_{rad}\, \Delta T_{rad} \qquad \text{Equation 5.6}$$

Where: ΔT_{rad} is the temperature difference between the heater element and the insulation material of the gauge. This value is recorded by the datalogger from the radial thermocouple measurements. The thermal conductivity of the gauge materials (K_{rad}) is an estimated value. It is also determined from the heat balance equation when the sap flow component (Q_f) is assumed to be zero, hence:

$$K_{rad} = \frac{Q_h - Q_{up} - Q_{down}}{\Delta T_{rad}} \qquad \text{Equation 5.7}$$

K_{rad} is assumed to be a constant for individual gauge installations (Dynamax inc., 1997; Grime and Sinclair, 1999). The sap flow calculation software supplied with the Dynagages produces a K_{app} value for each recorded measurement (i.e. every 10 minutes in the case of this experiment). The K_{app} is the calculated value for K_{rad} and assumes Q_f is always zero. From these calculated K_{app} values the user is advised to select a K_{app} for a period when it is assumed Q_f is zero (suggested to be early in the morning before sunrise). The original values of K_{rad} used in this experiment, shown in Table 5.2, were based on approximate mean values of K_{app} observed between 01.00 and 03.00 hrs for the first five nights post installation as recommended in the user manual (Dynamax 1999). Sap flow was assumed to be close to zero at this time.

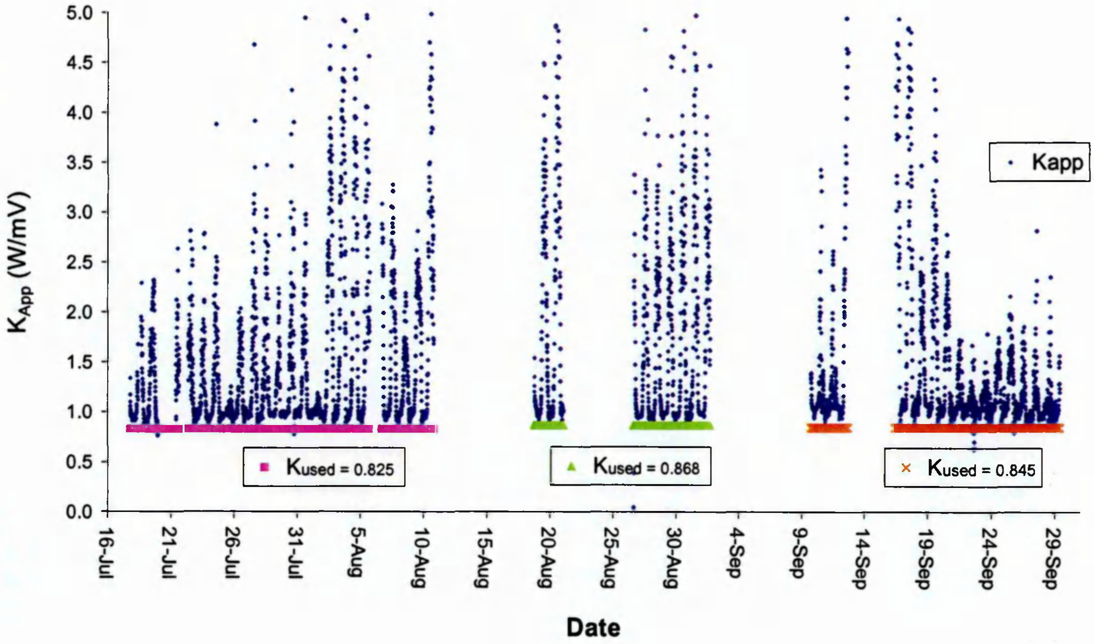
Table 5.3 Original values of $K_{rad\text{-}used}$ ($J\ s^{-1}\ °C^{-1}$) selected for the thermal conductivity of the gauges, applied as a constant in each calculation of sap flow throughout the whole measurement period 17/07/03-29/09/03, together with the temporal changes in $K_{rad\text{-}used}$ based on minimum K_{app} observed.

Stem number	Gauge Size (mm)	Original K_{rad}	$K_{rad\text{-}used}$ 17/07 – 28/07	$K_{rad\text{-}used}$ 29/07 – 10/08	$K_{rad\text{-}used}$ 16/08 – 01/09	$K_{rad\text{-}used}$ 01/09 – 12/09	$K_{rad\text{-}used}$ 12/09 – 29/09
2	19	1.550	**1.201**	**1.201**	1.298	**1.840**	**1.840**
4	19	1.100	0.787	**0.956**	**0.956**	**0.956**	**0.956**
5	16	1.060	**0.803**	**0.803**	0.850	**0.868**	**0.868**
8	16	1.020	**0.825**	**0.825**	0.868	**0.845**	**0.845**
20	13	1.015	**0.794**	**0.794**	0.874	**0.657**	**0.657**
7	10	0.960	0.842	0.903	0.911	**0.989**	**0.989**
16	10	1.010	**0.918**	**0.918**	0.922	1.095	0.990
13	9	1.147	1.097	0.998	0.995	**1.007**	**1.007**
15	9	1.288	1.030	1.101	1.154	**0.794**	**0.794**

Note: emboldened values remained unchanged between consecutive periods.

Closer examination of the range of the K_{app} values generated indicated that, for the majority of gauges, there were changes in the thermal conductivity during the course of the experiment. Re-evaluation of K_{app} was carried out in order to determine a specific value of K_{rad} for the distinct time periods when these changes occurred. Grime *et al.*, (1995) used minimum K_{app} values to estimate $K_{rad\text{-}used}$ when small amounts of night-time sap flow was apparent (i.e. there was no true zero sap flow state) and concluded that the resultant error in daytime sap flow was not significant. To avoid using extreme minimum K_{app} outliers it was decided to use the median of the minimum K_{app} values calculated for each time period (Table 5.2). Figure 5.12 shows the K_{app} values generated over the monitoring period for Stem 8 (16 mm gauge) and Stem 15 (9 mm gauge), together with the $K_{rad\text{-}used}$ values selected for each distinct time period. The $K_{rad\text{-}used}$ values for Stem 8 remained relatively constant compared to those of Stem 15.

a)



b)

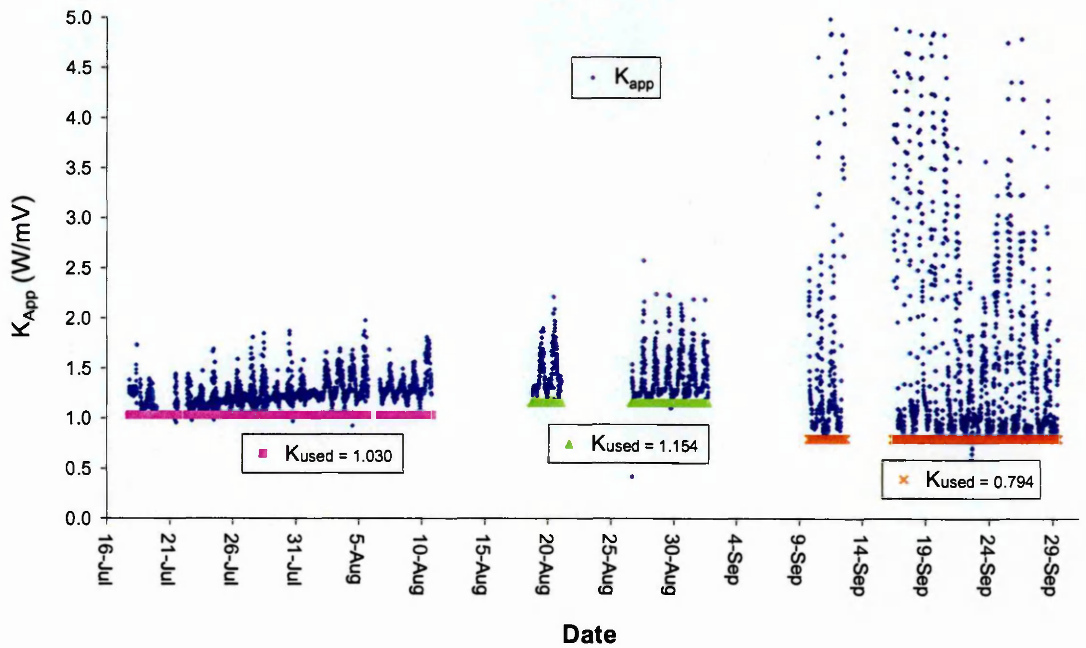


Figure 5.11 Values for the coefficient of radial heat loss (K_{app}) calculated by assuming is sap flow (Q_f) is always zero, for the monitoring period 17/07/03-29/09/03 for: a)Stem 8 (16 mm gauge) and b) Stem15 (9 mm gauge). The values for the coefficient of radial heat loss ($K_{rad-used}$) selected for the calculation of Q_f, for each gauge, are also indicated for each distinct time period (17 July - 10 Aug; 16 Aug - 01 Sept; 09 Sept - 29 Sept).

Correction factor for K_{rad}

A second possible source of error is in the choice of the K_{rad} value. In order to achieve the 'best fit' between the load-cell and sap flow values of water use, a correction function "*C*" was introduced. This was defined as the ratio of $K_{rad\text{-}best\ fit}$ to the $K_{rad\text{-}used}$ value (determined from K_{app} described above and presented in Table 5.2).

$$C = \frac{Used\ value\ of\ K_{rad}}{Original\ value\ of\ K_{rad}} \qquad \text{Equation 5.8}$$

A series of values for *C* ranging from 0.8 to 1.4 were used to determine the resulting correlation between calculated sap flow rates and the load-cell estimate of tree water use. The effect of changing *C* on the calculation of sap flow for the measurements taken on 17 August 2003 is illustrated in Figure 5.12. When using a value for *C* of 1.0 (i.e. the $K_{rad\text{-}used}$ values for each stem equal the values shown in Table 5.2 for the period 16/08 – 01/09), the calculated sap flow was greater than the water-use recorded by the load-cell. Decreasing the value of *C* to 0.8 resulted in increased discrepancy between sap flow and lysimeter estimates of water use; which was most acute when the load cell indicated plant water use close to zero. Increasing the value of *C* to 1.2, 1.3 and 1.4 resulted in progressively lower estimates of total water use for the day, although for periods of low or zero sap flow rates a closer match to the lysimeter data is achieved. A value for *C* of 1.1 gave the 'best-fit' between sap flow and lysimeter estimates of water-use, in terms of the total amount of water used on this particular day.

When a correction factor (*C*) of 1.1 was applied to the K_{rad} values, the correlation between sap flow (kg d^{-1}) and load-cell measurements of tree water use (kg d^{-1}) gave Equation 5.9 (Fig 5.14).

$$\text{Sap flow} = 0.69\ \text{Load-cell estimate} + 0.848\ (r^2 = 0.88) \qquad \text{Equation 5.9}$$

This indicates that the sap flow method still underestimated water use relative to the load-cell data. Use of the best-fit value for *C* also created an 'offset', in that when the load cell recorded zero water-use, the sap flow measurements would indicate water use of about 0.85 kg d^{-1}. Further refinement of the sap flow calculation is therefore needed to reconcile sap flow measurements with the lysimeter water balance.

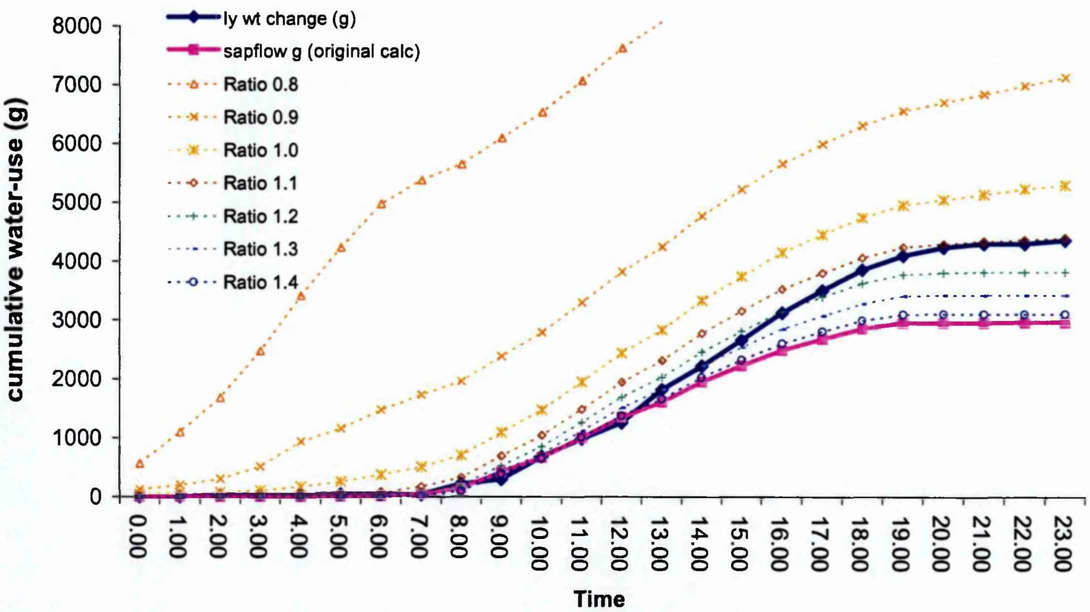


Figure 5.12 Cumulative change in lysimeter mass recorded by the lysimeter load cell and estimates of daily water-use calculated from sap flow measurements for a range of C values together with the original calculation of sap flow 17 August 2003.

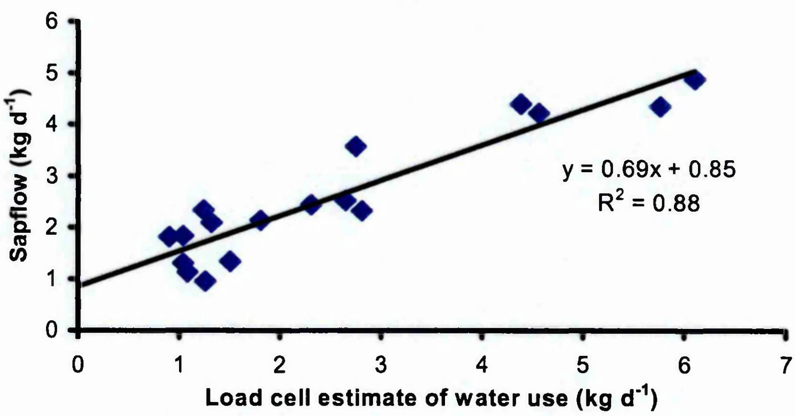


Figure 5.13 Relationship between calculated plant water use calculated using the load cell data with that from sap flow measurements, for the period 31 July to 29 September 2003 (excluding values from the 'flood phase' from 27-31 August) using revised values of $K_{rad\text{-}used}$, and a correction factor of 1.1

Excluding error associated with low sap flow-rates

During periods when sap flow rates approach zero the change in sap temperature, dT, measured by averaging the temperature measurements made by the Ha and Hb thermocouples (Figure 2.4) approaches zero. During these periods, a small residual of convected heat-flow (Q_f), accounting for only a small proportion of the power input (P_{in}) can predict exaggerated sap flow values (Dynamax Inc., 1997). A "low-flow filter" is therefore incorporated into the sap flow calculation to assume a zero sap flow rate when the ratio of Q_f to P_{in} goes above a prescribed value as dT approaches zero. The "filter" default setting is $Q_f < 20\% \, P_{in}$ and dT is less than a minimum value (dT_{min}) of 0.75°C.

In an attempt to remove the low-flow discrepancies reported above, various manipulations of the low flow filter were made. To be consistent for each stem, the maximum level of the filter was set to $Q_f < 100\% \, P_{in}$ for all stems. The dT_{min} value was set at 1.0°C for the 19 mm and 16 mm gauges and 0.8°C for the 13, 10, and 9 mm gauges (i.e. proportional to the power input supplied to the different sized gauges). By applying this low flow filter, significant improvements were made to the relationship between estimates of water use derived from the lysimeter water balance and sap flow calculations (Figure 5.15). The linear regression had gradients close to 1, based on values for C of 1.0 to 1.15, with r^2 values ranging from 0.93 to 0.94. With the low flow filter applied, the best fit sap flow results were achieved using a value for C of 1.1, giving Equation 5.10.

Sap flow estimate= 0.986 lysimeter estimate (r^2 =0.94). Equation 5.10

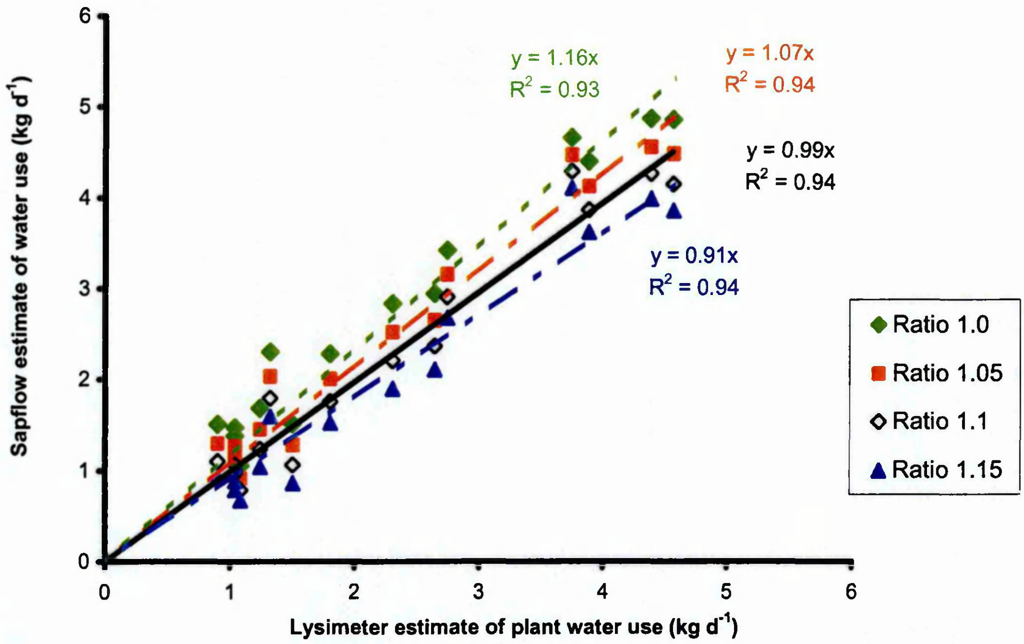


Figure 5.14 Relation between daily plant water use estimated from load cells with that from sap flow measurements, for the period 31 July to 29 September 2003 (but excluding values from the 'flood phase' from 27-31 August) using revised values of $K_{rad\text{-}used}$ ($K_{rad\text{-}best\,fit}$) with adjusted 'low flow filter'

5.4. Conclusions

1) In validating the sap flow measurements against the lysimeter water balance a coefficient of determination of 0.94 was achieved by careful selection of $K_{\mathrm{rad\text{-}used}}$ values and the imposition of a 'low flow filter'.
2) The default values for the low flow filter did not give the best correlation with lysimeter data and adjustments were needed to improve the relationship. The filter was applied differently to the larger gauges (19 and 16 mm gauges) than to the smaller gauges to provide the best fit relationship between sap flow and lysimeter estimates of plant water use.
3) Calculations and observations made in Chapter 6, of individual stem responses throughout the monitoring period, are based on sap flow measurements generated using the low flow filter as described in this chapter and using K_{rad} ratio of 1.1.

6. AUTONOMY OF INDIVIDUAL STEM SAP FLOW RESPONSES

This chapter describes further the experimental programme undertaken in 2003, with a focus on the sap flow responses of individual stems.

6.1. Introduction

As stated in Chapter 5, the aim for the study in 2003 was to assess the reliability of using sap flow measurements to estimate plant water use of a multi-stemmed plant. The objective in this chapter is to determine the level of variation between measurements of sap flow made on individual stems of a multi-stemmed plant when subjected to a wide range of soil moisture conditions and the impact of any such variation on scaled up estimates of plant water use. The research hypothesis, based on observations reported in chapter 3, is that transpiration from individual stems of a multi-stemmed plant is not consistently proportional to the leaf-area and/or the basal area of each stem across a broad range of soil water availability

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6.2. Method

The main method is fully described in chapter 5. This was broadly similar to the 2002 experiment but with the lysimeter being mounted on a load cell. Also, in response to the recommendations made in chapter 4, the range of soil water conditions imposed in this experiment was greater than that achieved in 2002. Daily sap flow measurements for the whole plant were validated against the lysimeter water balance as described in Chapter 5 with a high degree of correlation ($r^2 = 94\%$).

The sap flow of individual stems were measured and used to determine the contribution made by each stem to the total daily water use of the whole plant throughout the monitoring period. Estimates of water-use made for individual stems were then expressed as a proportion of both stem basal area and leaf area for the whole plant. These adjusted values were then scaled up to give estimates of water use for the whole plant based on single stem sap flow measurements. Comparison with lysimeter plant water-use estimates were then made to evaluate the reliability of single stem sap flow measurements in estimating total plant water-use through a range of growing conditions.

Stem basal area measurements

Initial measurements of stem diameter were taken on 8 July at gauge height and stem base for each stem, using digital vernier callipers (Table 6.1). Subsequent measurements of stem base diameter were taken on 15 July and 29 September 2003. These measurements were used to calculate stem basal area allowing plant growth rate to be estimated (Figure 6.3).

Table 6.1 Initial diameters recorded 8 July 2003 of stems selected for sapflow measurement. Showing nominal size of gauge installed, diameter at gauge height and at stem base for each stem.

Stem no.	gauge size	Diameter at gauge (mm)	Diameter at base (mm)
2	19 mm	21.2	19.0
4	19 mm	25.2	19.6
5	16 mm	18.5	16.0
8	16 mm	20.2	17.9
20	13 mm	17.8	14.1
7	10 mm	15.3	12.1
16	10 mm	9.9	9.7
13	9 mm	10.0	9.9
15	9 mm	10.1	9.6

Leaf area calculations

The cluster lengths of the leaves on each stem were measured on 29 July, 7 August and 26 August. The leaf area throughout the measurement period was then calculated using the relationship between cluster length and leaf area established in the 2002 experiment (Figure 4.6). Additional measurements were taken from clusters harvested from the stems removed from the plant at the beginning of the experiment. These extra measurements increased the range and number of data points included in this relationship. This method was adopted to provide a relatively quick estimate of the leaf area which avoided the necessity of measuring large numbers of leaves per stem, which would have been prohibitively laborious and time consuming.

6.3. Results

The daily totals of sap flow for the individual stems shows that through most of the monitoring period the sap flow of individual stems were ranked in order of stem diameter, with daily sap flow totals increasing with increasing stem size (Figure 6.1). This remained true through the period when the soil was maintained at field capacity and also through most of the first drying phase. One exception to this was stem 20 (13 mm gauge) which during the first few days of the monitoring period occasionally recorded daily sap flow rates less than the 10 mm gauge on stem 16. On 10 August, during the first dry phase, the sap flow measured for the 13 mm gauge on stem 20 (501 g d^{-1}) was greater than that of stem 5 (16 mm gauge) (473 g d^{-1}). At the end of the dry phase and for the first three days of the subsequent re-wetting phase, the sap flow rates for stems 20 and 5 were similar.

At the end of the first 're-wetting' phase and through the first five days of the 'flooded' phase the daily sap flows per stem were clearly ranked in order of stem size. However by the end of the 'flooded' phase the order was disrupted when stem 2 (19 mm) and stem 15 (9 mm) changed their rank. This changed order was maintained for the rest of the monitoring period (Figure 6.1a).

On 22 September, i.e. at the end of the second, more severe drying phase, stem 2 (19 mm), stems 7 and 16 (10 mm) and stem 13 (9 mm) recorded zero sap flow. A partial recovery of sap flow was observed in stem 2 during the subsequent re-wetting phase, but this was slower than that found with stems 7 and 16. Only a very limited recovery was observed for stem 13. Stem 15 (9 mm gauge) showed a markedly different response to the flood phase, in that the sap flow rate recorded at the end of the flood phase (284 g d^{-1} on 10 September) was greater than that recorded at the beginning (125 g d^{-1} 0n 30 August).

Throughout the second drying and rewetting phases, Stem 15 responded with sap flow rates that corresponded closely to the 16 mm gauged stems and for much of the time recorded the highest sap flow rates of all stems through these periods.

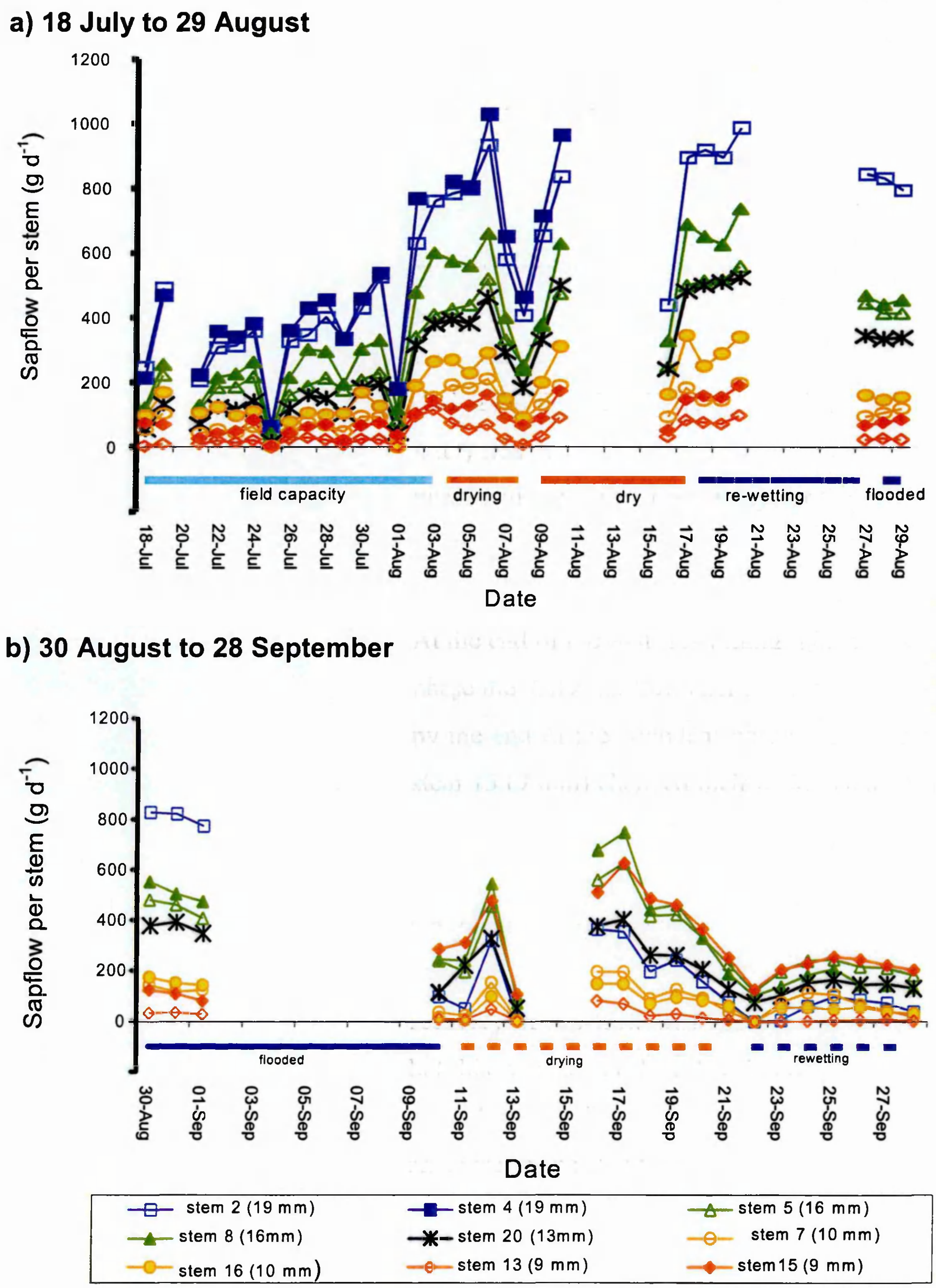

Figure 6.1 Estimates of daily water-use calculated from sap flow measurements for the individual stems of the coppice willow plant for the various phases of wetting and drying imposed from 18 July to 29 September 2003. (Gaps in data throughout the study period were due to temporary system failure caused by a variety equipment faults).

The relationship between sap flow for each individual stem (g d^{-1}) and sap flow of the whole plant (g d^{-1}) was plotted for: a) the whole monitoring period, b) the 'unstressed' phases including; the field capacity phase, the first drying and dry phase, the re-wetting phase and the first five days of the flood phase (i.e. 18 July to 1 August 2003), c) the 'stressed' phases including; the end of the flood phase, the second (more severe) drying phase and the second rewetting phase (i.e. 10 August to 28 September 2003) (Figure 6.2). An analysis of variance (StatSoft, Inc, 2005) indicated that for each stem, except stem 13, there was a significant ($p < 0.05$) change in relationship between the sap flow response (g d^{-1}) of the individual stems and that of the whole plant for the 'stressed' and 'unstressed' periods. For stems 2 (19 mm), 5 and 8 (16 mm), 20 (13 mm) and 15 (9 mm) the differences were very significant ($p < 0.001$).

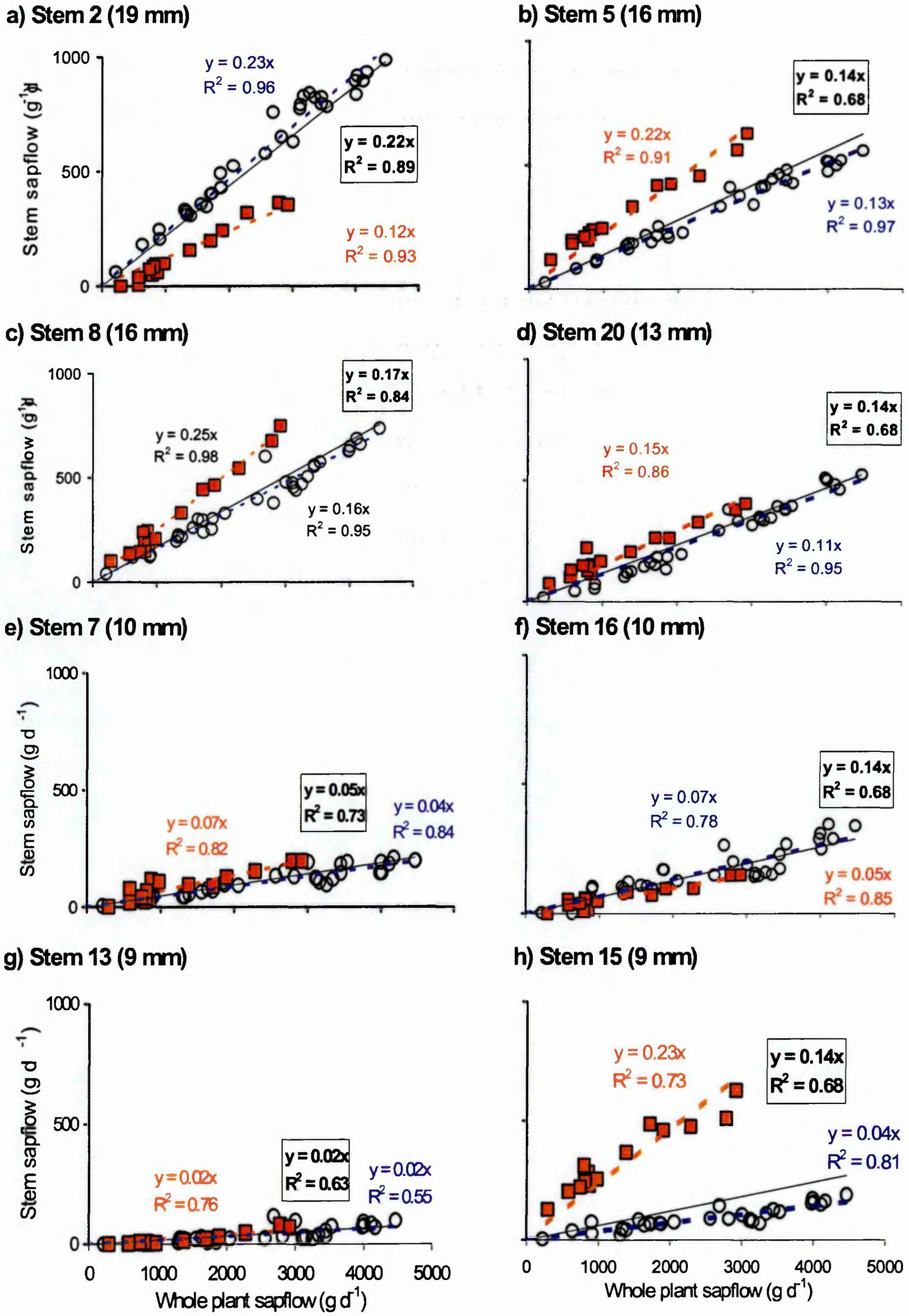

Figure 6.2 Relationships between daily sap flow for each individual stem and daily sap flow for the whole plant, plotted for: a) the whole monitoring period,(solid line) b) the 'unstressed' phases (open circles) (i.e. 18 July to 29 August 2003), c) the 'stressed' phases (solid squares) (i.e. 30 August to 28 September 2003).

Stem basal area measurements

Using the stem diameter measurements taken at the beginning and end of the monitoring period stem basal area (SBA) was calculated for each stem. The growth rate of each stem was estimated using a linear regression (Figure 6.3). These equations were then used to estimate SBA on a daily basis through the monitoring period.

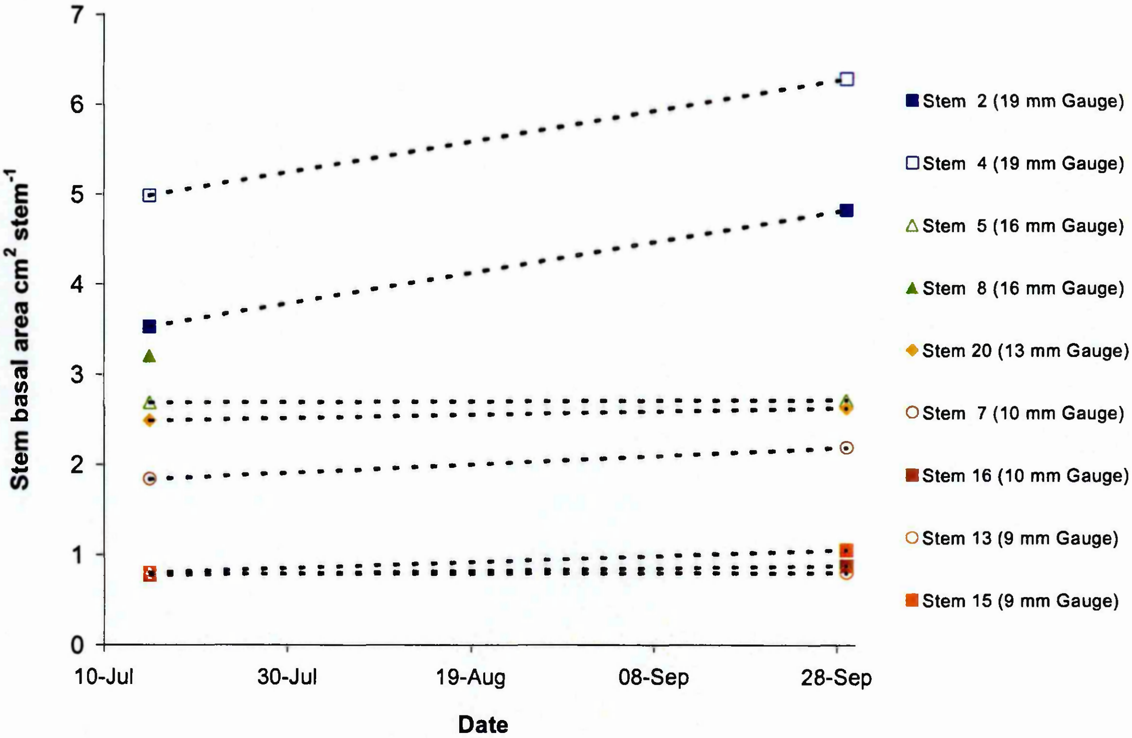

Figure 6.3 Linear regression used to estimate individual stem basal area growth rates for the period 15 July – 29 September 2003

The daily sap flow rate per unit stem basal area (units: g d^{-1} cm^{-2}) of each stem showed substantial variation above and below that calculated for the whole plant (Figure 6.4). As a general observation, the small stems (9 and 10 mm) tended to show the greatest deviations in daily sap flow per unit stem basal area from the mean value for the whole plant.

The relationship between sap flow rates for each individual stem and sap flow of the whole plant (g d^{-1} cm^{-2} SBA) were plotted for: a) the whole monitoring period, b) the 'unstressed' phases (i.e. 18 July to 1 August 2003), c) the 'stressed' phase (i.e. 10 August to 28 September 2003), (Figure 6.5).

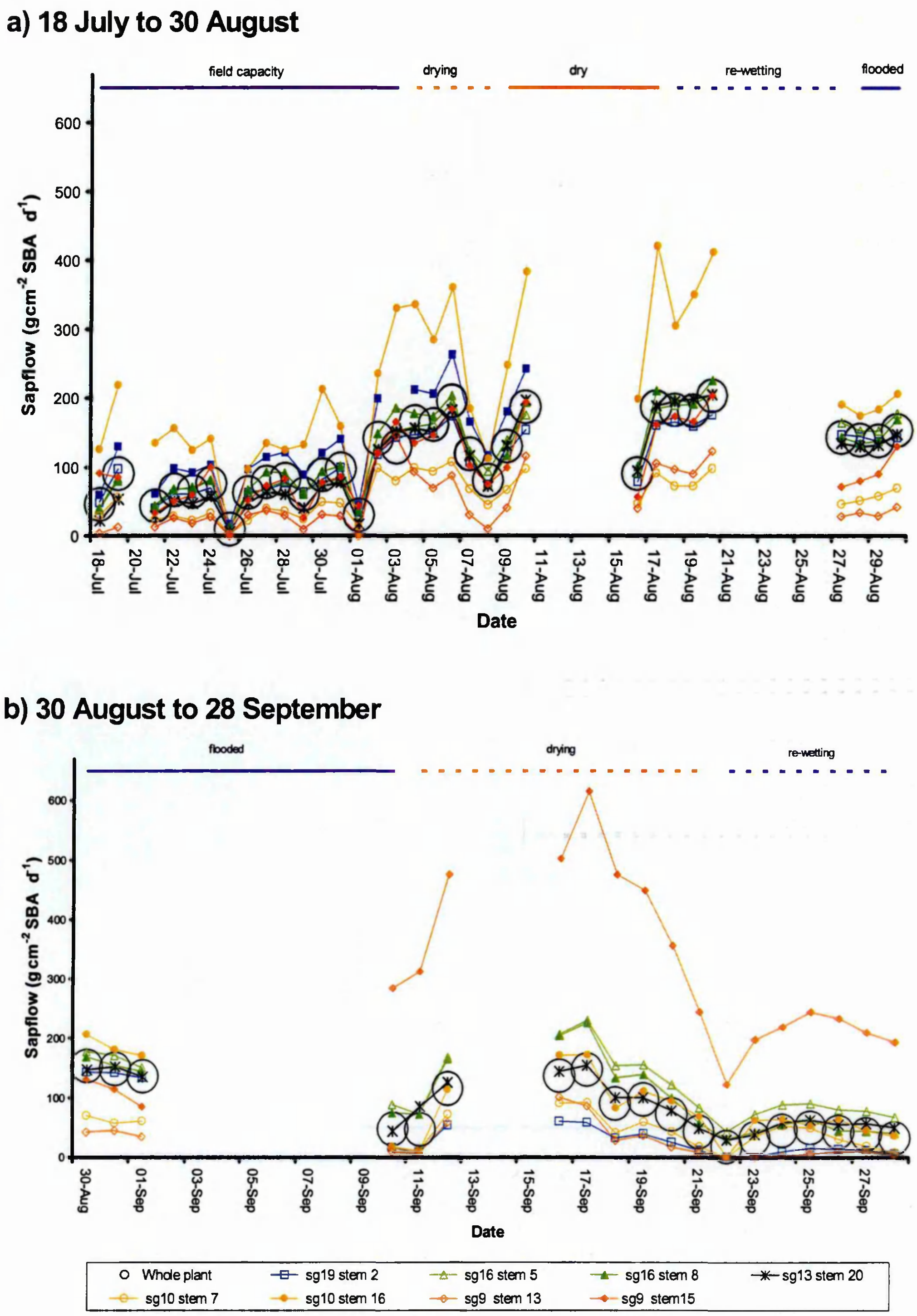

Figure 6.4 Daily totals of sap flow for individual stems, together with summed values for the whole plant, adjusted for Stem basal area for the period 18 July - 28 September 2003

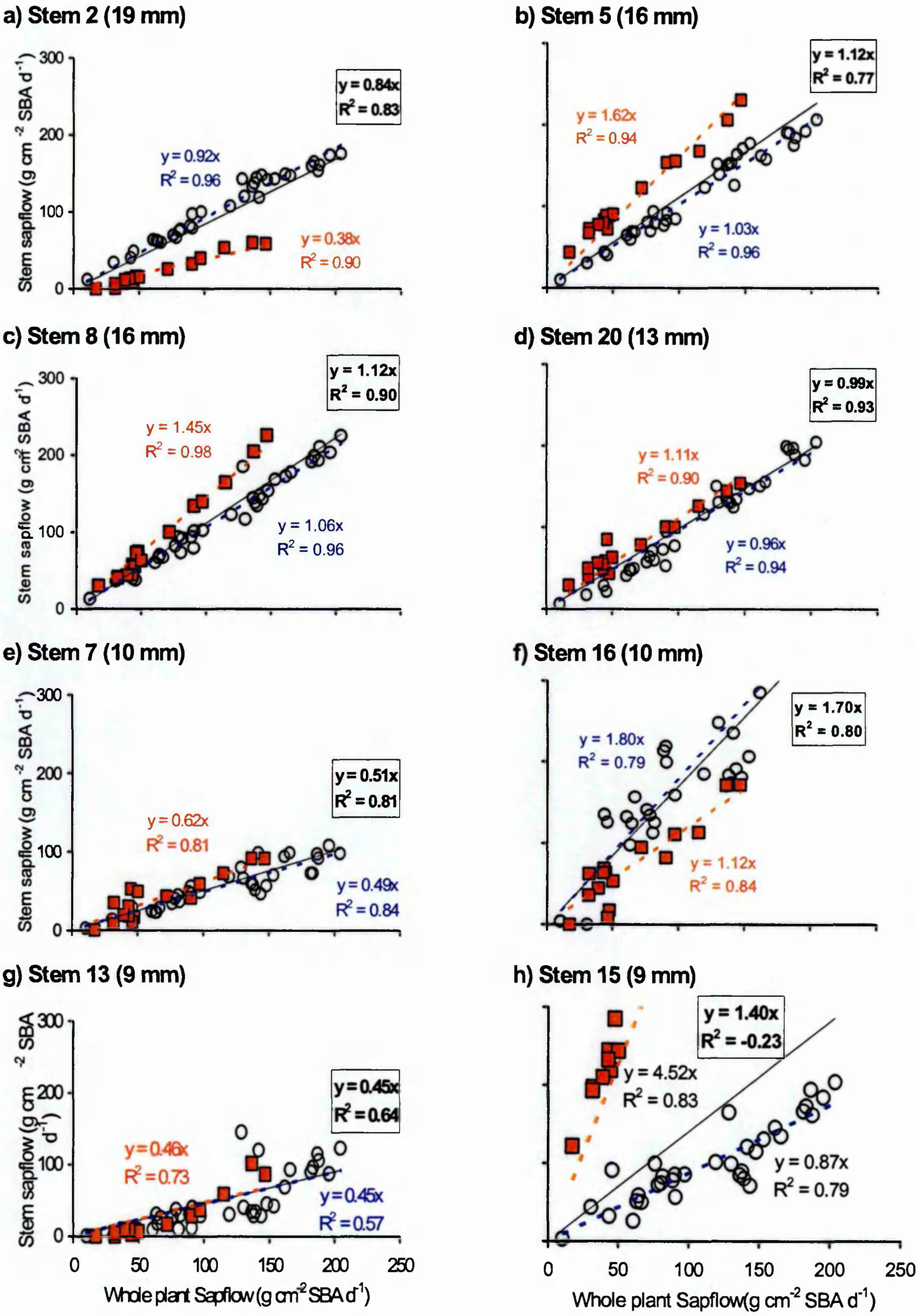

Figure 6.5 The relationships between sap flow per cm² stem basal area for each individual stem relative to that for the whole plant, plotted for: the whole monitoring period (solid line), the 'unstressed' phases from 18 July to 29 August 2003 (open circles), and the 'stressed' phases from 30 August to 28 September 2003 (solid squares).

For the whole monitoring period, Stem 20 (13 mm gauge) gave values of sap flow per unit stem basal area that matched that the whole plant ($y = 0.99\ x$; $r^2 = 0.93$). The values for the 19 mm and two 16 mm gauged stems were also similar to that for the whole plant ($y = 0.91$ to $1.11\ x$; $r^2 > 0.8$).

An analysis of variance (ANOVA) (StatSoft, Inc, 2005) showed that the sap flow per unit stem basal area relative to that for the whole plant remained similar *($p<0.05$)* between the 'stressed' and 'unstressed' phases for three of stems (Stem 8 (16 mm), stem 20 (13 mm) and stem 7 (10 mm). However the other stems showed significant *($p<0.05$)* (i.e. stem 13 (9 mm)) or very significant *($p<0.001$)* differences between the 'unstressed' and the 'stressed' period (Stems 2, 5, 16 and 15).

Leaf area calculations

Using the leaf cluster length measurements taken on 29 July, 7 August and 26 August 2003 together with observations made on 22 and 26 September (for which estimates of whole plant leaf loss of approximately 25% and 30%, respectively, were noted), estimates of leaf area (LA) were made for each stem. In making these estimates an algorithm was devised that assumed leaf loss was initially greatest from the leaf clusters of greatest length (> 200 mm) and also assumed leaf area development continued to occur on the smaller (younger) leaf clusters (<100 mm). These assumptions were made on the basis of observations made over the course of the experiment. Linear regression equations were generated, from which daily changes in LA of each stem to be estimated (Figure 6.6).

Daily sap flow rates per stem were then expressed as sap flow cm^{-2} LA. The sap flow rates of each stem as a proportion of LA are plotted in Figure 6.7 together with total sap flow cm^{-2} LA for the whole plant. As with the sap flow rates per unit stem basal area (Figure 6.4), the sap flow rates per unit leaf area of the small stems (i.e. 9 and 10 mm) show the greatest variation from the mean value for the whole plant (Figure 6.7).

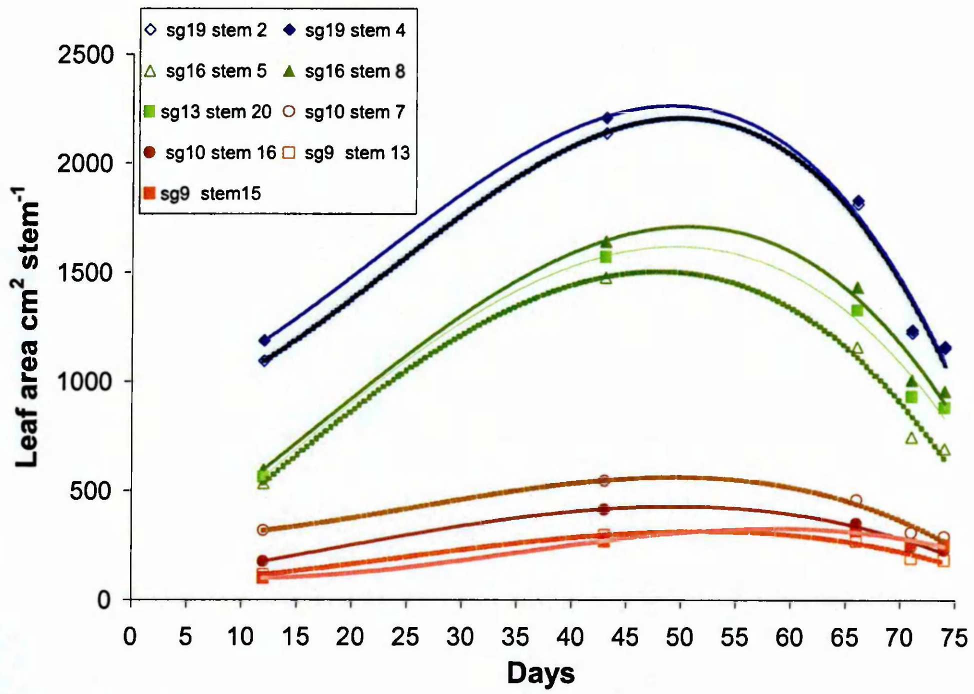

Figure 6.6 Leaf area estimates for period of sap flow measurement 18 July to 29 September 2003.

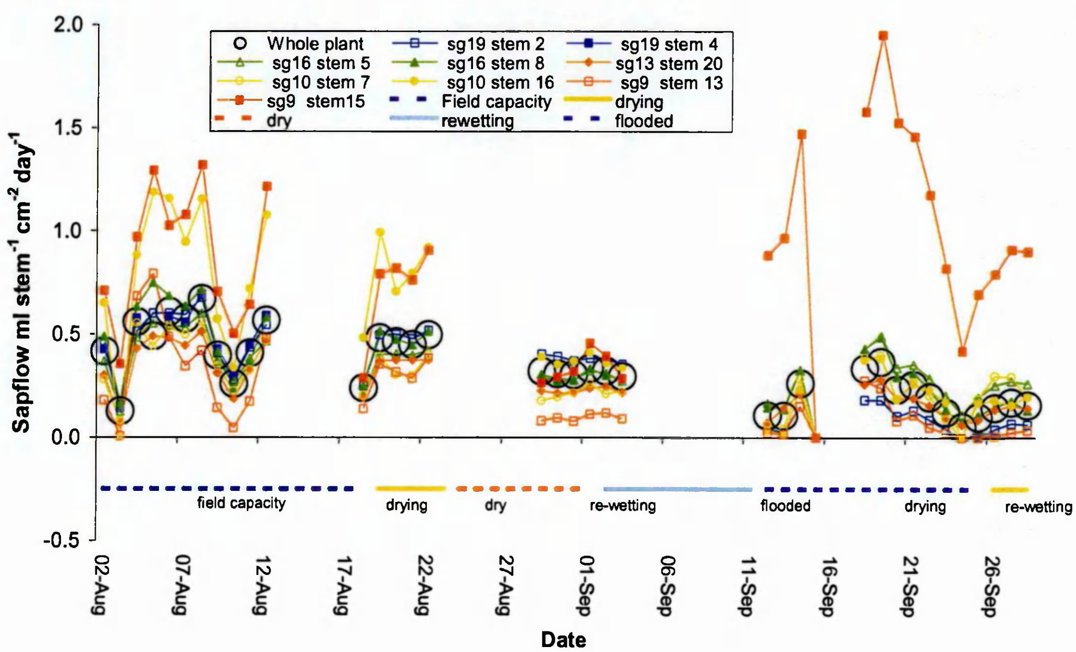

Figure 6.7 Daily totals of sap flow for individual stems, together with summed values for the whole plant, adjusted for leaf area for the period 2 August to 28 September 2003

The relationship between sap flow rates per unit of leaf area of each individual stem and for the whole plant (g d^{-1} cm^{-2} LA) were plotted for: the whole monitoring period, the 'unstressed' phase (i.e. 18 July to 1 August 2003), and the 'stressed' phase (i.e. 10 August to 28 September 2003) (Figure 6.8). As with the per unit stem basal area measurements, the sap flow per unit leaf area of stem 20 (13 mm) was well correlated to that for the whole plant (y = 0.79 x; $r^2 = 0.95$). The values for stem 2 (19 mm) and stem 8 (16 mm gauge) also correlated well with the totals for the whole plant (y = 0.98 to 1.10 x; $r^2 > 0.86$).

As for the sap flow per unit stem basal area measurement, an analysis of variance (StatSoft, Inc, 2005) showed that the sap flow per unit stem basal area relative to that for the whole plant remained similar ($p = 0.05$) between the 'stressed' and 'unstressed' phases for stem 7 (10 mm). The response was also similar for stem 5 (16 mm). All of other stems showed significant ($p = 0.001$) differences between the 'unstressed' and the 'stressed' period.

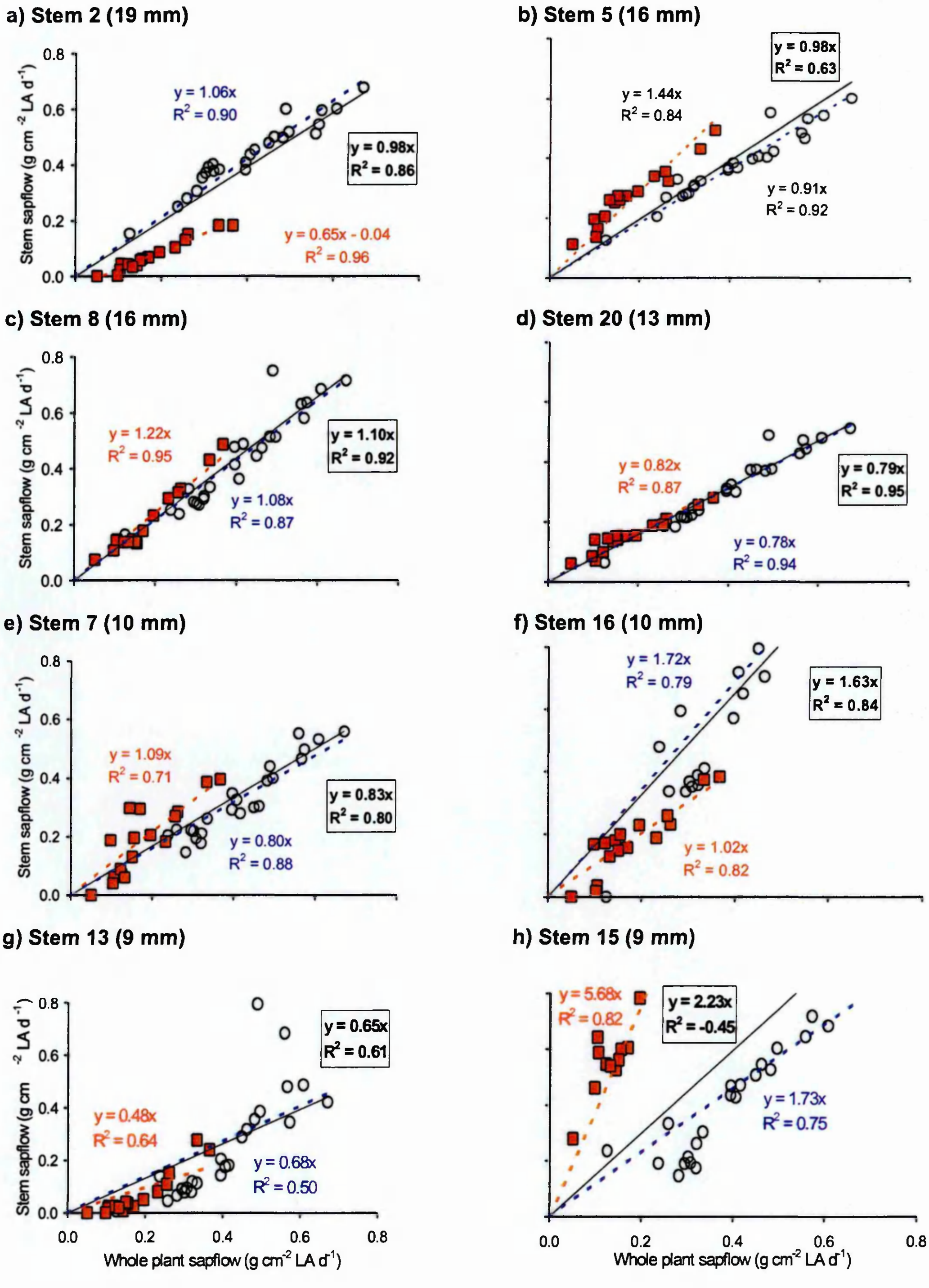

Figure 6.8 Relationships between sap flow per cm^2 LA for each individual stem and sap flow per cm^2 LA for the whole plant, plotted for: a) the whole monitoring period, b) the 'unstressed' phases (open circles) (i.e. 29 July to 29 August 2003), c) the 'stressed' phases (solid squares) (i.e. 30 August to 28 September 2003).

6.4. Scaling up stem values to estimate plant water-use

Estimates of water-use for the whole plant were made by scaling up the sap flow measurements calculated for the individual stems on the basis of SBA and also LA. These estimates were then compared to the values of daily water-use generated from the sum of the sap flow measurements recorded from all stems for: a) the 'unstressed period', b) the 'stressed period, c) the whole monitoring period (Table 6.2).

Table 6.2 Estimates of plant water-use (kg) for the whole monitoring period, the stressed period and the unstressed period, derived from sap flow measurements of individual stems scaled-up on the basis of a) stem basal area and b) leaf area.

Stem number	2	5	8	20	7	16	13	15	Whole
Diameter (mm)	19	16	16	13	10	10	9	9	plant
a) Plant water use derived from sap flow per unit stem basal area									
Unstressed	79	88	90	80	41	150	37	73	85
Stressed	9	44	36	30	15	28	9	124	26
Whole period	88	131	126	110	56	178	46	197	110
Difference from value derived for the whole plant (%)									
Unstressed	-7	3	6	-5	-52	77	-57	-15	
Stressed	-66	71	41	17	-39	11	-64	388	
Whole period	-21	19	15	0	-49	61	-58	79	
b) Plant water use derived from sap flow per unit leaf area									
Unstressed	81	68	78	57	57	121	44	119	74
Stressed	11	38	31	22	27	25	10	153	26
Whole period	92	106	108	79	84	146	54	272	100
Difference from value derived for the whole plant (%)									
Unstressed	9	-9	4	-23	-23	62	-41	61	
Stressed	-57	48	21	-15	6	-2	-60	499	
Whole period	-8	6	9	-21	-16	46	-46	173	

Table 6.2 shows that estimates of total plant water-use, for the whole period, calculated from the sap flow per unit basal area of individual stems varied greatly. For the whole period the 9 and 10 mm stems gave values ranging from an underestimate of 58% to an over-estimate of 79%. Generally, the large stems (16 and 19 mm) gave better estimates of total plant water-use, with a range from an underestimate of 21% to an over-estimate of 19%. The 13 mm stem gave an estimate that matched the value calculated for the whole plant.

For the 'unstressed' phase, the values derived from the larger stems (16 and 19 mm) closely matched the whole plant values, ranging from an underestimate of 7% to an overestimate of 3-6%. However during the 'stressed' phase, the values ranged from an underestimate of 66% to an over-estimate of 71% (Table 6.1). The small stems showed large discrepancies from the whole plant values for both the 'stressed' and 'unstressed' phases, although an improved water-use estimate was achieved from Stem 16 (10 mm) for the 'stressed' phase, with an overestimate of only 11%. Whilst stem 15 (9 mm) underestimated plant water use by 15% for the 'unstressed' phase, it overestimated water use by 388% in 'stressed' phase.

Estimates of water-use for the whole period, generated from individual stems on the basis of leaf area also vary greatly (Table 6.1b). As with estimates based on SBA, the 9 mm stems gave values that showed the greatest discrepancy from the whole plant value, ranging from an under-estimate of 46% to an over-estimate of 173%. The estimates with the 10 and 13 mm stems tended to be better, ranging from an under-estimate of 16-21% to an overestimate of 46%. The 16 and 19 mm stems gave good overall estimates ranging from underestimates of 8% to over-estimates of 6-9%.

When plant water-use was calculated separately for the two phases ('stressed' and 'unstressed') there were changes in the success with which the sap flow per unit leaf area of an individual stem matched that for the whole plant. This was most apparent for the 'stressed' phase, for which the 9 mm stems showed variation ranging from an underestimate of 60% to an over estimate of about 500%. For the large stems (16 to 19 mm), the variation ranged from an underestimate of 57% to an overestimate of 48%. By contrast the 10 and 13 mm stems maintained reasonable estimates during the 'stressed' phase ranging from an underestimate of 15% to an over-estimate of 6%.

6.5. Discussion

The experiment carried out in 2003 again demonstrated that it is possible for a single SHB sap flow gauge installed on a multi-stemmed woody perennial to provide satisfactory (< ±10%) estimates of plant water use. However, a high degree of variation in the accuracy of estimates made from individual gauges was also evident. This variation could be a function of: a) error attributed to the SHB technique and its implementation; b) scaling up of individual stem estimates to whole-plant estimates; and c) the responses of individual stems to changes in soil water status. Issues related to a) and b) are discussed in more detail in Chapter 7. The differing response of individual stems to changes in soil water status and the effect on water-use estimates is considered below.

Effect of soil-water status on relative contribution of individual stems to plant water-use

The mechanisms involved in controlling stomatal response to changes in available soil-water, are still not fully understood (Sperry *et al.,* 2003; Ahuja *et al.*, 2006) and are still under debate (Liu *et al.*, 2001; Yao *et al.*, 2001; Perks *et al.*, 2002). It has long been considered that the plant growth regulating hormone, abscisic acid (ABA), transported in xylem sap is the primary mechanism for root-to-shoot signalling (Sauter et al., 2001). In conditions of drought, ABA concentrations in an intact willow (*Salix sp.*) have been shown to increase in roots, xylem sap and subsequently leaves, resulting in significant reduction in stomatal conductance (Liu *et al.*, 2001). Many authors consider this mode of plant response is too simplistic to account for a plant's ability to close stomata under drought conditions (Sauter et al., 2001; Yao *et al.*, 2001; Perks *et al.*, 2002; Sperry *et al.,* 2003; Ahuja *et al.*, 2006). In a study of Scots pine (*Pinus sylvestris L.),* Perks *et al.* (2002) concluded that: '... short term stomatal response to soil drying, based solely on the action of a chemical messenger from the roots, is not applicable in mature conifer trees because signal transmission is too slow.' Also, Yao *et al.* (2001), in a study using bell pepper plants conclude that: 'our results are in contrast to those of other studies on herbaceous plants, which suggest that chemical messengers from the root bring about stomatal closure when plants are in water stress' They suggest that their work indicates a hydraulic control mechanism for stomatal closure. Sperry (2000) cautions against the temptation to assume there is likley to be single process controlling stomatal response but supports the concept of stomatal control being based on 'the hydrualic conductance of the soil-leaf continuum.' The processes involved in a hydraulic control mechanism are not fully understood but the suggestion is that, under drought conditions, resistances to water movement within the continuum vary to different degrees and act to trigger stomatal closure. Cavitation and embolism within xylem has been demonstrated under drought conditions and shown to be reversible on re-wetting (provided PWP is not exceeded) (Sperry *et al.,* 2003). Xylem cavitation is considered to be a major factor in changes in plant hydrualic resistances which subsequently govern stomatal response (Sperry *et al.,* 2003). It is conceivable that the larger the xylem vessel, the more prone it is to cavitation. It would not be unreasonable therefore, to propose that stems of a multi-stemmed plant may show 'apparent automomous' behavior, in response to drought stress, by virtue of their differing sizes and the resultant differences in hydrualic conductance for each soil-leaf pathway.

Brooks *et al.*, (2003), using a combination of porometry and sap flow techniques on Douglas-fir trees *(Pseudotsuga menziesii),* investigated the hydraulic connectivity of branches. They asked the question: "do branchlets within a branch have autonomous water supplies or do they share a common water supply?." They considered two conceptual hydraulic pathway models based on circuit resistances: a) where lateral branches are completely interconnected and b) where lateral branches function as independent units supplied by different sections of xylem in the main branch (Figure 6.9). Their assumption for a), based on Ohms law, is that: "variation in flow to the competing sinks is driven simply by the pathway resistance and the driving gradient".

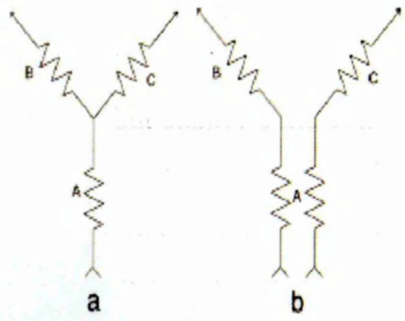

Figure 6.9 Conceptual models of hydraulic pathways from main branch (A) to lateral branches (B and C). Two extremes of conectivity are illustrated: a) where lateral branches are completely interconnected and b) where lateral branches function as independent units supplied by different sections of xylem in the main branch A. (Brooks et al., 2003)

For b), the 'pipe model theory' (Shinozaki *et al.*, 1964a, 1964b), flow to each unit of foliage is supplied by separate, independent pipes and assumes water in one pipe is not available to a competing sink. The two extreme conectivity models were used to predict changes in flow as a response to excision of one lateral branch. They found that flow through the remaining lateral branch was much lower than predicted by the complete interconnection model (Figure 6.9a). These findings and observations of flow responses to patial shading of foliage, led them to conclude that the true model of connectivity, for *P. menziesii*, lies somewhere between these two extremes. The suggestion is that branches of the tree have some degree of autonomous behaviour in terms of water flow.

Evidence supporting the pipe model theory was reported in a study investigating sap flow responses in evergreen oak (*Quercus ilex L.*) (Infante *et al.*, 2001). In this study gauges were installed in three orientations on the trunk (northeast, northwest and south). Significant differences in sap flow were observed between orientation (sap flow was higher in the northeast and northwest orientations than in the south). These differences occured on both a daily and a seasonal time scale and were reported to be less pronounced on cloudy days and at the end of a drought period.

The individual coppice willow stems reported in this chapter (and to some degree in the two previous chapters) appear to demonstrate a level of autonomy when responding to changes in soil water status. Figures 6.5 and 6.7 illustrated that the relative sap flow rate of individual stems, scaled up on the basis of either SBA or LA, changed in response to extreme changes in soil water status. These changes varied in magnitude for the different stems although relationship between stem diameter and response to water stress is not clear.

Stem responses: Impact on water-use estimates

The potential for partial or complete autonomous behaviour of individual stems in relation to xylem flow has implications when using sap flow techniques to estimate water-use of a multi-stemmed plant. The apparent unpredictability of these responses prohibit definitive recommendations for stem selection based on stem size. However, as reported in this study the best estimates of water-use compared to the lysimeter water balance were achieved from the larger stems for the 'unstressed phase' and from the intemediate stem sizes for the 'stressed phase.'

6.6. Conclusions

1) The results presented in this chapter appear to confirm the findings of the 2001 experiment recorded in Chapter 3, in that the relative sap flow rates of individual stems of a multi-stemmed plant, when expressed as a proportion of the whole plant, can change in response to changes in soil water content.
2) These changes in relative contribution of individual stems to the total daily water-use of the plant have the potential for creating large errors in the scaled-up estimates of plant water-use.
3) These relative changes in sap flow are not easily predicted. The most reliable estimates of water-use were generally achieved from the scaled-up values of sap flow recorded from the larger stems through the 'unstressed' phase. This was true when scaled up on the basis of both stem basal area and leaf area. During the 'stressed' phase the most reliable measurements came from the intermediate-sized stems.

7. SYNTHESIS AND RECOMMENDATIONS

The aims of the thesis were to evaluate the reliability of stem heat balance (SHB) sap flow gauges as a means of estimating water-use and to determine best practice for use on multi-stemmed woody plants. This chapter synthesises the results of the preceding chapters and proposes guidelines for using a single SHB sap flow gauge to estimate the water-use of a multi-stemmed woody plant.

7.1. Evaluation of the reliability of sap flow measurements

A major problem in validating the accuracy of sap flow measurements is determining the "actual" value of water use that the "measured" sap flow value must be compared with. In this thesis, over a period of three years, improved accuracy in the determining the "actual" value was achieved by improving the reliability of values for the individual components of a lysimeter water balance. In 2001, there was limited measurement of changes in soil water content, and in 2002 there were limited measurements of daily changes in soil water content. In 2003 a load-cell was used to allow relatively accurate (± 20 g) measurements of daily water-use.

In 2003, with modifications of low sap flow rates and the radial heat coefficient (Chapter 5), daily estimates of total sap flow closely matched that from the lysimeter water balance (Equation 5.10)

$$\text{Sap flow estimate} = 0.986 \text{ lysimeter estimate } (r^2 = 0.94) \qquad \text{Equation 7.1}$$

Hence it is possible to derive estimates of plant water-use from SHB gauges that correspond well to lysimeter water balance estimates. The general conclusion from literature appears to be that SHB gauges are capable of yielding good results (Baker and van Bavel, 1987; Steinberg *et al.*, 1989; Senock and Ham, 1993; Smith and Allen, 1996; Kjelgaard *et al.*, 1997; Grime and Sinclair, 1999).

Whilst it is possible to modify components of the sap flow calculation to provide a "reliable" estimate of plant water-use when output from individual gauges can be calibrated against "actual" values, in situations where the "actual" value is unavailable, an accurate estimate will depend on minimising all major sources of error. In the case of determining plant water-use of a multi-stemmed plant, from a single stem, this means following best practice for determining accurate measurements of sap flow for the individual stem and also for determining reliable whole plant water-use estimates. These are covered in turn.

7.2. Best practice to improve stem sap flow measurements

In their review of sources of error in SHB sap flow measurements, Grime and Sinclair (1999) used data from SHB gauges installed on a range of stem sizes and for a wide range of growing conditions. They concluded that 'the magnitude and relative importance of different errors is highly dependent on operating conditions.' From literature and from the results in this thesis, five areas of best practice in terms of obtaining an accurate sap flow measurement from an individual stem are considered.

- installation of the gauge
- determination of the co-efficient of radial heat conductance (K_{rad}),
- measurement of 'low' sap flow rates,
- measurement of 'high' sap flow rates,
- consideration of stem heat storage.

Installation and operation of the gauge

The Dynagage SHB sap flow gauges used in this study are delicate instruments. This, together with the inherent problems of using battery powered data logger equipment with the associated wiring, in field conditions increases the risk of equipment failure and subsequent data loss. This was evident over the duration of this study (2001- 2003) with the failure of two of the ten gauges used and loss of data at key stages of the final experiment. In a study using SHB gauges on poplar, Hall *et al.* (1998) reported three out of four gauges malfunctioning. Grime and Sinclair (1999) also reported problems associated with operational reliability especially with larger gauges (> 25 mm).

Erratic readings and damage to the electrical components of the gauge may result from water penetrating the gauge especially when power is turned on. This results in short-circuiting of the thermopile and thermocouples with the potential of permanent damage. Precautions to prevent this include installing a plastic collar above the gauge and sealing with grafting wax, as recommended by Smith and Allen (1996). Damage may also result from repeated flexing of the gauges installed on stems exposed to windy conditions. It is therefore important to ensure all gauges and connecting wires are fitted securely and in such a way so as to prevent flexing and 'tugging' of delicate gauge connections.

The instruction manual also recommends daily monitoring of the gauge to enable fine-tuning of power input especially if large changes in daily sap flow rates are anticipated.

Coefficient of radial heat conductance

The co-efficient of radial heat conductance (K_{rad}) is difficult to establish and, as illustrated in chapter 5, this value has a significant effect on the water-use estimate. The recommendation given in the user manual of evaluating K_{rad} from the K_{app} values recorded at night for the first few days post installation is complicated by the potential for night-time sap flow, in that a true zero sap flow state is difficult to determine. Alternative methods of determining K_{rad} from periods of zero sap flow include covering or removing leaves and excision of the stem above the gauge (Grime and Sinclair, 1999). However these methods are invasive.

The most practical solution for determining K_{rad}, as used in Chapter 5, appears to be the assessment of minimum K_{app} assessed over the duration of the study. This approach allows for adjustment of K_{rad} if step changes are observed for individual gauge installations during the study. In Chapter 5, in order to match the lysimeter estimate of water-use, it was necessary to increase the K_{rad} calculated in this way, by 10% (Figure 5.15). Although the exact value chosen may be somewhat subjective it is likely to prove satisfactory (estimates within 16% of the lysimeter values were achieved without this final adjustment) unless conducting highly detailed studies. For studies investigating relative, rather than absolute, values of sap flow, as a measure of plant response to growing conditions, the accuracy of K_{rad} is less critical. To overcome these difficulties an alternative design of SHB gauge has been proposed using dual heaters, which eliminates the need to measure K_{rad} (Peressotti and Ham, 1996) and may prove to be a significant improvement on single heater SHB gauge design.

Measurement of low sap flow rates

Under conditions of low sap flow rate the difference in temperature between the thermocouples above and below the gauge (dT) approaches zero, and the calculated Q_f value becomes less than 20% of the power supplied (P_{in}), the calculated sap flow rates become unstable. Hence a 'low-flow' filter is used, which states that when dT is less than a specified value and Q_f is less than a certain proportion of P_{in}, the sap flow rate is assumed to be zero.

Chapter 5 showed that the 'low-flow filter' setting also has an important influence on water-use estimates. By raising the 'low flow filter' threshold for dT_{min} from 0.75°C to 0.80°C for the small stems (9, 10 and 13 mm) and to 1.00°C for the large stems (16 and 19 mm), for situations when Q_f was less than 100% of P_{in}, gave an improved fit with the water use data from the lysimeter measurements. The default settings in the manual were dT_{min} = 0.75°C, and Q_f < 20% P_{in}. Grime and Sinclair (1999) recognised these as acceptable values for 25 to 50 mm stems. The suitability of the amended 'low-flow filter' setting, of dT_{min} = 0.8-1.0°C, and Q_f <100% P_{in} was only validated by reference to the lysimeter load cell measurements. This presents problems for field studies requiring precise sap flow outputs.

Measurement of high sap flow rates

Stem heat balance gauges have been shown to produce erroneous outputs at high sap flow velocities and as such a 'high flow filter' is incorporated into the sap flow calculation recommended by Dynamax (1997). Grime and Sinclair (1999) question the default setting in the manufacturer's instruction manual of a 'high flow filter' threshold of 152 cm h^{-1} for the maximum measurable sap flow velocity. They reported the potential for substantial overestimates of sap flow rate when sap flow velocity exceeded

100 cm h^{-1} and reported similar findings by Ham and Heilman (1990), Senock and Ham (1993), Heilman *et al.*, (1994), and Grime *et al.*, (1995b). The suggestion is that at high flow rates, the thermocouples mounted on the stem surface are incapable of giving a true measure of xylem fluid temperature.

Fichtner and Schulze (1990), Weibel and de Vos (1994), and Knan and Ong (1995) have shown improvements in sap flow measurements for a range of tree species by inserting the thermocouples into the stem. However, Kjelgaard *et al.* (1997) found no consistent differences in performance of SHB gauges with thermocouples inserted into the stem compared to those with surface-mounted thermocouples. They concluded that any differences were generally small and of no practical significance except where flow rates reached 200 g h^{-1}. However, they did acknowledge that 'further research was needed to determine whether inserted thermocouples should be recommended when measuring large flows". Inserting thermocouples into the stem complicates gauge installation and as an invasive technique has the potential of damaging the stem, which in turn may result in erroneous measurements.

Since sap flow velocity (cm h^{-1}) is a function of stem cross-section area and flow rate (g h^{-1}), the maximum flow rate that can be measured is a function of the stem diameter, i.e. a greater absolute range of flow rates can be measured with increasing stem size. The implication of this for studies investigating water-use of multi-stemmed plants where high sap flow rates are anticipated is that, by choosing stems with the largest diameter, measurement of the largest range of sap flow rates is permitted before 'high flow' threshold is exceeded.

In each experiment reported in this thesis the default threshold of 152 cm h^{-1} was used. The effect of this would have been minimal in the work presented here, as there were few instances where sap flow exceeded 100 cm h^{-1}. The greatest sap flow velocity was observed in stem 15 (9 mm) in 2003. Which for 20 minutes on 12 September, 30 minutes on 16 September and 80 minutes on 17 September exceeded 100 cm h^{-1} with a maximum of 124 cm h^{-1} recorded for one 10 minute period. Possible error associated with these relatively high velocities could explain in part, the 'extremely' high flow rates per unit stem basal area and per unit leaf area observed for that stem.

Stem heat storage

As discussed in section 4.4, sap flow rate errors can be associated with changes in stem heat storage. The error is likely to increase with increasing stem size and/or conditions of low sap flow rate. Grime *et al.* (1995) reported that inclusion of a heat storage term (Q_s) can significantly improve the measurements derived from large stems. In order to calculate Q_s, an additional thermocouple should be located on the stem at the mid-point of the heater. This again further complicates gauge installation and requires extra data logger capacity. Grime and Sinclair (1999) suggest that 'since errors introduced by neglecting heat storage increase with increasing stem size, errors may be reduced by installing a larger number of small gauges on branches, as opposed to a small number of large gauges on main stems'. This is pertinent to measurements on multi-stemmed plants. Where there is a choice of stem sizes on a multi-stemmed plant, selecting small stems would seem most appropriate if Q_s is not calculated. This appeared to be the case in 2002 (Chapter 4), where the 10 and 13 mm gauges, rather than the 9 mm or 16 or 19 mm gauges gave sap flow readings closest to the lysimeter derived estimates. However in 2003 (Chapter 5 and 6) the most reliable estimates of water-use in the 'unstressed' phase were generally achieved from large stems (16 and 19 mm). A possible reason for this is that in 2003 the mean daily sap flow rates were generally higher than those recorded in 2002 (e.g. for the 'unstressed' phase, 19 mm gauge mean flow was approximately 600 g d^{-1} compared to 450 g d^{-1} in 2002). This could have reduced the effect of stem heat storage allowing better estimates to be made from the larger stems.

7.3. Best practice to derive plant water-use from a single stem

Assuming accurate stem sap flow measurement, there are two further issues regarding best practice when deriving plant water-use of a multi-stemmed plant from a single stem. These are: choice of stem to be measured and the method of scaling-up.

Choice of stem

To measure the sap flow of a multi-stemmed plant a decision is required when selecting the most appropriate stem to use. There are two main issues for consideration: a) does the stem represent a high proportion of the total plant, b) is the sap flow rate of the stem representative of the whole plant.

a) High proportion

The contribution made by individual stems to whole plant water-use varies with stem size. This is illustrated in Figure 6.2 which shows the linear regression for individual stem sap flow against whole plant sap flow. The gradient of these lines indicates the relative proportion of total sap flow made by each stem. This shows that for the 'unstressed phases' the contribution made by the 19 mm stem was 23% of the total flow compared to 5% or less by each of the 9 and 10 mm stems Choosing a small stem under these conditions would result in measurements that represented a small proportion of the total measurement and as such increases the risk of error in estimates of whole plant water-use. Choosing a large stem under these conditions would therefore seem most appropriate.

b) Is the sap flow representative?

From the three experiments reported in this thesis there is evidence that individual stems of a multi-stemmed plant can show different responses to changes in soil water status. Under 'normal' growing conditions, where the plant has sufficient available water (soil matric potential > -50 kPa) *sap* flow appears to be consistently proportional to both leaf area and stem basal area. However through periods of water stress (either drought or flood) there can be a departure from this relationship. Hatton and Wu (1995) in a study of eucalypts subjected to drought stress noted that large trees transpired proportionally less per unit leaf area than the small trees through periods of drought stress. During the 'stressed phase' in the 2003 experiment the largest (19 mm) stem showed a greater relative decline in sap flow rate than the other stems. By contrast one of the small 9 mm stems (stem 15) showed a large increase in its contribution to total plant water use (although the two 16 mm stems also showed increases). One reason for this is that the hydraulic connection between the roots and the xylem of individual stems may vary as discussed in Chapter 6. The effect of such changes has the potential for creating large errors in scaled-up estimates of plant water-use.

Under conditions of water stress, flow rates of individual stems are less predictable. Results from the 'stressed' phase in Chapter 6 indicated that choosing an intermediate sized stem (10 and 13 mm) may give best estimates of whole plant water-use, although these findings are not conclusive.

Method of scaling up

In this study, the two methods for scaling up the sap flow of individual stems to calculate total plant water use were stem basal area (SBA) and leaf area (LA). In the 2003, for the whole measuring period, scaling up on the basis of the leaf area of large stems (16 and 19 mm) generally gave closer estimates (± 10%) to the total plant water use derived from a lysimeter water balance, than those scaled up using stem basal area (± 21%).

Estimates based on the stem basal area and the leaf area of large stems (16-19 mm) were both within 10% of that for the whole plant during the 'unstressed' phase of the 2003 experiment. However during the 'stressed' phase the precision of the stem-basal-area-derived estimates (±71%) was less than the leaf-area-based measurement (±57%). Leaf loss recorded at the end of second drought phase could account for the poorer estimates generated from stem-basal-area derived measurement for the 'stressed' period. This reinforces comments made by Wullschleger *et al.* (1998) referring to problems associated with using scalars having 'short-term temporal dependence' such as leaf area and stem basal area, giving rise to a poor correlation between the scalar and water-use. Caution is therefore needed when choosing a scalar and appropriate adjustments may need to be made during the course of a given study. This choice is also greatly influenced by the practicalities of accurately determining values from the scalar sample that truly represent the distribution of the whole plant or stand. Leaf area is significantly more complicated and time consuming to measure than stem basal area and therefore has greater potential for error.

7.4. Recommendations

When using a single stem heat balance sap flow gauge to estimate water-use of a multi-stemmed woody plant the results may be influenced significantly by the choice of stem selected for measurement. It is suggested therefore, that:

- In conditions where soil water availability is unlikely to impose stress on the plant and where flow rates are expected to be high a 'large' stem should be selected.
- In conditions where soil water availability is likely to impose stress on the plant and where flow rates are expected to be perturbed by changing conditions, selecting an 'intermediate' sized stem is recommended.
- Values for scalars need to be accurately determined and adjusted appropriately in response to temporal variation.

Recommendations for further work are:

- For improved accuracy of the heat balance calculation, there is a requirement for improved strategies for determining values of radial heat loss and threshold values of the 'high' and 'low' flow filters.
- Greater understanding of the reasons for differences in stem responses to stress is needed to enable relative changes in flow rates to be predicted, thus allowing increased confidence in plant water-use estimates.

8. REFERENCES

Ahuja, L. R., Ma,L. and Timlin, D.J. (2006). Trans-disciplinary soil physics research critical to synthesis and modelling of agricultural systems. Soil Science Society of America Journal 70: 311-326.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M. (1998). Crop evapotranspiration: Guidelines for computing crop requirements. *Irrigation and Drainage Paper No. 56, FAO*, Rome, Italy, 300 pp.

Allen, R.G. (2000). Using the FAO-56 dual crop coefficient method over an irrigated region as part of an evapotranspiration intercomparison study. *Journal of Hydrology* 229:27-41.

Allen, S. J. and Grime, V.L. (1995). Measurements of transpiration from Savannah shrubs using sap flow gauges. *Agricultural and Forest Meteorology* 75: 23-41.

Ansley, R.J., Dugas, W.A., Heuer, M.L. and Trevino, B.A. (1994). Stem flow and porometer measurements of transpiration from honey mesquite (Prosopis glandulosa). *Journal of Experimental Botany* 45: 847-856.

Baker, J. M. and van Bavel, C.H.M. (1987). Measurement of mass flow of water in the stems of herbaceous plants. *Plant, Cell, and Environment.* 10: 777-782.

Baker, J.M., and Neiber, J.L. (1989). An analysis of the steady-state heat balance method for measuring sap flow in plants. *Agricultural and Forest Meteorology* 48: 93-109.

Bauerle, W.L., Whitlow, T.H., Pollock, C.R. and Frongillo, E.A. (2002). A laser-diode-based system for measuring sap flow by the heat-pulse method. *Agricultural and Forest Meteorology* 3004: 1-10.

Bonneau, L.J.G. (2004). Drought resistance of willow short rotation coppice genotypes. PhD thesis. Silsoe, Bedfordshire: Cranfield University.

Bosch, D., (2004). Comparison of capacitance-based soil water probes in coastal plain soils. *Vadose Zone Journal* 3: 1380-1389.

Brierley, E.D.R., Tyrrel, S.F., Thorn, P., Stephens, William (2001). Application of landfill leachate to willow short rotation coppice. *Aspects of Applied Biology 65: Biomass and energy crops II. York.* 65:321-328.

Brooks J.R., Schulte, P.J., Bond, B.J., Coulorabe, R., Dornec, JC., Hinckley, T.M., McDowell, N. And Phillips, N. (2003). Does foliage on the same branch compete for the same water? Experiments on Douglas-fir trees. *Trees* 17: 101-108.

Burgess, S.S., Adams, M.A., Turner, N.C., Beverly, C.R., Ong, C.K., Khan, A.A., and Bleby, T.M. (2001). An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. *Tree Physiology* 21(9): 589-598.

Cermák, J., Deml, M., and Penka, M. (1973). A new method of sap rate determination in trees. *Biologia Plantarum (Praha)* 15: 171-178.

Cermák, J., Jenik, J., Kučera, J. and Zidek', V. (1984). Xylem water flow in a crack willow tree (*salix fragilis* L.) in relation to diurnal changes in the environment. *Oecologia* 64: 145-151.

Cermák, J., Jenik, J., Kučera, J., Lindroth, A. and Bednárová, E. (1995). Individual variation of sap-flow rate in large pine and spruce trees and stand transpiration: a pilot study at the central NOPEX site. *Journal of Hydrology* 168: 17-27.

Cermák, J., Kučera, J. and Nadezhdina, N. (2004). Sap flow with some thermodynamic methods, flow integration within trees and scaling up from sample trees to entire forest stands. *Trees* 18:529-546

Cermák, J., Kučera, J. and Penka, M. (1976). Improvement of the method of sap rate determination in full-grown trees based on heat balance and direct electric heating of xylem. *Biologia Plantarum (Praha)* 18: 105-110.

Cienciala, E. and Lindoth, A. (1995) Gas-exchange and sap flow measurements of *Salix viminalis* trees in short rotaion forest. *Trees: Structure and Function* 9:289-294

Cranfield University (2002). AWSET v3.0 (14) software. Cranfield University Silsoe, Bedfordshire, UK.

Cuenca, R.H., Stangel, D.E. and Kelly, S.F. (1997). Soil Water Balance in a Boreal Forest. *Journal of Geophysical Research - BOREAS Special Issue*, Vol. 102, No. D24, pp. 29,355 - 29,365.

Daamen, C.C., Simmonds, L.P., Wallace, J.S., Laryea, K.B. and Sivakumar, M.V.K., (1993). Use of microlysimeters to measure evaporation from sandy soils. *Agricultural and Forest Meteorology* 65: 159-173.

Delta-T Devices Ltd (1999). Dynagage Installation and Operation manual. Burwell, Cambridge, UK: Delta-T Devices Ltd.

Department of Trade and Industry, (2000). The Energy Report. London, Stationery Office.

Dugas, W.A., Wallace, J.S., Allen, S.J. and Roberts, J.M. (1993). Heat balance, porometry and deuterium estimates of transpiration from potted trees. *Agricultural and Forest Meteorology* 64: 47-62.

Dunin, F.X. (1991). Extrapolation of point measurements of evaporation: some issues of scale. *Vegetation* 91: 39-47

Dynamax Inc., (1997). Dynagage Users Manual. Houston, Texas

Dynamax Inc., (2005). http://www.dynamax.com/sap flow.htm

Edwards, W.R.N. (1986). Precision weighing lysimetry for trees using a simplified tared balance design. *Tree Physiology* 1: 127-144.

Forrester, D., Bauhus, J. and Connell, M. (2003). Competition in thinned silvertop ash (Eucalyptus sieberi L. Johnson) stands from early coppice growth. *Forest Ecology and Management* 174: 459-475.

Fritschen, L.J., Cox, L. and Kinerson, R. (1973). A 28-meter Douglas fir in a weighing lysimeter. *Forest Science* 19: 256-261

Fichtner, K. and Schulze, E.D. (1990). Xylem water flow in tropical vines as measured by a steady-state heating method. *Oecologia* 82: 355-361.

Giambelluca, T.W., Ziegler, A.D., Nullet, M.A., Truong, D.M. and Tran, L.T. (2003). Transpiration in a small tropical forest patch. *Agricultural and Forest Meteorology* 117(1-2): 1-22.

Goulden, M. L. and Field, C.B. (1994). Three methods of monitoring the gas exchange of individual tree canopies: ventilated-chamber, sap-flow and Penman-Monteith measurements on evergreen oaks. *Functional Ecology* 8: 125-135.

Granier, A. (1985). Une nouvelle mèthode pour la mesure du flux de sevè brute dans le tronic des arbres *Annales des Sciences Forestières* 42: 193-200.

Grime, V.L. and Sinclair, F.L. (1999). Sources of error in stem heat balance sapflow measurements. *Agricultural and Forest Meteorology* 94: 103-121.

Grime, V.L., Morison, J.I.L. and Simmonds, L.P. (1995). Including the heat storage term in sap flow measurements with the stem heat balance method. *Agricultural and Forest Meteorology*. 74: 1-25.

Groot A. and King, K.M. (1992). Measurement of sap flow by the heat balance method:numerical analysis and application to coniferous seedlings. *Agricultural and Forest Meteorology* 59: 289-308.

Groves, S.J., Rose. S.C. (2004). Calibration equations for Diviner 2000 capacitance measurements of volumetric soil water content of six soils. *Soil Use and Management* 20: 96-97.

Hall, R. L., Allen, S. J., Rosier, P.T.W., Hopkins, R. (1998). Transpiration from coppiced poplar and willow measured using sap-flow methods. *Agricultural and Forest Meteorology* 90: 275-290.

Hall, R. L., Allen, S. J., Rosier.P.T.W., Smith, D.M, Hodnett, M.G., Roberts, J.M., Hopkins, R., Davies, H., Kinniiburgh, D.G. and Gooddy, D.C. (1996). Hydrollogical effects of short rotation energy coppice. ETSU B/W5/00275/REP. ETSU for DTI. Harwell. pp 226.

Ham, J.M. and Heilman, J.L. (1990). Dynamics of a heat balance stem flow gauge during high flow. *Agronomy Journal* 82: 147 152.

Hatton, T.J. and Wu, H.I.. (1995). Scaling theory to extrapolate individual tree water use to stand water use. *Hydrological Processes* 9: 527-540.

Hatton, T.J., Moore, S.J. and Reece, P.H. (1995). Estimating stand transpiration in a *Eucalyptus populnea* woodland with the heat pulse method: measurement errors and sampling strategies. *Tree Physiology* 15: 219-227.

Haydon, S.R., Benyon, R,G. and Lewis, R. (1996). Variation in sapwood area and throughfall with forest age in mountain ash (*Eucalyptus regans* F. Muell.) *Journal of Hydrology*:187: 351-366.

Heilman, J.L., McInnes, K.J., Savage, M.J., Gesch, R.W. and Lascano, R.J. (1994). Soil and canopy energy balances in a west Texas vineyard. *Agricultural and Forest Meteorology* 71: 99-114.

Huber, B. and Schmidt, E. (1937). Eine kompensationsmethode zur thermoelektirschen messung langsamer saftstrome. *Bericht der Deutschen botanischen Gesellschaft*. 55: 512-529

Infante, J.M., Meuchamp, A., Fernandez-Ale, R., Joffre, R. and Rambal, S. (2001). Within-tree variation in transpiration in isolated evergreen oak trees: evidence in support of the pipe model theory. *Tree Physiology* 21 issue 6: 409-414.

Ishida, T., Campbell, G..S. and Calissendorff, C. (1991). Improved heat balance method for determining sap flow rate. *Agricultural and Forest Meteorology* 56:35-48.

Knan, A.A.H. and Ong, C.K. (1995). Correction of systematic errors in estimates of transpiration obtained using a constant temperature heat balance technique. *Experimental Agriculture* 31: 461-472

Kline, J.R., Martin J.R. Jordan, C.F. and Koranda, J.J. (1970) Measurement of transpiration in tropical trees with tritiated water. *Ecology* 5:1068-1073.

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. *Agricultural and Forest Meteorology* 85: 239-250.

Li, F., Cohen, S., Naor, A., Shaozong, K. And Erez, A. (2002). Studies of canopy structure and water use of apple trees on three rootsocks. *Agricultural Water Management* 55: 1-14.

Lindroth, A. (1995). Sap flow by the heat balance method applied to small size Salix trees in a short-rotation forest. *Biomass & Bioenergy* 8(1): 7-15.

Lindroth, A. and Iritz, Z. (1993). Surface energy budget dynamics of short-rotation willow forest. *Theoretical and Applied Climatology* 47: 175-185.

Lott, J.E., Khan, A.A.H., Ong,C.K. and Black, C.R. (1996). Sap flow measurements of lateral tree roots in agroforestry systems. *Tree Physiology* 16: 995-1001.

Lui, L., McDonald, A.J., Stadenberg, I. and Davies, W.J. (2001). Stomatal and leaf growth responses to partial drying of root tips in willow. *Tree Physiology* 21 Issue 11:765-770.

Martin, P.J. (2001). Growth of willow on Oxford clay; Personal communication.

Martin, P. J. and Stephens, W. (2001). The potential for biomass production on restored landfill caps. *Aspects of Applied Biology 65, Biomass and Energy Crops II,* pp. 337-344.

Martin, P.J. and Stephens, W. (2002). Growth of willow on Oxford clay. II. Growth and biomass production report. Silsoe, Bedfordshire: Cranfield University.

Martin, P.J. and Stephens, W. (2006a). Willow growth in response to nutrients and moisture on a clay landfill cap soil. I. Growth and biomass production. *Bioresource Technology* 97(3): 437-448.

Martin, P.J. and Stephens, W. (2006b). Willow growth in response to nutrients and moisture on a clay landfill cap soil. II: Water use. *Bioresource Technology* 97(3): 449-458.

Meiresonne, L., Sampson, D.A., Kowalski, A.S., Janssens, I.A., Nadezhdina, N., Cermák, J., van Slycken, J., Ceulemans, R. (2002). Water flux estimates from a Belgian Scots pine stand: a comparison of different approaches. *Journal of Hydrology* 270:230-252

Nadezhdina N. and Cermák, J.(1998). The technique and instrumentation for estimating the sap flow rate in plants (in Czech). Patent No. 286438 (PV-158-98). Bureau for Inventions and Discoveries, Prague.

Nadezhdina N., Cermák, J. And Nadezhdin, V. (1998) Heat field deformation method for sap flow measurements . In: Cermák, J., Nadezhdina N. (eds) Measuring sap flow in intact plants. Proceedings of 4th International Worshop, Zidlochovice, Czech Republic, IUFRO Publ. Brno, Czech Republic: Mendel University, p 72-92

Nadezhdina, N. (1992) Apple tree water relations studies by means of the relative rate of water flow in the trunk xylem. *Biologia Plantarum (Praha)* 34: 431-437.

Nadezhdina, N. (1999) Sapflow index as an indicator of plant water status. *Tree Physiology* 19:885-891.

Paltineanu, I,, and Starr, J. (1997). Real-time soil water dynamics using multisensor capacitance probes: laboratory calibration. *Soil Science Society of America Journal* 61: 1576-1585.

Pearcy, R.W., Schulze, E.D. and Zimmermann, R. (1989). Measurement of transpiration and leaf conductance. In: Pearcy, R.W., Ehleringer, J., Mooney, H.A., Rundel, P.W. (eds). *Plant Physiological Ecology, Field Methods and Instrumentation.* 153-158. London: Chapman & Hall.

Peressotti, A. and Ham, J.M. (1996). A duel heater gauge for measuring sap flow with an improved heat balance method. *Agronomy Journal* 88: 149-155.

Perks M.P., Irvine, J. And Grace J. (2002). Canopy stomatal conductance and xylem sap abscisic acid (ABA) in mature Scots pine during a gradually imposed drought. *Tree Physiology* 22 Issue 12:877-883.

Sakuratani, T. (1981). A heat balance method for measuring water flux in the stem of intact plants. *Journal of Agricultural Meteorology* 37: 9-17.

Sakuratani, T. (1984). Improvement of the probe for measuring water flow rate in intact plants with the stem heat balance method. *Journal of Agricultural Meteorology* (Japan) 40: 273-277.

Sauter, A., Davies, W.J. and Hartung, W. (2001). The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. *Journal of Experimental Botany* 52, no 363: 1991-1997.

Sellami, M.H. and Sifaoui, M.S. (2002). Estimating transpiration in an intercropping system: measuring sapflow inside the oasis. *Agricultural Water Management* 1785: 1-14.

Senock, R.S. and Ham, J.M. (1993). Heat balance sap flow gauge for small diameter stems. *Plant, Cell and Environment.* 16:593-601.

Shackel, K.A., Johnson,R.S., Medawar, C.K. and Phene, C.J. (1992) Subsantial errors in estimates of sap flow using the heat balance technique on woody stems under field conditions. *American Journal of Horticultural Science.* 117: 351-356.

Shinozaki K., Yoda, K., Hozumi, K. and Kira, T. (1964b) A qualitative analysis of plant form – the pipe theory model. II. Further evidence of the theory and its application in forest ecology. *Japanese Journal of Ecology* 14: 133-139.

Shinozaki, K., Yoda, K., Hozumi, K. and Kira, T. (1964a) A qualitative analysis of plant form – the pipe theory model. I. Basic analysis. *Japanese Journal of Ecology* 14: 97-105.

Smith, D.M. and Allen, S.J. (1996). Measurement of sap flow in plant stems. *Journal of Experimental Botany* 47 No 305: 1833-1844.

Souch, C. A., Martin, P. J., Stephens, W. and Spoor, G. (2004). Effects of soil compaction and mechanical damage at harvest on growth and biomass production of short rotation coppice willow. *Plant and Soil* 263:173-182.

Souch, C.A., Stephens, W., Spoor, G. (2000). The effects of soil compaction on biomass production in short rotation coppice of willow. *Third annual report to MAFF. Cranfield Universtiy.*

Sperry, J.S. (2000) Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology* 104: 13-23.

Sperry, J.S., Stiller, V. and Hacke, U.G. (2003). Xylem hydraulics and the soil-plant-atmosphere continuum: opportunities and unresolved issues. *Agronomy Journal* 95: 1362-1370.

StatSoft, Inc. (2005). STATISTICA (data analysis software system), version 7.1. www.statsoft.com.

Steinberg, S.L., van Bavel, C.H.M., and McFarland, M.J.(1989). A gauge to measure mass flow rate of sap in stems and trunks of woody plants *Journal of the American Society for Horticultural Science* 114: 466-472.

Steinberg, S.L., McFarland, M.J. and Worthington, J.W. (1990a). Comparison of trunk and branch sap flow with canopy transpiration in Pecan. *Journal of Experimental Botany* 41: 653-659.

Steinberg, S.L., van Bavel, C.H.M., and McFarland, M.J.(1990b). Improved sap flow gauge for woody and herbaceous plants. *Agronomy Journal* 82: 851-854.

Stephens, W., Bonneau, L.,Groves, S., Armstrong, A., Lindegaard, K. (2003). Improving the water use efficiency of short rotation coppice (SRC) willows. London : *DTI pub. URN 03/1619.*

Stephens, W., Hess, T. and Knox, J., (2001). Review of the effects of energy crops on hydrology. Silsoe, Bedfordshire: Cranfield University.

Swanson, R.H. and Whitfield D.W.A. (1981). A numerical-analysis of heat pulse velocity theory and practice. *Journal of Experimental Botany* 32: 221-239.

Vertessy, R.A., Benyon, R.G., O'Sullivan, S.K. and Gribben, P.R. (1995). Relationships between stem diameter, sapwood area, leaf area and transpiration in a young mountain ash forest. *Tree Physiology* 15: 559-567.

Waring, R.H. and Roberts, J.M. (1979). Estimating water flux through stems of Scots pine with tritiated water and phosphorous-32. *Journal of Experimental Botany* 30: 459-471.

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

Wilson, K.B., Hanson, J.H., Mulholland, P.J., Baldocchhi, D.D., Wullschleger, S.D., (2001). A comparison of methods for determining forest evapotranspiration and its components: sap-flow, soilwater budget, eddy covariance and catchment water balance. *Agricultural and Forest Meteorology* 106: 153-168.

Wullschleger, S.D., Meinzer, F.C. and Vertessy, R.A. (1998). A review of whole-plant water use studies in trees. *Tree Physiology* 18: 499-512.

Yao, C., Moreshet, S. And Aloni, B. (2001). Water relations and hydraulic control of stomatal behaviour in bell pepper plant in partial soil drying. Plant, Cell, and Environment. 24 no.2: 227-235.

Zhang, H., Simmonds, L.P., Morison, J.I.l. and Payne, D. (1997). Estimation of transpiration by single trees: comparison of sap flow measurements with a combination equation. *Agricultural and Forest Meteorology* 87: 155-169.