VOLUME 1

VALIDATION AND CALIBRATION OF SAP FLUX DENSITY MEASUREMENTS IN CITRUS

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Validation and Calibration of Sap Flux Density Measurements in Citrus

(Volume 1)

Report to the
WATER RESEARCH COMMISSION and
DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES

by

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The publication of this report emanates from a project entitled *Quantifying Citrus Water Use and Water Stress at Orchard Level* (WRC Project No. K5/2275), which was conducted by Citrus Research International for the Water Research Commission in collaboration with the University of Pretoria.

This report forms part of a series of two reports. The other report is *Measurement and Modelling of Seasonal Citrus Water Use for Different Growth Stages and Canopy Sizes − Volume 2* **(WRC Report No TT 772/2/18).**

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EXECUTIVE SUMMARY

BACKGROUND

This study presents information on the validation and calibration of SFD techniques that were used to measure citrus water use. The most appropriate SFD technique to quantify transpiration in *Citrus sinensis* was identified and then used to gather information on citrus water use. A unique set of data on citrus water use was compiled and comprises of the following:

i. Hourly measurements of transpiration.
ii. Multi seasonal. The largest dataset consists of water use measurements, of a single orchard, for 718 days.
iii. Water use information on three different sized (small, medium and mature) canopies.
iv. Information on water use for the following species: *Citrus sinensis, Citrus paradisi and Citrus reticulata*.
v. Results on citrus water use from both the summer (Letsitele area) and winter rainfall (Citrusdal area) regions of South Africa.
vi. Relationships between selected plant physiological measurements, such as stomatal conductances, leaf and stem water potentials and transpiration.
vii. Impact of water stress on yield and fruit quality.

RATIONALE

The citrus industry is the largest exporter of fresh produce, in South Africa, in terms of volume and one of the highest for earning of foreign exchange with more than 100 million 15 kg cartons exported annually. The 70 917 hectare citrus industry, which depends on irrigation, provides more than 125 000 jobs that support more than 750 000 people. Citrus is a perennial crop which requires a constant supply of water in order not to limit yields and returns on investment. Due to climate change, established production areas are likely to become drier, which will place increasing pressure on water resources and irrigation management to maintain productivity. A previous WRC research project (K5/1770//4) used a sap flow technique to quantify water use of mature citrus, deciduous fruit and nut tree cultivars under best management practices. Findings from this project indicate results that were contrary to expectations, specifically for citrus. In addition, an external international review recommended more in depth research to first validate measuring techniques; and secondly to quantify water use over all growth stages for different cultivars. The more detailed research should investigate water use over seasonal growth stages, from planting to mature canopy size and water stress in relation to fruit yield and quality.

In order to provide effective advice to both established and emerging commercial farmers on irrigation methods and scheduling, accurate knowledge is required on water use. The emerging commercial farmers, who comprise approximately 300 of the 2 700 citrus growers and are supported by the industry through bursaries, mentoring and extension, are especially in need of this information. All citrus fruit producers are faced with a major challenge in maintaining high yields per hectare and fruit quality, whilst simultaneously achieving viable returns and ensuring sustainability. Given the increase in competition for water between irrigation agriculture, secondary industry and domestic water use, more knowledge is required on citrus water use for growers to remain competitive and justify future production.

AIMS AND OBJECTIVES

The objectives and aim for this WRC project are given below. The results from this study are published in two volumes. The first volume addresses the validation and calibration of the SFD techniques (Objective 1) that were used to measure and model the water use of different sized citrus trees in the summer and winter rainfall region of South Africa (Objective 2). Results from the infield water use and modelling and from the study on the impact of water stress on yield and fruit quality (Objective 3) are covered in volume 2.

General

To analyse the water use, yield, fruit size and quality of a selected Valencia, navel, grapefruit and/or soft citrus cultivar for different canopy architectures in summer and/or winter rainfall regions; including a detailed analysis of water stress in relation to yield and quality for a selected cultivar at a single location.

Specific

1. To validate citrus water use by comparing different SFD techniques with an appropriate technique such as lysimetry, cut stem and/or eddy covariance.

2. To measure and model citrus water use and water use efficiency according to seasonal growth stages from planting to mature canopy size.

3. To determine the influence of water stress on fruit set, fruit yield, and pre- and post-harvest fruit quality for a selected cultivar and single location.

METHODOLOGY

One of the aims of the study was to calibrate and test the most appropriate SFD (SFD) technique to quantify transpiration in citrus. Three approaches were taken. The first approach consisted of a laboratory study, where a wide range of citrus species were evaluated with the

stem perfusion method. In the second approach, tree water use measurements from different SFD techniques were compared to tree water use measurements conducted with a weighing lysimeter, which is considered to be a reference method for water use studies. The third approach involved infield validation and calibration of the heat ratio (HR) and the compensation heat pulse (CHP) methods that were tested against micrometeorogical measurements in a commercial orchard.

Validating SFD measurements using the stem perfusion method

Calibration and evaluation of the HR, CHP and thermal dissipation (TD) methods, with the stem perfusion method, was done at the Environmental Biophysics laboratory of the Department of Plant and Soil Sciences at the University of Pretoria. Stems and branches of four different species of citrus trees, between 7 and 11 years old, were used in the experimental work, namely *Citrus sinensis* (orange), *Citrus reticulata* (soft citrus), *Citrus paradisi* (grapefruit) and *Citrus limon* (lemon). A minimum of two branches (replicates) of each cultivar (treatment), with a circumference of approximately 15 cm, were selected for the experiments.

Stem perfusion setup for validation and calibration of SFD techniques

A modified Mariotte-based verification system was used for the validation and calibration of various SFD techniques in cut citrus branches or stems, according to the method described by Steppe et al. (2010). High flow rates were achieved by applying pressure, not exceeding 100 kPa, to a 0.8 m plastic tube filled with water and attached to a 35 cm branch or stem. This design also allowed for the preparation of stems and calibration to be completed within 12 hours, which significantly reduced xylem damage and blockage.

Two HR and two CHP sets were installed simultaneously on one branch or stem, at opposite sides of the branch or stem and separated vertically by at least 2 cm. The TD probes were installed into separate stems. The probes were coated with petroleum jelly to ease probe insertion and to ensure good thermal contact between the probes and the xylem tissue. Each probe set was thermally insulated with foam.

A sharp blade was then used to re-open any closed xylem vessels resulting from the sawing. Both sides of the branch and stem samples were covered with a wet cloth during stem preparation to avoid dehydration and the formation of embolisms. At both ends of the branch and stems, 4 cm bark was removed to ensure water flow through the xylem and not the phloem and that a tight fit was obtained between the rubber hose connecting the stem and the pressurised container with water. All joints were checked for any leakages.

The baseline for the measurements was determined from zero flow conditions. Data were logged from the installed probe sets for approximately one hour prior to the start of the experiment, when no water flow occurred. The branch or stem was then flushed with distilled water for one hour once the readings had stabilized. Various flow rates were achieved by applying different pressures, using a CO_2 regulator. Each flow rate was maintained for at least 45 min, before changing the pressure. Water passing through the stem was collected in a glass beaker and weighed every 10 min during the testing of the HR and CHP methods and every 5 min for the TD method using an electronic balance (Mettler Toledo model PB3002-S or Precisa model 800M both manufactured in Switzerland).

At the end of each experiment Safranin O dye was added to the water in the column and pressure was increased so that sap flow occurred. This resulted in the staining of the active xylem in the stem. A cross section of the stems was made at the probe insertion positions. Photographs were taken of the cross section and digitally analysed using Adobe® Photoshop® CS5 Extended (Version 12.0x32) to determine conducting sapwood area.

Heat ratio and compensation heat pulse methods

Each probe set consisted of two type-T copper-constantan thermocouples, embedded in polytetrafluoroethene tubing with an outside diameter (OD) of 2 mm, and a 1.8 mm OD stainless steel heater probe. For the HR method, thermocouples were placed equidistant (0.5 cm) upstream and downstream of the heater probe, whilst for the CHP method the upstream thermocouple was placed 0.5 cm from the heater probe and the downstream thermocouple 1 cm from the heater probe. Depending on the circumference of the stem, more than one probe set were installed at different depths within the xylem of each stem or branch to account for the radial variation in sap flux. Heat pulse velocities were measured and logged at specified intervals using a CR1000 data logger and an AM16/32B multiplexer (Campbell Scientific Inc., Logan, Utah, USA). The thermocouples, heater probes and relay control modules were manufactured locally (Andrew Everson, Pietermaritzburg).

Thermal dissipation method

The temperature difference, ΔT (K), in the TD method was measured between a heater probe that emitted heat constantly and an unheated reference probe located approximately 4 cm from each other. According to Vandegehuchte and Steppe (2013) exact spacing is less important in this method, as long as the reference probe is not influenced by the heated probe. The TD probe set (model TDP30, Dynamax Inc., Houston, TX, USA) consisted of two 30 mm long stainless steel needles with an OD of 1.2 mm. Holes were drilled into the branch using

a drill guide supplied by the manufacturer. The probes were attached to a Dynamax FLGS-TDP XM1000 sap velocity system, which consisted of a CR1000 logger, an AM16/32B multiplexer and an adjustable voltage regulator that was set at 3V for the two TDP30 probes. Data were logged every 5 min.

Validating SFD measurement methods using weighing lysimeters

All experiments were conducted in a glasshouse at the University of Pretoria's Hatfield experimental farm (25°45'7.13'' S, 28° and 15'32.89'' E), where the HR equipment and methodology was first tested in a potted *Eucalyptus marginata* tree. This was done to ensure that reliable and accurate measurements of tree water use were made with the locally manufactured equipment. For the validation and calibration experiments, two potted disease-free 13-year-old 'Midknight' Valencia trees (*Citrus sinensis* L. Osbeck) grafted onto Carrizo citrange rootstock were used. The trees were grown in 74 L black dustbins in a mixture of sand and coir and were part of the collection of parent material kept at the foundation block of Citrus Research International in Uitenhage, Eastern Cape.

Experimental design and measurement protocol

Two cantilever-type weighing lysimeters were used in these experiments to determine mass loss (transpiration) from *E. marginata* and *Citrus sinensis* trees. The exposed soil surface of the pots as well as the bottom of the pots was covered with plastic sheeting to eliminate evaporation and to prevent drainage. The load cells used had a range of 0-500 N (0-51 kg) and a theoretical resolution of 4.3 g. Calibration of the load cells, using a known mass of water, was conducted to convert the load cell measurements (mV) into mass (kg). The effect of hysteresis was determined by adding mass at 25 kg intervals and then removing the mass again at 25 kg intervals, from 0-150 kg. The stability of the load cells with a constant mass was also assessed, as this would be critical when calibrating the SFD techniques over a number of days. Recalibration of the lysimeter was conducted once the trees were placed on the lysimeter, using a 2 L beaker which was placed on the soil surface of the plastic bag. Water was then added in 100 ml intervals and the mV reading from the load cell was recorded to establish regression equations.

Transpiration (T_{sap}) from the potted trees were determined with the HR and CHP methods. The equipment and the installation thereof are described in the relevant HR and CHP sections. In order to calculate SFD ($cm^3\ cm^{-2}\ h^{-1}$), sapwood moisture content (m_c), the sapwood density (ρ_b, $g\ cm^{-3}$), wound width (mm) and sapwood area (cm^2) were determined.

Data from the HR and CHP methods were compared (regression analysis) to the mass loss measured with the weighing lysimeter at an hourly interval. Total daytime transpiration, when the plants were actively transpiring, was calculated as the sum of hourly sap flow between 06:00 and 18:00. The same was also done for the weighing lysimeter and a comparison (regression analysis) was made between the daily mass loss measured with the weighing lysimeter and the daily transpiration measured with the HR and CHP methods.

Infield validation of the SFD measurements using eddy covariance measurements

Infield calibration and validation of the HR and CHP methods was conducted in the winter rainfall region of South Africa. The trial site consisted of a 4.1 ha, 9-year-old commercial orchard of 'Washington' navels (*Citrus sinensis*), grafted on 'Carrizo' citrange rootstock which was planted in 2006. An open path eddy covariance (EC) system was used for the measurement of orchard ET for the calibration period (3-18 March 2015). Measurements were performed by the Council for Scientific and Industrial Research (CSIR), Natural Resources and Environment unit based in Stellenbosch. Soil evaporation (E) was measured daily from 9-14 March 2015 using cylindrical microlysimeters. Transpiration from the weeds was assumed to be negligible since the orchards were described as clean orchards, also there were no cover crops. A set of twelve microlysimeters were installed considering the dry and wet areas on the orchard floor and movement of shade throughout the day within the orchard. Transpiration (T_{res}) for the orchard was then taken as the residual between ET and E.

Sap flow measurements, to determine transpiration (T_{sap}), were conducted in trees in the proximity of the EC tower. Four trees were instrumented with the HR method equipment and three additional trees with the CHP method equipment to quantify and compare transpiration measurements. One of the major challenges faced infield, was the onset of gumming which hastened the rate of corrosion of the heater probes soon after the trees were instrumented. This problem was solved by inserting brass collars (2.5 mm in diameter) in the tree to accommodate the heater probes, thus reducing the occurrence of corrosion. At the end of the experiment sections of the tree trunk, where probes were inserted, were removed from the four HR method measurement trees. The exposed, fresh face was shaved smooth using a chisel, after which the wound width was clearly identified by its darker colour. Wound width at its widest point was measured for each tree using a digital vernier calliper and an average wound for the orchard determined, which was used for the calculation of the SFD. Sapwood depth was determined through staining conducting tissue with Safranin O dye.

RESULTS AND DISCUSSION

Sap flux density techniques provide direct measurements of transpiration rates and have been successfully used to determine xylem sap flow and transpiration rates of woody plants (de Oliveira Reis et al., 2006). However, Smith and Allen (1996) advised that the techniques should be calibrated for each species in which they are to be used, due to uncertainties associated and assumptions made with the use of the techniques.

Validating SFD measurements using the stem perfusion method

Sap flux densities were underestimated in all the species and cultivars, except for one of the 'Eureka' lemon stems measured. Fairly good linear correlations ($R^2 > 0.7$) between SFD measured with the HR method and gravimetric method were observed in most of the 'Star Ruby' grapefruit, 'Delta' Valencia, 'Bahianinha' navel and 'Eureka' lemon stems, but poor correlations ($R^2 = 0.19$) were found for 'Nadorcott' mandarin. The slope of the regression equations were inconsistent between stems within the same cultivar and also between species and cultivars, with the slope of the regression curves ranging from 0.012 to 1.153. Thus, a single correction factor for the HR method for citrus could not be obtained with this calibration technique.

As with the HR method, the SFD was underestimated with the CHP method, in all species, compared to the gravimetric method. Relatively good correlations ($R^2 > 0.7$) between SFD measured with the CHP and gravimetric methods were observed in some branches from 'Star Ruby' grapefruit, 'Delta' Valencia, and 'Eureka' lemon, but once again, poor correlations were found for 'Nadorcott' mandarin ($R^2 = 0.27$). The slope of the regression equations differed within the same cultivar and also between species and cultivars and ranged from 0.015-0.538.

As was observed with the HR and CHP methods, SFD was also underestimated using the TD method. Despite the observed underestimation, good linear correlations were found for 'Star Ruby' grapefruit, 'Delta' Valencia and 'Eureka' lemon ($R^2 > 0.7$), although there was some variation in the slopes (0.019-0.194) of the regression equations within the same species and between species and cultivars.

The variations in conducting sapwood was determined by staining of the sapwood, which was not uniform in all species. The percentage surface area of conducting sapwood varied within the same cultivar and between different species. A large proportion of the sapwood in the 'Nadorcott' mandarin sample was not stained and following analysis it was found that only 8% of the sapwood area was able to conduct water. Cross sections of the sapwood of 'Delta' Valencia, 'Star Ruby' grapefruit, 'Bahaininha' navel, 'Nadorcott' mandarin and 'Eureka' lemon were prepared to expose the xylem vessels in both mature and flush leaf wood (FLW) for

further analyses. The xylem vessels in mature leaf wood (MLW), which is the wood produced early in the season, were widely distributed, with greater distances between the vessels. On the other hand in the FLW, xylem vessels were more closely packed, with smaller distances between the vessels and were arranged into groups of two to three vessels. Mature and FLW was evident in sapwood samples taken from 'Delta' Valencia, 'Star Ruby' grapefruit and 'Eureka' lemon, whilst only mature wood was found in sapwood samples from 'Bahaininha' navel and 'Nadorcott' mandarin. In general, the xylem vessels in MLW were more widely spaced in 'Delta' Valencia samples (554 µm in stems and 474 µm in branches), whilst in the sapwood of the 'Nadorcott' mandarin they were more closely spaced (415 µm in stems and 396 µm in branches). Although differences between species were not always significant ($p < 0.05$), it was found that for the stems in this study the distance between vessels exceeded the limit (400 µm) determined by Swanson (1983) for thermal homogeneity, except for the branches of the 'Nadorcott' mandarin.

Validating SFD measurement methods with a weighing lysimeter

Sap flux densities measured at different depths over a period of four days were characterised by early morning increases, midday depressions and virtually zero flows at night for both the HR and CHP methods. Hourly measurements with the HR method determined diurnal patterns of sap flow with great sensitivity. T_{sap} was overestimated at low flows and underestimated at high flow rates for 'Midknight' Valencia trees compared to the mass loss measured with the lysimeters. Regression analysis showed that the correlation between the HR and gravimetric methods for the hourly measurements was highly significant (P value < 0.0001) and the linear relationship accounted for 84% (lysimeter 1) and 87% (lysimeter 2) of the variation in the data. When day time whole-plant water use was calculated, the HR method measurements corresponded closely with gravimetric measurements. Overall values of RMSE and MAE were 0.10 and 0.09 (L day^{-1}) respectively. T_{sap} was 0.02 L day^{-1} (from MBE calculation) lower than the water loss measured with the weighing lysimeter. The HR method therefore tended to underestimate tree transpiration by 1.08% on a day time water use basis. A highly significant linear relationship existed between day time T_{sap} and day time water loss measured with the gravimetric method. This was indicated by a P value of less than 0.001, a R^2 of 0.98 and with the intercept not significantly different from zero and the slope not significantly different from unity (according to t-test).

A comparison between hourly transpiration, measured with the CHP method and gravimetric method, was conducted for two different calibration windows, 27-30 April 2015 and 9-12 September 2015. A good and highly significant (P value < 0.001) correlation ($R^2 = 0.83$ and $R^2 = 0.73$) was found between the hourly transpiration determined with the CHP method and

transpiration determined gravimetrically. However, at low flow rates (< 0.15 L h^{-1} for tree 1) transpiration was overestimated and at high flow rates (> 0.15 L h^{-1} for tree 1) transpiration was underestimated by the CHP method. When day time water use was calculated and compared with the day time mass loss determined with the lysimeters, a highly significant (P value < 0.001) linear relationship (R^2 = 0.91) was obtained with a slope of unity and an intercept of zero. Statistical analyses (RMSE of 0.1 and MAE of 0.08 L day^{-1}) revealed that T_{sap} was 0.02 L day^{-1} (from MBE calculation) higher than the mass loss measured with a weighing lysimeter. The CHP method, therefore, slightly overestimate transpiration by 1.23% day^{-1}.

Infield validation of the SFD measurements using eddy covariance measurements

Transpirational sap flow (T_{sap}) determined with the HR and CHP methods were compared to transpiration (T_{res}) determined as a residual of ET and E from EC measurements, assuming negligible transpiration from cover crops (clean orchard). When a wound correction factor of 2.5 mm (width of the widest probe) was used the HR and CHP method yielded similar results, with both methods underestimating transpiration by a similar magnitude. The HR method was lower than the T_{res} by 42%, whilst the CHP method was 36% lower than the T_{res}. When a comparison was made between the two methods of T estimation, a highly significant linear relationship between T_{sap} determined by HR method and T_{res} was observed (P value < 0.01, R^2 = 0.97, RMSE=0.008, MAE=0.42 and MBE= -0.42), whilst a moderately strong significant linear correlation was observed between T_{sap} from the CHP method and T_{res} (P value < 0.05, R^2 = 0.80, RMSE = 0.01, MAE = 0.36 and MBE = -0.36).

As a result of the underestimation of T_{res} by the two SFD measurement methods, it was deemed necessary to calibrate the techniques. A significant relationship (P value < 0.05) was obtained using a wound size of 3.6 mm for the CHP method and a highly significant (P value < 0.01) relationship for the HR method was obtained with a wound width of 4.4 mm. In this case the analysis resulted in an MBE of 0.01 mm day^{-1}, a RMSE value of 0.03 mm day^{-1} and MAE of 0.05 mm day^{-1} for the CHP method and an MBE of -0.01 mm day^{-1}, a RMSE value of 0.02 mm day^{-1} and MAE of 0.03 mm day^{-1} for the HR method. Although the CHP and HR methods both provided good estimates of transpiration once calibrated, frequent missing data and outlier data points were observed with the CHP method. These missing and outlier data points were replaced by averaging the previous and the succeeding value. Some CHP method probes also registered noisy data which required a significant amount of data patching and baseline adjustment to minimise the “noise”. As the main aim of this study was to determine the most appropriate method for the quantification of transpiration in citrus, the CHP method was not considered to be an ideal method for long term measurements because of the overall quality of the data obtained. In contrast, the HR method provided an almost

complete record for the calibration period. Therefore the HR method is proposed to be a better suited technique for long term measurements, when considering raw data quality. Further, when T_{sap} (HR method) was determined using the measured wound width (4.7 mm) and heartwood and sapwood radii there was a close match between T_{sap} and T_{res} (R^2=0.85), with a MBE of -0.05 mm day^{-1}, a RMSE of 0.04 mm day^{-1} and MAE of 0.05 mm day^{-1}. Orchard transpiration was underestimated by 5% on average per day, which is considered reasonable. The close match of the HR method to HR measurements show that if the parameters (wound width, sapwood depth and heartwood radius) for determining SFD with the HR method are measured accurately, accurate measurements of transpiration in *Citrus sinensis* can be achieved.

CAPACITY BUILDING

A number of students benefitted from this research project and used the data generated throughout the project to obtain post graduate qualifications. Three students received their MSc degrees and two their honours degrees. Information on and results from the project were communicated to the growers community through a series of information sessions held at the CRI Research Symposium, farmer's days, study group meetings and workshops. Results from this research project were also presented at local and international conferences and published in conference proceedings and as a chapter in a book named Citrus. The detail of the capacity building activities are listed below.

MSc

i. Ms Mpaballeng Sam. 2016. Calibration of sap flow techniques in citrus using the stem perfusion method. University of Pretoria
ii. Mr M Banda. 2017. Validating SFD measurement methods in *Citrus sinensis*. University of Pretoria
iii. N Shongwe. 2018. Measuring and modelling canopy size of *Citrus sinensis* in relation to water use. University of Pretoria. In press

Honours projects

i. Ms A Bresler. 2016. Environmental control of transpiration of different Citrus spp. University of Pretoria
ii. Mr N Neethling. 2016. Seasonal variation in water relations and transpiration of Valencia oranges in summer and winter rainfall regions. University of Pretoria

Information dissemination and study groups

i. A special session titled “Water use of citrus orchards” was held at the 8th Citrus Research Symposium on 19 August 2014. This symposium is held every two years and is the main event, with more than 500 delegates, for the exchange of knowledge and experience between researchers, industry and farmers in South Africa
ii. A farmer’s day for upcoming citrus farmers was arranged by Mr Andrew Mbedzi and held in conjunction with the CRI at the farm of Mr Chauke, close to Thohoyandou in the Limpopo Province, on 6 August 2015. The meeting was attended by 48 delegates that consisted of emerging citrus farmers, the Chief of the local area, and officials from the local municipality and Agriculture Research Council
iii. Mr MC Pretorius, an extension officer of the Citrus Research International (CRI) arranged an information day in conjunction with the CRI and Letsitele Constantia Study Group in the Letsitele region on 5 April 2016. The meeting was attended by approximately 40 commercial citrus farmers. Information on the project was well received, especially in the light of the current drought

Study group meetings

i. Citrusdal Study Group, Patrysberg. Citrus Water Use and Irrigation Scheduling, JT Vahrmeijer. 7 February 2017
ii. Benede-Oranje River Study Group. Field visits to Mosplaas Sitrus, Loveren & Renosterkop. Presentation at Lake Grapa. Citrus Water Use and Irrigation Scheduling, JT Vahrmeijer. 9 February 2017

Workshops

i. CRI production workshops held at Swadini, Loskop Dam, Nelspruit, Jeffreys Bay and Citrusdal, JT Vahrmeijer during September 2015
ii. Drought management, Allée Bleue, Simondium. Citrus Water Use and Management, JT Vahrmeijer. 27 October 2017

Popular articles

i. Water research in citrus. JT Vahrmeijer, TG Grout and NJ Taylor. South African Fruit Journal. June 2015, p 88-90
ii. Irrigation scheduling made easy. JT Vahrmeijer, and NJ Taylor. AgriCulture, April 2017

Conference presentations

i. Are sap flow measurements useful for determining water use of fruit orchards, when absolute values are important? Taylor NJ, Ibraimo NA, Annandale JG, Everson CS, Gush MB, Vahrmeijer JT. 9th International Workshop on Sap Flow Ghent, Belgium. June 2013

ii. Calibrating sap flow systems: is it really necessary? Taylor NJ, Vahrmeijer JT, Everson CS, Sam MC, Teklemichael B, Gilfillan RG, Annandale JG. South African Society of Crop Production/Soil Science Society of South Africa/Southern African Society of Horticultural Sciences Combined Congress, Grahamstown, January 2014

iii. Are citrus trees thirsty? Vahrmeijer JT, Annandale JG, Everson CS and Taylor NJ. 8th Citrus Research Symposium, Champagne Sports Resort, Drakensberg 17-20 August 2014

iv. Are all citrus trees created equal? Taylor NJ, Annandale JG, Everson CS and Vahrmeijer JT. 8th Citrus Research Symposium, Champagne Sports Resort, Drakensberg 17-20 August 2014

v. Validating SFD measurement methods in potted *Citrus sinensis*. Banda M, Vahrmeijer JT and Taylor NJ. South African Society of Crop Production/Soil Science Society of South Africa/Southern African Society of Horticultural Sciences Combined Congress, George, January 2015

vi. Calibration of sap flow techniques using the stem perfusion method. Sam MC, Taylor NJ and Vahrmeijer JT. South African Society of Crop Production/Soil Science Society of South Africa/Southern African Society of Horticultural Sciences Combined Congress, George, January 2015

vii. Validating SFD measurement methods in potted *Citrus sinensis.* Banda M, Vahrmeijer JT and Taylor NJ. South African Society of Crop Production/Soil Science Society of South Africa/Southern African Society of Horticultural Sciences Combined Congress, George, January 2015

viii. Modelling water use of subtropical fruit the crops: the challenges. Taylor NJ, Annandale JG, Vahrmeijer JT, Ibraimo NA, Mahohoma W, Gush MB, Allen RG. X International Symposium on Modelling in Fruit Research and Orchard Management, June 2-June 5, 2015. Agropolis international, Avenue Agropolis. Montpellier, France

ix. Testing the heat ratio method for sap flow estimates in *Citrus sinensis*. Vahrmeijer JT, Taylor NJ, Everson CS and Banda M. X International Symposium on Modelling in Fruit Research and Orchard Management, June 2-June 5, 2015. Agropolis international, Avenue Agropolis. Montpellier, France

x. Validating SFD measurement methods in potted *Citrus sinensis.* Banda M, Taylor NJ, Everson CS and Vahrmeijer JT. South African Society of Crop Production/Soil Science Society of South Africa/Southern African Society of Horticultural Sciences Combined Congress, Bloemfontein, 18-21 January 2016

xi. Modelling transpiration of citrus orchards. Taylor NJ, Vahrmeijer JT, van der Merwe S, Ibraimo NA, and Annandale JG. South African Society of Crop Production/Soil Science

Society of South Africa/Southern African Society of Horticultural Sciences Combined Congress, Bloemfontein, 18-21 January 2016

xii. Can crop coefficients be used to schedule irrigation in citrus? Taylor NJ, Mahohoma W, Vahrmeijer JT, Gush MB, Allen RG and Annandale RG. 9th Citrus Research Symposium held at Champagne Sports Resort, Drakensberg on 21-25 August 2016

xiii. Citrus water use in a winter rainfall region. Vahrmeijer JT, Taylor NJ, Banda M and Sam CM. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 21-25 August 2016

xiv. Validating SFD measurement methods in potted Citrus sinensis. Banda M, Taylor NJ and Vahrmeijer JT. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 21-25 August 2016

xv. Validating SFD measurement methods in potted *Citrus sinensis.* Banda M, Taylor NJ, Everson CS and Vahrmeijer JT. International Citrus Conference, Foz do Iguaçu, Brazil, 18-23 September 2016

xvi. Citrus water use in a Mediterranean climate. Vahrmeijer JT, Taylor NJ, Banda M, Sam M and Everson CS. International Citrus Conference, Foz do Iguaçu, Brazil, 18-23 September 2016

Conference proceedings

i. Citrus Water Use in South Africa. Vahrmeijer JT, Annandale JG, Gush MB and Taylor NJ. Acta Horticulture. Proceedings. International Citrus Congress, November 2012, Valencia, Spain

ii. Are sap flow measurements useful for determining water use of fruit orchards, when absolute values are important? Taylor NJ, Ibraimo NA, Annandale JG, Everson CS, Gush MB, Vahrmeijer JT**,** Acta Horticulture 991. Proceedings: 9th International Workshop on Sap Flow. Editor K Steppe

iii. Modelling water use of subtropical fruit the crops: the challenges. Taylor NJ, Annandale JG, Vahrmeijer JT, Ibraimo NA, Mahohoma W, Gush MB, Allen RG. Proceedings of the X International Symposium on Modelling in Fruit Research and Orchard Management, June 2-June 5, 2015. Agropolis international, Avenue Agropolis. Montpellier, France. Editor E. Costes

Book chapter

Vahrmeijer JT and Taylor, NJ. 2018. Citrus Water Use. Citrus, Intechopen, London

CONCLUSIONS

There are a number of reliable methods for quantifying orchard level water use, including EC measurements and weighing lysimeters. Lysimetry methods, which are considered as the reference method, take years to be established before measurements are conducted (Subedi et al., 2013) and lysimeters sufficient to house large citrus trees are costly to construct and maintain. The only realistic and relatively inexpensive methods available for transpiration (T) measurements are SFD techniques (Smith and Allen, 1996). Whilst the theory behind the different methods differs, all the techniques use heat as a tracer to estimate water movement in a stem. It was, therefore, decided to focus on SFD measurement methods, which can be used for a wide range of stem sizes. The major concern with these techniques is that they tend to underestimate T_{sap} (Green and Clothier, 1988; Jones et al., 1988; Steppe et al., 2010), and as a result species-specific calibration is often required (Smith and Allen, 1996).

Results from the stem perfusion technique showed that SFD was consistently underestimated by the HR, CHP and TD methods for the citrus cultivars and species tested. Underestimation of SFD with the HR method was less compared to the other two methods. Although fairly good correlations between SFD, determined with HR, CHP and TD methods, and that determined gravimetrically were obtained, the correlation coefficients were inconsistent within a cultivar or between species. As a result a single empirical calibration factor could not be calculated to correct sap flow measurements from SFD techniques to that determined gravimetrically. However, the data showed that all three methods can potentially be used to estimate water use in citrus, provided corrections are made for thermal inhomogeneity of the sapwood and that the area of conducting sapwood is accurately determined. Our analysis of the performance of SFD techniques showed that the HR method should perhaps be considered before the CHP and TD methods, as the underestimations of SFD were less with this method. Capturing low flow rates is one of the strengths of the HR method.

The large variation in calibration factors between stems of an individual species and between species was attributed to a number of factors. Firstly, it was noted that on a number of occasions the inserted probes were placed in non-conducting tissue. Large variations in conducting sapwood were noted in some stems, which was impossible to identify prior to the insertion of probes. Secondly, the mature sapwood of citrus branches in this study were not thermally homogenous and therefore deviated from the ideal heat pulse velocity (HPV) theory. However, the FLW adhered to the definition of a thermally homogenous medium, but was not found in all species. Thirdly, the xylem anatomy of the stem will have a large influence on measurements and this must be taken into account with the installation of the HR and CHP probe sets.

Initial evaluation of the HPV equipment and method against a weighing lysimeter, in a *Eucalyptus marginata* tree (model specie for HR method), gave reliable results. This gave the assurance that the locally manufactured HPV systems and methods are reliable and accurate. Both the HR and CHP methods were then tested on 'Midknight' Valencia trees in the glasshouse, where they performed equally well when compared to the weighing lysimeters in a glasshouse. Over a period of 37 days for the HR and 30 days for the CHP method, an error less than 2% was found between the HR and CHP methods and the weighing lysimeter, when a wound width of 2.0 mm (width of the widest probe) was used in the SFD calculations. However, on an hourly basis both the HR and the CHP method overestimated T_{sap} when flow rates were less than 0.1 L h^{-1} and underestimated T_{sap} at flow rates greater than 0.1 L h^{-1}. A time lag between sap flow measurements and mass loss measured with the lysimeter was observed. The measurement of sap flow and the actual process of T are spatially separated on the tree, which may contribute to the time lags between the two measurements techniques. The lag was more pronounced at low flow rates towards the end of the day when the weighing lysimeter registered virtually no mass loss, whilst the sap flow methods continued to record sap movement. Another process that may be involved is the refilling of the tree at the end of the day. After cessation of T (closing of stomata) no mass loss is registered with the lysimeter, but sap continues to flow (recorded with HR method) in order to rehydrate the plant tissues (Coelho et al., 2012).

The HR and CHP methods were calibrated and validated in a commercial 'Washington' navel orchard against EC measurements combined with estimates of soil evaporation. The wound width was initially assumed to be 2.5 mm (width of the widest probe), which was valid for the glasshouse experiment. Brass collars were used in the field experiments to house the heater probes in order to protect them against corrosion. However, this resulted in a severe underestimation of T_{res} by approximately 42% with the HR method and 36% with the CHP method when compared to T_{res}. Wound width is one of the most difficult parameters to determine in the field and an attempt to measure the actual wound width resulted in a 37% coefficient of variation (CV) in the field, while for the glasshouse a CV of 1% was observed. The exact cause of the large variation in wound width in field experiments is not known, but one can expect a greater wound response in fast growing trees when compared to the potted slower growing 13 year old trees in the glasshouse. Nonetheless, a large variation in wound width for the infield experiments led to the calibration of the techniques focusing mainly on a wound width correction factor. An agreement of 94% was observed between T_{sap} (HR method) and T_{res}, underestimating orchard T by only 0.4%, when a virtual wound width correction factor of 4.4 mm was used. On the other hand, the CHP method also performed reasonably well against T_{res} with a correlation coefficient of 78%, overestimating orchard T by 1.4%, when a

virtual wound width of 3.6 mm was used. Of the two methods, the HR method performed better than the CHP method, though the CHP is known to perform well under high flow rates (> 5 $cm^3\ cm^{-2}\ h^{-1}$ and > 100 $cm^3\ cm^{-2}\ h^{-1}$). The HR method was tested within its limits, with a maximum SFD observed of approximately 20 $cm^3\ cm^{-2}\ h^{-1}$, which is below its upper limit of 45 $cm^3\ cm^{-2}\ h^{-1}$ (Vandegehuchte and Steppe, 2013). The wound width correction factor (CHP 3.6 mm and HR 4.4 mm) determined in this experiment exceeded the wounding expected by Barrett et al. (1995) who suggested that wound extends 0.3 mm either side of the widest probe. However, observations of wound width at the end of the study suggest that this is a real value for citrus, as the observed wound width was on average 4.7 mm. Coupled with the results from staining of the conducting sapwood, T_{sap} values within 5% of T_{res} were obtained for the HR method. These were good results considering the potential large sources of error associated with this validation and it is concluded that SFD methods, especially the HR method, can be used in citrus orchards, if the parameters such as wound width, sapwood conducting area and heartwood radius are determined accurately.

RECOMMENDATIONS FOR FUTURE RESEARCH

When using the stem perfusion method it is suggested that a better way is found to achieve different flow rates, as these sudden changes in flow are registered by the gravimetric readings but are not necessarily taken into account by the SFD techniques. Future research should also focus on improved practical infield measurements and assessment of the wound widths.

In this study a technique to measure tree water use was validated and rigorously tested. This technique can give detailed insights into tree water use and changes in tree water use due to external factors, such as changes in canopy size and irrigation management. Therefore, in the light of the recent droughts, future research should focus on quantifying tree water use when water saving practices are implemented, such as the reduction in canopy size and changes of irrigation practices and systems. The research should take into account the impact of reduced tree water use on yield, fruit quality and the time it takes to recover to previous yield levels under optimal conditions.

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LIST OF ABBREVIATIONS

A_n	Conducting Sapwood Area
AWS	Automated Weather Station
CHP	Compensation Heat Pulse
C_s	Heat Capacity of Wood (4 182 J kg^{-1} $^oC^{-1}$)
CV	Coefficient of Variation
C_w	Heat Capacity of Water (1 200 J kg^{-1} $^oC^{-1}$)
D	Willmott Index of Agreement
E	Evaporation
EC	Eddy Covariance
ET	Evapotranspiration
ET_o	Reference Evapotranspiration
E_s	Evaporation from Soil Surface
fc	Fractional Canopy Cover
G	Soil Heat Flux (W m^{-2})
FLW	Flush Leaf Wood
HFD	Heat Field Deformation
HPV	Heat Pulse Velocity
HR	Heat Ratio
k	Fresh Wood Thermal Diffusivity (2.5 x 10^{-3} cm^2 s^{-1})
MAE	Mean Absolute Error
MBE	Mean Bias Error
m_c	Sapwood Moisture Content
MLW	Mature Leaf Wood
OD	Outside Diameter
RH	Relative Humidity
RMSE	Root of the Mean Square Error
R_n	Net Radiation (W m^{-2})
SFD	Sap Flux Density (cm^3 cm^{-2} h^{-1})
SHB	Stem Heat Balance
T	Transpiration
T_a	Air Temperature
TD	Thermal Dissipation
t_e	Same temperature measured at upstream and downstream sensors at time t_e
THB	Trunk Heat Balance

T_{res} $ET - E_s$

T_{sap} Transpiration determined with the sap flow technique

V_1 and V_2 Temperature increases

V_h HPV (cm h^{-1})

V_h' Corrected HPV (cm h^{-1})

VSF Volumetric Sap Flow (L h^{-1})

ρ_s Density of Water (g cm^{-3})

ρ_b Sapwood Density

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1 INTRODUCTION

Quantification of plant transpiration (T) is essential to facilitate the understanding of water and energy balances of orchards and field crops in relation to environmental conditions (Bauerle et al., 2002). Various techniques have been developed to quantify T through plant physiological measurements (chamber system and sap flow methods), hydrological methods (weighing lysimeters) and micrometeorological measurements based on aerodynamic and eddy covariance (EC) methods (Rana et al., 2005). Of these techniques weighing lysimetry is considered as the reference method to measure crop water use (Subedi et al., 2013). This method is preferred because it gives a direct measure of whole plant water use. However, in many circumstances, this technique is mainly restricted to potted plants and is not feasible for large trees and infield measurements due to the cost and the limited number of trees that can be studied. One of the major disadvantages is that once constructed the equipment cannot be moved around the orchards (Bauerle et al., 2002). On the other hand, sap flow measurement techniques are relative cheap and easily moveable between trees and can be installed in a multifaceted heterogeneous terrain. They are also powerful tools for scaling from an individual tree to an entire plant community (Vertessy et al., 1997). Sap flow measurement techniques work on the principle that the ascent of sap in plants *via* the xylem vessels is equivalent to T (Sun et al., 2012). This assumption is considered valid, since approximately 5% of the total water that is absorbed from the soil is assimilated into the plant. The remaining 95% is lost to the atmosphere by T through stomata in the leaves (Schroeder et al., 2001).

Sap flow measurement techniques are thermally based techniques, as they rely on heat as a tracer of sap flow in plants (Kool et al., 2014). These thermometric techniques have been utilised in the study of plant water relations for the past 80 years (Phillips et al., 2009) and can be categorised into: (i) heat balance methods, (ii) constant heating methods and (iii) heat pulse methods (Kool et al., 2014). The heat balance techniques depend on the measurement of different components of heat transported from a continuous constant heat supply (Smith and Allen, 1996). It comprises of the trunk heat balance (THB) and stem heat balance (SHB) methods. The major advantage of the heat balance methods is that no calibration is required prior to measurements. However, the major disadvantage of these methods is that they cannot be used on trees with large stems, where the trunk diameter exceeds 12.5 cm. Gauges should also be installed on sections of the stem that are straight and have no swellings or lumps, as this could cause poor contact between the stem surface and the heater or thermocouples (Smith and Allen, 1996). Constant heating methods are used to measure sap flow velocity based on heat dissipation from a constant heating source into the stem. They

comprise the thermal dissipation (TD) method (Granier, 1985) and the heat field deformation (HFD) method (Smith and Allen, 1996).

The heat pulse methods are centred on quantifying the sap velocity with the application of a heat-pulse and determining the increase in temperature in a thermocouple downstream from the heater needle. Heat pulse techniques include the heat ratio (HR) and the compensation heat pulse (CHP) methods (Ortuno et al., 2006). Heat pulse techniques are often preferred due to their simple installation and low power requirements (Burgess et al., 2001). They have proved to be reliable, convenient and non-destructive methods of continuously measuring sap flow (Green et al., 2003). They can be used to quantify water use without calibration in plants, which are classified as thermally homogeneous (Smith and Allen, 1996). The heat pulse methods have been used intensively to estimate T_{sap} of individual trees (Dunn and Connor, 1993; Barrett et al., 1995; Bauerle et al., 2002), but it is essential, however, to calibrate the technique in plants with thermally inhomogeneous xylem like citrus. Xylem is considered to be thermally homogenous if sap-conducting vessels are uniformly distributed in the sapwood and the distances between the xylem vessels do not exceed 0.4 mm (Swanson and Whitfield, 1981). A distinction can be made within the existing sap flow methods, between those measuring sap flux density (SFD) such as TD, CHP and HR methods and those measuring volumetric sap flow rate such as SHB and THB. Sap flux density has a unit of $cm^3\ cm^{-2}\ h^{-1}$ and describes the amount of sap flowing through a certain stem surface area per time unit, while total sap flow refers to the volumetric sap flow rate in a plant stem or stem section with units of $g\ h^{-1}$ (Vandegehuchte and Steppe, 2013). Sap flux density methods can discern spatial differences in SFD within the plant and allow more detailed investigation of hydraulic plant traits. The latter are very suitable for estimating whole-plant water use; but are less suited to the investigation of variation in sap flow within the plant (Vandegehuchte and Steppe, 2013). This is important because sap velocity varies both radially across the stem and circumferentially (Wullschleger and King, 2000).

Sap flux density measurement techniques are intrusive in that they require the insertion of thermocouples coupled with a linear heater into the sapwood, at the position where the sap flow is to be measured (Fernández et al., 2006). The insertion of probes causes sap stream disruptions, which can modify the thermal homogeneity of the adjacent sap-conducting wood, and ultimately leads to a systematic underestimation of measured HPV (Fernández et al., 2001). As a result, calibration coefficients which relate the measured HPV to real sap flow are required to account for the influences of probe thermal properties, flow blockages, differences between species and wounding (Bauerle et al., 2002). In addition, sapwood structure of some species does not adhere to the original definition of a homogenous and porous material

(Marshall, 1958). If the distance between the xylem vessels is too large, the time required for thermal equilibrium between the sap and woody matric becomes significant and affects the transmission and measurement of the heat pulse (Smith and Allen, 1996). As citrus has diffuse porous sapwood, meaning that it has narrower vessels which are packed more densely per unit wood area (Taneda and Sperry, 2008), this may need to be taken into account when using these techniques. Fernández Luque et al. (1999), highlighted that theoretical calibration coefficients have not been widely tested and that further tests are required. Steppe et al. (2010) also pointed out that the calibration of the techniques should be conducted for different species. A number of methods have been used to test and calibrate the SFD techniques. These include the cut tree method (Dunn and Connor, 1993), stem perfusion (Fernández et al., 2001; Steppe et al., 2010), micrometeorological methods (Poblete-Echeverría et al., 2012), and weighing lysimeters (Bleby et al., 2004). With the weighing lysimeters, two assumptions are employed namely, (i) water loss only occurs through the plant, and; (ii) the plant loses no mass other than that of water (Cirelli et al., 2012).

One of the aims of the study was to calibrate and test the most appropriate SFD technique to quantify T in *Citrus sinensis*. Three approaches were taken. The first approach consisted of a laboratory study, where a wide range of citrus species were evaluated with the stem perfusion method. In the second approach, tree water use measurements from different SFD techniques were compared to tree water use measurements conducted with a weighing lysimeter, which is considered to be a reference method for water use studies. Infield validation of the HR method and the CHP method was then conducted against EC measurements in a commercial orchard to assess the validity of the calibration performed under controlled conditions in the third approach.

Objectives for the first approach – the calibration and testing of the most appropriate SFD technique for citrus using the stem perfusion method:

i. To measure SFD in stem segments of ‘Delta’ Valencia, ‘Bahaininha’ navel, ‘Nadorcott’ mandarin, ‘Star Ruby’ grapefruit and ‘Eureka’ lemon using SFD techniques, whilst determining the rate of water moving through the stem gravimetrically.
ii. To conduct measurements under a wide range of sap flow densities in a number of cut stems.
iii. To examine the potential and adequacy of each technique in different citrus species.
iv. To calculate the correction factors for each citrus variety/cultivar.
v. To determine if xylem anatomy of citrus is the same across cultivars and species and if this impacts the empirical calibration.

Objectives for the second approach – determination and validation of an appropriate SFD technique with weighing lysimetery:

i. To test the performance of the CHP and HR methods in potted *Eucalyptus marginata* trees.
ii. To compare tree water use measurements, conducted with the SFD methods, to tree water use measured with a weighing lysimeter in potted *Citrus sinensis* trees.
iii. To calculate calibration coefficients of a number of test trees of the same species in the glasshouse and test if these coefficients would be applicable to infield measurements.

Objectives for the third approach – infield validation of SFD methods with micrometeorological measurements.

i. Infield validation of the HR method with EC measurements.
ii. Infield validation of the CHP method with EC measurements.

2 LITERATURE OF SAP FLUX DENSITY MEASUREMENT METHODS

2.1 Sap flux density techniques

Sap flux density techniques are among the most widely accepted and appropriate methods for determining T in woody plants and although the theory between the various SFD techniques differs, they have in common the use of heat as a tracer to estimate sap flow (Vieweg and Ziegler, 1960). These systems are easy to automate, the data is fairly easily interpreted, they do not alter the microclimate of the plant and they can estimate T over extended periods of time (Smith and Allen, 1996). A number of SFD techniques are available and a distinction needs to be made between methods which measure sap flow rates (g h^{-1}) and methods which measure SFD (cm^3 cm^{-2} h^{-1}, also expressed as cm h^{-1}) (Vandegehuchte and Steppe, 2013). Methods measuring total sap flow in a plant stem or stem section are very useful for determining whole plant water use, whilst SFD measurements estimate the amount of sap flowing through a specific surface area per unit time. Sap flux density methods are well suited to investigate the variation of sap flow within plants and are divided into three major categories: i) methods that use a pulse of heat and include the HR, CHP, Tmax, calibrated average gradient and “Sap flow+” methods, ii) those that apply continuous heat include the TD or Granier method and iii) the HFD method (Vandegehuchte and Steppe, 2013). Three techniques (HR, CHP and TD) that have been used successfully in a number of studies within the stem, and even within the branches of woody plants, will be the focus of this study. In Table 2.1 the advantages and disadvantages related to accuracy, required parameters, ease of installation and use, ease of data interpretation, cost, power requirements, systematic error and available expertise of the three different methods are summarised. The major disadvantage of these methods is the underestimation of sap flow, which occurs as a result of wounding caused by the implantation of sensors into the sapwood. It is therefore important to understand how each technique works under different circumstances and to test and calibrate the various SFD techniques prior to the measurement phase, as it is imperative that accurate T rates are captured. This chapter will provide the basic theory of the various SFD techniques and how these techniques can be calibrated using other independent methods, such as stem perfusion, weighing lysimeters and micrometeorological measurements (Fernández et al., 2001; Bush et al., 2010; Hultine et al., 2010).

Table 2.1 Advantages and disadvantages of sap flux density (SFD) techniques and parameters required for determining SFD

Method	Advantages	Disadvantages	Parameters to be measured
Heat ratio method	Measures low flow rates and even reverse sap flow. Has a simple function to describe wound effects. Low power requirements. Simple instrumentation. Data is generated as electrical signals, suitable for further processing or storage on data loggers. Less susceptible to natural temperature gradients within the sapwood.	Performs poorly at high flow rates (> 45 cm^3 cm^{-2} h^1). Wounding results from drilling of holes for sensor installation, which must be accounted for by wounding correction coefficients. Accuracy depends on the correct spacing during sensor installation. Requires species-specific calibration.	Sapwood density. Sapwood water content. Wound width. Area of sapwood. Axial thermal diffusivity of wood. Specific heat capacity of the woody matrix. Specific heat capacity of the sap.
Compensation heat pulse method	Low power requirements. Simple instrumentation. Data is generated as electrical signals, suitable for further processing or storage on data loggers. Less susceptible to natural temperature gradients within the sapwood. Independent of thermal diffusivity.	Incapable of resolving reverse, low or very high flux densities (< 5 and > 100 cm^3 cm^{-2} h^{-1}). Wounding results from drilling of holes for sensor installation, which must be accounted for by wounding correction coefficients. Accuracy depends on correct spacing during sensor installation. Requires species-specific calibration.	Sapwood density. Sapwood water content. Wound width. Area of sapwood. Specific heat capacity of the woody matrix. Specific heat capacity of the sap.
Thermal dissipation method	Can be used in large diameter trees. Allows estimation of low, average and high sap flux densities (0-80 cm^3 cm^{-2} h^{-1}). Simple and reliable method. Low cost. Accuracy does not depend on probe spacing, provided the reference probe is not influenced by the heated probe.	Wounding results from drilling of holes for sensor installation. Natural temperature gradients in the sapwood affect measurement accuracy. Difficult to detect zero flux. High power requirement. Not capable of detecting reverse flow. Requires species-specific calibration.	Zero flow rate is required for calculations. Natural temperature gradients. Proportion of the length of the heater probe in contact with the sapwood and inactive xylem.

Methods which measure sap flow rates include the SHB technique. However, for the purposes of this study, where sap flow systems were to be calibrated for mature citrus orchards, the SHB technique will not be discussed further. This is due to the fact that the method is not readily applicable to stems greater than 15 cm in diameter and different stem sizes require different size sensors, which is expensive and has a high power usage (Smith and Allen, 1996).

2.2 Heat pulse techniques

Heat pulse techniques have been utilised for over 80 years, dating back to 1932 when the use of heat as a way of tracking sap flow was first developed by Bruno Huber (Marshall, 1958). In his experiments, Huber applied heat to a 4 mm tropical liana stem for 1-5 seconds and noticed that heat could be detected at a thermocouple located 30 cm downstream (Marshall, 1958). The velocity of the heat pulse was calculated by dividing the distance between the heater and the thermocouple with the time it took for the thermocouple to register an increase in temperature. Huber initially concluded that the method was only suitable for sap velocities greater than 60 cm h^{-1}, but later realised the significance of differentiating between the transport of heat by thermal conduction and the influence of convection by the moving sap. Thermocouples were then positioned upstream and downstream from the heater to distinguish between these two effects, and this became the early form of the CHP method (Marshall, 1958). The period of highest warming of the upstream sensor when related to the downstream sensor was used for 'compensating' for the special effects of thermal conduction (Green et al., 2003). The method was then later modified by Marshall (1958) who developed analytical solutions for the heat pulse techniques. The limitations of the CHP method to measure low sap velocities were later realised by Becker (1998). At low sap flow rates the heat pulse dissipated by conduction before reaching the thermocouples when the sap velocities were lower than 0.01-0.02 mm s^{-1} and were indistinguishable from zero flows (Becker, 1998). In such circumstances, the up- and downstream sensors record the same temperatures, since the temperatures have reverted to initial values (Burgess et al., 2001). This limitation has serious consequences, because the contribution of low flow rates to water movement is important during both daytime and night-time in tropical overstory and understory trees. As a result, the CHP method was modified and the HR method was developed to detect reverse and low flow rates. In this method, the up- and downstream thermocouples are placed equidistant (0.5 cm) from the heater (Becker, 1998; Burgess et al., 2001).

2.2.1 Theory of compensation heat pulse method

The CHP method described by Swanson and Whitfield (1981) and Smith and Allen (1996) has been used to determine water use in apples and kiwi fruits (Cohen et al., 1981), *Citrus*

sinensis, plum and olives (Fernández et al., 2006), *Eucalyptus (Dye and Olbrich, 1993)*, Asian pear (Caspari et al., 1993), *Eucalyptus maculate, Doryphora sassafras and Ceratopetalum apetalum* (Barrett et al., 1995), *Pinus radiate* (Teskey and Sheriff, 1996), willow (Green et al., 2003) and lemon (Alarcón et al., 2006), amongst many others. This method has the advantage that it is independent of thermal diffusivity, a sapwood characteristic that has to be determined for the HR method (Vandegehuchte and Steppe, 2013). The CHP method, however, is unable to quantify reverse, low or very high sap flux densities (< 5 $cm^3\ cm^{-2}\ h^{-1}$ and > 100 $cm^3\ cm^{-2}\ h^{-1}$ respectively), because under these conditions no equality between the upstream and downstream temperatures occur (Vandegehuchte and Steppe, 2013). The CHP method is based on a heater probe that is inserted between two thermocouples, which are in line with the axis of the stem and inserted radially to the same depths in the sapwood (Burgess et al., 2001). In a normal arrangement (Figure 2.1), designated as "5, 0, 10 mm" formation, the upstream thermocouple is installed 5 mm and the downstream thermocouple 10 mm from the heater probe (Swanson and Whitfield, 1981). Short heat pulses from the heater probe are periodically released into the sap stream and the thermocouples are used to detect an increase in sap temperature, which is used to compute the rate of transfer of the heat pulse as it travels in the sap stream (Smith and Allen, 1996).

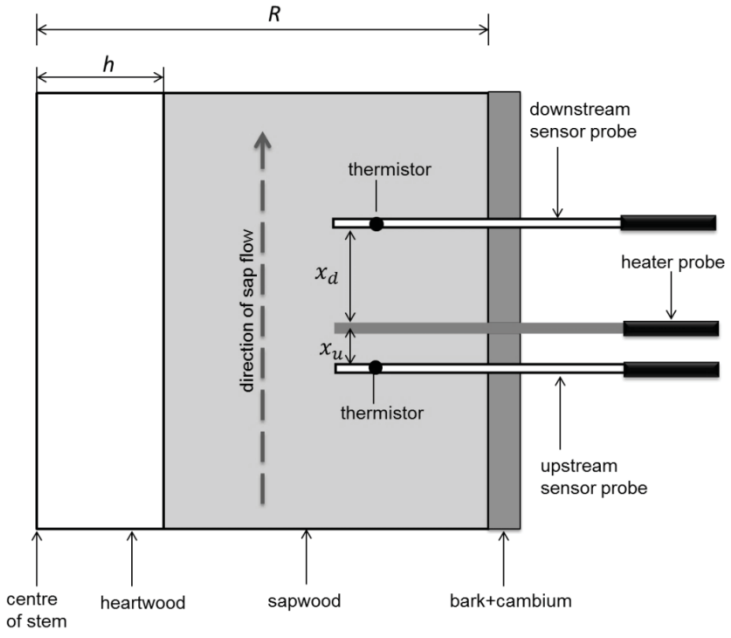

Figure 2.1 Configuration of the compensation heat pulse (CHP) method, with probes inserted into a stem of radius R and the heartwood boundary at distance h from the centre of the stem. Adapted from Smith and Allen (1996)

Within 1-2 seconds after application of the heat pulse the upstream thermocouple, which is closer to the heater probe, detects the increase in temperature first. This increase in temperature is due to conduction, while the increase in temperature measured by the downstream thermocouple is due to convection (sap flow). At time t_e both thermocouples register the same temperature. This has the physical meaning that the heat has travelled 2.5 mm from the heater probe, which is midway between the thermocouples (Figure 2.2). The rate of heat transfer is determined by measuring the time it took the heat pulse to travel to the midpoint between the thermocouples by convection and conduction (Burgess et al., 2001) and can be calculated as follows (Swanson and Whitfield, 1981):

$$V_h = \frac{X_u - X_d}{2t_e} 3600 \quad (1)$$

Where t_e = time taken to reach thermal equilibrium at the thermocouples at x_u and x_d distances (mm) from the heater probe and 3 600 converts seconds to hours.

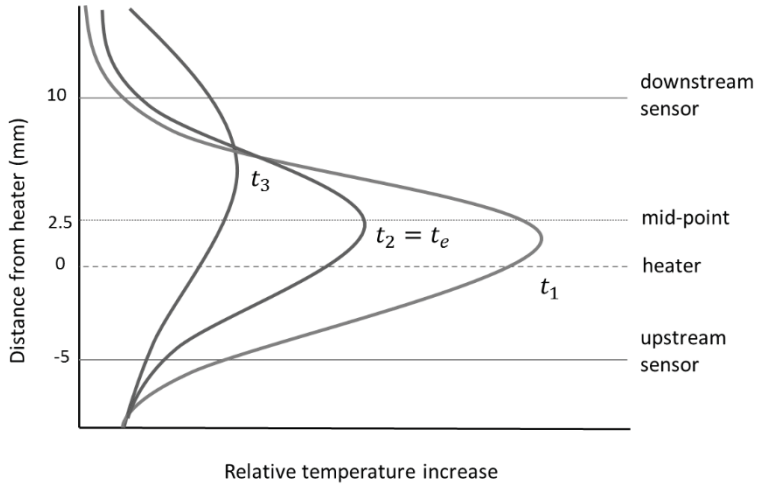

Figure 2.2 Dissipation of a heat pulse released from the heater probe into a stem containing moving sap for the configuration shown in Figure 2.1. The distribution of relative temperature at times t_1, t_2 and t_3 after release of the heat pulse is illustrated, with the temperature of the upstream and downstream sensor probes equal at time t_e. Adapted from Smith and Allen (1996)

Equation 1 is applicable if the assumption holds that the influence of the heater probe and thermocouples on the sapwood and sap flow is insignificant (Alarcón et al., 2006) and that sufficient sap flow occurs so that the heat pulse does not dissipate by conduction before being detected by the thermocouples (Burgess et al., 2001). In practice, however, the transportation of heat by convection is disrupted by the drilling of holes into the xylem tissue and the insertion of the heater probes (Green et al., 2008) that leads to the underestimation of the HPV. The

HPV measurements can be corrected (V_h', cm h^{-1}) for the induced effect of wounding and to account for the influence of the materials used to construct the heater and sensor probes, by using the wound coefficients as calculated by Swanson and Whitfield (1981):

$$V_h' = a + bV_h + cV_h^2 \quad (2)$$

Where a, b, c and d are the correction factors calculated for specific wound widths (z cm):

$$a = -11.744z^2 + 14.59z - 1.6424 \quad (3)$$

$$b = 7.2088z^2 - 6.4412z + 2.2024 \quad (4)$$

$$c = 2.3935z^2 - 0.3194z + 0.0259 \quad (5)$$

After wound correction, SFD (cm h^{-1}) can be calculated from equation 6 (Barrett et al., 1995):

$$SFD = \frac{\rho_b V_h'}{\rho_s C_s}(C_w + M_c C_s) \quad (6)$$

where C_w and C_s are the heat capacities of water and wood respectively, with C_w = 1 200 J kg^{-1} $^{\circ}C^{-1}$ at a temperature of 20°C (Edwards et al., 1997), C_s = 4 182 J kg^{-1} $^{\circ}C^{-1}$ at a temperature of 20°C. M_c is sapwood water content, $\boldsymbol{\rho}_b$ is the wood density (g cm^{-3}) and $\boldsymbol{\rho}_s$ is density of water (g cm^{-3}).

Integration of individual sap flow velocity measurements to obtain whole stem sap flux was performed according to the method of the weighted sum of SFDs with the associated sapwood area for each insertion depth (Hatton et al., 1995). Finally, the volumetric sap flow (VSF) is determined by integration of individual SFDs at different depths in the stem which are weighted according to the area of conducting sapwood for each insertion depth (Steppe et al., 2010):

$$VSF = \frac{(\sum_{i=1}^{n} A_n SFD)}{1000} \quad (7)$$

where VSF is the volumetric sap flow L h^{-1}, which can be considered as the product of sapwood conducting area and SFD (Pfautsch et al., 2010), 1 000 represents the conversion factor from cm^3 to L, n is the number of thermocouples for each heater probe and A_n is different conducting sapwood areas (cm^2) for each insertion depth.

Calibration of the compensation heat pulse method in citrus

The CHP method was compared with a gravimetric method (weighing balances) to estimate T in 2-year-old *Citrus limon* trees (Alarcón et al., 2006). A good correlation (R^2 = 0.97) was

found between the CHP method and the gravimetric measurements. Fernández et al. (2006) also calibrated the CHP method for oranges using an excision experiment and a forced-flow perfusion experiment. In this study the CHP method clearly overestimated sap flow, especially at high perfusion rates, but qualitatively tracked the actual sap flow, which was measured by collecting the perfusion solution from the distal end. The overestimation observed was attributed to larger lumen diameters when compared to other tested species. The results from the two calibration experiments conducted in citrus shows the possibility of using the CHP method to quantify citrus water use, hence further tests are required to confirm the applicability of CHP method in quantifying citrus water use.

2.2.2 Theory of the heat ratio method

Becker (1998) highlighted the limitations of the CHP method in measuring low sap flow rates if the sap flow velocities are between 0 and 0.02 mm s^{-1}. This led to the development of the HR method, which can measure reverse and low flow rates, but has limited accuracy for flux densities greater than 45 $cm^3\ cm^{-2}\ h^{-1}$ (Burgess et al., 2001; Vandegehuchte and Steppe, 2013). The HR method works on the same principle as the CHP method, but with the difference that the thermocouples are placed equidistant from the heater (Figure 2.3). The HR method has frequently been used to compute water use in *E. marginata* (Burgess et al., 2001; Madurapperuma et al., 2009) and olive trees (Williams et al., 2004) according to the equation developed by Marshall (1958):

$$V_h = \frac{k}{x} \ln \frac{V_1}{V_2} 3600 \quad (8)$$

where V_h is the HPV, x is the distance (5 mm) between the heater and the thermocouples , V_1 and V_2 are temperature increases (from initial temperatures) at the same distance, upstream and downstream of the heater probe (Figure 2.3), k is the fresh wood thermal diffusivity (2.5 x 10^{-3} $cm^2\ s^{-1}$) and 3 600 converts seconds to hours.

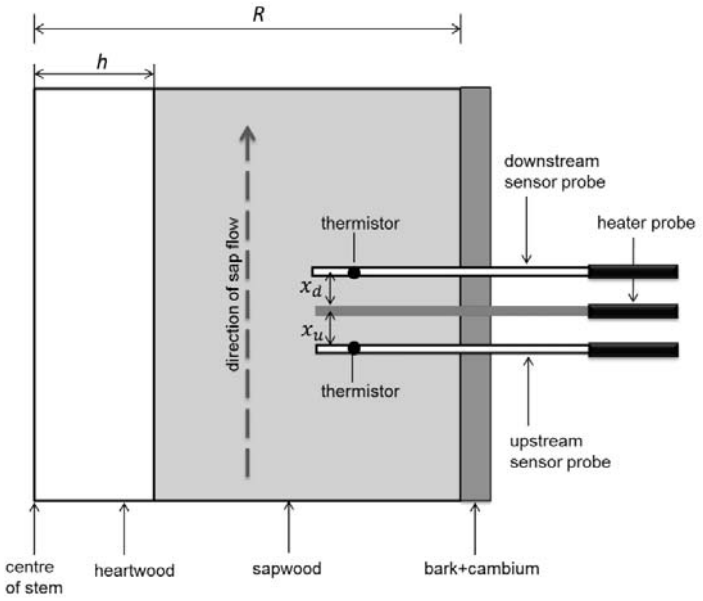

Figure 2.3 Configuration of the heat ratio method, with a heater probe and thermocouples inserted into a stem of radius R and the heartwood boundary at distance h from the centre of the stem. Adapted from Burgess et al. (2001)

As with the CHP method installing the heater probe and thermocouples results in wounding and consequently modifies the flow of sap. In addition, tyloses which are outgrowths, can form on the parenchyma cells of the xylem, which can block the vessels and disrupt sap flow (Barrett et al., 1995). Burgess et al. (2001) modified the empirical model of Swanson and Whitfield (1981) for the correction of wounding, because solutions did not pass through the origin and the resulting corrections yielded a poor approximation of low and reverse sap flow rates.

$$V_C = bV_h + cV_h^2 + dV_h^3 \quad (9)$$

where V_c is the corrected HPV, V_h is the HPV and b, c and d are the correction coefficients to adjust for wound width (cm) calculated as follows:

$$b = 6.6155x^2 + 3.332x + 0.9236 \quad (10)$$

$$c = -0.149x^2 + 0.0381x - 0.0036 \quad (11)$$

$$d = 0.0335x^2 - 0.0095x + 0.0008 \quad (12)$$

Sap flux density is then computed according to Marshall (1958), which was later modified by Barrett et al. (1995):

$$SFD = \frac{V_c \rho_b}{\rho_S C_s} (C_w + m_c C_s) \quad (13)$$

where C_w =1 200 J kg^{-1} $^{\circ}$C^{-1} and C_s = 4 182 J kg^{-1} $^{\circ}$C^{-1} are respectively the specific heat capacities of water and wood at a temperature of 20°C, m_c the sapwood moisture content, ρ_b the wood density (g cm^{-3}) and ρ_s the density of water (g cm^{-3}). Volumetric sap flow is then calculated as described for the CHP method using equation 7.

Calibration of the heat ratio method in citrus

There have not been many studies on the calibration of SFD techniques, specifically the HR method, in *Citrus sinensis*. A literature search has revealed that the HR method has been used in *Citrus sinensis* ('Delta' Valencia, 'Bahianinha' navel and 'Rustenburg' navel) by Taylor et al. (2015) to quantify T. In their study, T determined via sap flow measurements was scaled up to orchard T and compared with evapotranspiration (ET) during periods of negligible evaporative water loss from the soil and cover crops. A nearly 1:1 relationship was observed after correction. However, the calibration focused on determining a wound width correction factor that would result in a 1:1 relationship between ET, when determined with an EC system, and T when determined with the HR method. Their study did not focus on the validity of HR method when compared to an independent measure of T, which left room for research on the accuracy of the HR method compared to the golden standard (gravimetric method) of T measurement in *Citrus sinensis*.

2.3 Methods for calibration and validation of sap flux density techniques

There is a general perception that sap flow methods tend to underestimate tree water use (Steppe et al., 2010), due to the drilling and insertion of probes in the stem, which obstruct and block the flow of sap. As a result, a number of authors have stressed the importance of determining species-specific calibration curves prior to measurements (Fernández Luque et al., 1999; Steppe et al., 2010). Several independent methods are available to calibrate SFD techniques, which relate the measured HPVs to real sap flows. These independent methods include weighing lysimetry, whole plant gas exchange chambers, cut-tree, stem perfusion and micrometeorological techniques.

2.3.1 Weighing lysimeters

For crop ET measurements, weighing lysimeters are considered to be the most reliable, due to direct and simple measurements without damage to the plants (Aboukhaled et al., 1982). Evapotranspiration is estimated directly through changes in mass and ET can be quantified over short periods, without the need for any interpretation or scaling (Beeson, 2011). Transpiration can also be determined by minimising evaporation (E_s) from the soil surface

using a soil cover, e.g. a plastic sheet (Burgess et al., 2000). Lysimeters are, however, expensive to install and to maintain and have therefore not been used extensively for crop water use measurements, especially tree crops. Historically, weighing lysimeters have always been considered as a suitable tool to correctly measure ET (Aboukhaled et al., 1982; Tanner and Sinclair, 1983). This should, however, be considered the "gold standard" for tree T to which sap flow measurements should be compared, as it is a measure of whole plant T, that does not cause injury to the plant or embolisms in the xylem tissue.

Many types of scale systems have been used in weighing lysimeter designs. Lever-load cell scales are commonly used, because counterbalancing is easy and load cell signals can be recorded with high precision electronic data recorders (Howell et al., 1995). Weighing lysimeters are generally calibrated by adding known quantities of mass to the lysimeter (Howell et al., 1995) and have been used to quantify T in citrus (Green and Moreshet, 1979; Vellame et al., 2010) and apples (González-Altozano et al., 2008). Validation of the CHP method with a weighing lysimeter was conducted by Alarcón et al. (2006) in well-watered apricot trees. A strong linear relationship (regression line was within 5% of the 1:1 line) was found between sap flow and T measured with the weighing lysimeter. Vellame et al. (2010) reported that the SHB method proved to be reliable in estimating daily T in citrus trees, but underestimated tree T by 4.6% when compared to a weighing lysimeter. Madurapperuma et al. (2009) validated the HR method with gravimetric measurements and found that the HR method corresponded very closely with the gravimetric measurements ($R^2 = 0.92$) with a slope close to unity and an intercept of zero. Bleby et al. (2004) validated the HR and CHP methods simultaneously in *E. marginata* saplings using weighing lysimeters and found an agreement of 99% and 96%, respectively. Slopes of one and intercepts of zero on an hourly basis were achieved between the HR method and weighing lysimeter and the CHP and the weighing lysimeter (Bleby et al., 2004). Whilst there have been examples of sap flow calibration in large weighing lysimeters (Shackel et al., 1992; Nortes et al., 2008), calibration is often performed in potted trees on smaller balances or specially constructed lysimeters with load cells (Burgess et al., 2000; Alarcón et al., 2006). However, potted trees with stem diameters large enough for installation of sap flow sensors are uncommon, hence calibrations are typically done at lower SFD's.

2.3.2 Stem perfusion method

The stem perfusion method is usually performed under laboratory conditions and involves the forcing of water through a section of a stem or branch in which probes are inserted, as shown in Figure 2.4 (Fernández et al., 2001; Steppe et al., 2010). The stem is attached to a cylinder and the sap that moves through the stem is collected in a beaker and weighed using an

electronic balance. Pressure is applied and maintained during the measurements to achieve the desired flow rates, which are comparable to the flow rates obtained in the field. The gravimetric measurements made, with an electronic balance, are compared with those measured with sap flow sensors, following normalisation for conducting sapwood area.

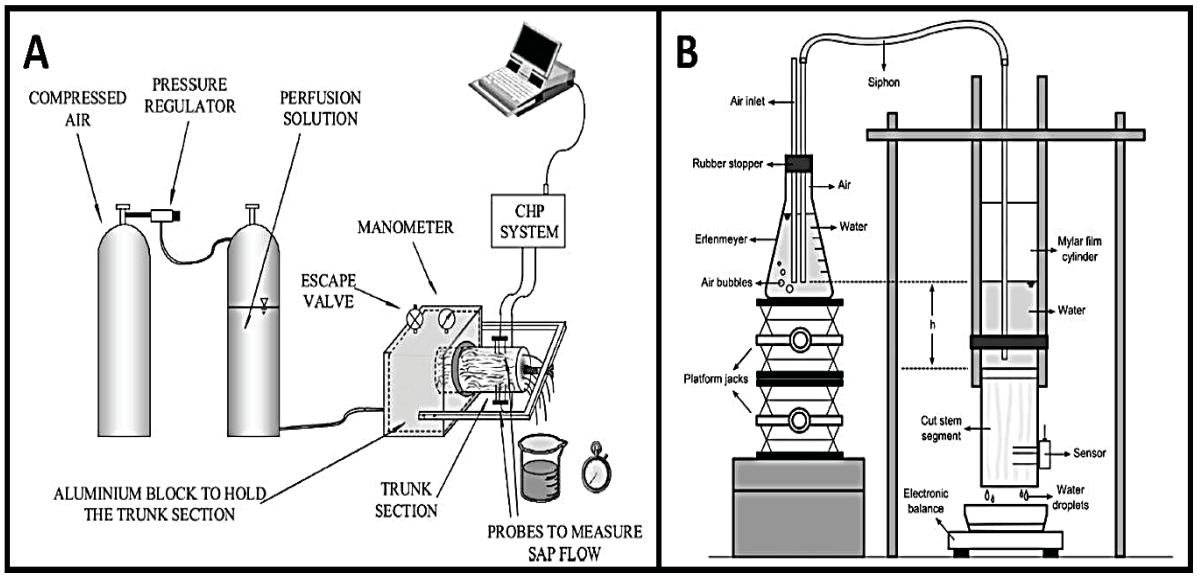

Figure 2.4 Experimental set-up for calibrating sap flow systems using the stem perfusion method. A) A pressurised system described by Fernández et al. (2001) and B) the Mariotte's bottle principle described by Steppe et al. (2010)

The advantage of this method is that it allows the direct comparison of SFDs and calibration can be performed fairly quickly in a number of species, using excised branches. The stem characteristics, such as sapwood conducting area, wood density and stem water content that are required for calculation of SFD, can be easily obtained from the cut stem or branch segments. The disadvantage of this method is that some xylem vessels can become occluded during cutting of the stems or branches.

2.3.3 Micrometeorological methods

Infield calibration of SFD techniques can also be performed against measurements of ET using micrometeorological methods, i.e. EC measurements (Conceição and Ferreira, 2008; Poblete-Echeverría et al., 2012). This calibration method can be done in the field without using lysimeters. Although the lysimeters provide more accurate measurements they are expensive to construct and the period it takes for the tree to grow on a lysimeter can be substantial before measurements can be conducted (Kramer and Boyer, 1995). In comparison to other methods of calibration such as the potometer and stem perfusion, this method is non-destructive. Even though the measurements from EC are associated with

some error, these errors can be evaluated and reduced through the careful analysis of data (Allen et al., 2011). Furthermore, a weighing lysimeter is used to quantify water use of a single tree, whereas the EC measurements represent a number of trees and a much larger area. It is also important to bear in mind that the size and shape of the area sampled are not fixed in time and vary with wind speed and direction (Horst and Weil, 1992; Baldocchi, 1997). For calibration, T_{res} is calculated as the residual of ET measurements with the EC technique and soil evaporation (E_s) determined with microlysimeters:

$$\text{Transpiration} = \text{ET} - \text{E}_\text{s} \tag{14}$$

Validation includes the linear regression analysis between T determined as the residual of ET and E_s and T_{sap} determined with the sap flow technique (Figure 2.5). A strong linear correlation (R^2 = 0.88) was found by Poblete-Echeverría et al. (2012) when the CHP method was calibrated with micrometeorological data and E_s data using microlysimeters (Figure 2.5). Microlysimeters are generally considered the most reliable method to measure E_s and often serve as a validation for other methods (Kool et al., 2014). Some researchers, however, have reported some limitations in the use of microlysimeters, which include the inability to measure during irrigation or rain (Thompson et al., 1997) and reduced representation of field conditions due to small sample size (Daamen et al., 1993). Micrometeorological measurements represent crop ET and the fetch over a large portion of the orchard. Therefore, calibration of sap flow systems against this method will include possible errors in the upscaling process from individual tree T_{sap} to stand or orchard T.

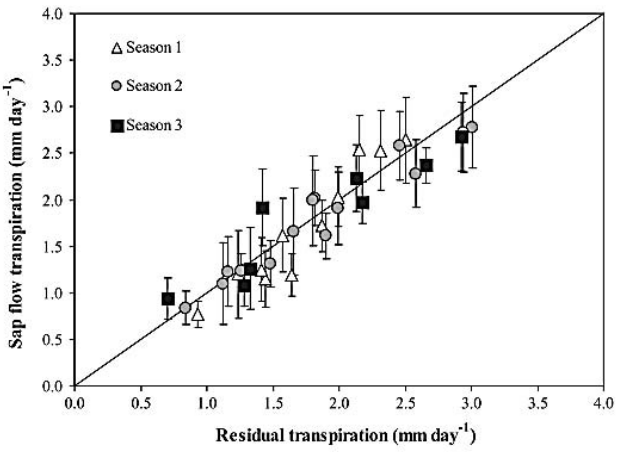

Figure 2.5 Comparison between residual transpiration (T_{res}) measured with a eddy covariance system and evaporation (E) measured with microlysimeters and transpiration (T_{sap}) determined using the compensated heat pulse (CHP) method for three seasons in a Merlot vineyard (Poblete-Echeverría et al., 2012)

3 MATERIAL AND METHODS

3.1 Validating SFD measuring methods with the stem perfusion method

3.1.1 Selection of plant material

Calibration and evaluation of the HR, CHP and TD methods, with the stem perfusion method, was done at the Environmental Biophysics Laboratory of the Department of Plant and Soil Sciences at the University of Pretoria. Stems and branches of four different species of citrus trees were used in the experimental work, namely *Citrus sinensis* (sweet orange), *Citrus reticulata* (soft citrus), *Citrus paradisi* (grapefruit) and *Citrus limon* (lemon). These samples were collected from different orchards in the Mpumalanga Province. 'Delta' Valencia and 'Star Ruby' grapefruit were collected from Golden Frontiers Farm in Hectorspruit, 'Bahianinha' navel from Dirishana Farm and 'Nadorcott' mandarin were collected from Naranja Farm, both in Burgersfort. 'Eureka' lemon was collected from the Hatfield Experimental Farm at the University of Pretoria in Gauteng. The trees were between 7 and 11 years old and the characteristics of the branches are given in Table 3.1 and Table 3.2. A minimum of two branches (replicates) of each cultivar (treatment), with a circumference of approximately 15 cm, were selected and cut at the base close to the main axis of the tree. The samples were marked to indicate direction of sap flow before they were immediately submersed into water to maintain moisture and to prevent embolism formation. The samples were then taken back to the laboratory to start with the experiments without further delay.

Table 3.1 Characteristics of the branches used in the calibration experiments of the heat ratio (HR) and compensation heat pulse (CHP) methods

Species	Replicate	Length (cm)	Circumferences (cm)	Cross sectional area (cm^2)	Area of conducting sapwood (%)	Area of conducting sapwood (cm^2)
Citrus sinensis 'Delta'	1	28.0	15.0	17.90	10.5	1.88
	2	26.0	14.0	15.60	31.6	4.93
	3	25.0	14.0	15.60	16.2	2.53
Citrus paradisi 'Star Ruby'	1	29.0	16.5	21.66	28.9	6.26
	2	29.0	15.0	17.90	32.5	5.82
	3	24.5	15.0	17.90	38.7	6.93
Citrus reticulata 'Nadorcott'	1	37.0	18.0	25.78	12.8	3.30
	2	36.0	17.5	24.37	8.8	2.14
Citrus sinensis 'Bahianinha'	1	34.0	15.0	17.90	39.8	7.13
	2	30.0	14.5	16.73	4.8	0.80
Citrus limon 'Eureka'	1	33.0	14.5	16.73	62.2	12.83
	2	33.5	15.0	17.90	47.4	8.72
	3	35.0	15.0	17.90	48.0	9.78
	4	33.5	14.0	15.60	34.4	7.82

Table 3.2 Characteristics of the branches used in the calibration experiments of the thermal dissipation (TD) method

Species	Replicate	Length (cm)	Circumferences (cm)	Cross sectional area (cm^2)	Area of conducting sapwood (%)	Area of conducting sapwood (cm^2)
Citrus sinensis 'Delta'	1	28.0	15.0	20.37	12.3	2.51
	2	26.0	14.0	16.73	14.3	2.39
	3	25.0	14.0	15.60	8.6	1.34
Citrus paradisi 'Star Ruby'	1	29.0	16.5	21.66	18.9	4.09
	2	29.0	15.0	16.73	7.5	1.25
	3	24.5	15.0	23.00	14.3	3.29
Citrus limon 'Eureka'	1	25.0	16.1	17.90	46.6	8.34
	2	25.0	15.2	20.37	36.6	7.46
	3	25.0	16.0	20.37	32.1	6.54

3.1.2 Experimental setup – Stem perfusion

Heat pulse velocity

Each probe set consisted of two type-T copper-constantan thermocouples, embedded in polytetrafluoroethene tubing with an outside diameter (OD) of 2 mm, and a 1.8 mm OD stainless steel heater probe. For the HR method, thermocouples were placed equidistant (5 mm) upstream and downstream of the heater probe, whilst for the CHP method the upstream thermocouple was placed 5 mm from the heater probe and the downstream thermocouple 10 mm from the heater probe. Two probe sets were installed, one set at a depth of 10 mm and the other set 15 mm below the bark, within the xylem of each branch. This was done to account for the radial variation in sap flux in the conducting sapwood. Holes were carefully drilled using a drill jig to ensure that the probes were correctly spaced and the holes drilled parallel to each other. To generate a heat pulse, heat was applied for 0.4 seconds every 10 minutes. The heat pulse velocities were then measured and logged at 10 min intervals using a CR1000 data logger and an AM16/32B multiplexer (Campbell Scientific Inc., Logan, Utah, USA). The thermocouples, heater probes and relay control modules were manufactured locally (Andrew Everson, Pietermaritzburg).

Thermal dissipation

The temperature difference, ΔT (K), in the TD method was measured between a heater probe that emitted heat constantly and an unheated reference probe located approximately 40 mm from each other. According to Vandegehuchte and Steppe (2013) exact spacing is less important in this method, as long as the reference probe is not influenced by the heated probe. The TD probe set (model TDP30, Dynamax Inc., Houston, TX, USA) consisted of two 30 mm long stainless steel needles with an OD of 1.2 mm. Holes were drilled into the branch using a drill guide supplied by the manufacturer. The probes were attached to a Dynamax FLGS-TDP XM1000 sap velocity system (Dynamax Inc., Houston, TX, USA), which consisted of a Campbell CR1000 logger, an Campbell AM16/32B multiplexer and an adjustable voltage regulator that was set at 3V for the two TDP30 probes. Data were logged every 5 min.

Stem perfusion calibration setup

A modified Mariotte-based verification system (Figure 3.1 A) was used for the calibration of various SFD techniques using cut citrus branches, according to the method described by Steppe et al. (2010). Different designs were tested before choosing a design that was able to achieve the desirable head of water pressure on the stems. For our initial design, an approximately 300 mm high cylinder made of plastic film was fixed directly to the stem with a polymer and double-sided adhesive tape to ensure a good tight fit and to avoid any leakage (Figure 3.1). A 4 L flask was filled with water and sealed with a plastic stopper in which two

glass tubes were installed. One of the glass tubes acts as an air inlet and the other, attached to a third glass rod by flexible tubing, acts as a siphon. By adjusting the height of the flask, the siphon maintained the flow of water to the column, which allowed a constant head of water on the stem segment, regardless of flow rate through the stem. Although Steppe et al. (2010) found good flow rates without the use of complicated equipment in their experiment, high flow rates were not achieved in the citrus branches and significant leakage occurred from the plastic column during the initial stages of this study.

The design was therefore modified with the aim of achieving high flow rates by applying pressure on a stem segment. A bicycle tyre pump was used to pump air into an 800 mm plastic tube filled with water and with valves fitted to both ends of the tube, to increase the pressure on the column of water (Figure 3.1 B). With this design, a maximum pressure of approximately 100 kPa was achieved. This design also allowed for the preparation of stems and calibration to be completed within 12 hours, which significantly reduced xylem damage and blockage. However, the pressure applied with the bicycle type pump was not constant and this had a great impact on sap flow measurements. The design was changed and a CO_2 regulator was installed to achieve a constant pressure with reasonably constant flow rates.

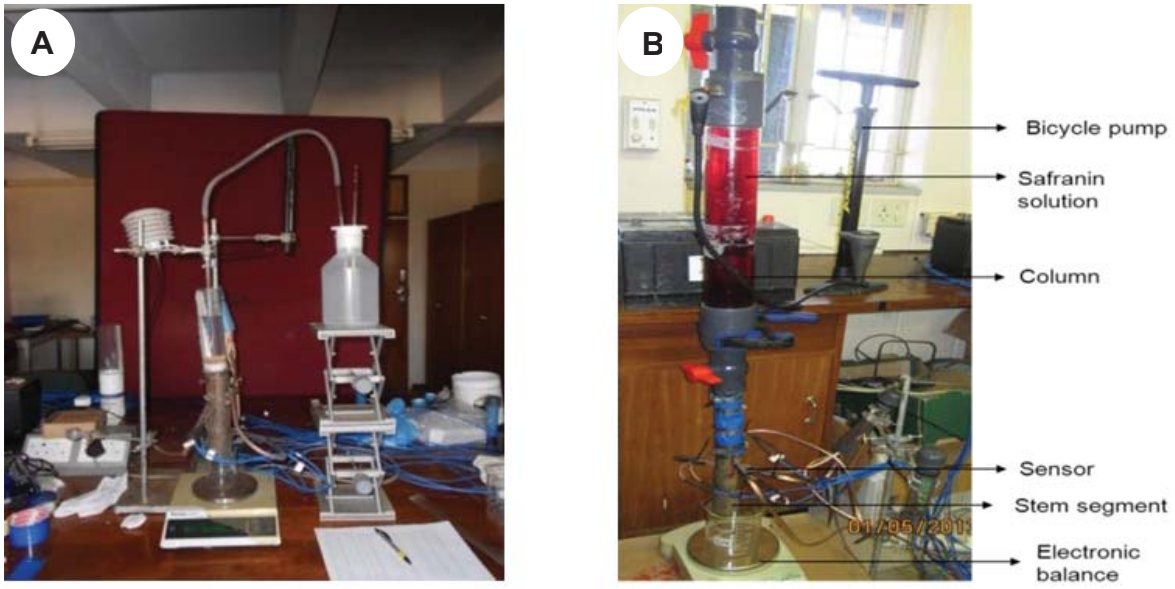

Figure 3.1 (A) Initial and (B) modified experimental set-up for the calibration of sap flow using the stem perfusion method

Branches, taken from the orchards, were cut at both ends with a circular saw to a sample length of 350 mm. Holes for probe insertion were drilled at 90° angles to each other, using a grill guide to ensure that the holes were spaced correctly vertically and parallel and to avoid any misalignment. Two HR and two CHP probe sets were installed simultaneously on one

stem at opposite sides of the stem and separated vertically by at least 20 mm. The TD probes were installed into separate stems. The probes were coated with petroleum jelly to ease probe insertion and to ensure good thermal contact between the probes and the xylem tissue. Each probe set was thermally insulated with foam.

A sharp blade was then used to re-open any closed xylem vessels resulting from the sawing. Both sides of the stem sample were covered with a wet cloth during stem preparation to avoid dehydration and the formation of embolisms. At both ends of the stem, a section of 40 mm bark was removed. This was done to ensure that water flowed through the xylem and not the phloem and that a tight fit was obtained between the rubber hose connecting the stem and pressurised container with water. All joints were checked for any leakages.

The baseline for the measurements was determined from zero flow conditions. Data were logged from the installed probe sets for approximately one hour prior to the start of the experiment, when no water flow occurred. The stem was then flushed with distilled water for one hour and once the readings had stabilized, different flow rates were achieved by applying different pressures, using the CO_2 regulator. Each flow rate was maintained for at least 45 min, before changing the pressure. Water passing through the stem was collected in a glass beaker and weighed every 10 min for the HR and CHP methods and every 5 min for the TD method using an electronic balance (Mettler Toledo model PB3002-S or Precisa model 800M both manufactured in Switzerland).

At the end of each experiment Safranin O dye was added to the water in the column and pressure was increased so that sap flow occurred. This resulted in the staining of the active xylem in the stem (Figure 3.2). A cross section of the stems was made at the probe insertion positions. Photographs were taken of the cross section and digitally analysed using Adobe® Photoshop® CS5 Extended (Version 12.0x32). The cross sectional stem area and the total number of pixels in this area was determined. From the colour range menu the number of pixels corresponding to that particular RGB range was determined, as shown in Figure 3.2. The percentage conducting sapwood was then calculated from the total number of pixels representing the staining divided by the total number of pixels in the stem section multiplied by 100.

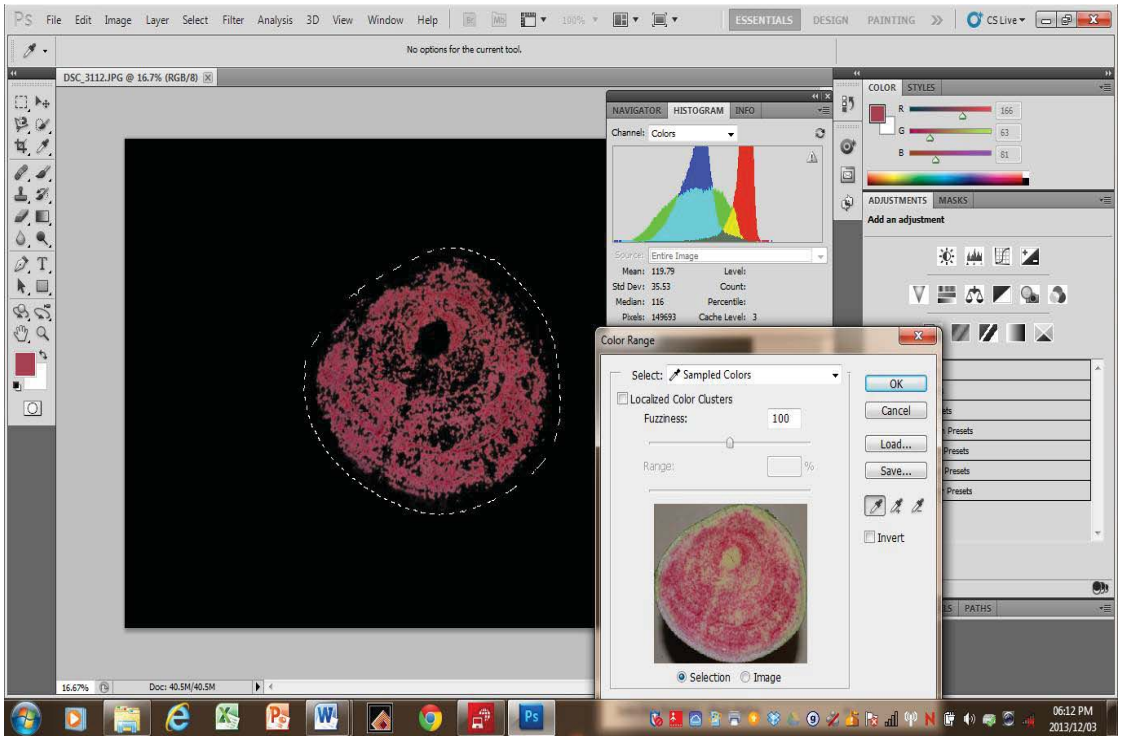

Figure 3.2 Determination of sapwood conducting area using pixel analysis in Adobe® Photoshop®

Gravimetric SFD was calculated as the rate of water passing through the stem segment divided by the cross section area of the conducting sapwood. The wound width for the HPV techniques was considered to be 2 mm, which is the diameter of the thermocouples. It was assumed that the wound would not extend beyond this distance, as calibration was completed within 5 hours of probe insertion. The temperature difference during the period of zero flow (ΔT_o), required for the TD method calculations, was determined at least one hour prior to the start of calibration when no water was forced through the stem.

The linear relationship between SFD determined gravimetrically and the SFD techniques was evaluated using correlation and regression analyses. A correction factor was then calculated as the reciprocal of the slope of this relationship, as performed by Steppe et al. (2010). The sap flow, measured with the HPV and TD techniques, was then corrected using this correction factor to assess the ability of a single correction factor to provide accurate estimates of sap flow.

3.1.3 Thermal interference

The possible thermal inference between probe sets installed together on the same branch was evaluated in order to ensure that good quality data were obtained. Two probe sets of the HR method and six additional thermocouples were installed in one stem according to the layout

in Figure 3.3. Probes 1U, 1L, 3 and 4 were positioned 0.5 cm from the heater probe, whilst probes 1 and 2 were positioned 1 cm and probes 5 and 6 positioned 2 cm from the heater probe. The two HR probe sets were placed opposite each other in the stem and the heater probe was set to release heat for 0.3, 0.5 and 0.8 seconds. Temperatures were recorded for 60 s after the release of the heat pulse.

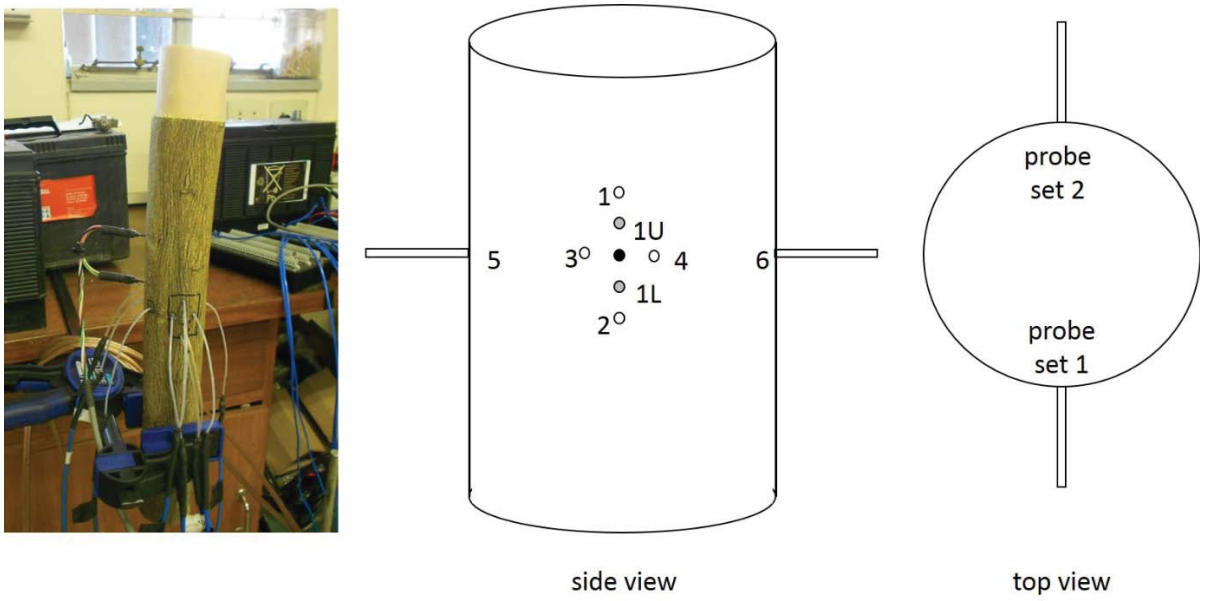

Figure 3.3 Positioning of sensors in the stem to determine possible thermal interference between the different techniques. Grey circles indicate the positioning of the heat ratio thermocouples for probe set 1, whilst the black circle indicates the positioning of the heater. The white circles indicate additional thermocouples used to determine the distance the heat pulse travels in the sapwood

3.1.4 Sapwood properties

Physical properties

Sapwood characteristics are important parameters required for the calculation of SFD. Sapwood density and sapwood water content were determined following the procedures outlined by Steppe et al. (2010). Bark was removed from small pieces of wood and the fresh mass was determined. The samples were then submerged in water for at least 30 minutes to ensure adequate swelling and saturation with water. It was then removed from the water, and submerged into a known mass of water in a beaker on a balance and the mass was recorded immediately. This was done carefully without touching the bottom or side walls of the beaker for estimation of the volume from the fresh mass of the sapwood sample. Thereafter the samples were oven dried at 60°C. Samples were removed periodically and weighed until no

further loss in mass occurred. The volume of sapwood and fresh and dry mass were used to determine sapwood density and water content. Sapwood density (ρ_b) was calculated as:

$$p_b = \frac{w_d}{v_f} \quad (15)$$

where w_d (g) is the oven dry mass and v_f is the volume of a freshly excised section of wood.

The sapwood moisture (m_c) content was calculated as:

$$m_c = \frac{w_f - w_d}{w_d} \quad (16)$$

where w_f (g) is the fresh mass.

In Table 3.3 the m_c and ρ_b are given for different species of citrus, which were used for calculation of SFD in this study and the broader project. The average m_c content was 0.61 ± 0.08 g and the ρ_b was 0.74 ± 0.04 g cm^{-3}, which shows that these parameters are fairly conservative for citrus species.

Table 3.3 **Sapwood densities and moisture content for citrus samples collected from Mpumalanga, Gauteng and the Western Cape**

Cultivar	Fresh mass (g)	Dry mass (g)	Sapwood volume (cm^3)	Sapwood density (g cm^{-3})	Sapwood moisture content
Citrus sinensis 'Delta'	28.664	19.766	25.164	0.79	0.45
Citrus sinensis 'Delta'	17.225	11.563	15.610	0.74	0.49
Citrus reticulata 'Nadorcott'	31.662	20.847	27.022	0.77	0.52
Citrus reticulata 'Nadorcott'	41.706	26.878	35.146	0.76	0.55
Citrus paradisi 'Star Ruby'	12.881	8.026	11.054	0.73	0.61
Citrus paradisi 'Star Ruby'	17.776	11.062	15.522	0.71	0.61
Citrus sinensis 'Bahianinha'	13.165	9.605	12.648	0.75	0.43
Citrus sinensis 'Bahianinha'	17.335	10.819	14.105	0.77	0.60
Citrus sinensis 'Bahianinha'	20.966	13.418	17.572	0.76	0.56
Citrus limon 'Eureka'	29.500	18.230	25.94	0.70	0.62
Citrus limon 'Eureka'	26.300	15.290	23.73	0.64	0.72
Citrus limon 'Eureka'	19.710	12.510	16.53	0.76	0.58
Citrus limon 'Eureka'	25.910	16.210	21.85	0.74	0.60
Citrus limon 'Eureka'	25.303	15.110	21.71	0.70	0.68
Citrus limon 'Eureka'	20.500	12.430	17.32	0.72	0.65
Citrus sinensis 'Midknight'	35.092	21.477	28.81	0.75	0.63
Citrus sinensis 'Midknight'	26.807	16.843	20.348	0.83	0.59
Citrus sinensis 'Midknight'	7.876	4.773	6.364	0.75	0.65
Citrus sinensis 'Midknight'	8.324	4.635	6.081	0.76	0.80
			Average	**0.74**	**0.61**
			Standard Deviation	**0.04**	**0.08**

3.1.5 Anatomical analysis

Plant material and sample preparation

Xylem anatomy was assessed in stems and branches of the different varieties of citrus used in this study namely: 'Delta' Valencia, 'Star Ruby' grapefruit 'Bahianinha' navel, 'Nadorcott' mandarin and 'Eureka' lemons. Samples were taken from the branches used for calibration of the sap flow methods and from samples collected at Patrysberg Farm in Citrusdal, Western Cape. Core samples from stem and branches were collected from three trees per cultivar, using a 5.15 mm diameter incremental borer (Haglöf Sweden AB, Långsele, Sweden). The position of the cambium and inner sapwood were carefully marked on each core. The cores were sealed in air tight Ziploc bags for transportation to the laboratory, where they were removed from the bags and preserved in formalin:acetic acid:ethanol solution (FAA, 1:1:1.8 v/v) for a few days. The samples were subsequently removed from the solution and rinsed with water before cross-sections were made using a sliding microtome. Three subsamples per sample were taken from both the inner and outer part of the sapwood and placed on a microscope slide with water and glycerine to avoid drying out.

To assist with better contrast and tissue identification, the subsamples were dehydrated in a sequential series of 30%, 50%, 70% and 100% ethanol and then stained with Safranin O solution for one hour. The subsamples were then stained again with fast green and rinsed with a sequential series of 30%, 50%, 70% and 100% xylene and mounted in DPX on microscopic slides. The subsamples were then observed and analysed under a Wild Leitz GMBH light microscope (Abbott Laboratories, Illinois, USA). The images were obtained with a Power Shot A630 digital camera (Canon Inc., USA) mounted on the microscope. Images were subsequently merged together using the Microsoft Image Composite Editor.

Lumen diameter of xylem vessels and distance between groups of vessels from both spring flush and summer flush wood of the inner and outer part of the sapwood were measured using the UTHCA Image Tool for Windows version 3.00. The xylem vessels were chosen by randomly selecting one xylem vessel and measuring the distances around that selected vessel to the nearest vessels, as indicated in Figure 3.4. The lumen diameter of these vessels was also measured. The sampling of each stem and branch from the inner and outer part was replicated three times.

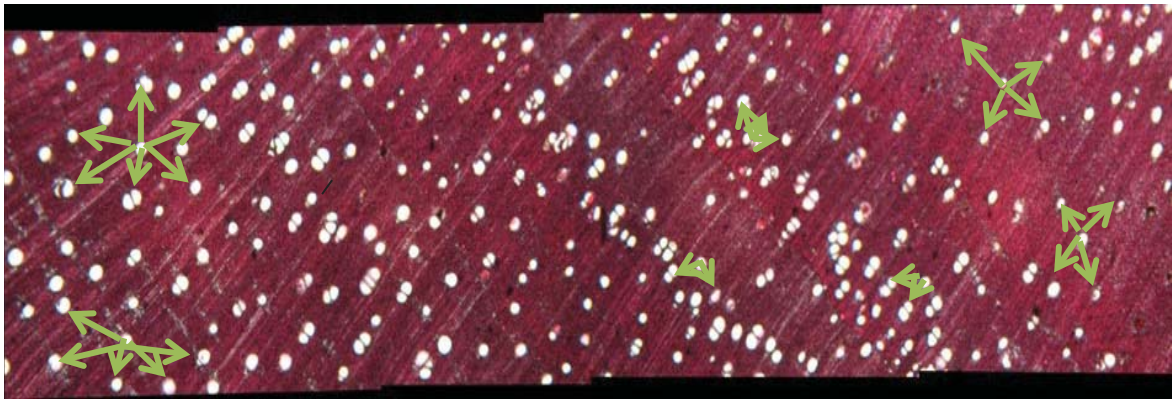

Figure 3.4 Cross sectional area of a core from a citrus tree showing how the distance between the xylem vessels was measured, as indicated by the arrows

3.1.6 Statistical analysis

The performance of the SFD techniques was evaluated with the aid of statistical parameters, such as the coefficient of determination (R^2), mean absolute error (MAE), root of the mean square error (RMSE) and Willmott index of agreement (D) (Willmott et al., 2012). The performance of each sap flow technique was considered accurate when R^2 >0.8, MAE <20% and D >0.8 according to (De Jager, 1994). The SAS program version 9.3 was used to determine the P values for the correlations between the SFD determined by SFD techniques and that determined gravimetrically for each stem of each variety.

3.2 Validating SFD measurement methods in a glasshouse using weighing lysimeters

3.2.1 Experimental site

All experiments were conducted in a glasshouse at the University of Pretoria's Hatfield experimental farm (25°45'7.13'' S and 28°15'32.89'' E). Glasshouse air temperature (T_a) and relative humidity (RH) were recorded every 15 minutes using an HPM50 sensor (Vaisala Oyj, Vantaa, Finland) attached to a Campbell CR10X data logger. Water vapour pressure deficit of the air (VPD) was calculated from T_a and RH using the functions presented by Campbell and Norman (1998):

$$e_s = 0.611 * Exp\left(\frac{17.502*T_a}{T_a+240.97}\right) \quad (17)$$

$$VPD = e_s - \frac{e_s*RH}{100} \quad (18)$$

where, e_s (kPa) is the saturated vapour pressure at T_a (°C).

3.2.2 Plant material

The HR method was first tested in *E. marginata* by Burgess et al. (2001) and was shown to accurately estimate T in this species. Therefore, to test the equipment and methodology, a potted *E. marginata* tree was used. Details of the *E. marginata* tree grown in a 20 L plastic bag and weighing approximately 30 kg, are provided in Table 3.4.

Table 3.4 Details of the *E. marginata* tree used for testing the heat pulse velocity equipment

Stem diameter at probes (mm)	Bark thickness (mm)	Probe depths (mm)	Canopy width (m)	Tree height (m)
65.2	3.0	8.0 and 12.0	1.2	3.2

Two potted disease-free 13-year-old 'Midknight' Valencia trees (*Citrus sinensis* L. Osbeck) grafted onto a Carrizo citrange rootstock were used for validation of the different SFD techniques. The trees were grown in 74 L black dustbins in a mixture of sand and coir and were part of the collection of parent material kept at the foundation block of Citrus Research International in Uitenhage, Eastern Cape. The trees with their containers weighed approximately 133 kg. Details of the trees are given in Table 3.5.

Table 3.5 'Midknight' Valencia citrus trees used for testing of the heat ratio and compensation heat pulse methods

Stem diameter at probes (mm)	Bark thickness (mm)	Probe depths (mm)	Canopy width (m)	Tree height (m)
103.7	1.0	8.0, 12.0 and 15.0	1.8	2.7
73.8	1.0	8.0, 12.0 and 15.0	2.4	2.6

Initially citrus trees on the lysimeter were irrigated on a three-day interval. The irrigation volume was matched to the volume of water lost from the pots as determined using the lysimeter measurements. However, water stress started to develop in the trees which was noticeable in the HPV data and the irrigation regime was changed to twice a day using an automated irrigation system during the night from 01:00-02:00 and 20:00-21:00. No irrigation was performed during the day as this would affect lysimeter measurements. The trees were supplied with nutrients in solution using a full strength Hoagland solution every three days. Pest and disease scouting was conducted every day and no pests were recorded in *E. marginata*. Mealy bug infestation was frequently recorded in the 'Midknight' Valencia trees and it was controlled by spraying an insecticide which was registered for mealy bug (Ripcord) on a weekly basis.

3.2.3 Experimental design and measurement protocol

Two cantilever-type weighing lysimeters were used in these experiments to determine mass loss from *E. marginata* and *Citrus sinensis* trees (Figure 3.5). The load cells used had a range of 0-500 N (0-51 kg) and a theoretical resolution of 4.3 g.

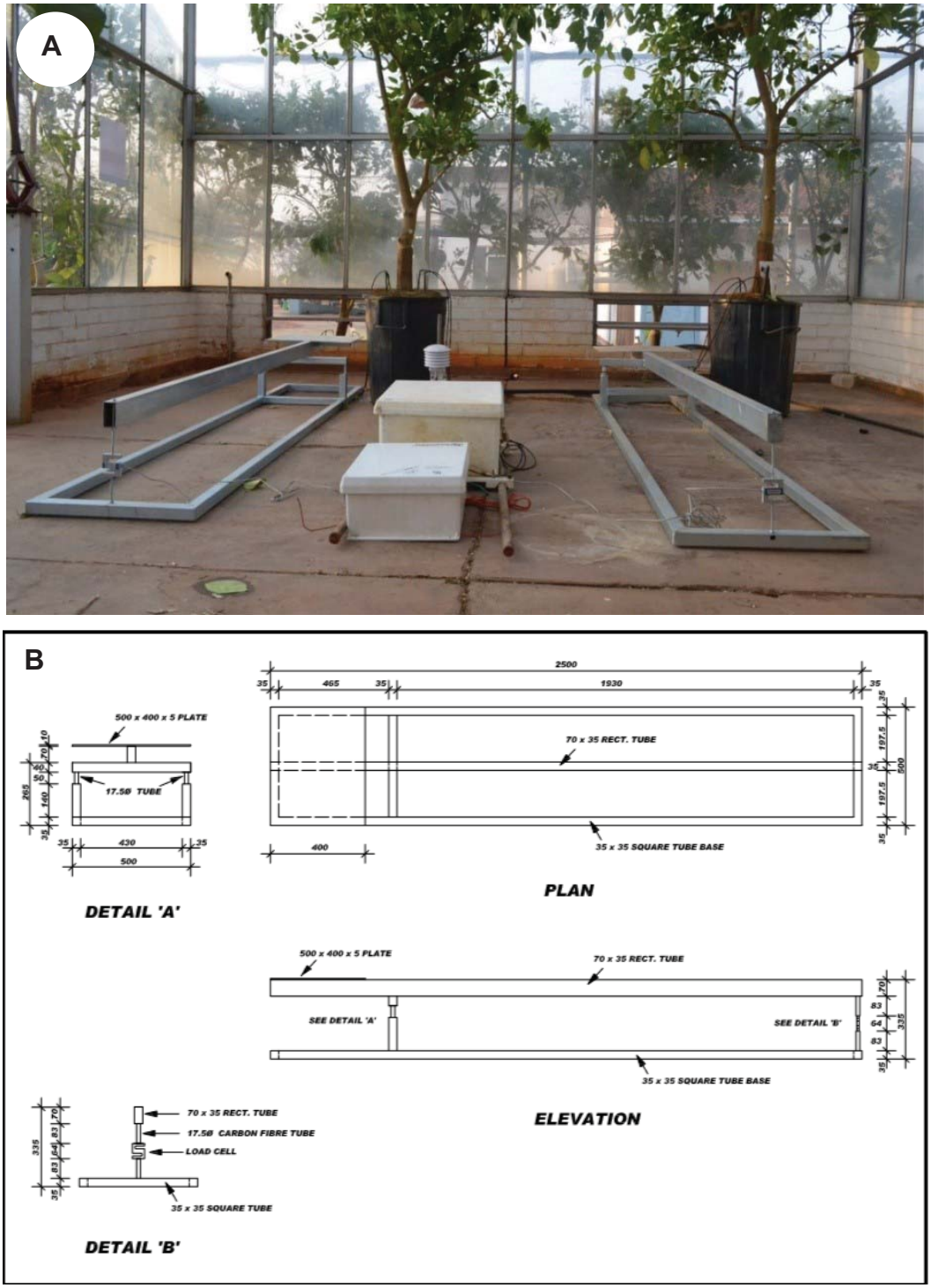

Figure 3.5 (A) Picture (B) scale drawing of and cantilever weighing lysimeters for checking the calibration of sap flow sensors

Power was supplied to the load cells via a 12 V constant regulated power supply. The output signal from the two load cells of the cantilever-type lysimeters was recorded separately using a Campbell CR10X logger, at one second intervals and then averaged and logged every 15 minutes.

3.2.4 Calibration of the load cell on the weighing lysimeters

Calibration of the load cells using a known mass of water was conducted to convert the load cell measurements (mV) into mass (kg). Water was added at 100 mL intervals from 0-1 L, then at 1 L intervals from 1-50 L, 2 L intervals from 50-148 L and then again at 100 mL intervals from 148-150 L. T_a was recorded and the relationship between the volume of water at a known temperature and mass was used to convert the water volume to mass. The mV reading from the load cell was recorded for each addition of water and a calibration curve was drawn, as indicated by the blue regression line in Figure 3.6 A and B. The calibration results for the two lysimeters showed a perfect positive linear relationship ($R^2 = 1$) between the load cell output (mV) and calibration mass (kg).

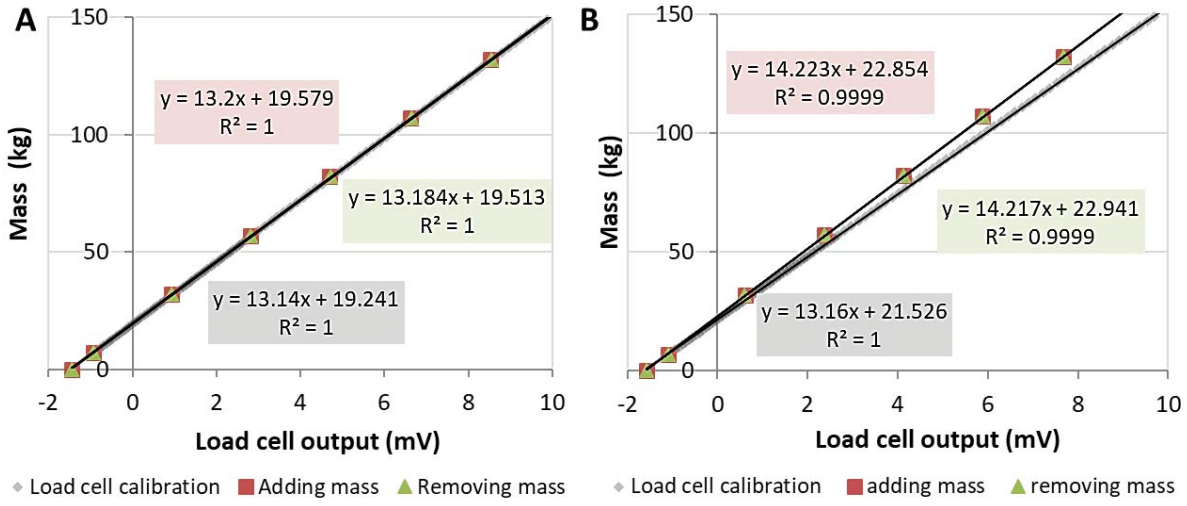

Figure 3.6 Regression analysis for the calibration of (A) lysimeter 1 and (B) lysimeter 2 at the start of the experiment without the trees on the lysimeter

The effect of hysteresis was determined by adding mass at 25 kg intervals from 0-150 kg as indicated by the red regression line and removing mass also at 25 kg intervals from 150-0 kg as indicated by the green regression in Figure 3.6 A and B. No hysteresis was observed. The stability of the load cells with a constant mass was also assessed, as this would be critical when calibrating the SFD techniques over a number of days. For this purpose two plastic dustbins (74 L) were used. The bin on lysimeter 1 was filled with sand and a non-living branch planted in the middle and watered to mimic the experimental conditions. The exposed soil

surface of the pot was covered with plastic to eliminate water loss by E. A dry empty plastic dustbin was placed on lysimeter 2 and the outputs from the load cells were logged every 15 minutes for 7 days. The results showed that the two load cells were stable over time (Figure 3.7).

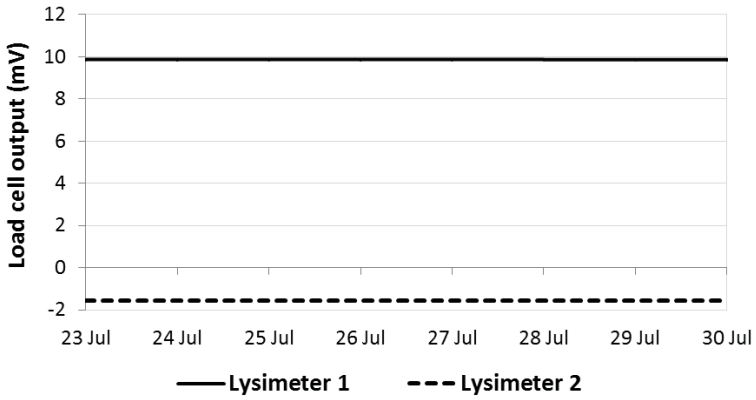

Figure 3.7 Stability of the load cell output over a seven day period (23-30 July 2014) as observed from the mV readings

Measurement of E. marginata and citrus water use using weighing lysimeters

In order to determine T from the potted trees, the exposed soil surface of the pots was covered with plastic sheeting to eliminate E from the soil surface. The bottom of the pots was also sealed with plastic sheeting and duct tape to prevent drainage as conducted by Bleby et al. (2004) and as shown in Figure 3.8. Recalibration of the lysimeter was conducted once each tree was placed on the lysimeter, using a 2 L beaker that was fasten to the tree trunk of the plastic bag containing the *E. marginata* tree and the dustbin containing the citrus trees. One litre of water was then added in 100 ml intervals and the mV reading from the load cell was recorded for each addition of water to establish regression equations as shown in Figure 3.9.

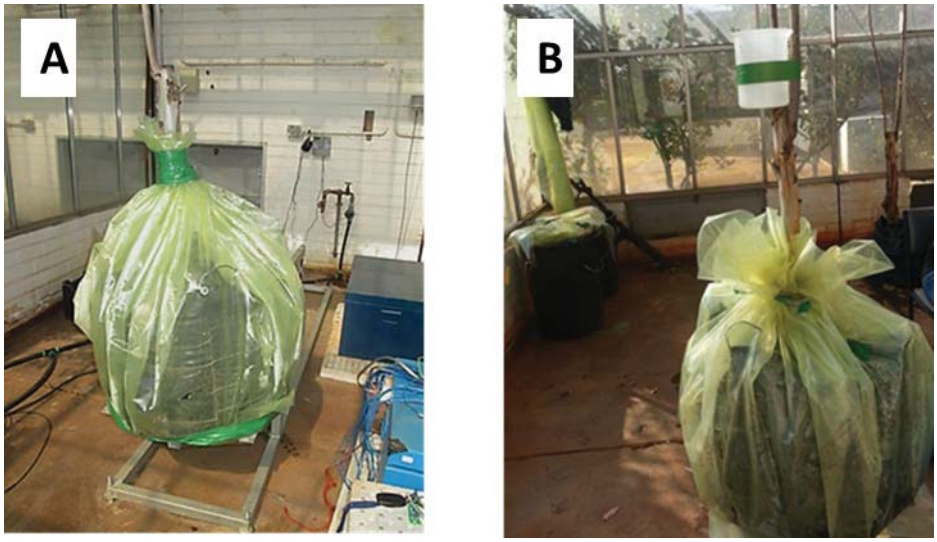

Figure 3.8 (A) Placement of the *E. marginata* trees on the weighing lysimeters and (B) attachment of 2 L beaker for recalibration. Note the plastic covering to eliminate evaporation and drainage from the pots

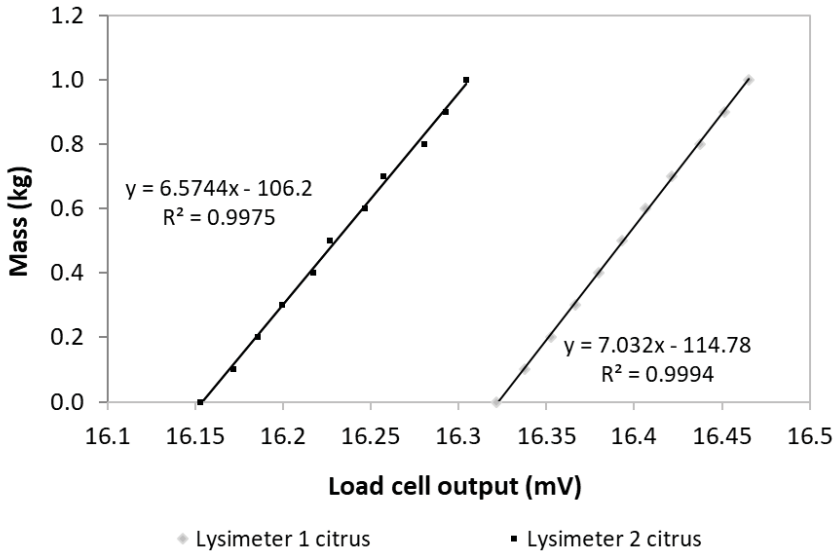

Figure 3.9 Regression analysis for the calibration of lysimeter 1 and 2 after the placement of trees on the weighing lysimeters

Mass lost from the lysimeter, which was assumed to be T from the trees, was calculated for 15 minute intervals as the difference in mass at the beginning and at the end of a 15 minute interval. The results from the 15 minute intervals were then summed to calculate hourly water use.

3.2.5 Sap flow equipment and measurements

Heat ratio method

Sap flow measurements in *E. marginata* using the HR method were conducted from 8-16 August 2014 and for 'Midknight' Valencia from 23 September-20 December 2014 and 28 August-28 November 2015. For the HR method two thermocouples were placed equidistant from the heater probe at 5 mm downstream and upstream of the heater probe (Figure 3.10).

Figure 3.10 Placement of the 'Midknight' Valencia trees on the weighing lysimeters and probe installation. Note the plastic covering to eliminate evaporation and drainage from the pots

For the *E. marginata* tree, HR method probe sets (1 heater and 2 thermocouples) were installed at two depths (8 and 15 mm). Initially probes were installed in the scion of the 'Midknight' Valencia trees but poor results were obtained (data not shown). Subsequently the probe sets were installed in the rootstock at three depths (8, 12 and 15 mm). Heater probes consisted of a 1.8 mm OD stainless steel heater probe, whilst Type-T copper-constantan thermocouples were embedded in 2 mm OD polytetrafluoroethene tubing. Three probe sets were used for each citrus tree, as these trees had larger stem diameters than the *Eucalyptus* tree. A steel drilling guide was strapped to the tree to ensure that the holes were drilled parallel and at a fixed spacing along the plant stem-root axis. A 2 mm drill bit was used to drill holes for the insertion of thermocouples and a 1.8 mm drill bit was used for the heater probe. As HPV techniques are very sensitive to misalignment, care was taken to ensure that the

upstream and downstream thermocouples were properly aligned. Petroleum jelly was used to ease probe insertion and maintain thermal contact between the probe and wood tissue (Barrett et al., 1995). Individual thermocouples were wired to a Campbell AM16/32B multiplexer. Heat pulse velocities, calculated according equation 8, were logged at 15 min interval on a Campbell CR1000 logger. Voltages of batteries powering the load cells and HPV systems was carefully monitored to ensure they did not decrease below 12 V.

Employing the assumption that at night zero flow should be recorded, the heat pulse velocities from the logger were below zero (negative values) indicating misalignment of probe sets. This was corrected by a constant factor so that at night flows of close to zero are adjusted as shown in Figure 3.11. High abnormal flows were also adjusted and corrected by averaging the two preceding or two following readings. The adjusted data were corrected using the wounding correction equations and assuming a wound width of 2 mm equal to the width of the widest probe, which was also used by Swanson and Whitfield (1981) and Burgess et al. (2001). The SFD and final sap flow volumes were calculated with equation 13. The area representing each probe was determined using the perfusion experiment as described in Section 3.2.7, following which the HPV from each probe was multiplied by the specific area represented by the probe, which yielded the VSF per 15 minutes. This was later averaged to sap flow per hour (L h^{-1}) and compared to the weighing lysimeter at hourly intervals.

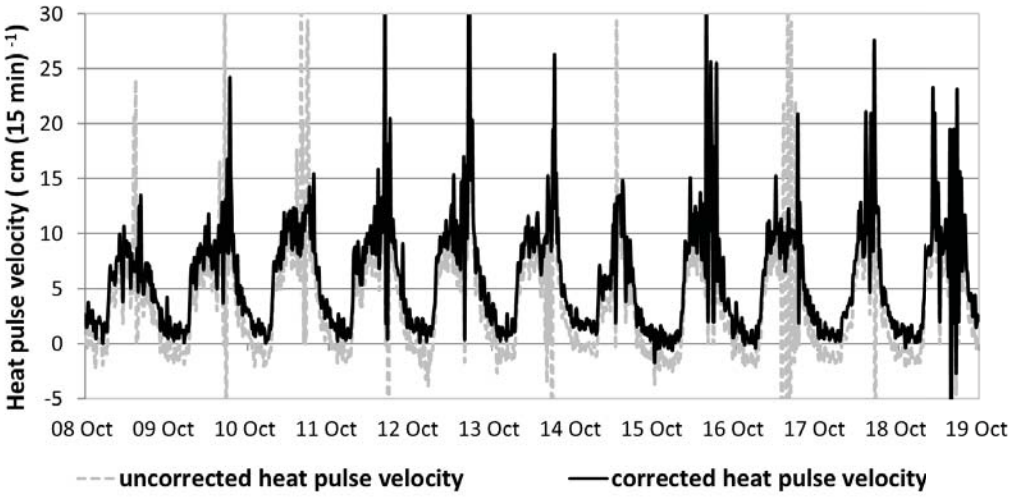

Figure 3.11 Heat pulse velocity determined at 8 mm below the cambium for the heat ratio method, bold line is the adjusted baseline and dashed line is the raw data from the logger for citrus from 8-19 October 2015

Compensation heat pulse method

Sap flow measurements using the CHP method were only conducted in 'Midknight' Valencia trees for the period of 24 April-24 May and 9 September-29 November 2015. The installation

of the probes and data correction for the CHP method was the same as for the HR method. The only difference was the orientation of the probes; where the downstream thermocouple was located 10 mm from the heater and the upstream thermocouple at 5 mm from the heater. For each probe set, sap velocities were sampled at 8, 12 and 15 mm depths in the rootstock. Baseline adjustments were also conducted for the CHP method as shown in Figure 3.12, corrected sap velocities were calculated according to equation 6 and multiplied by the cross-sectional area represented by each probe to determine the VSF (L h^{-1}) according to equation 7.

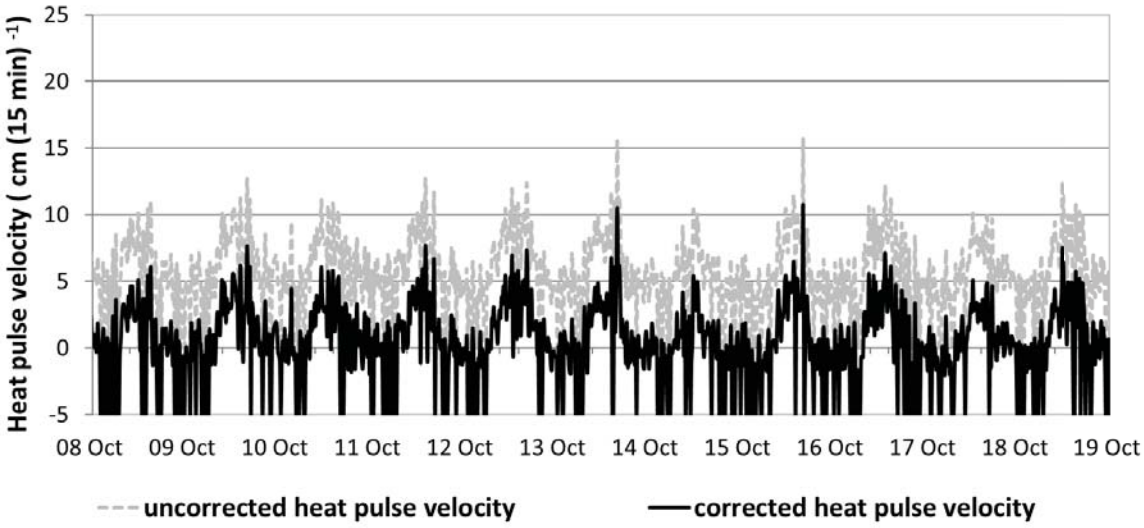

Figure 3.12 Heat pulse velocity (HPV) determined at 8 mm below the cambium for the compensation heat pulse (CHP) method. The bold line is the adjusted baseline and the dashed line is the raw data from the logger

3.2.6 Data analysis

Data from the HR and CHP methods were compared by means of regression analysis to the mass loss measured with the weighing lysimeter on an hourly interval and for daytime totals. Total daytime T, when the plants were actively transpiring, was calculated from the sum of hourly sap flow and for weighing lysimeter losses between 06:00 and 18:00.

3.2.7 Additional parameters needed to calculate SFD

To be able to calculate SFD additional parameters were determined, which included m_c, the ρ_b (g cm^{-3}), wound width (mm) and sapwood area (cm^2).

Sapwood area

The area of sapwood conducting tissue was determined for the 'Midknight' Valencia trees using the potometer method described by Barrett et al. (1995) and the perfusion experiment

for *E. marginata* as conducted by Steppe et al. (2010). In Figure 3.13 a schematic outlay of the potometer experiment is presented. Trees were cut early in the morning before dawn and they were quickly placed in a bucket full of water to prevent the formation of embolisms. The excised stem was recut under water using a sharp blade to prevent the closure of the xylem vessels, which could have occurred when the tree was roughly cut with a saw. The cut tree was then placed in water containing Safranin O dye for three days. After three days the trees were removed from the dye solution and stems were then cut into smaller pieces and taken to the laboratory, where 10 mm cross sections were cut for analysis.

Figure 3.13 Schematic outlay of the process for determining the sapwood conducting area

The stained cross section of the stem was photographed together with a scale bar and the area of stained tissue determined using Adobe Photoshop CS5™ (Figure 3.14). A measurement scale was created and customized in Adobe Photoshop CS5™, where 312 pixels equated to 10 mm. The customised measurement scale enabled the calculation of the perimeter of the heartwood which is the unstained area.

The heartwood radius was then computed from the formula for calculating the circumference of a circle using equation 19.

$$\text{Circumference of a circle} = 2\pi r \qquad (19)$$

where *r* (cm) is the radius.

Knowing the heartwood radius enabled the determination of the sapwood radius, which was the difference between the stem radius and the heartwood radius combined with the bark thickness. This was subsequently used to correct sap flow values.

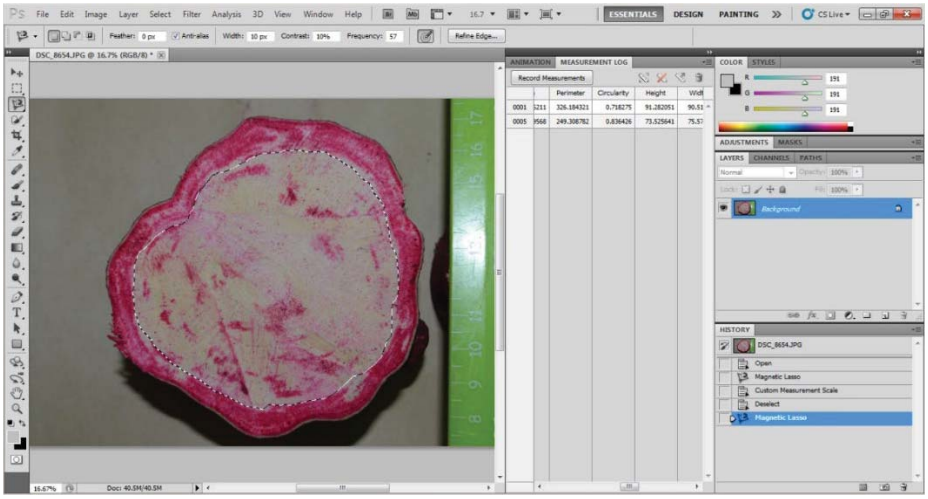

Figure 3.14 Determination of the sapwood (pink stained) and the heartwood (white unstained area) using Adobe Photoshop

Sapwood density and moisture content

The m_c content and ρ_b was determined as described in Section 3.1.4.

Wound width

At the end of the experiment, sections of the tree trunk containing probe implantation holes were excised from two measurement trees instrumented with the HR and the CHP method. Each block was recut longitudinally at the particular depth below the cambium where the probes were originally positioned. The exposed, fresh face was shaved smooth using a microtome, after which the wound width was clearly identified by its darker colour as shown in Figure 3.15. Wound width of the widest probe was measured for each tree using a digital vernier calliper and the average wound size was determined, which was then used for the calculation of the SFD.

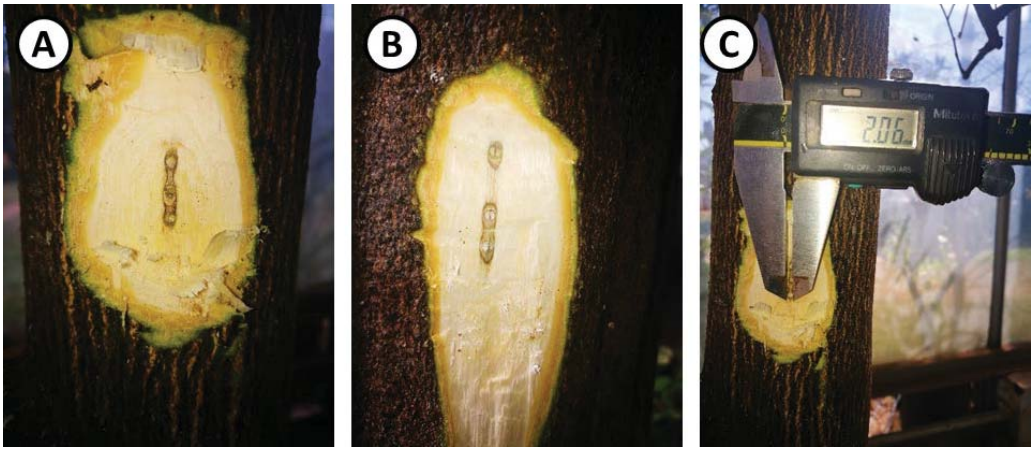

Figure 3.15 Wounding response in 'Midknight' Valencias after (A) heat ratio probe installations and (B) for the compensation heat pulse probe installations. (C) Measurement of the wound width with a Vernier calliper

3.2.8 Heat ratio and compensation heat pulse method error analysis

Data from the HR and CHP method validation experiments were used to simulate the effects of different sources of error on the quantification of daily sap flow as described by Steppe et al. (2010). Sources of possible error that were used in the error analysis include parameters to calculate SFD such as m_c, heartwood radius, ρ_b and wound width. The resultant errors in daily sap flow were expressed as a percentage of the overestimates or underestimates of the measured value as determined by equation 20.

$$\text{Resultant error} = \left(\frac{\text{Measured value} - \text{Simulated value}}{\text{Measured value}}\right) 100 \quad (20)$$

where the measured value is the actual measured value and the simulated value is the value when an error is made.

Keeping all the other variables in equation 6 and 13 at actual measured values, separate simulations were done by increasing errors of each parameter (±0, 10, 20, 30, 50, 70, 100%). A negative value indicates that the daily sap flow is overestimated, while a positive value indicates an underestimation of the daily sap flow.

3.3 Infield validation of the SFD measurements using eddy covariance measurements

3.3.1 Description of the experimental orchard

Infield calibration and validation of the HR and CHP methods was conducted in the winter rainfall region of South Africa. The trial site consisted of a 4.1 ha, 9-year-old commercial orchard of 'Washington' navels (*Citrus sinensis*), grafted on 'Carrizo' citrange rootstock that

was planted in 2006. The trees were planted in a north-south orientation at Patrysberg farm (32°27'44.30"S and 18°59'1.83"E) in the Western Cape Province near Citrusdal (Figure 3.16). Tree spacing was 2.5 x 5 m (12.5 m^2 $tree^{-1}$) and the average height of the trees was 2.6 m. The orchard was drip irrigated with two dripper lines per tree row using pressure compensating emitters, spaced 0.8 m apart with a discharge rate of 1.8 L h^{-1}. Typically the orchard was irrigated daily in a single irrigation event of 2-3 h. An automated weather station (AWS) (32°27'2.82"S and 18°58'6.22"E) was installed on Patrysberg farm (Figure 3.16). The area receives an average annual rainfall of 200 mm and has average minimum and maximum temperature of 10 and 24°C respectively. Weather variables measured included wind speed, wind direction, rainfall, solar radiation, T_a and RH. Measurements were stored at hourly intervals on a Campbell CR200 datalogger that was powered by a 12 V battery connected to a solar panel.

Figure 3.16 Location of the 'Washington' navel orchard and the automated weather station

3.3.2 Evapotranspiration measurements

An open path EC system was used for the measurement of orchard ET for the period of calibration (3-18 March 2015). Measurements were performed by the Council for Scientific and Industrial Research (CSIR), Natural Resources and Environment unit based in Stellenbosch. Micro-meteorological instruments were mounted on a lattice mast, which was

erected in the centre of the orchard with a fetch of approximately 200 m, based on the prevailing N-S wind direction (Figure 3.17). An extended Open Path EC (OPEC) system, comprising a Campbell CSAT3 three-dimensional sonic anemometer, a fast response LI-7500 open path infrared gas (H_2O and CO_2) analyser (IRGA) (LI-COR Inc., Lincoln, NE, USA), was mounted at 5 m above ground (2 m above average canopy height) to determine ET of the orchard.

Figure 3.17 Lattice mast in the 'Washington' navels orchard showing position of eddy covariance sensors

Other components of the EC system included air temperature and humidity sensors, Vaisala HMP45C temperature and humidity probe (Vaisala Oyj, Vantaa, Finland) that was mounted 4.5 m above ground. Net radiation (Rn) was measured using an NR-Lite (Kipp and Zonen, Delft, The Netherlands) net radiometer mounted 8 m above ground and soil heat flux (G) was determined using two HFP01SC (Hukseflux, Delft, Netherlands) soil heat flux plates buried 80 mm below the soil surface. Lastly, Campbell TCAV-L soil temperature averaging probes were installed at 2 locations representing within-row and between-row conditions and were positioned 20 and 60 mm below the soil surface to correct the measured soil heat flux data for the energy stored above the plates. Eddy covariance measurements were sampled at a frequency of 10 Hz and logged on a Campbell CR5000 data logger every 30 minutes. The quality of data obtained from the EC measurements was analysed by determining the energy balance closure (EBC) for the 30 min interval measurements as conducted by Wilson et al. (2001):

$$EBC = \frac{H+LE}{Rn-G} \quad (21)$$

where H is sensible heat flux, LE is latent heat flux, R_n is net radiation, G is soil heat flux expressed in MJ m^2 day^{-1} and the EBC is the energy balance closure ratio (dimensionless).

Using all valid half-hourly data, the slope between the available energy flux (R_n − G) and the sum of sensible and latent heat fluxes (LE + H) was 0.67, intercept of 15.4 W m^{-2}, and the coefficient of determination (R^2) was 0.90, as shown in Figure 3.18. The available energy (R_n – G) exceeded turbulent fluxes of energy (LE + H) for most of the measurement period (underestimation of 33%), which was of the same order as reported by Zhang et al. (2014).

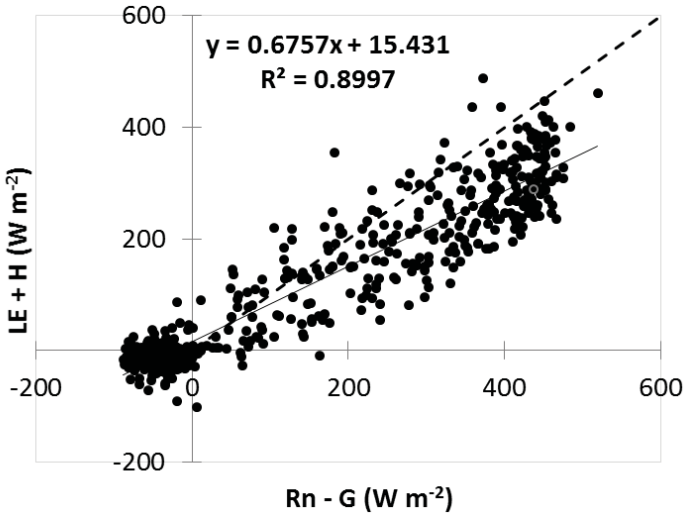

Figure 3.18 Energy balance closure for the period 2-18 March 2015 in the 'Washington' navel orchard. The 1:1 line is indicated by a dashed line

3.3.3 Soil evaporation

Soil evaporation was measured daily from 9-14 March 2015 using cylindrical microlysimeters. The microlysimeters, were made of 2 mm thick wall PVC pipe, 100 mm deep and had an internal diameter of 85 mm. Each microlysimeter was equipped with one external cylinder made of 3 mm thick wall PVC pipe, which had an internal diameter of 100 mm and was 100 mm deep. A set of twelve microlysimeters (Figure 3.19) were installed taking into account the dry and wet areas on the orchard floor and movement of shade throughout the day within the orchard. Seven microlysimeters (numbers 3, 6, 7, 8, 9, 10 and 11) were installed under the drippers, three were placed midway between the canopy edge and the tree trunk (numbers 2, 4 and 12), two on the canopy edge (numbers 5 and 13) and the last one (number 1) in between the trees (Figure 3.19). Removal of undisturbed cores from the top soil layer was conducted as described by Daamen et al. (1993). The microlysimeter was gently tapped into the soil with a rubber hammer and an undisturbed soil core was then removed. A sheet metal base plate was placed at the bottom of the microlysimeter, which was secured in place with waterproof

tape. The undisturbed cores were then placed on top of the metal plate within the external cylinder, flush with the soil surface.

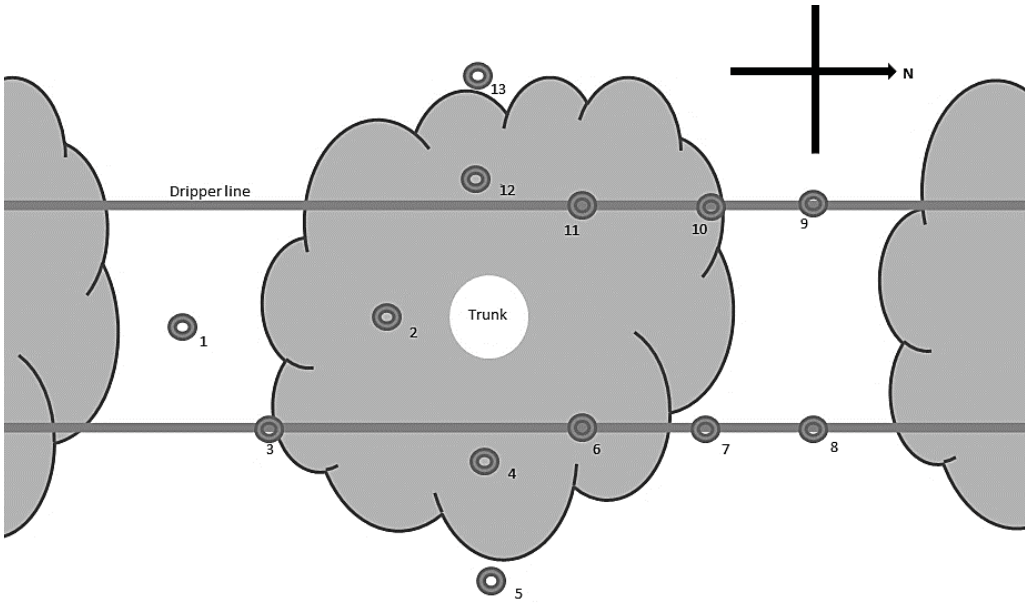

Figure 3.19 Diagrammatic representation for the placement of microlysimeters (double circles) for evaporation measurements in the 'Washington' navel orchard

One hour after an irrigation event new similar soil samples were collected from other areas in the orchard that represented the same positions as indicated in Figure 3.19. The rate of E_s was calculated from the difference in mass between measurements divided by the surface area of the microlysimeter and weighted by the area in the orchard represented by each microlysimeter (Poblete-Echeverría et al., 2012):

$$E = (1 - f_c)\, E_{sr} + (f_c)\, E_{scan} \qquad (22)$$

where f_c is the fractional canopy cover, E_{sr} is the soil evaporation between row mm day^{-1} and E_{scan} is the soil evaporation below the canopy next to the drippers.

3.3.4 Sap flow measurements

Sap flow measurements were conducted in trees in the proximity of the EC tower. Four trees were instrumented with the HR method equipment and three additional trees with the CHP method equipment to quantify and compare T_{sap} measurements. One of the major challenges faced infield, was the onset of gumming which hastened the rate of corrosion of the heater probes soon after the trees were instrumented. This problem was solved by inserting brass collars (2.5 mm in diameter) in the tree to accommodate the heater probes, thus reducing the occurrence of corrosion.

Heat ratio method

Sensors for the HR method were installed as described in Section 3.2.5, with a slight modification in the insertion depths as these stems were larger (Table 3.6). For each tree, four probes sets (heater, downstream and upstream thermocouples) were inserted to measure sap velocity at 8, 13, 22 and 30 mm depths (Table 3.6). Heat pulse velocities were logged at one hour intervals on a Campbell CR1000 logger. Baseline adjustments were conducted as previously described for the glasshouse experiments. Hourly VSF was determined in the stem using the SFDs at the four different points over the total sapwood conducting area represented by each probe using a wound width of 2.5 mm, measured ρ_b of 0.66 g cm^{-3} and m_c of 0.61. Average VSF was determined for the four measuring trees, which was upscaled to orchard water use, using a weighted average based on a tree circumference survey of 50 trees in the orchard. This was done to compare between T_{sap} measured with the HR method and T_{res} determined as the residual of ET, measured with the EC system, and E_s, measured by the microlysimeters, ($T_{res} = ET - E_s$).

Table 3.6 Trunk circumference (mm) and probe insertion depths for trees selected for sap flow measurements using the heat ratio method

Orchard	Stem circumference (mm)	Insertion depth of the probes (mm)
'Washington' Navel	Tree 1 = 273 Tree 2 = 321 Tree 3 = 295 Tree 4 = 302	Probe 1 = 8 Probe 2 = 13 Probe 3 = 22 Probe 4 = 30

Compensation heat pulse method

Sensors for the CHP method were installed as described in Section 3.2.5, also with a slight modification in the insertion depths. Four probe sets were inserted to measure sap velocity at 10, 15, 25 and 35 mm depths (Table 3.7). Heat pulse velocities were logged at one hour intervals on a Campbell CR1000 logger and orchard T_{sap} was calculated as described in the HR method section.

Table 3.7 Trunk circumference (mm) and probe insertion depths for trees selected for sap flow measurements using the compensation heat pulse method

Orchard	Stem circumference (mm)	Insertion depth of the probes (mm)
'Washington' navel	Tree 1 = 311 Tree 2 = 250 Tree 3 = 292	Probe 1 = 10 Probe 2 = 15 Probe 3 = 25 Probe 4 = 35

3.3.5 Empirical determination of wound correction coefficient

Initially a wound width of 2.5 mm (the width of the widest probe, the same specification was used to determine the wound width in the glasshouse experiment) was used for infield calibration, but this led to an underestimation of T_{sap} by both CHP and HR methods when compared to T_{res}. As a result an empirically determined wound width correction factor was used to correct sap flow measurements. T_{sap} was calculated, for each day for which T_{res} values were available, with wound widths ranging from 2-5 mm. Regression equations were determined from 12 points as done by Poblete-Echeverría et al. (2012) (Figure 3.20). The regression equation for each day was used to back calculate the wound width, which would match the T_{res} on that specific day. The calibrated wound widths for the five measuring days were averaged to obtain an empirical wound width correction factor.

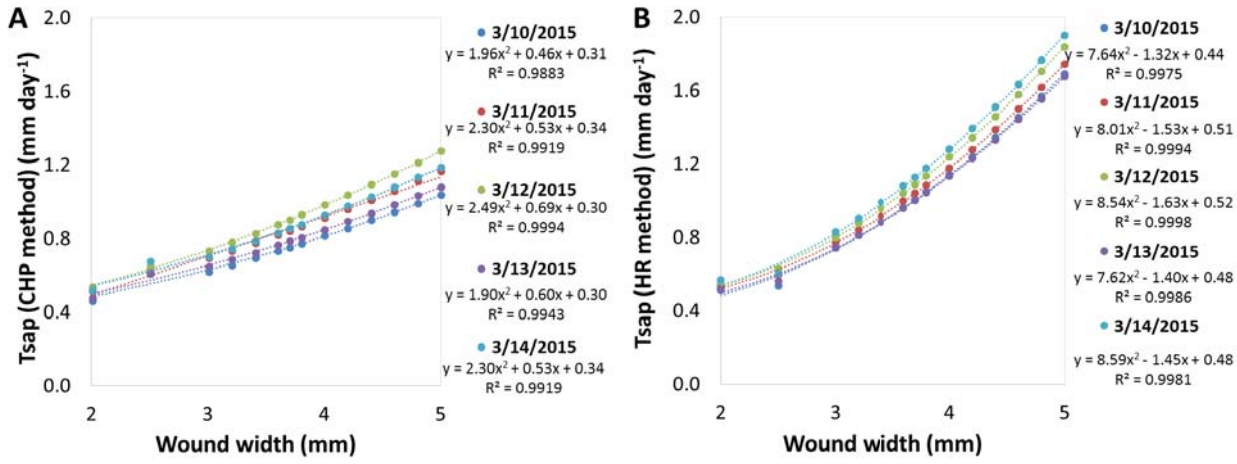

Figure 3.20 Regression analysis of wound width against the resulting T_{sap} for (A) the CHP method and (B) the HR method

3.3.6 Infield determination of the wound correction coefficient and sapwood depth

Wound correction coefficients

At the end of the experiment sections of the tree trunk, where probes were inserted, were excised from the four HR method measurement trees. The exposed, fresh face was shaved smooth using a chisel, after which the wound width was clearly identified by its darker colour, as shown in Figure 3.21. Wound width at its widest point was measured for each tree using a digital Vernier calliper. An average wound width was then determined for the orchard that was used for the calculation of the SFD.

Figure 3.21 Wounding response in 'Washington' navels at the end of the measurements (24 months)

Sapwood depth and heartwood radius

Sapwood depth was determined through the staining of the conducting tissue with Safranin O dye, as shown in Figure 3.22. A container filled with Safranin O dye was suspended in the tree to allow the free flow of the solution into the stem via a drilled hole. After 36 hours a stem sample was extracted 2 and 6 cm directly above the point where the dye was administered using an incremental borer. The sapwood conducting tissue was heavily stained as shown in Figure 3.22 and the width of the heavily stained area was measured using a digital Vernier calliper. The measured sapwood depth was then subtracted from the radius of the tree trunk at the probe insertion to determine the heartwood radius. These two parameters were then used in the calculation of SFD.

Figure 3.22 Schematic outlay of the process for determining sapwood depth

4 RESULTS – STEM PERFUSION

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4.1 Possible heat interferences from adjacent heater probes

Increase in temperature was measured by the upper and lower thermocouples of the two HR probe sets (1U and 1L, together with 2U and 2L), and the thermocouple to the left (TC 3), 5 mm away from the heater for all three heating settings (Figure 4.1). The increase in temperature measured with TC 3 is probably due to the angle at which the hole was drilled that caused the thermocouple to be closer to the heater than intended. No change in temperature was registered for the three different heating settings by thermocouples 4-6 (Figure 4.1). Therefore, it can be assumed that inserting the probe sets for the HR and CHP method at a 90° angle from each other has no influence on the measurements made by the different methods.

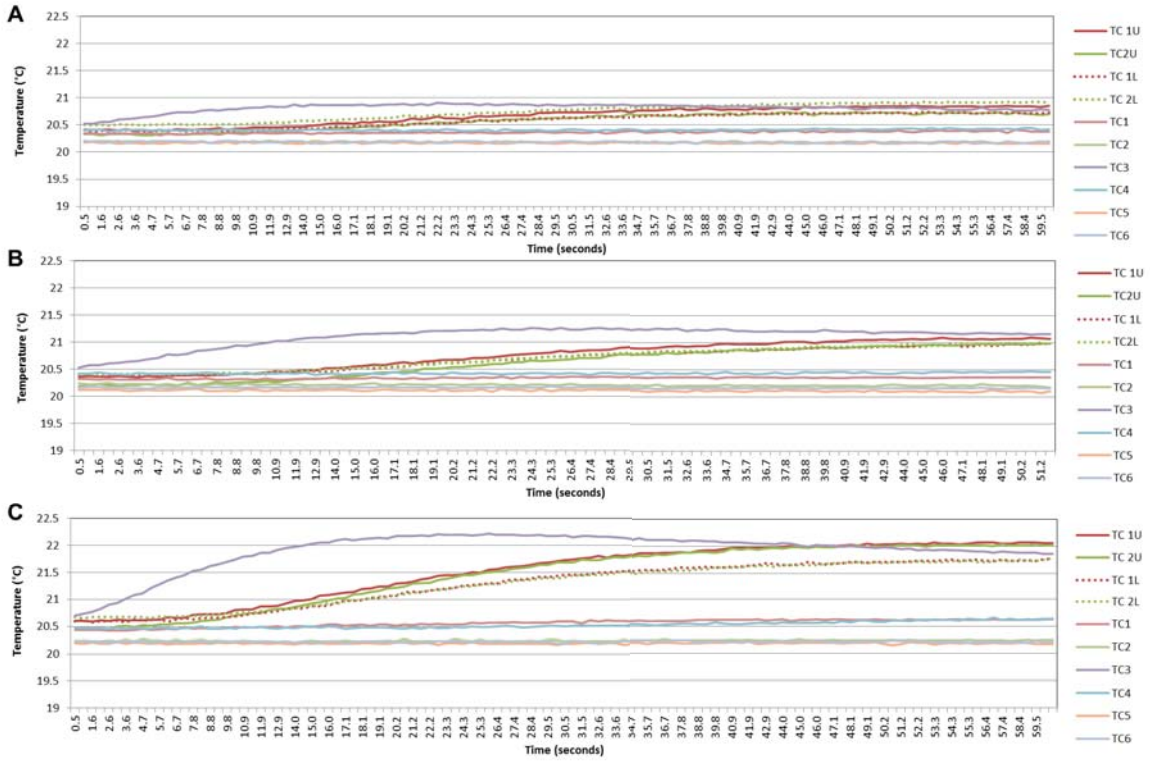

Figure 4.1 Temperature of the thermocouples at different positions in the stem following the release of a A) 0.3 s, B) 0.5 s and C) 0.8 s heat pulse

4.2 SFD measurements

SFD measured by the HR and CHP methods was compared to that determined gravimetrically (Figure 4.2). The same trend, with the gravimetric method recording higher SFD values than the HR and CHP methods, was observed for the three measurements, although it is evident that a constant flow could not be achieved as illustrated by the different SFDs. In this particular stem, the HR method was able to measure SFDs of approximately 30 $cm^3\ cm^{-2}\ h^{-1}$, whilst the CHP method was able to measure SFDs up to 75 $cm^3\ cm^{-2}\ h^{-1}$ (Figure 4.2). Throughout the study the highest SFD measured by the HR method was approximately 45 $cm^3\ cm^{-2}\ h^{-1}$ and 77 $cm^3\ cm^{-2}\ h^{-1}$ for the CHP method.

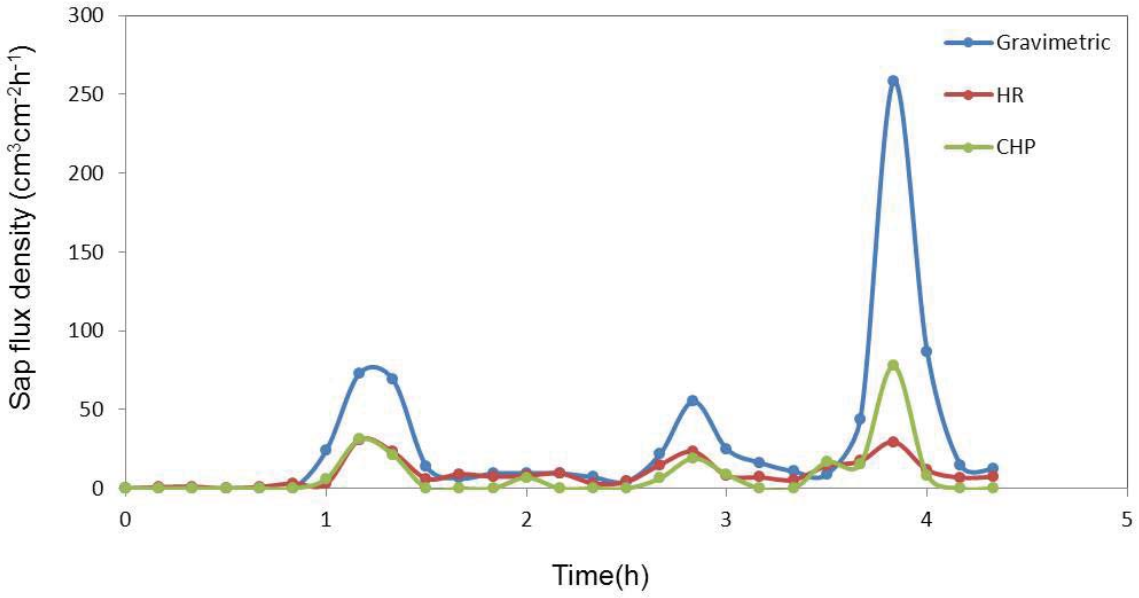

Figure 4.2 Comparison of sap flux density (SFD) determined with the heat ratio (HR) and compensation heat pulse (CHP) methods with that determined gravimetrically for 'Star Ruby' grapefruit. Sharp increases in the SFD indicate periods of pressure application to the column of water

The TD method was calibrated separately from the heat pulse methods, to avoid interference from the constant heat that was applied by the TD probe. The SFD measured by the TD method followed the same trend as the SFD determined gravimetrically for a 'Eureka' lemon sample (Figure 4.3). Large differences, however, were observed in the magnitude of the SFD measurements between the two methods, with the TD method measuring substantially lower SFD values than the gravimetric determined SFD. The TD method was able to measure a maximum SFD of approximately 45 $cm^3\ cm^{-2}\ h^{-1}$ in all stems measured, whilst SFD values of more than 300 $cm^3\ cm^{-2}\ h^{-1}$ was determined gravimetrically.

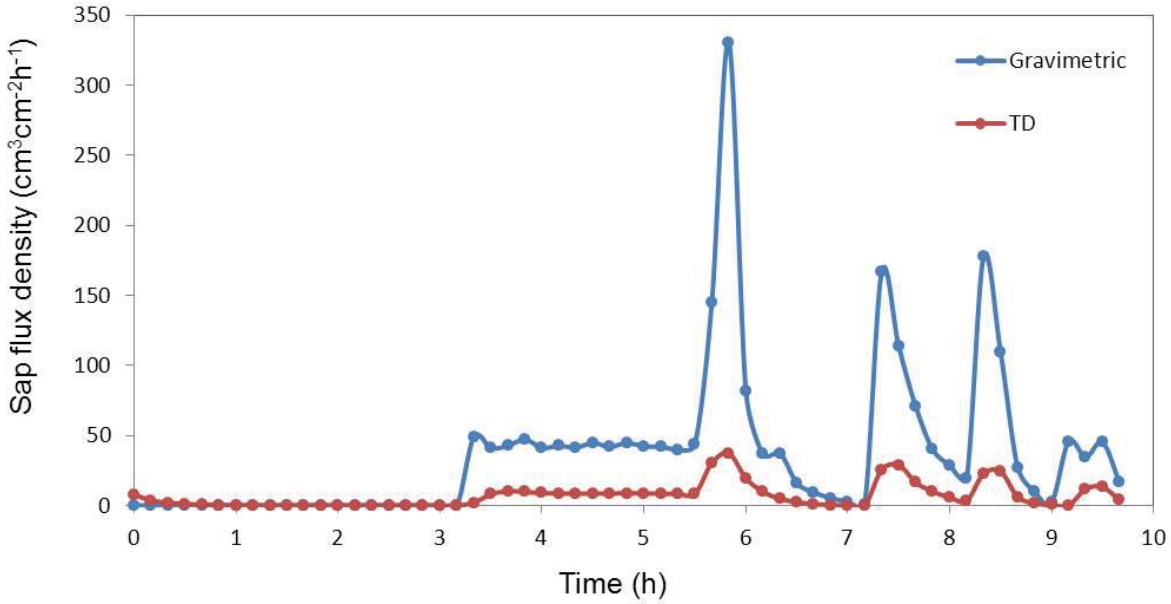

Figure 4.3 SFD measurements for the thermal dissipation (TD) and gravimetric methods for 'Eureka' lemon. Sharp increases in the SFD indicate periods of pressure application to the column of water

From Figure 4.2 and Figure 4.3 it is evident that the sudden increases in flow rate, as a result of increasing pressure, resulted in substantially higher SFD values measured gravimetrically than measured with the SFD techniques. Literature confirms that such high flow rates cannot be resolved by HPV and TD techniques and therefore SFDs above 250 $cm^3\ cm^{-2}\ h^{-1}$ for the HR, 300 $cm^3\ cm^{-2}\ h^{-1}$ for CHP and 200 $cm^3\ cm^{-2}\ h^{-1}$ for TD method were not considered in the calibration studies. These flow rates were also substantially higher than what has been measured in the different orchards during the project and were therefore probably unrealistically high for citrus.

4.3 Calibration of the heat ratio method

The relationship between SFD measured with the HR method and gravimetrically are presented in Figure 4.4 and Figure 4.5. Sap flux densities were underestimated in all the species and cultivars, except for one of the 'Eureka' lemon stems measured (Figure 4.4). Fairly good linear correlations ($R^2 > 0.7$) between SFD measured with the HR and gravimetric methods were observed in most of the 'Star Ruby' grapefruit, 'Delta' Valencia, 'Bahianinha' navel and 'Eureka' lemon stems, but poor correlations ($R^2 = 0.19$) were found for 'Nadorcott' mandarin (Table 4.1). Plotting these data on a single set of axes (Figure 4.5) revealed that the slope of the regression equations were inconsistent between stems within the same cultivar and also between species and cultivars, with the slope of the regression curves

ranging from 0.012 to 1.153 (Table 4.1). This indicates that a single correction factor for the HR method for citrus could not be obtained with this calibration technique.

Table 4.1 Linear regression coefficients (slopes and intercepts) between sap flux densities measured with the heat ratio method (y-axis) and that determined gravimetrically (x-axis). R^2 is the coefficient of determination and the CV of the slopes = 104%

Species	Replicate	Slope	Intercept ($cm^3cm^{-2}h^{-1}$)	R^2	P values
Citrus paradisi 'Star Ruby'	1	0.522	0.445	0.94	<.0001
	2	0.669	2.630	0.76	<.0001
	3	0.128	6.035	0.55	<.01
Citrus sinensis 'Delta'	1	0.032	1.915	0.60	<.0001
	2	0.012	4.037	0.75	<.0001
Citrus sinensis 'Bahianinha'	1	0.081	-0.109	0.67	<.0001
	2	0.408	3.903	0.83	<.0001
Citrus reticulata 'Nadorcott'	1	0.047	2.217	0.35	<.01
	2	0.026	2.765	0.19	n.s.
Citrus limon 'Eureka'	1	0.419	1.431	0.63	<.0001
	2	0.530	4.058	0.79	<.0001
	3	1.153	0.476	0.94	<.0001

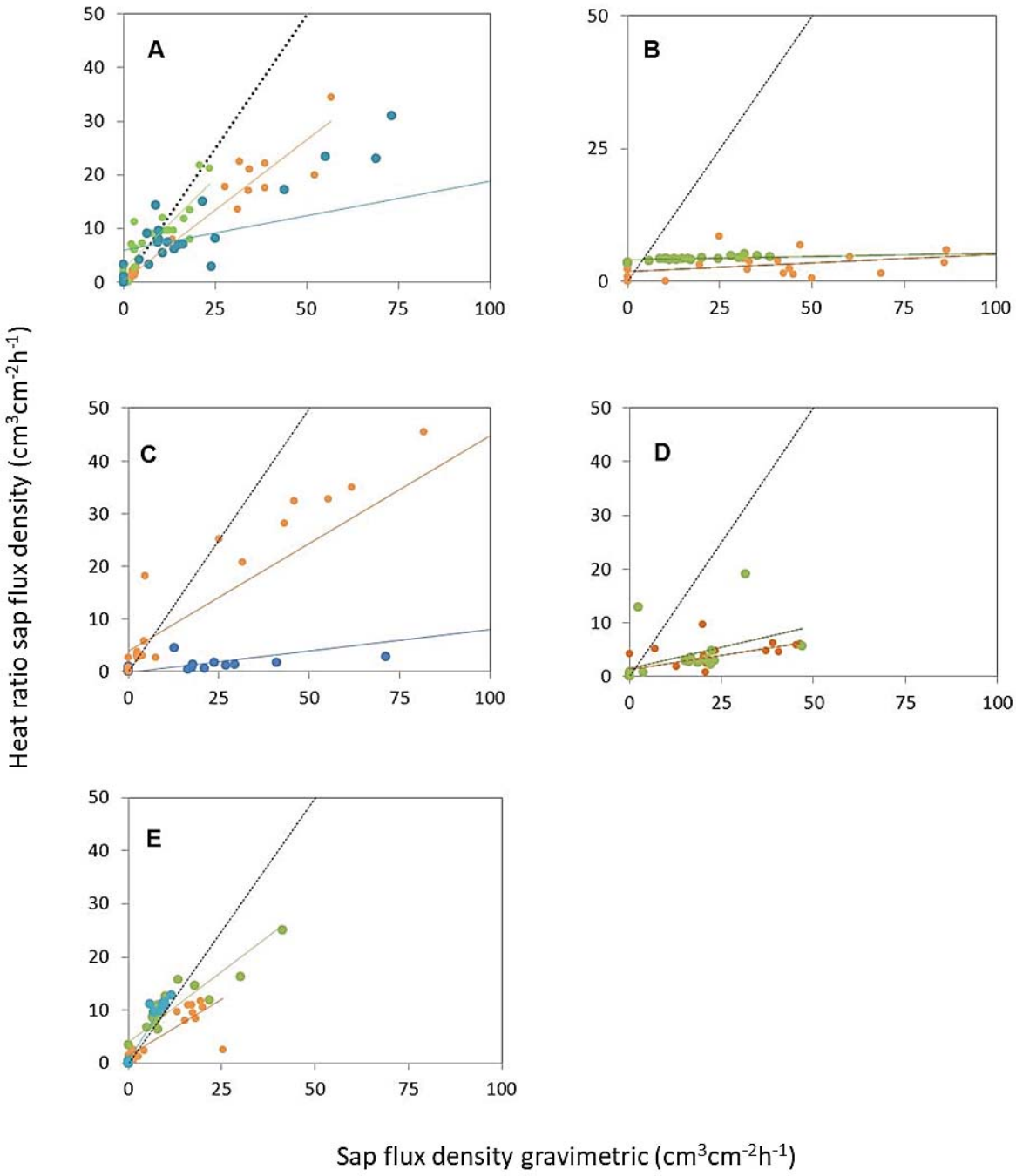

Figure 4.4 Relationship between the sap flux densities (SFD) measured with the heat ratio method and SFD measured gravimetrically for A) 'Star Ruby' grapefruit, B) 'Delta' Valencia, C) 'Bahianinha' navel, D) 'Nadorcott' mandarin and E) 'Eureka' lemon. Each colour represents a separate stem and the 1:1 line is indicated by the black dotted line

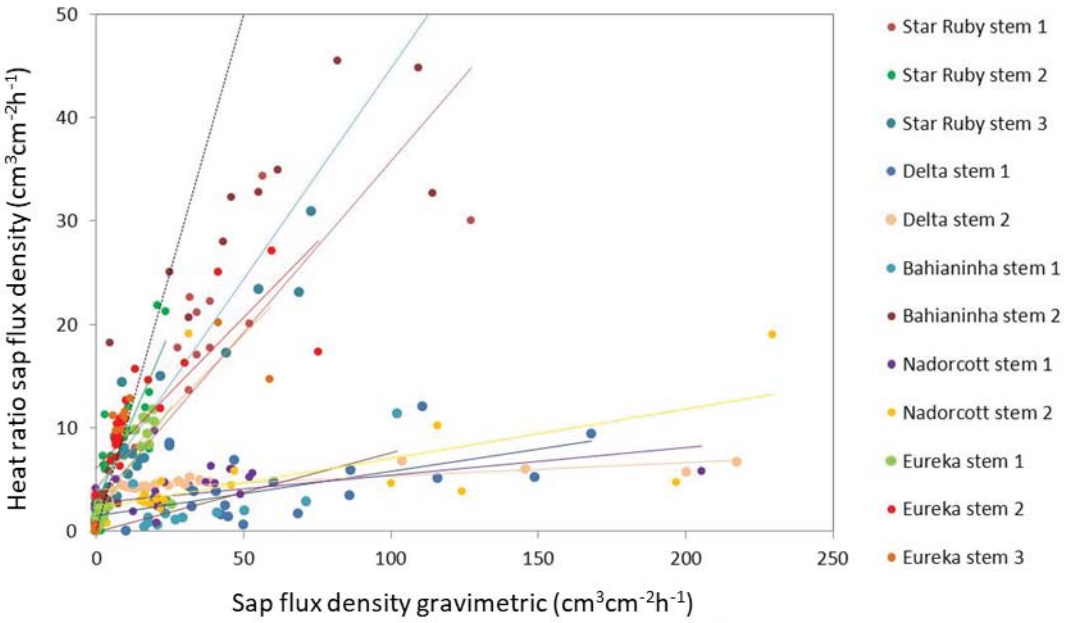

Figure 4.5 The relationship between the sap flux densities (SFD) measured using the heat ratio method for five citrus species and SFD determined gravimetrically. The 1:1 line is indicated by the black dotted line

4.4 Calibration of the compensation heat pulse method

As with the HR method, the SFD determined by the CHP method was also underestimated in all species, as compared to that determined gravimetrically. Relatively good correlations (R^2 > 0.7) between SFD measured with the CHP and gravimetric methods were observed in some stems of 'Star Ruby' grapefruit, 'Delta' Valencia, and 'Eureka' lemon, but once again, poor correlations were found for 'Nadorcott' mandarin (R^2 = 0.27) (Table 4.2 and Figure 4.6). When the species are plotted on the same axes (Figure 4.7) it was evident that the correlations differed between stems of the same cultivar. However, the slope of the best fit lines was more consistent between the five species than was observed with the HR method (Figure 4.5). The slope of the regression curves ranged from 0.015 to 0.538 (Table 4.2).

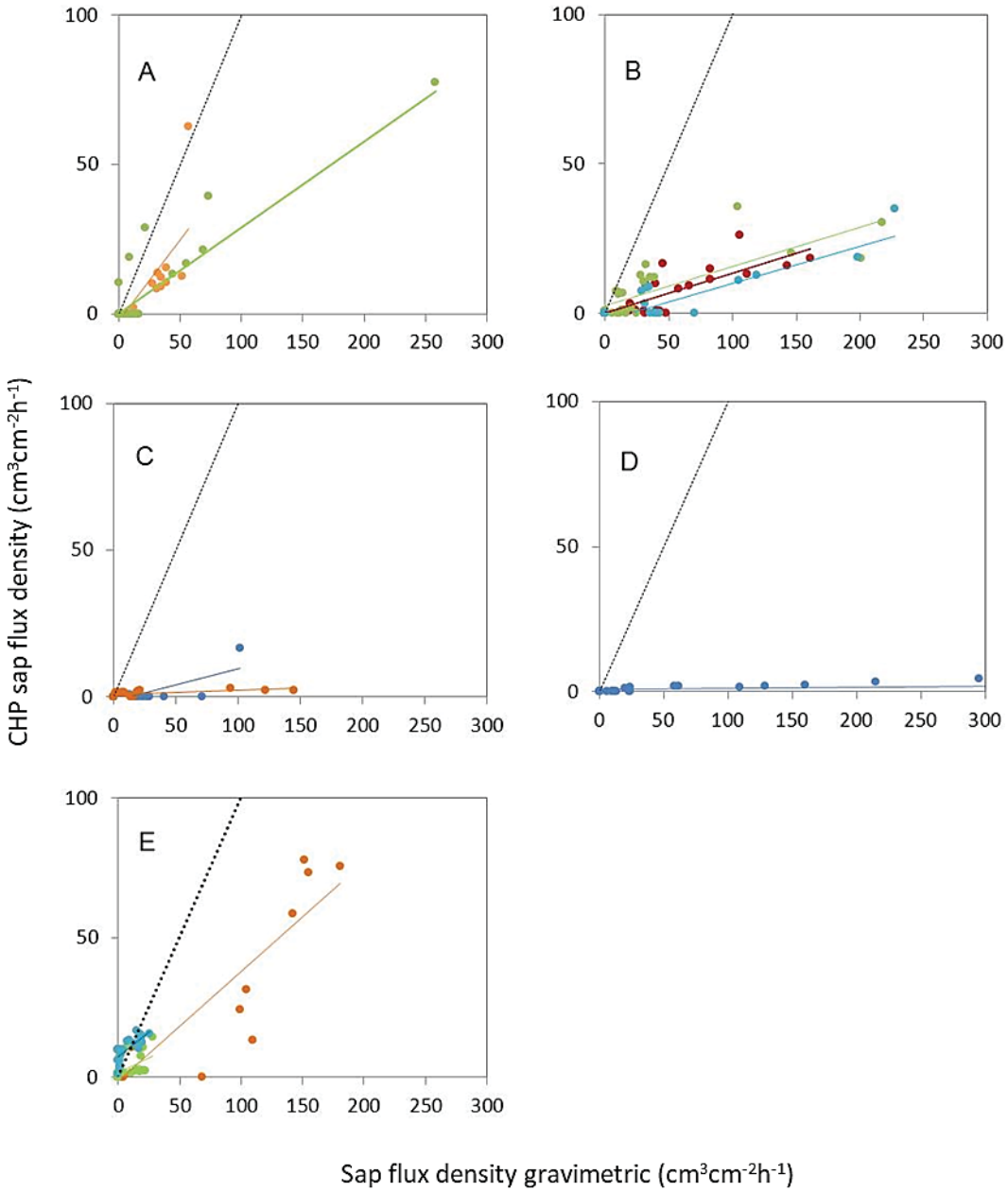

Figure 4.6 Relationship between the sap flux densities (SFD) measured with the compensation heat pulse method and SFD measured gravimetrically for A) 'Star Ruby' grapefruit, B) 'Delta' Valencia, and C) 'Bahianinha' navel, D) 'Nadorcott' mandarin and E) 'Eureka' lemon. Each colour represents a separate stem and the 1:1 line is indicated by the black dotted line

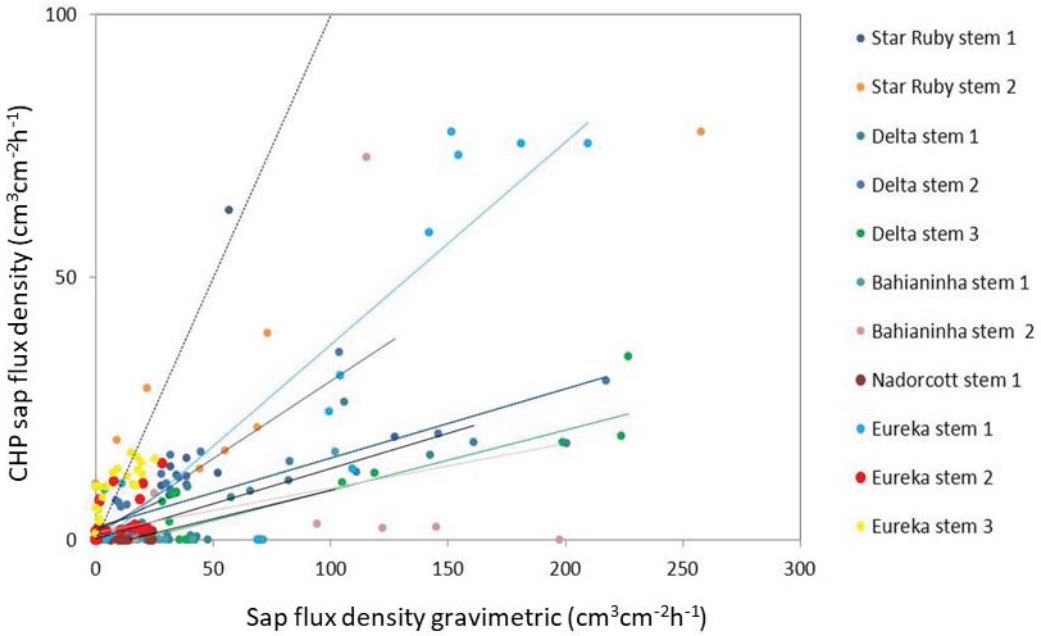

Figure 4.7 The relationship between the sap flux densities (SFD) measured using the CHP method for five citrus species and SFD determined gravimetrically. The 1:1 line is indicated by the black dotted line

Table 4.2 Linear regression coefficients (slopes and intercepts) between sap flux densities measured with the compensation heat pulse method (y-axis) and that determined gravimetrically (x-axis). Coefficient of determination (R^2), P values are given. The CV of the slopes = 77%

Species	Replicate	Slope	Intercept ($cm^3cm^{-2}h^{-1}$)	R^2	P values
Citrus paradisi 'Star Ruby'	1	0.538	-2.130	0.60	<.0001
	2	0.288	-0.032	0.73	<.0001
Citrus sinensis 'Delta'	1	0.134	0.110	0.61	<.0001
	2	0.132	2.414	0.64	<.0001
	3	0.124	-2.405	0.73	<.0001
Citrus sinensis 'Bahianinha'	1	0.107	-1.380	0.54	n.s.
	2	0.015	0.408	0.40	<.01
Citrus reticulata 'Nadorcott'	1	0.027	0.089	0.27	<.01
Citrus limon 'Eureka'	1	0.393	-1.566	0.84	<.0001
	2	0.206	1.730	0.23	n.s.
	3	0.343	7.379	0.50	<.0001

4.5 Calibration of the thermal dissipation method

The linear regression relationship between SFD measured with the TD method and SFD determined gravimetrically is presented in Figure 4.8, Figure 4.9 and Table 4.3. As was observed with the HR and CHP methods, SFD was also underestimated using the TD method. Despite the observed underestimation, good linear correlations were found for most of the stems of 'Star Ruby' grapefruit, 'Delta' Valencia and 'Eureka' lemon ($R^2 > 0.7$). Although there was some variation in the slopes (from 0.019 to 0.194) of the regression equations within stems of the same species (Figure 4.8) and between species and cultivars (Figure 4.9). The variation within the same species was approximately the same for that observed for the HR method (CV = 78%).

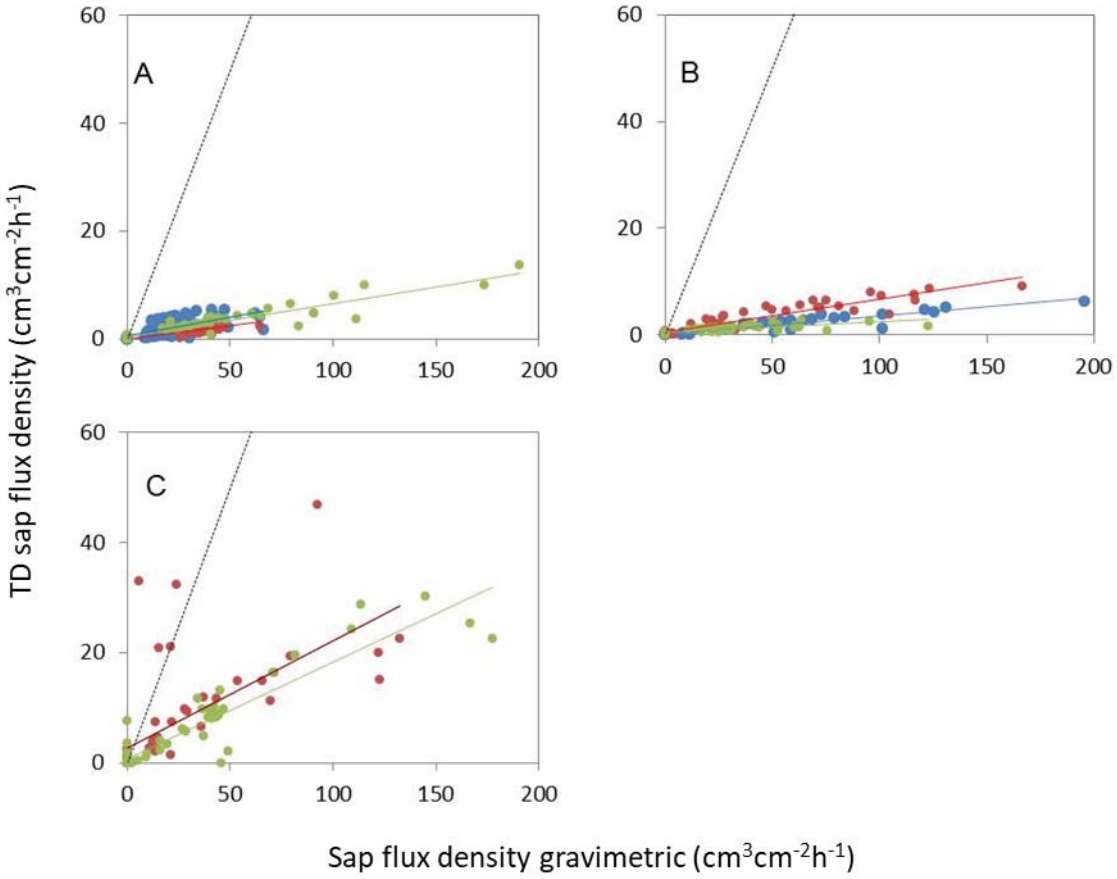

Figure 4.8 Relationship between the sap flux densities (SFD) measured with the thermal dissipation (TD) method and SFD measured gravimetrically for A) 'Star Ruby' grapefruit, B) 'Delta' Valencia and C) 'Eureka' lemon. Each colour represents a separate stem and the 1:1 line is indicated by the black dotted line

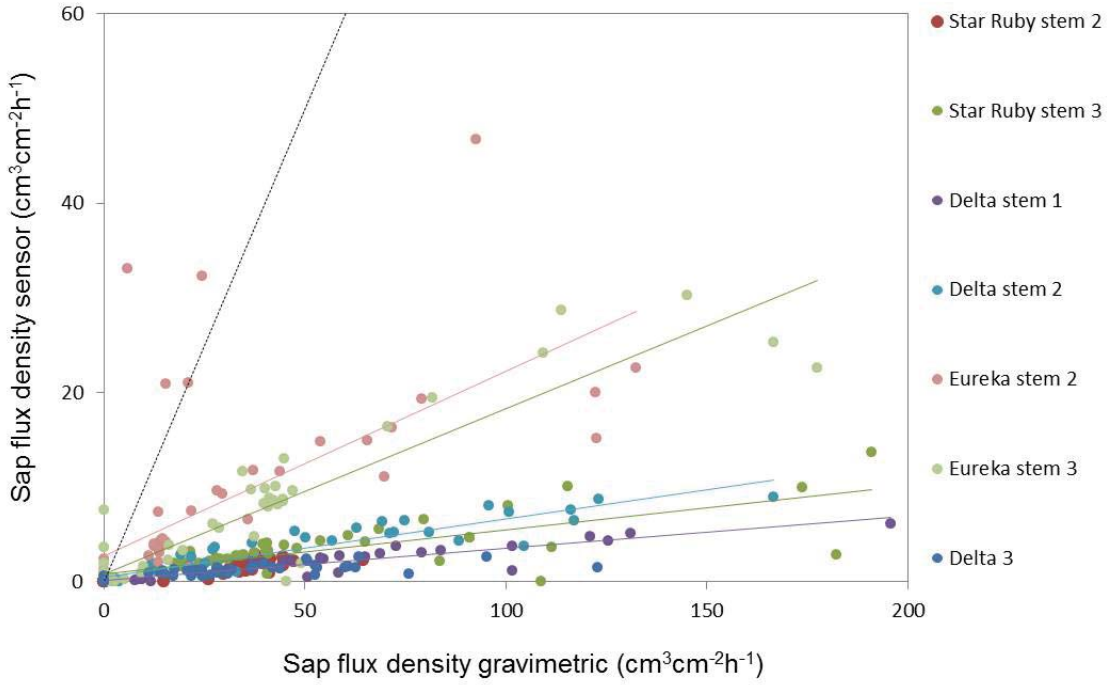

Figure 4.9 The relationship between the sap flux densities (SFD) measured using the thermal dissipation method for three citrus species and SFD determined gravimetrically. The 1:1 line is indicated by the dotted line

Table 4.3 Linear regression coefficients (slopes and intercepts) between sap flux densities measured by the thermal dissipation method (y-axis) and that determined gravimetrically (x-axis). Coefficient of determination (R^2) and P values are given. The CV of the slopes = 78%

Species	Replicates	Slope	Intercept ($cm^3cm^{-2}h^{-1}$)	R^2	P values
Citrus paradisi 'Star Ruby'	1	0.069	0.525	0.45	<.01
	2	0.048	0.036	0.79	<.0001
	3	0.061	0.452	0.85	<.0001
Citrus sinensis 'Delta'	1	0.033	0.167	0.86	<.0001
	2	0.061	0.480	0.87	<.0001
	3	0.019	0.443	0.54	<.0001
Citrus limon 'Eureka'	1	0.194	2.801	0.43	<.01
	2	0.174	0.859	0.85	<.0001

4.6 Corrected sap flux density

A substantial variation in the underestimation of actual SFD for the different sap flow methods for the different citrus species was found (Table 4.4). The HR method underestimate by 89% and CHP method by 99% the SFD for 'Nadorcott' mandarin compared to the SFD measured with the gravimetric method. The lowest percentage underestimation was for 'Eureka' lemon (50% for the HR method and 27% for the CHP method) compared to other species. The TD method was only assessed in 'Star Ruby' grapefruit, 'Delta' Valencia and 'Eureka' lemon and once again SFD was underestimated by 94% for 'Star Ruby' grapefruit, 72% for 'Delta' Valencia and 78% for 'Eureka' lemon.

Table 4.4 Summary of the degree of underestimation of sap flux densities (SFD) determined gravimetrically by the various SFD techniques for the different citrus varieties

Species	Underestimation (%)		
	Heat ratio method	Compensation heat pulse method	Thermal dissipation method
'Star Ruby' grapefruit	59	68	94
'Delta' Valencia	60	64	72
'Bahianinha' navel	70	91	ND*
'Nadorcott' mandarin	89	99	ND*
'Eureka lemon'	50	27	78
CV %	37	32	8

ND*TD method was not tested in these species

To correct the SFDs determined for the HR, CHP and TD methods to the SFD determined gravimetrically, correction factors for each sap flow method were calculated as the reciprocal of the slope of the linear regression of each variety, when forced through the origin. These correction factors were averaged to determine a single correction factor for each citrus species. Only stems where $R^2 > 0.5$ for the regression relationship were considered when determining the average correction factor. There was a substantial variation in correction factors between the species for each method (Table 4.5), confirming that the correction factor is species dependant and a single correction factor for citrus could therefore not be determined in this study. Correction factors were not determined in 'Nadorcott' mandarin, as reliable correlations ($R^2 > 0.5$) between SFD determined using the sap flow sensors and that determined gravimetrically were not achieved.

Table 4.5 Citrus species-specific correction factors for sap flux densities (SFD) determined by various SFD techniques

Species	Correction factors		
	Heat ratio method	Compensation heat pulse method	Thermal dissipation method
'Star Ruby' grapefruit	1.72 ± 0.52	2.66 ± 1.13	17.52 ± 3.66
'Delta' Valencia	8.77 ± 3.42	7.69 ± 0.32	21.40 ± 9.22
'Bahianinha' navel	7.22 ± 7.15	11.30 ± 2.86	ND*
'Nadorcott' mandarin	-	-	ND*
'Eureka' lemon	2.18 ± 0.21	3.43 ± 1.23	5.73 ± 3.94

ND*TD method was not tested in these species

Figure 4.10, Figure 4.11 and Figure 4.12 present the relationships between the SFDs determined by different SFD techniques and that determined gravimetrically, after the SFD for each technique was corrected using the average correction factors given in Table 4.5. In some species ('Star Ruby' grapefruit and 'Eureka' lemon) a single correction factor resulted in good agreement between SFD determined by each technique with that determined gravimetrically for that particular species. In other species measured ('Delta' Valencia and 'Bahianinha' navel) a single correction factor resulted in poor agreement between the two methods. Surprisingly, applying the derived correction factors resulted in the consistent overestimation of SFD in all species using all techniques. The performance of the techniques as determined by the statistical parameters are presented in Table 4.6, Table 4.7 and Table 4.8. Overall, the attempted calibration was poor, as the MAE was greater than 20% in all species using all techniques, however, D was satisfactory (> 0.80) in most species for each technique.

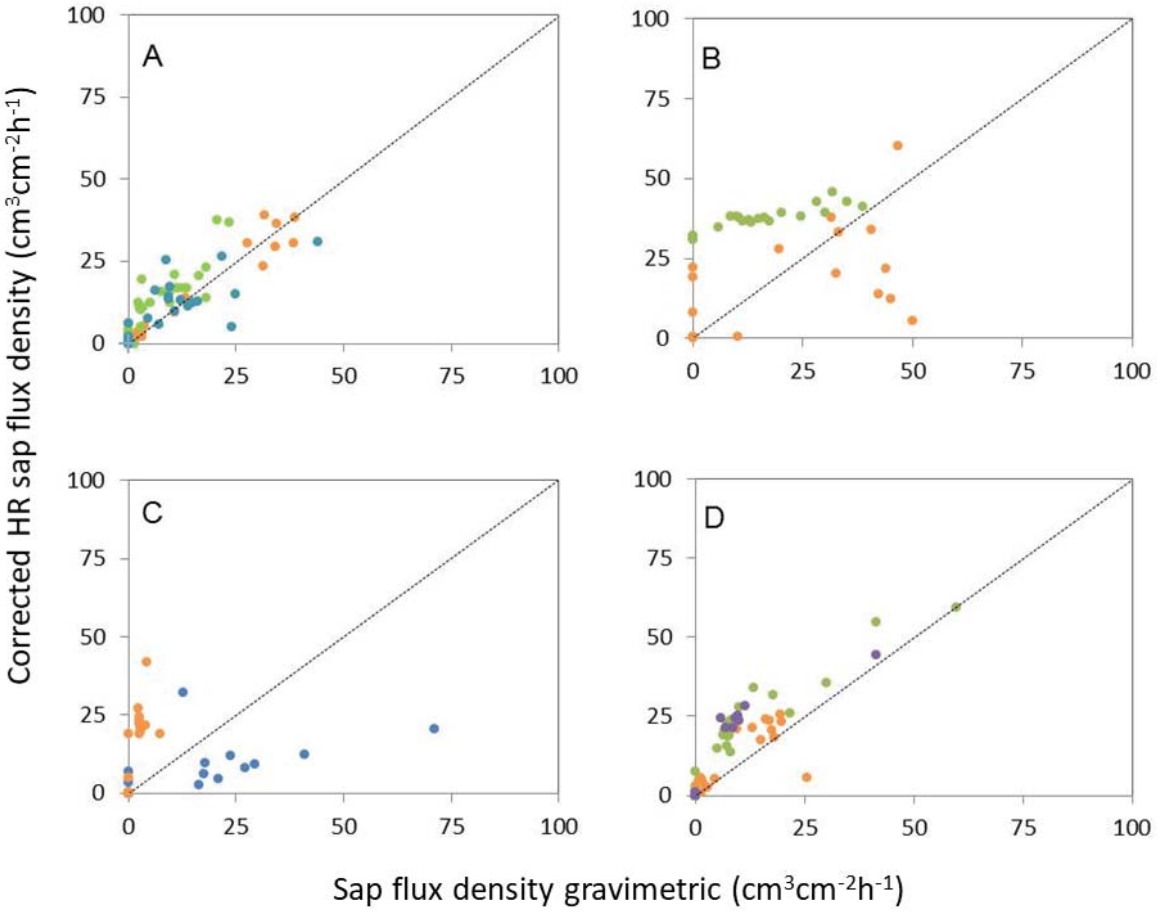

Figure 4.10 Corrected sap flux densities measured with the heat ratio (HR) method compared to that determined gravimetrically. Correction factors were calculated as the average of the reciprocal of the slope of each regression line for A) 'Star Ruby' grapefruit, B) 'Delta' Valencia, C) 'Bahianinha' navel, D) and 'Eureka' lemon. Each colour represents a separate stem and the 1:1 line is indicated by the black dotted line

Table 4.6 Performance of the corrected heat ratio method in various citrus species as indicated by the coefficient of determination (R^2), Willmott index of agreement (D), root of the mean square error (RMSE) and mean absolute error (MAE)

Species	Replicate	R^2	D	RMSE ($cm^3\ cm^{-2}\ h^{-1}$)	MAE (%)
Citrus paradisi 'Star Ruby'	1	0.94	0.98	6.58	49
	2	0.85	0.90	7.9	118
	3	0.59	0.86	16.5	120
Citrus sinensis 'Delta'	1	0.67	0.90	18.5	67
	2	0.76	0.78	22	142
Citrus sinensis 'Bahianinha'	1	0.74	0.89	22	86
	2	0.84	0.58	141	593
Citrus limon 'Eureka'	1	0.91	0.93	6	88
	2	0.82	0.93	7	84
	3	0.54	0.81	14	105

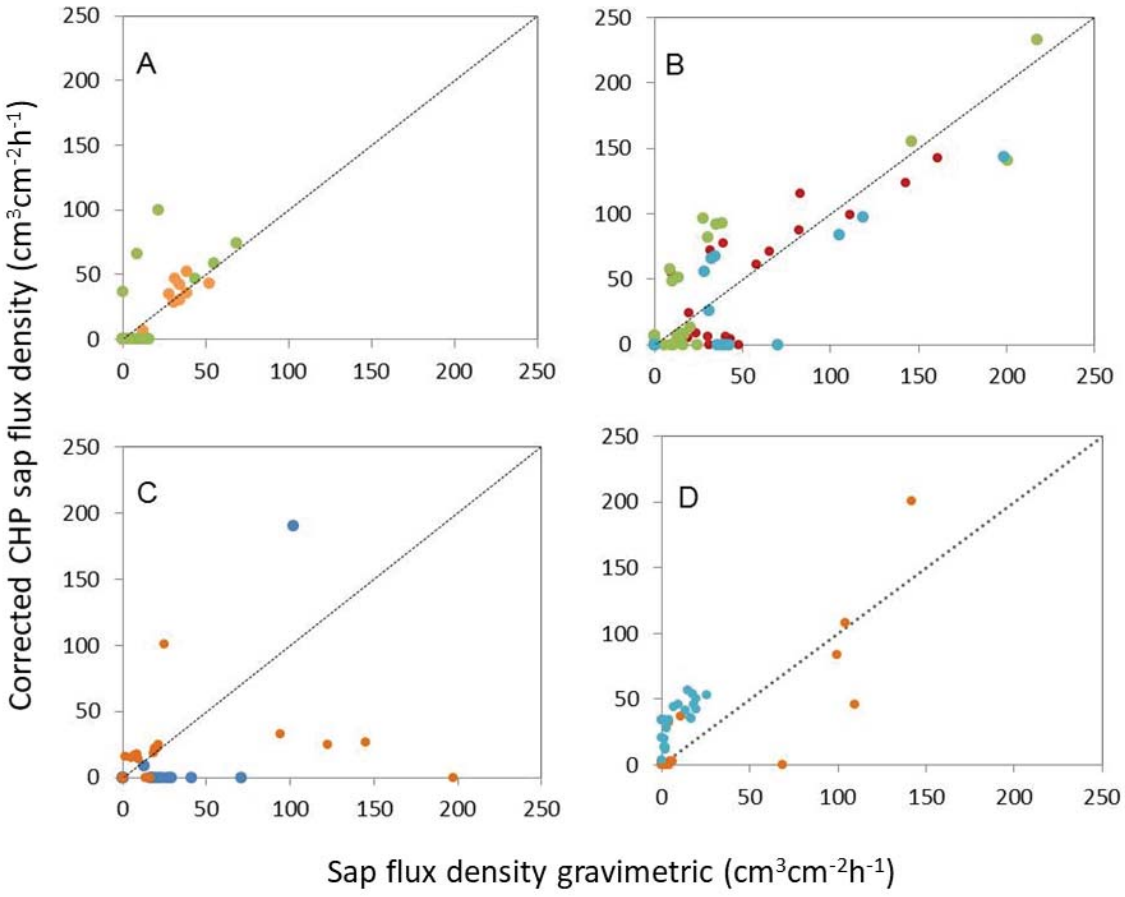

Figure 4.11 Corrected sap flux densities measured with the compensation heat pulse (CHP) method compared to that determined gravimetrically. Correction factors were calculated as the average of the reciprocal of the slope of each regression line for A) 'Star Ruby' grapefruit, B) 'Delta' Valencia, C) 'Bahianinha' navel and D) 'Eureka' lemon. Each colour represents a separate stem and the 1:1 line is indicated by the black dotted line

Table 4.7 Performance of the corrected compensation heat pulse method in various citrus species as indicated by the coefficient of determination (R^2), Willmott index of agreement (D), root of the mean square error (RMSE) and mean absolute error (MAE)

Species	Replicate	R^2	D	RMSE ($cm^3\ cm^{-2}\ h^{-1}$)	MAE (%)
Citrus paradisi 'Star Ruby'	1	0.92	0.96	8	55
	2	0.74	0.91	41	142
Citrus sinensis 'Delta'	1	0.82	0.92	62	82
	2	0.68	0.87	69	158
	3	0.77	0.89	59	136
Citrus sinensis 'Bahianinha'	1	0.02	0.61	40	148
	2	0.25	0.33	226	198
Citrus limon 'Eureka'	1	0.78	0.93	82	84
	2	0.106	0.50	26	250
	3	0.50	0.44	42	733

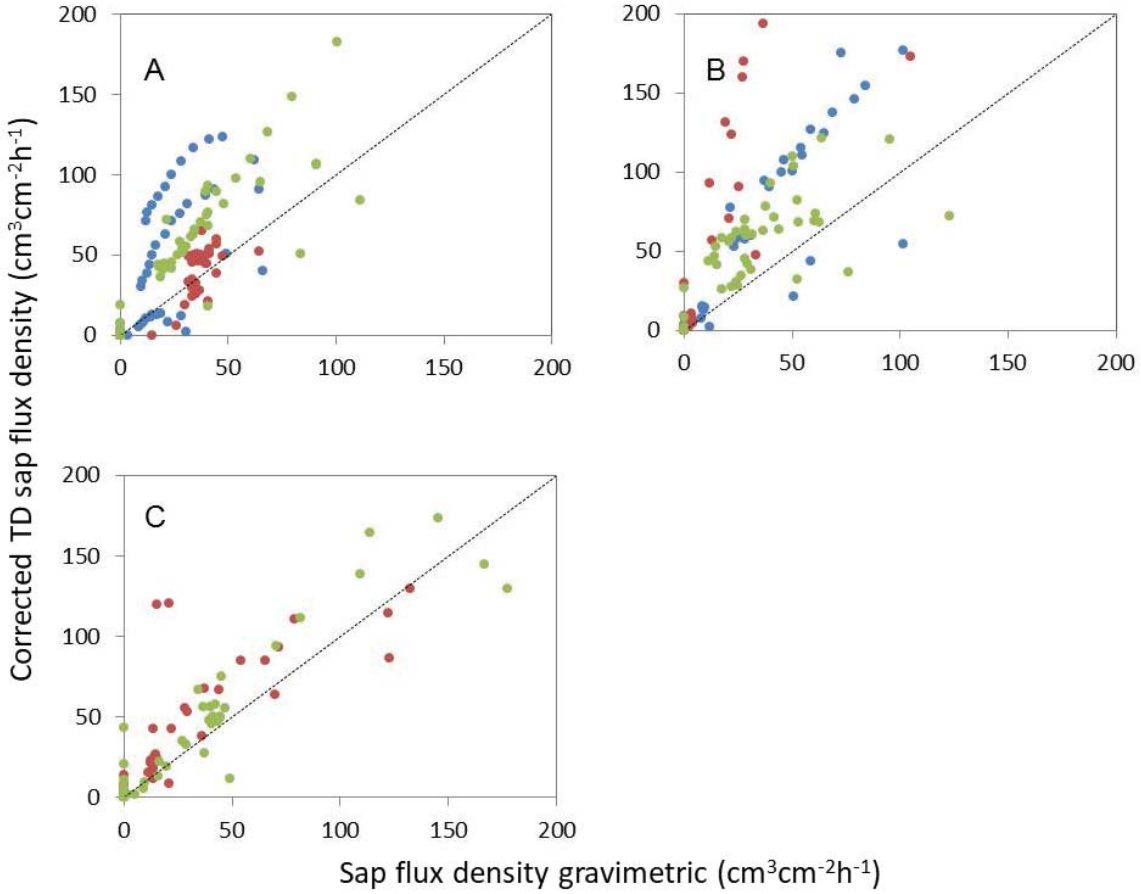

Figure 4.12 Corrected sap flux densities measured with thermal dissipation (TD) method as compared to that determined gravimetrically. Correction factors were calculated as the average of the reciprocal of the slope of each regression line for A) 'Star Ruby' grapefruit, B) 'Delta' Valencia and C) 'Eureka' lemon. The 1:1 line is indicated by the black dotted line. Different coloured markers represent different stems

Table 4.8 Performance of the corrected thermal dissipation method in various citrus species as indicated by the coefficient of determination (R^2), Willmott index of agreement (D), root of the mean square error (RMSE) and mean absolute error (MAE)

Species	Replicate	R^2	D	RMSE ($cm^3\ cm^{-2}\ h^{-1}$)	MAE (%)
Citrus paradisi 'Star Ruby'	1	0.46	0.63	70	523
	2	0.79	0.93	18	81
	3	0.81	0.92	44	168
Citrus sinensis 'Delta'	1	0.88	0.90	69	236
	2	0.87	0.94	38	91
	3	0.25	0.64	94	308
Citrus limon 'Eureka'	1	0.63	0.84	52	240
	2	0.87	0.96	38	149

4.7 Variation in conducting sapwood

The variations in conducting sapwood in various citrus species, which were used for the calibration of different SFD techniques, are illustrated in Figure 4.13. Staining of the sapwood was not uniform in all species, and the percentage area of conducting sapwood varied from branch to branch of the same cultivar and between different species. A large proportion of the sapwood in the 'Nadorcott' mandarin sample was not stained (Figure 4.13 B) and on average, for both 'Nadorcott' mandarin stems only 10.8% of the sapwood was able to conduct water. In addition, it was evident that one of the probes for one of the stems, for both the HR and CHP methods, was inserted into non-conducting sapwood (Figure 4.13 B). This could have contributed to the very poor correlations between the actual SFD determined gravimetrically and that determined by the sap flow sensors.

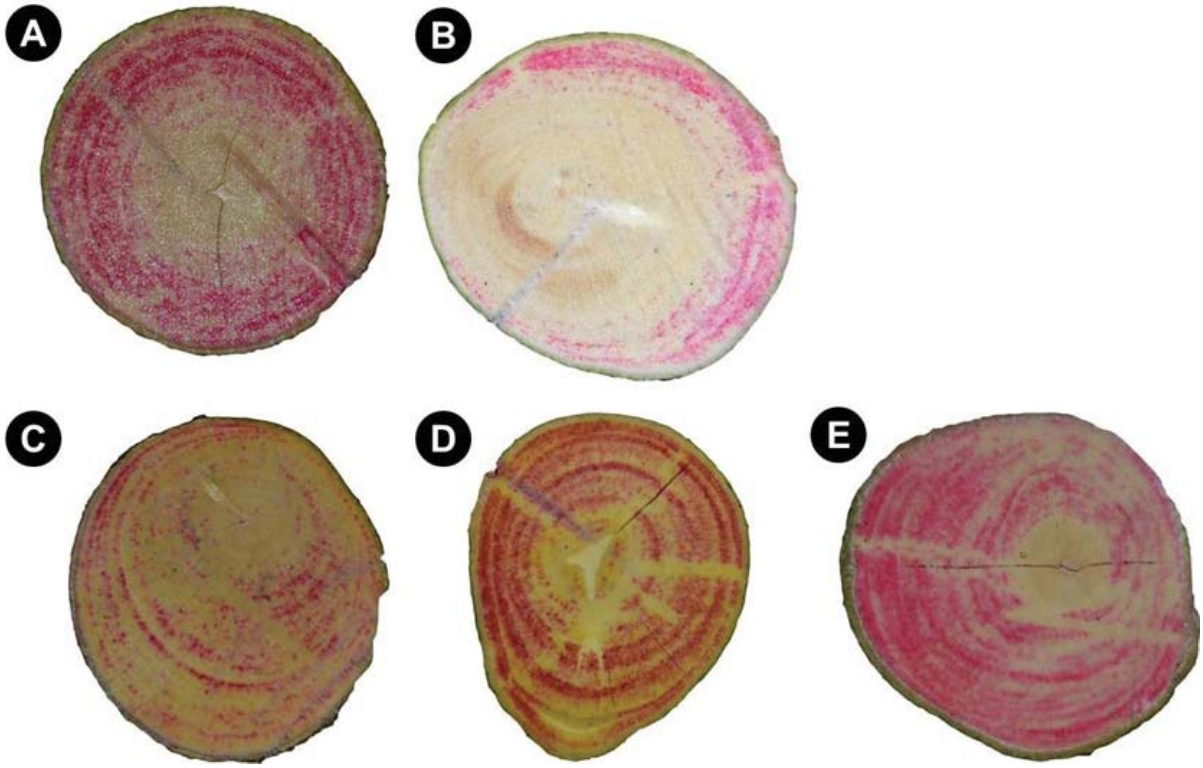

Figure 4.13 Cross sections of stem segments A) 'Star Ruby' grapefruit, B) 'Nadorcott' mandarin, C) 'Delta' Valencia, D) 'Bahianinha' navel, and E) 'Eureka' lemon used for sap flow calibration. The stems were stained with Safranin O dye to indicate the conducting sapwood as indicated by the pink colour

Clear evidence of the impact of the implantation of probes on the movement of water is shown in Figure 4.14. Stain is clearly evident on the upstream side of the drilled hole, but no stain is evident on the downstream side of the drilled hole. This clearly demonstrates that the insertion of probes prevents the movement of water past the probe and therefore it is necessary to take wounding into account when calculating the SFD. In this instance the wound width can be taken as the width of the thermocouple probe, i.e. 0.2 cm.

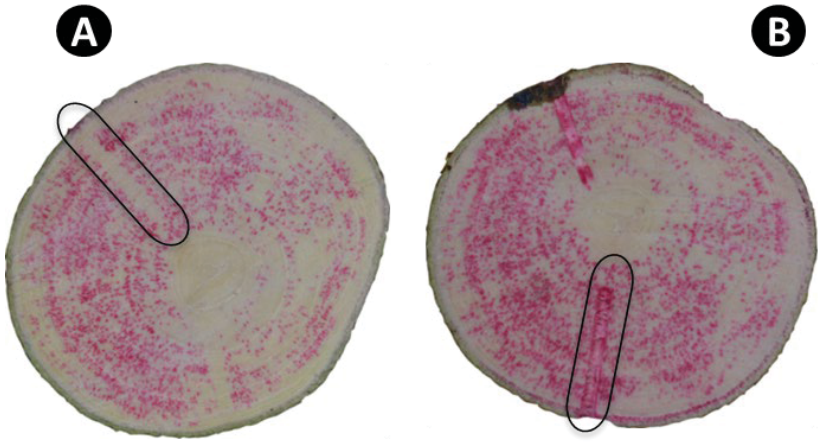

Figure 4.14 Cross section of stems indicating A) no water movement on the downstream xylem where the thermocouple was inserted and B) staining in the upstream drilled hole where the thermocouple was inserted, indicating direct water movement to the thermocouple, but not beyond

4.8 Discussion and conclusions

Sap flux density techniques provide direct measurements of T_{sap} rates and have been successfully used to determine xylem sap flow and T rates of woody plants (de Oliveira Reis et al., 2006). Smith and Allen (1996) advised that the techniques should be calibrated for each species in which they are to be used, due to uncertainties associated with the use of the techniques. The accuracy of these techniques are critically dependent on assumptions that do not apply to all species, and the determination of parameters that are required for the upscaling of heat pulse velocities to SFD and T. Accurate estimations of T are critical as T plays an important role in physiological processes in plants, affecting their growth and productivity by influencing water relations (Villalobos et al., 2013). Accurate measurements of T rates are important not only for research purposes, but also for irrigation scheduling and improving water use efficiency (Alarcón et al., 2005). It is therefore important that calibration of these techniques occurs before actual measurements in the field (Chen et al., 2012).

In this study the calibration of SFD techniques was conducted in a laboratory using the stem perfusion method. A method similar to the procedure described by Steppe et al. (2010) was used to obtain independent measurements of SFD. This allowed the direct comparison of SFD measured with the SFD techniques, to those measured gravimetrically. It was also possible to achieve a range of desirable flow rates and the testing of a wide range of citrus varieties within a short period of time. The stem perfusion method does, however, have some disadvantages. The method is associated with the cutting of the stem, which damages the tissue and creates embolisms in xylem vessels. This can affect the SFD measurements, as flow through the stem may not be comparable with the flow under natural conditions and whilst

the stem was still part of the living tree. The problem of tissue damage caused by cutting stems was highlighted by Bleby et al. (2004) and was the reason why these authors choose to conduct their experiment under controlled and natural conditions in a greenhouse using weighing lysimeters. However, potted citrus trees of a number of varieties and of sufficient size for the insertion of probes are not readily available for calibration of the SFD methods using weighing lysimetry.

Calibration of the HR, CHP and TD methods in citrus species conducted in the laboratory yielded large variations between species. These variations were evident in the different slopes of the linear regression equations (Table 4.1, Table 4.2 and Table 4.3), between the SFD using sap flow sensors and those determined gravimetrically. Furthermore, the slope and interception of the regression equations were inconsistent between stems of the same variety. However, significant correlations ($R^2 > 0.7$) were attained in some stems of each variety, indicating a good relationship between the two measurements. The performance of heat pulse techniques in our study is similar to reports by Pearsall (2011) who found that even though there were good correlations between the CHP and lysimeter measurements in grapevines, the nature of the relationship was inconsistent. Similarly, Bleby et al. (2004) found significantly different slopes and intercepts in the relationship between the CHP method and the gravimetric method in *E. marginata*. Large variations in SFD measured with the CHP method in stems of *Fagus grandifolia* were also observed, although the authors obtained a reasonably good relationship (Steppe et al., 2010).

Measured values of SFD across citrus species were in general higher with the TD and CHP methods, than with the HR method. As was expected, the HR method performed well under low flow rates, which is an advantage in these conditions (Burgess et al., 2001). However, unlike the HR method, the CHP method performed better under high flow rates as shown in Figure 4.1 and as previously reported by Becker (1998). The good performance of the HR method at low flow rates compared to the good performance of the CHP method at high flows rates was also previously observed by Bleby et al. (2004).

All three methods evaluated in this study consistently underestimated the sap flux densities, with large variation in percentage underestimation between techniques, species and individual branches. Previously it was not thought that calibration was necessary for the TD method. It was calibrated in five woody species and in sawdust and this was assumed to be valid for all species (Lundblad et al., 2001). However, other studies have demonstrated the necessity of species specific calibration in order to obtain accurate measurements (Vandegehuchte and Steppe, 2013). In this study the TD method underestimated SFD by between 50 and 90% in the three species evaluated. Similar underestimations were found by Steppe et al. (2010) in

Fagus grandifolia, where a 60% underestimation of actual sap flow was observed. Hultine et al. (2010) reported a 50% underestimation in excised branches of *Tamarix ramosissima* × *chinesis,* and underestimations of more than 50% were recorded in *Quercus gambelii* and *Acer grandidentatum* (Taneda and Sperry, 2008). In a study by Paudel et al. (2013) underestimations of 70% were found for apple in greenhouse trees and cut branches, 55% for peltophorum cut branches, 60% for a persimmon orchard tree and 60% for nectarine cut branches.

When comparing our results using the heat pulse methods, the underestimation percentages were higher (50-90% for the HR method and 27-99% for the CHP method) than what was found in previous reports by Cohen et al. (1981). Infield and laboratory calibrations of the T-max system for *Citrus sinensis* L.*, Pseudotsuga menziesii*, *Platanus orientalis L*. and *Populus alba L*. revealed a 45% underestimation of the HPV. Steppe et al. (2010) found that T was underestimated in *Fagus grandifolia* by 35% when using the CHP method. The results were quite similar to the findings of Green and Clothier (1988) in kiwifruit, where the CHP method underestimated transpiration by 62% (Sorensen et al., 1999). Smith and Allen (2006) also found serious underestimation of flow through branches of *Azdirachta indica* A. These authors attributed the underestimation to the diffuse porous nature of the sapwood.

In our study, the inconsistency in the regression coefficients and the large variation in the underestimation of values for the SFD measurements with the different sap flow methods were likely due to the nature of the sapwood, which is reported to be very influential in determining the accuracy of the techniques (Swanson and Whitfield, 1981; Green and Clothier, 1988; Fernández et al., 2006). Whilst the HPV techniques involved a measurement every 10 min in this study, the TD method involves a constant heat source with continual measurements, where the average of the temperature differences (ΔT) is calculated every 5 minutes. It was, therefore, expected that the TDP method would be better in capturing the sudden changes in flow rates. However, although R^2 values of greater than 0.8 were frequently observed in some stems, the TD method greatly underestimated the flow determined gravimetrically, with most slopes of the regression equations being smaller than 0.1. An additional source of error is also attributed to the contact of the probes with inactive xylem and the manner in which pressure was increased in the experiments, which resulted in water coming out of the probe insertion holes. This could have influenced the measurements of heat pulse velocities and may even have caused the sensors to shift, resulting in probe misalignment. Therefore, in future, when using the stem perfusion method it is suggested that a much better way is found to achieve different flow rates, as these sudden changes in flow are registered by the gravimetric readings, but are not necessarily taken into account by the SFD techniques.

5 RESULTS – CITRUS SAPWOOD ANATOMY

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5.1 Citrus sapwood anatomy

Cross sections of the sapwood of 'Delta' Valencia, 'Star Ruby' grapefruit, 'Bahaininha' navel, 'Nadorcott' mandarin and 'Eureka' lemon were prepared to show the xylem vessels in both mature and flush leaf wood (Figure 5.1). The xylem vessels in mature leaf wood (MLW), which is the wood produced early in the season, are widely distributed, with greater distances between the vessels. On the other hand the flush leaf wood (FLW), xylem vessels are more closely packed with smaller distance between the vessels (Figure 5.1 D). In the FLW the xylem vessels are grouped into groups of 2-3 vessels. Mature and FLW was evident in sapwood samples taken from 'Delta' Valencia, 'Star Ruby' grapefruit and 'Eureka' lemon (Figure 5.1 D, SR and E), whilst only MLW was found in sapwood samples from 'Bahaininha' navel and 'Nadorcott' mandarin (Figure 5.1 B and N).

Mean distances between the xylem vessels in MLW from both the inner and outer part of the stem and branch are shown in Table 5.1. In general, in MLW the xylem vessels were more widely spaced in 'Delta' Valencia samples (554 µm in stems and 474 µm in branches), whilst they were more closely spaced in the sapwood of the 'Nadorcott' mandarin (415 µm in stems and 396 µm in branches). Although differences between species were not always significant ($p < 0.05$), it was found that in all the stems investigated the distance between vessels exceeded the limit (400 µm) determined by Swanson (1983) for thermal homogeneity, except for the branches of the 'Nadorcott' mandarin.

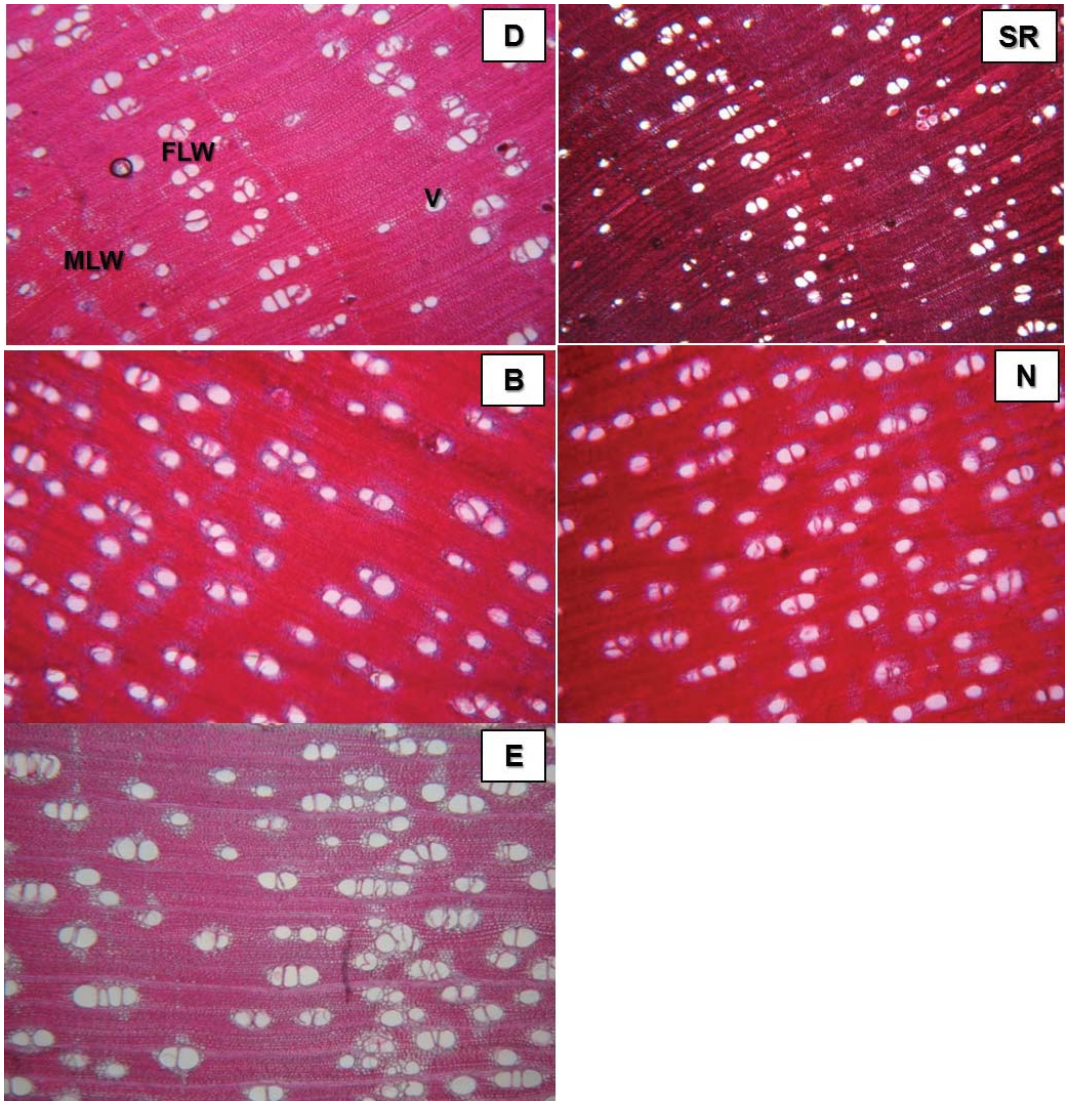

Figure 5.1 Cross sectional images of the xylem vessels for (D) 'Delta' Valencia, (SR) 'Star Ruby' grapefruit, (B) 'Bahaininha' navel, (N) 'Nadorcott' mandarin, and (E) 'Eureka' lemon, illustrating both mature wood (MLW) and flush wood (FLW) and xylem vessels (V)

Table 5.1 Distance between the xylem vessels in branches and stems of various citrus species in mature leaf wood. Values are means ± standard error

Species	Stem (µm)		Branch (µm)	
	Inner part	Outer part	Inner part	Outer part
Citrus sinensis 'Delta'	566.8 ± 49.9^{a}	542.2 ± 46.6^{a}	468.1 ± 26.1^{ab}	479.7 ± 65.5^{ab}
Citrus paradisi 'Star Ruby'	474.0 ± 29.9^{b}	539.1 ± 31.1^{a}	407.5 ± 33.5^{b}	436.5 ± 24.0^{ab}
Citrus limon 'Eureka'	458.5 ± 13.7^{b}	432.0 ± 6.7^{b}	425.7 ± 9.6^{ab}	427.4 ± 11.9^{ab}
Citrus sinensis 'Bahianinha'	460.4 ± 15.4^{b}	481.8 ± 16.8^{ab}	518.0 ± 16.9^{a}	454.2 ± 18.4^{a}
Citrus reticulata 'Nadorcott'	434.6 ± 21.1^{b}	396.0 ± 14.5^{b}	406.5 ± 23.2^{b}	385.1 ± 21.0^{b}

In each column, values followed by the same letter are not significantly different at ($p<0.05$)

In the inner part of the stem, the distance between the vessels was significantly larger in 'Delta' Valencia than all the other species investigated, which were not significantly different from each other. In the outer part of the stem, vessels were spaced significantly ($p < 0.05$) wider apart in 'Delta' Valencia and 'Star Ruby' grapefruit, compared with 'Eureka' lemon and 'Nadorcott' mandarin. Vessel spacing in the branches differed from spacing in the stems, when comparing the different species. In the inner part of the branches, the distances between vessels were significantly greater in 'Bahianinha' navel, as compared to 'Star Ruby' grapefruit and 'Nadorcott' mandarin. In the outer part of the branch, the vessels were more widely spaced in 'Bahaininha' navel as compared to 'Nadorcott' mandarin. In general there was a nonsignificant difference in the vessel spacing between the inner and outer sapwood in both stems and branches.

Measurements in the FLW were only performed for 'Delta' Valencia, 'Star Ruby' grapefruit and 'Eureka' lemon, as FLW was not readily evident in 'Bahaininha' navel and 'Nadorcott' mandarin. It was immediately evident that vessels were spaced much closer together in the FLW than in the MLW. For example, whilst xylem vessels were on average spaced 554 µm apart in the MLW of stems of 'Delta' Valencia, vessels were spaced on average 209 µm apart in the FLW of these stems. In the FLW the distance between vessels fell within the limit determined by Swanson (1983) for thermal homogeneity of 400 µm.

Table 5.2 Distance between the xylem vessels in branches and stems of various citrus species in flush leaf wood. Values are means ± standard error

Species	Stem (µm)		Branch (µm)	
	Inner part	Outer part	Inner part	Outer part
Citrus sinensis 'Delta'	186.8 ± 8[b]	232.2 ± 35[a]	200.0 ±11[a]	336.7± 132[a]
Citrus paradisi 'Star Ruby'	238.3 ± 9[a]	229.4 ±*[a]	197.6 ± 4[a]	169.8 ±15[ab]
Citrus limon 'Eureka'	151.4 ± 8[c]	159.2 ±12[a]	127.3 ± 13[a]	138.3 ± 5[b]
Citrus sinensis 'Bahianinha'	*	*	*	*
Citrus reticulata 'Nadorcott'	*	*	*	*

In each column, Values followed by the same letter are not significantly different at ($p<0.05$).*No flush wood was observed in these species

There were no significant differences between species in terms of the outer part of the stem and the inner part of the branch. However, in the inner part of the stem there were significant differences between all three species with 'Star Ruby' grapefruit having the most widely spaced vessels and 'Eureka' lemon the most closely spaced vessels. In the outer part of the branch the vessels were significantly wider apart in 'Delta' Valencia than the other two species.

The homogeneity of the sapwood is not only dependent on the number of xylem vessels or the distances between the vessels, but also on the size of the vessels (Steppe and Lemeur, 2007). Lumen diameter was, therefore, also determined in the various citrus species in both stems and branches selected randomly from both MLW and FLW (Table 5.3). 'Bahianinha' navel tended to have wider vessels (114 µm) on average, whilst 'Nadorcott' mandarin tended to have narrower vessels (92 µm). Vessel lumen diameter was significantly greater in 'Bahianinha' navel than in 'Nadorcott' mandarin in the inner and outer part of the stem, but did not differ significantly for the different branches. Vessels in the outer part of the stem of the 'Star Ruby' grapefruit were also significantly larger than the vessels of the 'Nadorcott' mandarin, but in the inner part of the branch the vessels from 'Star Ruby' grapefruit were significantly smaller than those from 'Bahaininha' navel.

Table 5.3 Diameter of the xylem vessels in branches and stem of various citrus species. Values are means ± standard error

Species	Stem (µm)		Branch (µm)	
	Inner part	Outer part	Inner part	Outer part
Citrus sinensis 'Delta'	103.7±11.2^{ab}	112.8± 10.4^{ab}	93.37 ± 6.5^{ab}	94.8 ± 8.5^{a}
Citrus paradisi 'Star Ruby'	94.0 ± 4.3^{ab}	121.7 ± 7.7^{a}	89.33 ±8.4^{b}	109.0 ± 18.0^{a}
Citrus limon 'Eureka'	94.1 ± 3.2^{ab}	106.1 ± 1.6^{ab}	106.80 ±3.0^{ab}	116.7 ± 11.2^{a}
Citrus sinensis 'Bihaininah'	106.9 ± 6.6^{a}	128.6 ± 3.1^{a}	109.32 ±5.0^{a}	110.4 ± 3.5^{a}
Citrus reticulata 'Nadorcott'	83.9 ± 4.5^{b}	94.6 ± 2.7^{b}	92.36 ±2.6^{ab}	95.8 ± 1.7^{a}

In each column, values followed by the same letter are not significantly different at ($p < 0.05$)

5.2 Discussion and conclusions

The estimation of SFD, both gravimetrically and using SFD techniques, requires accurate estimation of the cross-sectional area of the xylem that actively transports water. In the laboratory calibration this was accomplished by adding a dye solution to the water in the calibration experiments to stain the conducting area. The distribution of conducting sapwood may explain the variation between stems, as probes may be placed inadvertently in non-conducting tissue or in areas where conductance is low. This has important implications for studies in whole trees, as it is almost impossible to determine whether the probes are inserted in non-conducting tissue. But attempts should be made at the end of field studies to make accurate estimates of the conducting tissues relative to probe placement. Nadezhdina et al. (2002) demonstrated that errors of between 90 and 300% result from the assumption of uniform flow at all sapwood depths. In the current study, the largest variation in conducting sapwood within a stem was found in the 'Nadorcott' mandarin samples. Only sapwood towards the outside of the stem was stained with Safranin O dye, whilst the rest of the sapwood

was not stained (Figure 4.12). This could have accounted for the low R^2 values for this species for both the HR and CHP methods when compared to the SFD determined gravimetrically. The solution to this problem when conducting field measurements could be to insert more sensors per stem to account for the greater variability. In addition, measurements should also focus on the outer sapwood where conductance is higher. A survey of the variability in conducting sapwood area using core samples should also be done prior to probe insertion in order to gain an understanding of this variability in each orchard. This is important as the underestimation of sap flow due to inactive xylem increases with SFD.

The reasons for the large areas of inactive xylem within some stems are not clear, but could be attributable to the propensity for greater xylem embolism in some species than others (Eilmann and Rigling, 2012). Paudel et al. (2013) also encountered the problem of continuous inactive xylem and attributed it to the fact that in hot and dry regions, climate extremes often challenge xylem integrity, and as a result inactive xylem is, therefore, more common in these regions than in other places. This is, however, typically a problem in species with large vessel diameters (Taneda and Sperry, 2008) and as 'Nadorcott' mandarin samples had smaller vessels than most of the other samples, embolism formation may not have been the major contributing factor.

Parameters required for the upscaling of HPV to SFDs and then T_{sap}, also influence the accuracy of measurements. The conversion of HPV to sap velocity is very sensitive to the m_c content and ρ_b (Swanson and Whitfield, 1981; Green et al., 2003; Steppe et al., 2010). Sapwood moisture content and ρ_b did not exhibit much variation in a wide range of citrus varieties from different locations. These values can therefore be used with confidence for the determination of T_{sap} in citrus trees and this is unlikely to be a major source of error in the calculation of SFD for the HR and CHP methods.

The arrangement and spacing of xylem vessels within the sapwood can also influence SFD measurements. Smith and Allen (1996) suggest that heat pulse techniques can be used in species with thermally homogeneous sapwood without the need for calibration. A thermally homogenous wood is described as having distances between vessels of less than 400 µm (Swanson, 1994). This is the maximum distance between vessels where the time taken for thermal equilibrium between sapwood and woody matrix is considered to be negligible. However, if this distance is exceeded, errors in HPV calculations are expected and calibration of SFD techniques for such species should be considered. In this study the distances between the xylem vessels exceeded 400 µm in both the inner and outer part of the MLW sapwood for the stems and branches of 'Delta' Valencia, 'Star Ruby' grapefruit, 'Eureka' lemon and 'Bahaininha' navel. As a result the sapwood of these cultivars can be described as thermally

inhomogeneous. The one exception was 'Nadorcott' mandarin, where the distance between the vessels was close to 400 µm. The sapwood of this cultivar could therefore be considered thermally homogeneous. However, the variation and underestimation of SFD observed in 'Nadorcott' mandarin in this study was due to the insertion of probe in non-conducting tissue, as mentioned previously. According to Green and Clothier (1988), the lack of thermal homogeneity in the sapwood affects the transmission and the measurements of the heat pulse and results in underestimations of SFD. Empirical correction factors are therefore required for these species, to match measured SFD to actual SFD.

Importantly, the distribution of vessels within the sapwood of the 'Delta' Valencia, 'Star Ruby' grapefruit and 'Eureka' lemon samples was not consistent and marked differences between MLW and FLW were observed. Differences between MLW and FLW were, however, not observed in samples from 'Bahianinha' navel and 'Nadorcott' mandarin, but the techniques did not perform any better in these branches. The distances between vessels in the FLW was completely different to distances measured in MLW, with distance between vessels of less than 400 µm (ranging from 127-337 µm), which means it did not depart from the definition of a homogenous, porous material. As a result heat is expected to move uniformly through this part of the sapwood and inserting probes into this part of the sapwood is therefore likely to result in accurate measurements, which do not require empirical adjustment. The non-uniform nature of the sapwood in some citrus species could potentially complicate the determination of a conservative calibration for citrus, as it will depend heavily on probe placement i.e. whether probes are placed in mature or FLW. Zreik et al. (2000) found considerable variation in calibration factors between citrus trees (CV 20%) and warned that the calibration of SFD determined by the CHP method cannot be very accurate due to the inhomogeneous nature of the xylem vessel distribution in this tree species. Similar variation in calibration factors between stems of the same species was found in this study. One of the contributing factors to this variation was most probably the large variation in sapwood structure between MLW and FLW. Steppe and Lemeur (2007) also noted that differences in wood anatomy between beech and oak trees caused differences in the calibrated values of the hydraulic parameters of the stem.

Underestimation of T by SFD techniques has also largely been attributed to the mechanical damage to the sapwood as a result of probe insertion and wounding as a result of intermittent heating (Swanson, 1994). Swanson and Whitfield (1981) and Burgess et al. (2001) have both derived numerical solutions which allow the estimation of the impact of wounding on the measured heat pulse velocities. Whilst, the determination of wounding can be difficult in whole trees and may even vary with time, in cut stems it is easier to determine by including a stain

in the perfusion solution. In addition, as calibration is complete within a few hours, the wound was not expected to develop and using the width of the probe (2 mm) was probably sufficient to account for wounding. Longer term calibration experiments are therefore recommended to account for the impact of wounding on estimates of SFD.

Finally, branches were used in this study as opposed to stems, as these could be collected from commercial orchards without felling the entire tree. Whilst previous studies have also used branches to calibrate SFD techniques (Cohen et al., 1981; Clearwater et al., 1999; Nadezhdina et al., 2007; Burgess and Dawson, 2008; Taneda and Sperry, 2008; Paudel et al., 2013), it should be noted that the sapwood anatomy differs between stems and branches. Compression or tension wood in branches may have contributed to the variability in sap flow observed in this study.

6 RESULTS – VALIDATING SAP FLUX DENSITY MEASUREMENT METHODS IN A GLASSHOUSE USING WEIGHING LYSIMETERS

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6.1 Weather variables

Weather variables were measured throughout the calibration periods and results are presented in Figure 6.1 for the period 9-15 November 2015. Air temperature and VPD followed a typical diurnal trend, with maximum values obtained around midday and lowest values observed at night. The extraction fan used to suck air through a wet wall, to cool the glasshouse, was switched off, because the air movement interfered with the lysimeter measurements. As a result air temperatures of up to 44°C and very low RH of up to 6% were observed and this resulted in high VPD values (8 kPa).

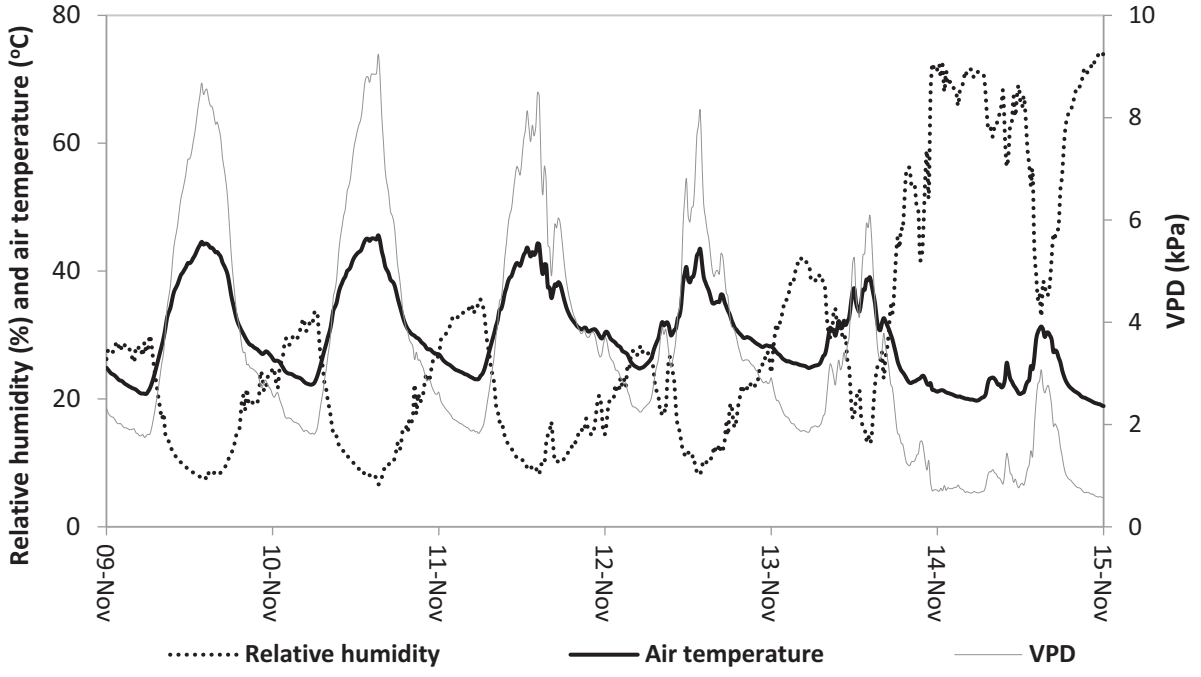

Figure 6.1 Hourly values of average air temperature (°C), relative humidity (%) and average VPD (kPa) for the period 9-15 November 2015

6.2 Testing the heat ratio method in *E. marginata*

6.2.1 Variations of sap flux density with depth in an *E. marginata* stem

The spatial distribution of the xylem vessels and variation in SFD within the sapwood conducting tissue was accounted for by installing the thermocouples of the HR method at various depths in the stem as described by Wullschleger and King (2000) and Poblete-Echeverría et al. (2012). Results from the two probe sets for the HR method at two installations depths (8 and 12 mm) over a four day period are presented in Figure 6.2.

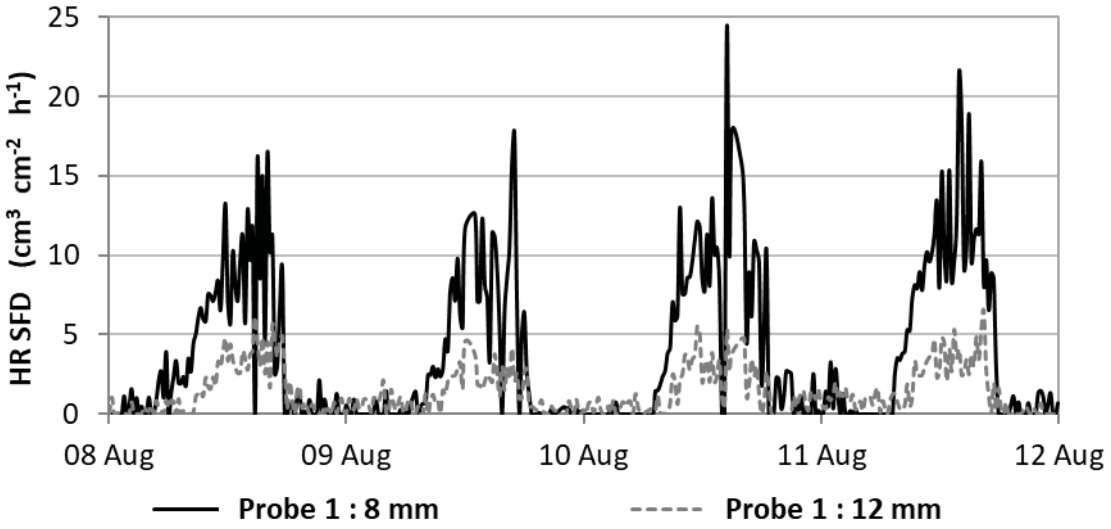

Figure 6.2 Hourly sap flux densities (SFD) of the heat ration (HR) method installed at 8 and 12 mm depth in *E. marginata*

Clear diurnal trends can be observed for both probe sets, with the lower SFDs recorded by the deeper probe set (Figure 6.2). The maximum SFD calculated for the calibration period was approximately 25 cm^3 cm^{-2} h^{-1} for the probes installed at 8 mm depth and 5 cm^3 cm^{-2} h^{-1} for the probe set installed at 12 mm depth. The behavior depicted by the SFDs in Figure 6.2 shows that xylem conductivity of the sap decreased with depth and that the peripheral part of the stem section was made up of very young and active xylem vessels, compared to the central part of the stem section (Fernández et al., 2001; Poblete-Echeverría et al., 2012).

6.2.2 Hourly sap flow

Hourly VSF was calculated for the stem and compared with the mass loss per hour measured gravimetrically using a weighing lysimeter (Figure 6.3). A clear diurnal trend in mass loss from the weighing lysimeter and T_{sap} (HR method) was observed (Figure 6.3 A). Hourly measurements with the HR method and the gravimetric method clearly shows diurnal patterns of transpiration with great sensitivity, including rapid early morning increases (07:00) in the

transpiration rate, midday depressions (10:00-14:00), afternoon recoveries, (16:00), evening decreases (17:00) and rates near to zero during the night (19:00-06:00).

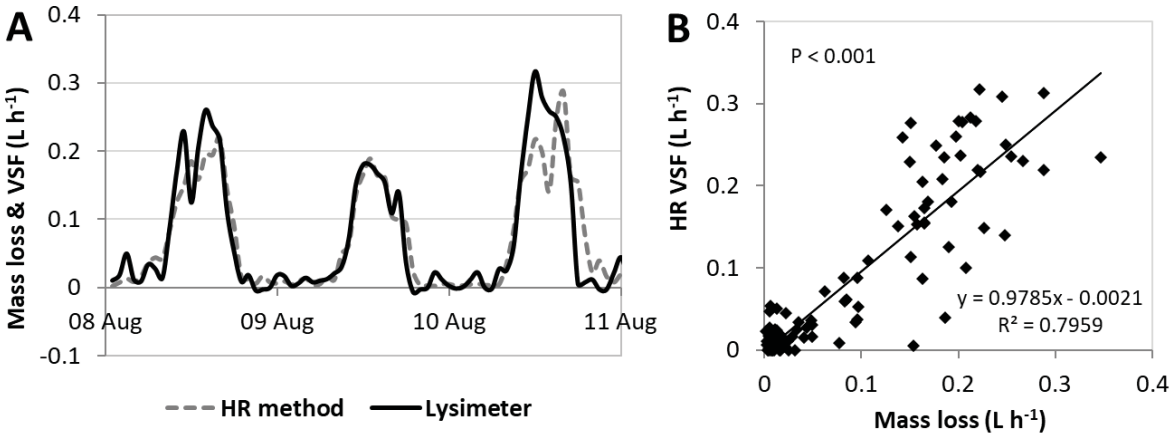

Figure 6.3 Diurnal trend for (A) hourly mass loss between the gravimetric method (lysimeter) and sap flow method (HR) on *E. marginata* and (B) relationship between hourly mass loss measured with a weighing lysimeter and HR method for *E. marginata*

Linear regression analysis of the HR and gravimetric method data showed a highly significant relationship (P value < 0.001) (Figure 6.3 B). Also, the t-test indicated that the intercept was not significantly different from zero and the slope was not different from unity at 99% level of significance.

6.2.3 Day time water use

Heat ratio method measurements corresponded very closely with gravimetric measurements with respect to day time whole-plant water use. Statistical analysis of the daily sap flow data indicated that T_{sap} was less than T determined with the gravimetric method with a mean biased error (MBE) of 0.04 L day^{-1}, root mean square error (RMSE) of 0.09 L day^{-1} and mean absolute error (MAE) of 0.04 L day^{-1} (Figure 6.4).

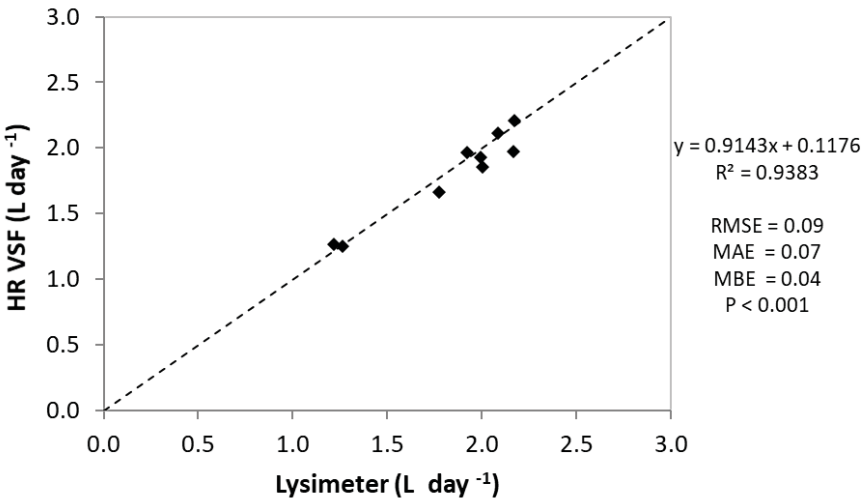

Figure 6.4 Comparison of daily mass loss of the weighing lysimeter to VSF determined by the heat ratio method (HR method) in *E. marginata* (dashed line is a 1:1 line)

The HR method tended to underestimate tree T by 1.9% on average per day, which was a very good result. The linear regression analysis indicated that the correlation (R^2 = 0.94) between the HR method and gravimetric estimates of daily whole-plant water use was highly significant (P value < 0.001) and very near to a 1:1 relationship (Figure 6.4). Also, the t-test indicated that the intercept was not significantly different from zero and the slope was not significantly different from unity (Appendix 1). These results are consistent with similar validations involving palm frond (Madurapperuma et al., 2009) and potted Eucalyptus trees (Bleby et al., 2004).

6.3 Validating sap flux density measurement methods in 'Midknight' Valencia (*Citrus sinensis* L. Osbeck)

6.3.1 Heat ratio method

Similar diurnal trends in the SFD to that of *E. marginata* were observed in 'Midknight' Valencia trees (Figure 6.5). Sap flux densities measured at three depths (8, 12 and 15 mm) over a period of four days are characterised by early morning increases, midday depressions and virtually zero flows at night.

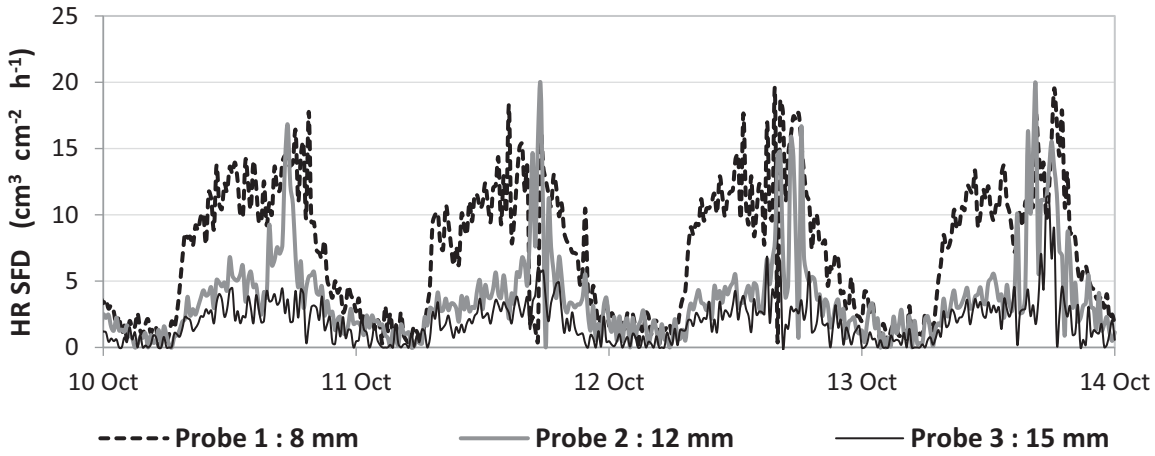

Figure 6.5 Corrected sap flux densities (SFD) measured at 8, 12 and 15 mm depths for a potted 'Midknight' Valencia tree

Greater SFDs were measured by the shallower probe (8 mm) than the deeper probe (15 mm) (Figure 6.5). These values indicate that xylem conductivity decreased with depth and the xylem closer to the surface of the trunk was very young and active compared with the xylem that are located deeper from the trunk surface (Poblete-Echeverría et al., 2012).

6.3.2 Hourly sap flow

Hourly VSF was calculated from the SFD measurements at three different depths and was compared to mass loss per hour from the weighing lysimeters. Both 'Midknight' Valencia trees showed a typical bell-shaped diurnal pattern as observed in Figure 6.6 A and C.

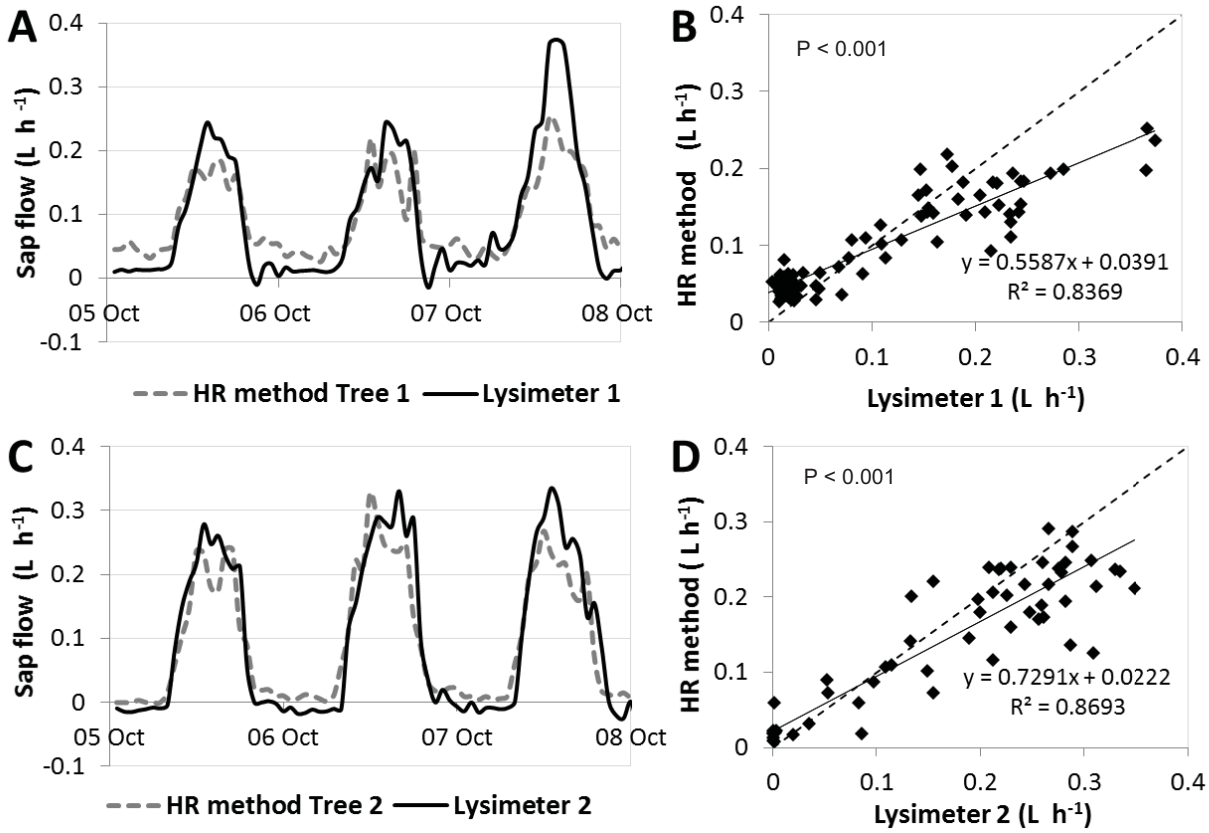

Figure 6.6 Hourly transpiration of 'Midknight' Valencia measured with the heat ratio method (HR) and the gravimetric method for (A) lysimeter 1 and (C) lysimeter 2. The relationship between hourly water use measured by the HR and gravimetric methods is shown for (B) lysimeter 1 and (D) lysimeter 2

Likewise, at an hourly time scale, the HR method measured diurnal patterns of sap flow with great sensitivity. The HR method overestimated T at low flows and underestimated T at high flow rates for both 'Midknight' Valencia trees (Figure 6.6 B and D). These results are similar to the findings of Barrett et al. (1995), who observed an overestimation of sap flow at low flows and an underestimation at high flow rates for eucalyptus. Regression analysis showed that the correlation between the HR and gravimetric methods for the hourly measurements was highly significant (P value < 0.001) for both trees. Approximately 84% (lysimeter 1) and 89% (lysimeter 2) of the variation in the lower HR measurements can be attributed to the linear regression relationship.

6.3.3 Night flows

During the night the weighing lysimeter recorded virtually zero flows starting around 20:00, while sap flow was still recorded by the HR method (Figure 6.6 A and C). The discrepancy between the two methods can be explained as follows: during the night the plant refills some

of the water that was lost during the day (Dawson et al., 2007), nearly no mass loss is recorded on the weighing lysimeter during this time since a large portion of water that is drawn from the soil is a result of a lower water potential in the plant created during the day (Fisher et al., 2007). As the stomata are typically closed during the night, this water is not lost to the atmosphere through T and therefore not registered by the lysimeter.

6.3.4 Day time water use

The HR method measurements corresponded closely with gravimetric measurements with respect to day time whole-plant water use. The comparison between day time tree T obtained from the gravimetric method and that obtained by the HR method (T_{sap}) is given in Figure 6.7. Overall values of RMSE and MAE were 0.10 and 0.09 (L day^{-1}) respectively. T_{sap} was 0.02 L day^{-1} (from MBE calculation) lower than the water loss measured with the weighing lysimeter. The HR method therefore tended to underestimate tree T by 1.08% on a day time water use basis. The linear regression analysis showed that the relationship between the day time T measured with the HR method and the day time water loss measured with the gravimetric was highly significant. This is indicated by a P value of less than 0.001, a R^2 of 0.98 and an almost 1:1 relationship (Figure 6.7). According to the t-test, intercept was not significantly different from zero and the slope was not significantly different from unity (Appendix 2).

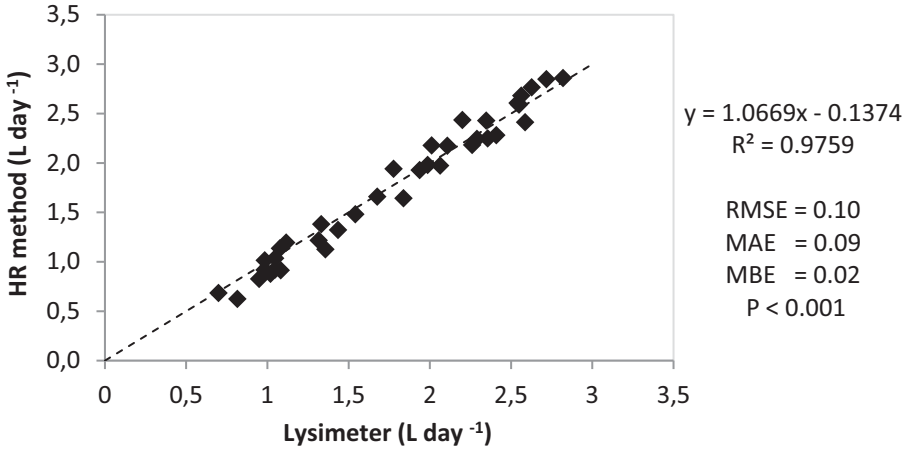

Figure 6.7 The relationship between total day time water use of a potted 'Midknight' Valencia tree, as determined with a weighing lysimeter and heat ratio method over 37 days. Total day time transpiration was calculated from the sum of hourly rates between 06:00 and 18:00

This was an exceptional result considering that the validations are associated with a number of possible sources of error, which includes the likelihood of E and condensation underneath the plastic covering the pots and additive errors from determination of sapwood area, wounding, wood density, etc. as mentioned for *E. marginata*. These results are consistent

with other similar validation experiments involving palm frond, where Madurapperuma et al. (2009) clearly demonstrated that the HR method provided accurate T measurements compared with results from a gravimetric method (R^2 = 0.92, slope of 1.01 and intercept of 0.04). Similarly Bleby et al. (2004) showed that there were no significant differences between the HR method and the gravimetric method when these authors assessed the HR method in potted eucalyptus trees (R^2 = 0.97, slope of 0.95 and intercept of 0.02).

6.3.5 Sap flow under water stress conditions

The comparison between sap flow measurements with the HR and gravimetric methods for a 'Midknight' Valencia tree experiencing water stress is given in Figure 6.8. Irrigation was then applied during the night of 2-3 and 4-5 September (Figure 6.8). Soon after irrigation water started to drain from the container and a sharp increase in sap flow was recorded at midnight by the HR method, but no mass loss was measured with the lysimeter. Thus, water moved from the irrigated soil into the tree, to rehydrate the plant tissues following water stressed conditions, but no T occurred. This spontaneous water movement into the tree can be explained by the large water potential difference between the inside of the tree (very low) and the irrigated soil (high) (Oren et al., 1999). Repair of embolized xylem conduits formed during water stress could also contribute to the increase in sap flow at night following irrigation (Zwieniecki and Holbrook, 2009). A clear lag between sap flow and T measured with the lysimeter is evident on 6 September (Figure 6.8). In the early morning hours water stored in the upper parts of the tree, such as the leaves, branches and stem is lost to the atmosphere, registering a mass loss with the lysimeter before sap flow is recorded with the HR equipment installed on the stem close to the soil surface. At the end of the day after the cessation of T (closing of stomata), no mass loss registered with the lysimeter, but the sap continues to flow (recorded with HR method) in order to rehydrate the plant tissues (Coelho et al., 2012).

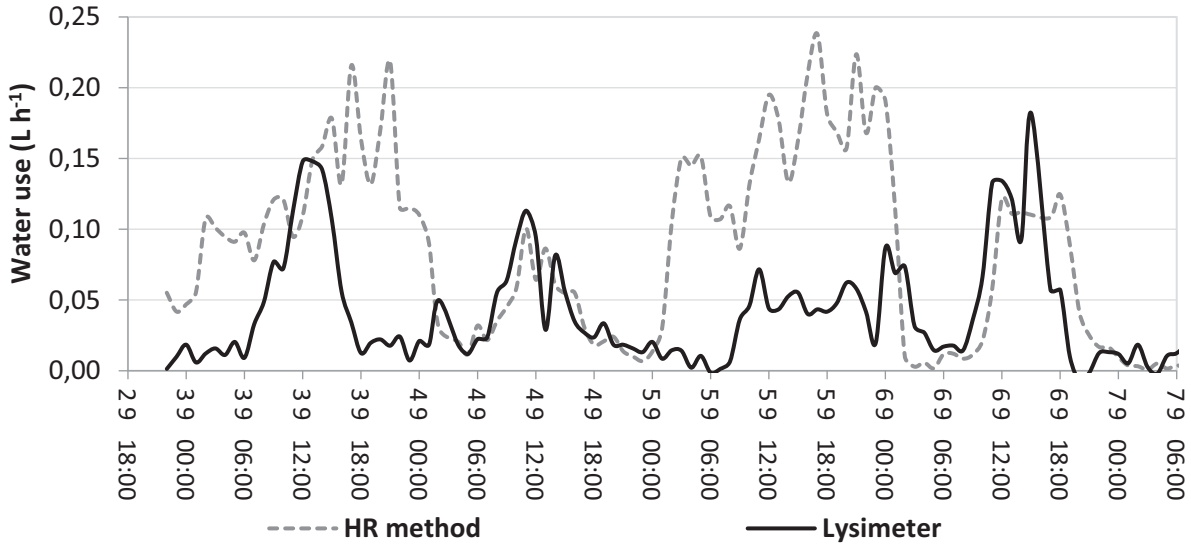

Figure 6.8 Comparison of hourly water use of citrus determined with heat ratio method and gravimetrically on irrigated and non-irrigated days

6.4 Compensation heat pulse method

The CHP method is not able to resolve low and zero flow rates (0-5 cm^3 cm^{-2} h^{-1}) (Becker, 1998; Burgess et al., 2001) and it was confirmed with our study that the sensors could not resolve SFDs of approximately 5 cm^3 cm^{-2} h^{-1} or less. In Figure 6.9 SFDs for four selected days are presented. Clear diurnal trends were recorded for the different measuring depths, with the highest SFDs of approximately 15 cm^3 cm^{-2} h^{-1} measured at a probe depth of 12 mm. The lowest SFD was measured with the probe inserted 15 mm into the sapwood (Figure 6.9).

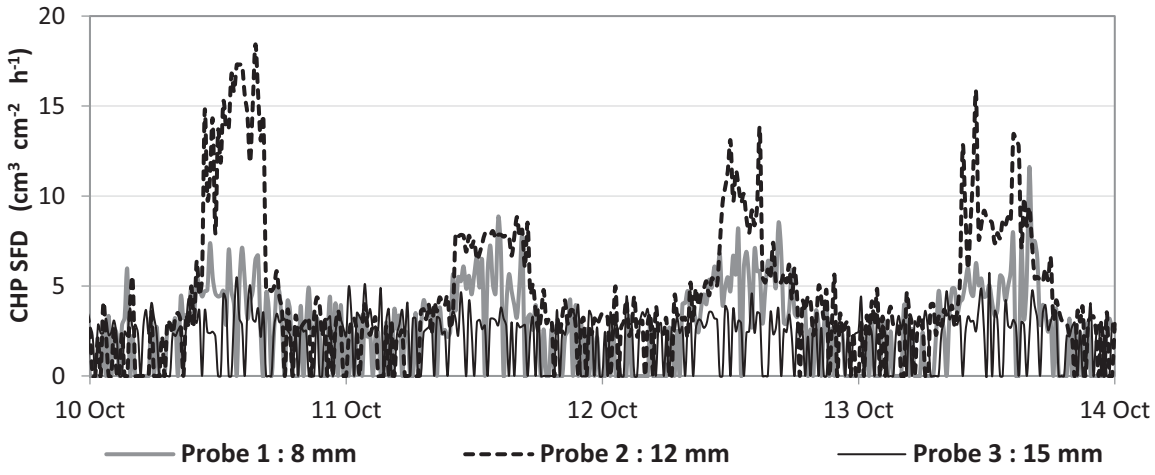

Figure 6.9 Sap flux densities (SFDs) measured using the compensation heat pulse (CHP) method at 8, 12 and 15 mm depths for a potted 'Midknight' Valencia tree

6.4.1 Hourly flows

A comparison between hourly T from the CHP method and gravimetric method was conducted for two different calibration windows, 27-30 April 2015 and 9-12 September 2015. A good and highly significant (P value < 0.001) correlation (R^2 = 0.83 and R^2 = 0.73) was found between the hourly T_{sap} determined with the CHP method and T determined gravimetrically (Figure 6.10). However, at low flow rates (< 0.15 L h^{-1} for tree 1) T was overestimated and at high flow rates (> 0.15 L h^{-1} for tree 1) T was underestimated by the CHP method. A lag between sap flow and mass loss measured with the weighing lysimeter was observed towards the end of the day (Figure 6.10 A and C), because of capacitance within the tree (discussed in Section 6.3.5).

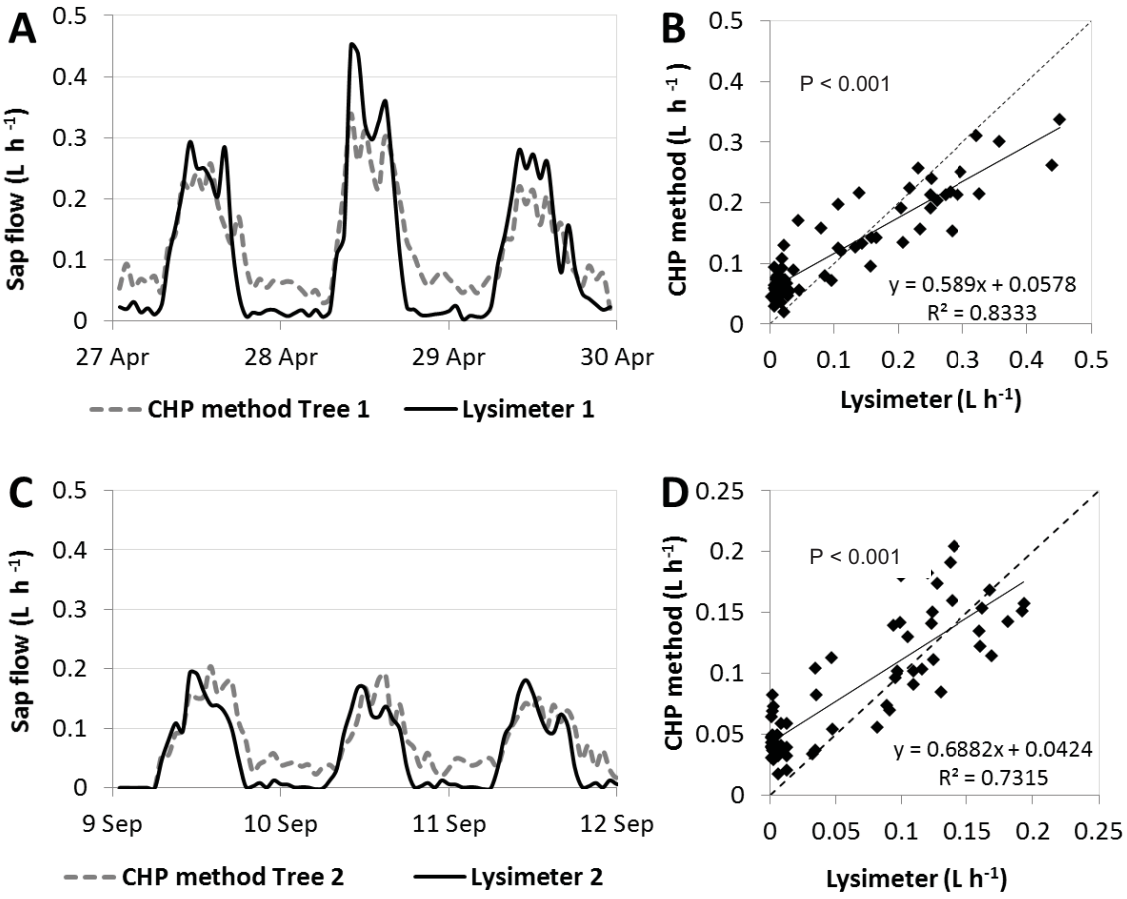

Figure 6.10 Hourly transpiration of 'Midknight' Valencia measured with the compensation heat pulse method (CHP) and the gravimetric method for (A) lysimeter 1 and (C) lysimeter 2. The relationship between hourly water use measured by the CHP and gravimetric methods is shown for (B) lysimeter 1 and (D) lysimeter 2

6.4.2 Day time water use

When day time (06:00-18:00) T_{sap} measured with the CHP method was compared with the day time mass loss determined with the lysimeters, a highly significant (P value < 0.001) linear relationship (R^2 = 0.91) was obtained with a slope of unity and an intercept of zero (Figure 6.11). Statistical analyses (RMSE of 0.1 and MAE of 0.08 L day^{-1}) revealed that T_{sap} was 0.02 L day^{-1} (from MBE calculation) higher than the mass loss measured with a weighing lysimeter. The CHP method, therefore, slightly overestimated transpiration by 1.23% day^{-1}, which is a credible result. The slight overestimation which was observed with the CHP method could be a result of errors incurred when determining the heartwood radius (an overestimation in the heartwood radius could result in overestimation of the T_{sap}).

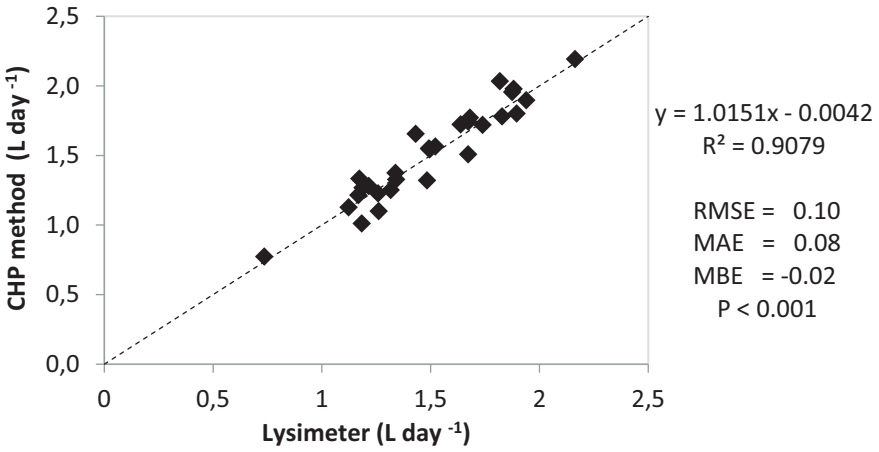

Figure 6.11 The relationship between day time (06:00-18:00) transpiration measured with a weighing lysimeter and transpiration measured with the CHP method for a 'Midknight' Valencia tree over a period of 30 days

6.4.3 Error analysis of heat ratio and compensation heat pulse methods

An error analysis (Figure 6.12) with ρ_b, m_c, heartwood radius and the wound correction was conducted to evaluate the influence of these parameters on SFD calculations. Results from this analysis indicated a linear relationship between error in ρ_b and m_c, used to calculate daily sap flow (%) in both HR and the CHP methods and the resultant error (%) in daily sap flow, whilst heartwood radius and wound correction factor, yielded a polynomial (order 2) relationship (Figure 6.12). Since these two SFD measurement techniques (HR method and CHP method) are based on the same principle, Figure 6.12 A and B shows that the determination of sap flow by the CHP method can be affected by the same magnitude of error when compared to the HR method, as a 10% overestimation in the wound width correction resulted in an 8.2% overestimation of sap flow, whilst a 10% underestimation of the wound correction also resulted in an 8.3% underestimation of sap flow for the CHP methods. In

general, an overestimation or underestimation of the m_c fraction and ρ_b by the same percentage resulted in approximately the same magnitude of error, as indicated by a positive direct linear relationship for both SFD methods (HR method and CHP method). These results are similar to what Bleby et al. (2004) observed when similar error analyses were conducted on *E. marginata*. Bleby et al. (2004) observed that a 10% overestimation in wound correction resulted in a 6.8% overestimation of sap flow, whereas a 10% underestimation of wound correction resulted in a 5.8% underestimation of sap flow. The error as a result of over- or underestimation of the wound correction was therefore very similar. The most sensitive parameter for both methods proved to be ρ_b as indicated by a slope of 1. This is consistent with the findings of Steppe et al. (2010), although the most sensitive parameter which they recorded was wood fresh mass, which is directly related to wood density. From these findings it is clear that there are many possible sources of error, from base line adjustment, data patching, and determination of wound width, wood moisture content, wood density and scaling up to whole tree water use. Therefore, preventive measures have to be taken into account when quantifying these parameters in order to ensure accurate estimates of T. These measures can include the use of many replications when determining a certain parameter and weighing the fresh wood mass as soon as possible after sampling. Other parameters not tested here which can cause erroneous results include bark thickness and proper selection of insertion depths.

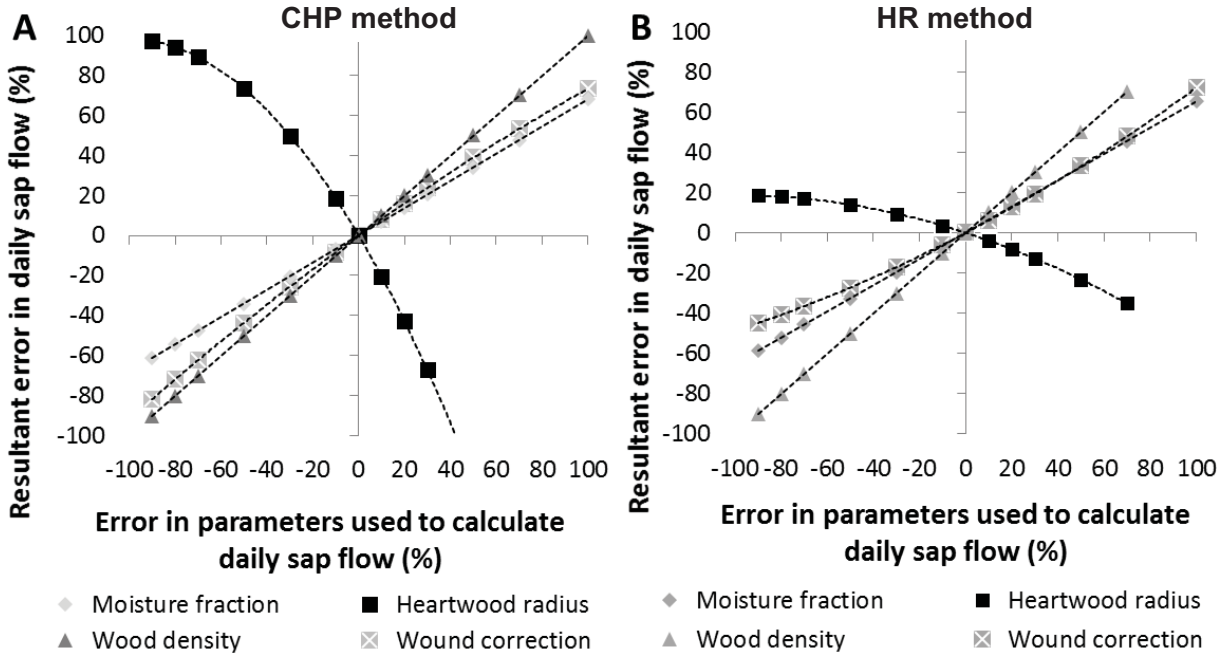

Figure 6.12 The resultant error in daily sap flow from the (A) compensation heat pulse (CHP) method and (B) heat ratio (HR) method due to errors in selected variables used in the determination of sap flow

6.5 Sap flow quantification in the scion versus the rootstock for potted 'Midknight' Valencias

In the initial experiment the probes of the various SFD techniques were installed in the scion, which resulted in poor correlations between the SFD technique and the weighing lysimeters. When the probes were installed in the rootstocks, the quality of the data improved and good correlations between T_{sap} measured with the SFD techniques and mass loss measured with the weighing lysimeters were found. The main reason behind the poor data quality was attributed to the very narrow band of actively transporting xylem near the cambium in the scion and in many instances it was likely that only one of the sap flow sensors was recording in this band. In the rootstock, however, the coloured area was much wider and this improved the chance of accurately capturing the correct sap velocities, as shown in Figure 6.13 A and B.

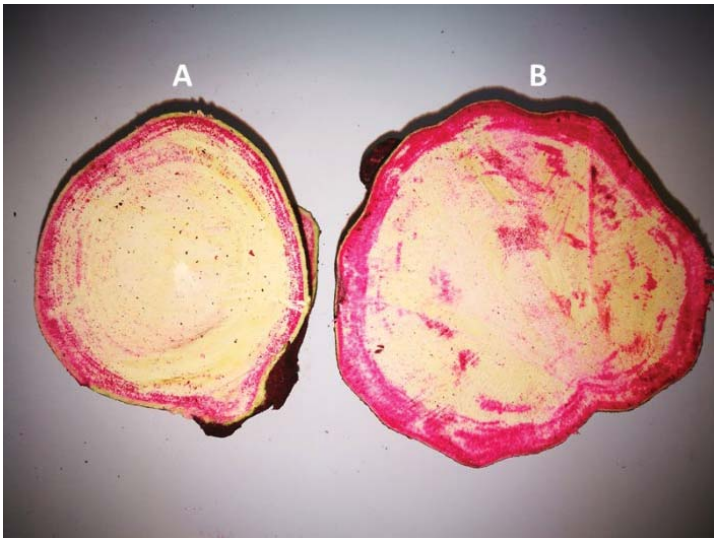

Figure 6.13 Active xylem pink coloured with Safranin O dye in the (A) scion and in the (B) rootstock of the same 'Midknight' Valencia tree

This observation was similar to what Olmstead et al. (2006) reported in sweet cherries, as these authors noted significant reduction in xylem vessel frequency from the rootstock to the graft union, with a further decrease in scion tissues. A further microscopic study revealed that the sapwood of the scion contained many more vessels differing in size (Figure 6.14 A). In the rootstock, active xylem vessels had a smaller diameter and were more evenly distributed (Figure 6.14 B), a feature which is favourable for the heat pulse techniques. Since the HPV calculated from the stem is a point estimate, there are higher chances of inserting the probes into the non-conducting part of the stem in the scion than the rootstock.

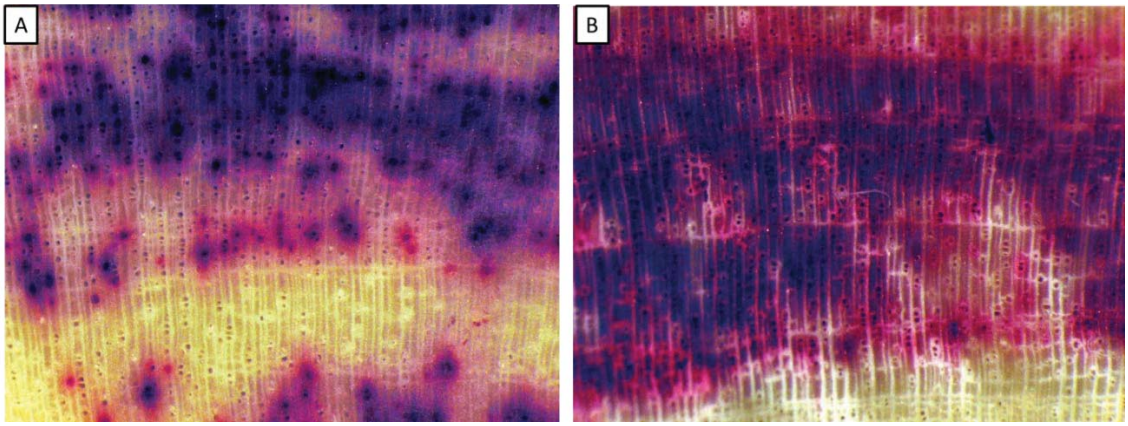

Figure 6.14 Active xylem (pink staining) in the (A) scion and in the (B) rootstock of the same 'Midknight' Valencia tree

6.6 Conclusions

The HPV equipment and methods were tested successfully in *E. marginata,* and therefore it was decided to proceed with the validation of the HPV techniques in *Citrus sinensis*. Results from the validation experiments showed that the HR method gave the most reliable and accurate results (R^2 = 0.98), underestimating transpiration by 1.08% day^{-1}. On the other hand, the CHP method was also satisfactorily accurate, as indicated by an R^2 value of 0.91 and an overestimation of daily tree transpiration by an average of 1.23% day^{-1}. These results suggest that both the CHP and HR methods were equally accurate in estimating T in a tree with a small canopy. There was also no need for calibration in order to achieve a 1:1 relationship between the sap flow method and the weighing lysimeter. However, patches of missing data were frequently observed with the CHP method due to its limitation in resolving low flow rates of less than 0.05 L h^{-1}. This poses a challenge in the quantification of tree water use over a long time period. In contrast, the HR method did not record any missing data and the nearly perfect agreement between the HR method and the gravimetric method on a daily basis suggested that this method can be used for measuring T for long periods of time.

7 RESULTS – INFIELD VALIDATION OF THE SAP FLUX DENSITY MEASUREMENTS WITH EDDY COVARIANCE

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7.1 Weather variables

In Figure 7.1 weather data for the period 10-14 March are presented. T_a, solar radiation and VPD followed a typical diurnal trend, with maximum values obtained around midday and lowest values observed at night. The average daily temperature for this period was 23°C. Only one rainfall event of 0.8 mm rain was recorded, which fell on 14 March. Daily VPD ranged from 0.8-4.7 kPa, with an average of 1.6 kPa. The highest average air temperature recorded for the calibration window (10-14 March), was 36 °C at 14:00 on 14 of March (close to solar noon in Citrusdal), which coincided with maximum VPD (4.7 kPa).

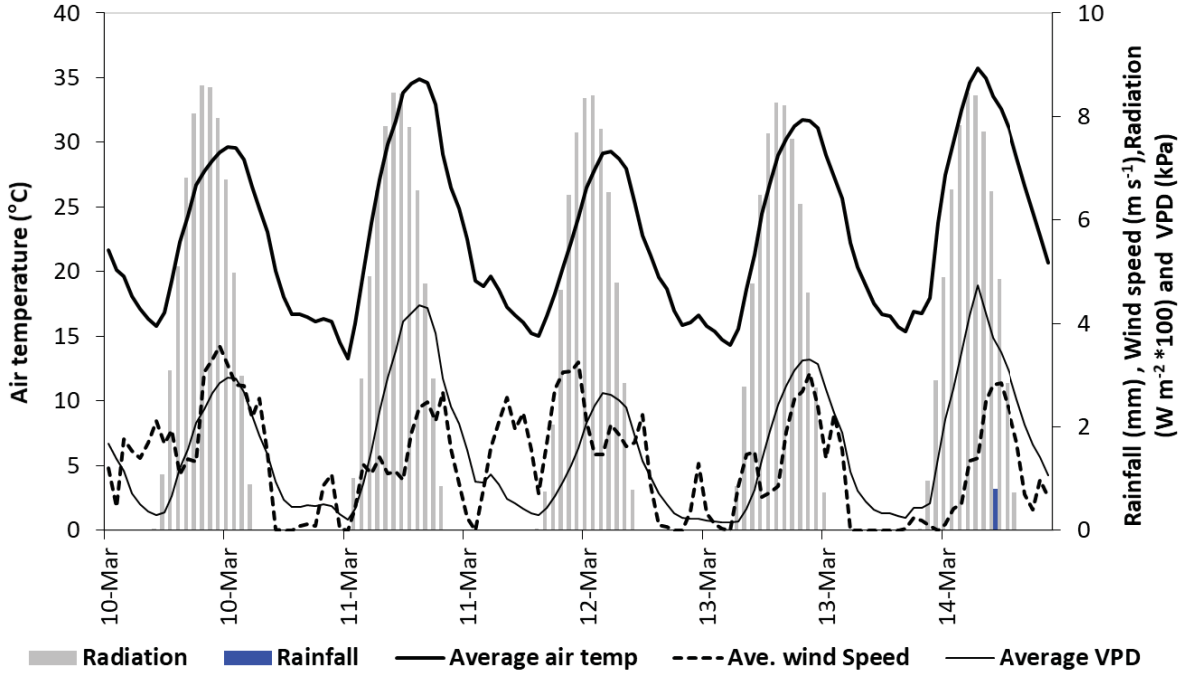

Figure 7.1 Hourly values of air temperature (°C), solar radiation (W m^{-2} x 100), rainfall (mm), wind speed (m s^{-1}) and VPD (kPa) at Patrysberg close to Citrusdal from 10-14 March 2015

7.2 Evaporative water loss from the soil

The data presented in Figure 7.2 are an example of the cumulative E_s determined for different areas in a 9-year-old 'Washington' navel orchard. Measurements were conducted during day time, at least every two hours from 10-14 March 2015. Most of the E_s occurred from the wetted areas on the west side of the trees, which received significantly more solar radiation during the hottest part of the day. Generally E is a two stage process, the first phase is energy limited (Boulet et al., 2004). This is evident in Figure 7.2 on the wetted shaded and sun exposed surfaces, where E increased from morning until solar noon (14:00) as the quantity of available energy increased. The second phase is a water limited phase, which is evident from the wetted sun area for the period 14:00-16:00, where limited water loss (E_s) was recorded relative to the wetted areas in the shade and sun exposed areas at 14:00 and 16:00. From 16:00 till 07:00 the following day virtually no E was observed, as there was insufficient energy during the night to drive E.

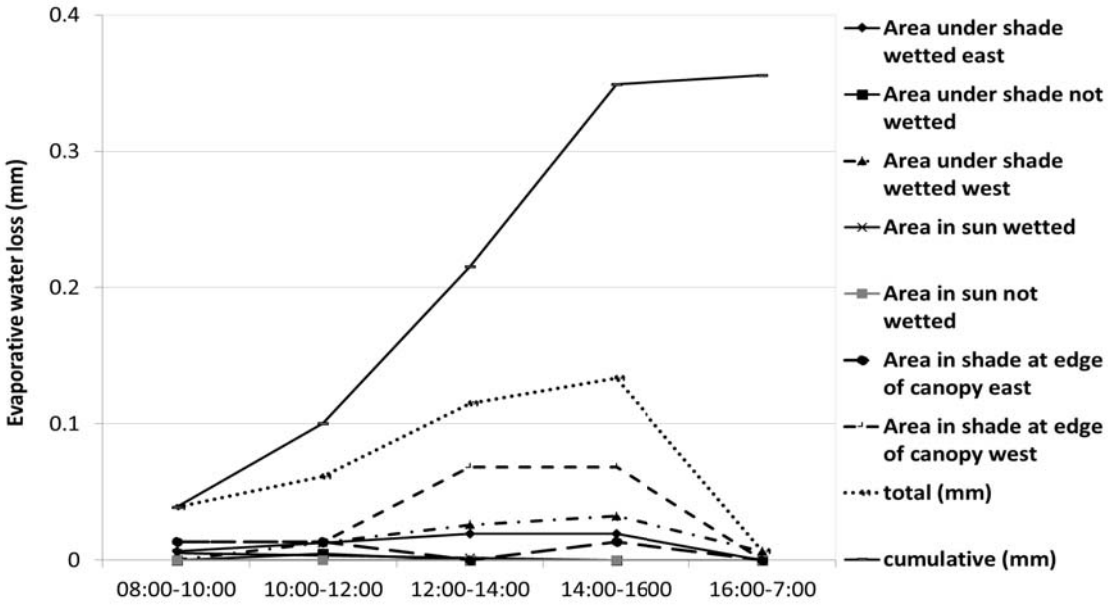

Figure 7.2 Cumulative soil evaporation in a 9-year-old 'Washington' navel orchard measured on 10 March

Daily total E_s and ET for the 9-year-old 'Washington' navel orchard for the period of 10-14 March 2015 is presented in Figure 7.3. The average daily E_s over the measuring period was 0.51 mm day^{-1} and the daily average for the ET measurements was 1.51 mm day^{-1}. Thus, E_s represented approximately 34% of ET. The day when the maximum values were measured for E_s (0.68 mm day^{-1}) and ET (1.75 mm day^{-1}), during the calibration period, coincided with the day when the highest temperature was recorded (14 March). The lowest E_s, ET, T_a and VPD were recorded on 10 March (Figure 7.3).

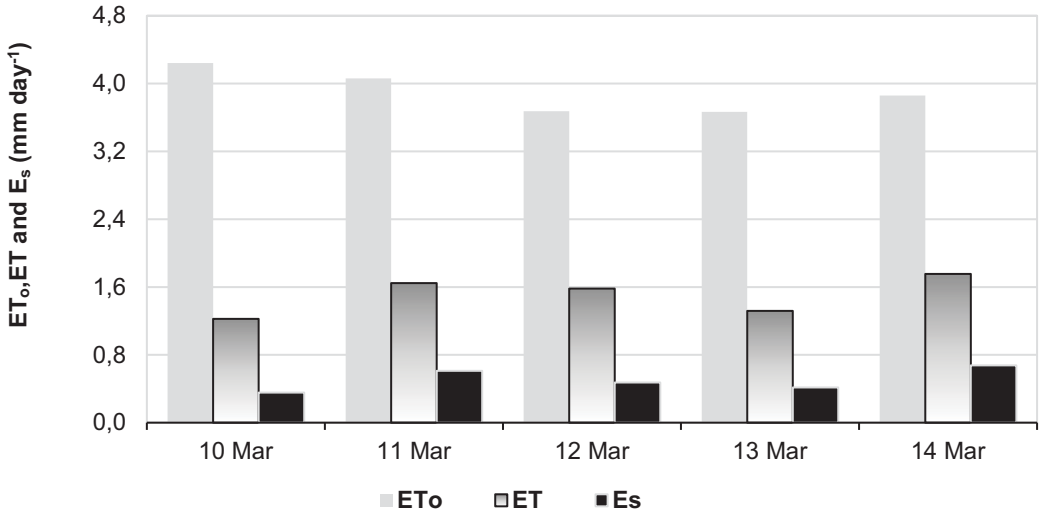

Figure 7.3 Total daily reference evapotranspiration (ET_o), soil evaporation (E_s) and evapotranspiration (ET) in mm day^{-1} is illustrated for a 'Washington' navel orchard for the period 10-14 March 2015

7.3 Hourly measured sap flux densities using the heat ratio method

Figure 7.4 the SFDs calculated with a 2.5 mm wound correction factor are given for each probe depth (8, 13, 22 and 30 mm). Clear diurnal trends were recorded for all probe sets (Figure 7.4), with SFDs characterised by an early morning increase (07:00-08:00), midday depressions (12:00-16:00), afternoon recoveries (16:00-18:00) and nearly zero flows at night (20:00-06:00). The variation in the SFD measurements, at different depths (8, 13, 22 and 30 mm), illustrates the variability in the conducting sapwood tissue. Sap flux densities of the same order (15-20 cm^3 cm^{-2} h^{-1}) were measured in probe sets 1, 2 and 3, with probe 3 often exhibiting the highest values, suggesting little variation in the distribution of active xylem vessels across this stem section i.e. 0-25 mm from the cambium. SFDs of approximately 5 cm^3 cm^{-2} h^{-1} was measured at probe set 4, which was installed at a 30 mm depth. SFD therefore decreased towards the centre of the stem, as found for a number of species (Poblete-Echeverría et al., 2012).

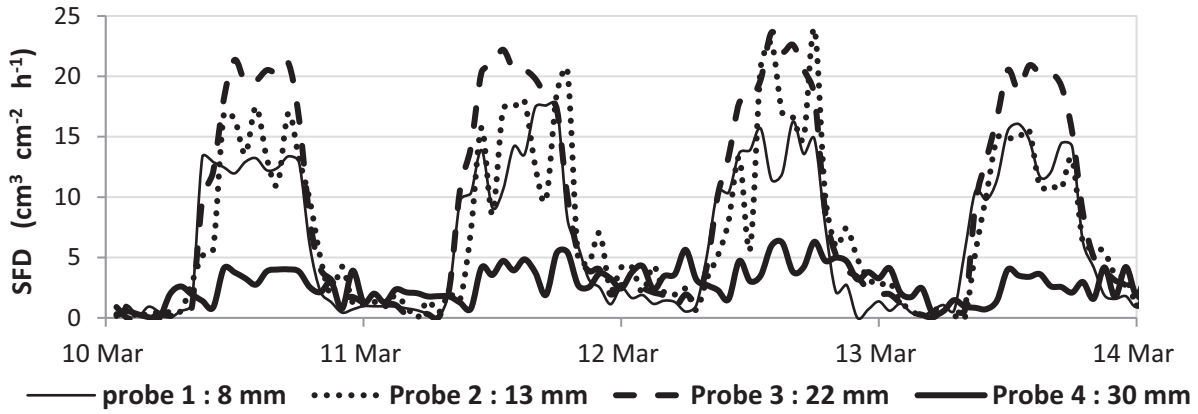

Figure 7.4 Sap flux densities (SFD) measured with the heat ratio method in a 'Washington' navel tree, with a wound correction factor of 2.5 mm at 8, 13, 22 and 30 mm depths

7.4 Hourly measured sap flux densities using the compensation heat pulse method

A bell-shaped diurnal trend was observed for all four probe sets of the CHP method (Figure 7.5). As with the HR method, SFD was characterised by an early morning increase (08:00), fluctuations in the middle of the day (10:00-16:00), a sharp decline in the evening (17:00-18:00) and nearly zero flows at night (21:00-06:00). On average maximum SFDs of approximately 25 cm^3 cm^{-2} h^{-1} were registered by the sensors at 10, 15 and 25 mm depths, also suggesting little variation in the xylem distribution across the stem section from 0-30 mm depth (Figure 7.5). SFDs of less than 5 cm^3 cm^{-2} h^{-1} were registered for probe set 4, suggesting lower conductivity of sapwood in that region. The lower conductivity can be due to a number of reasons, such as the vessels are older and potentially blocked with gum, thereby rendering them inactive (Poblete-Echeverría et al., 2012).

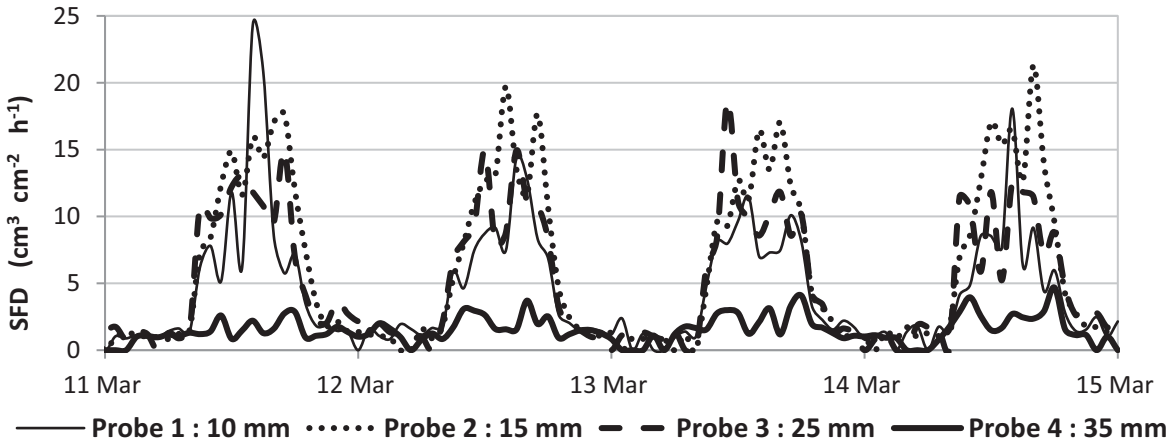

Figure 7.5 Sap flux densities (SFD) measured with the compensation heat pulse method in a 'Washington' navel tree, with a wound correction factor of 2.5 mm at 10, 15, 25 and 35 mm depths

7.5 Comparison of daily reference evapotranspiration, evapotranspiration and transpiration from the heat ratio and compensation heat pulse methods

Hourly T_{sap} (HR and CHP method), ET estimates with an EC system and ET_o, from a AWS, are shown for the period 10-14 March in Figure 7.6. A bell-shaped diurnal trend was evident for T_{sap}, ET, and ET_o mesurements. As observed by González-Altozano et al. (2008), sap flow rates increased exponentially early in the mornings, from approximately 06:00 to a maximum at about 14:00 and then a decrease to a minimum at 21:00. The maximum sap flow rates (0.05 mm h^{-1} for the HR method and 0.1 mm h^{-1} for CHP method) recorded at 14:00 coincided with the maximum ET_o (Figure 7.6).

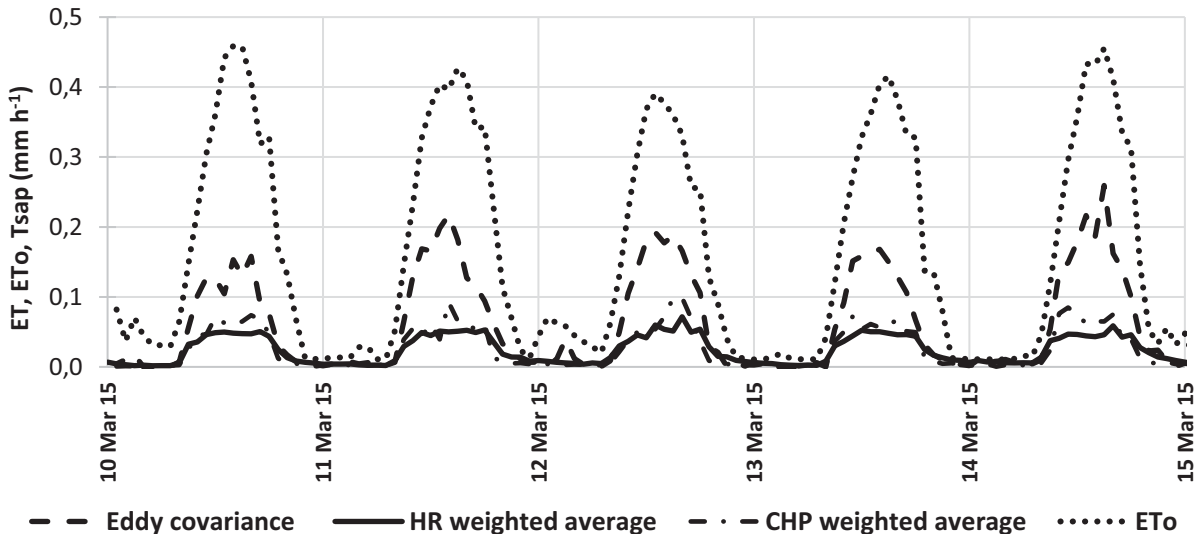

Figure 7.6 Comparison of hourly evapotranspiration (mm h^{-1}) from an eddy covariance system and transpiration (T_{sap}) measured with the heat ratio and compensation heat pulse methods, using a wound width of 2.5 mm, and reference evapotranspiration (ET_o) from an automatic weather station for a 'Washington' navel orchard for the period 10-14 Mar 2015

7.6 Comparison of daily sap flow versus residual transpiration

Transpirational sap flow determined with the HR and CHP methods were compared to transpiration (T_{res}) (Figure 7.7 A and B) determined as a residual of ET and E_s, assuming negligible transpiration from cover crops (clean orchard). When a wound correction factor of 2.5 mm (width of the widest probe) was used the HR and CHP method yielded similar results, with both methods underestimating transpiration by a similar magnitude. The T_{sap} measured with the HR method was 42% lower than T_{res}, whilst T_{sap} measured with the CHP method was 36% lower than T_{res} (Figure 7.7 A). When a comparison was made between the two methods of T estimation, a highly significant linear relationship between T_{sap} determined by HR method and T_{res} was observed (P value < 0.01, R^2 = 0.97, RMSE = 0.008 mm day^{-1}, MAE = 0.42 mm day^{-1} and MBE = -0.42 mm day^{-1}), whilst a moderately strong significant linear correlation was observed between T_{sap} from the CHP method and T_{res} (P value < 0.5, R^2 = 0.80, RMSE = 0.01 mm day^{-1}, MAE = 0.36 mm day^{-1} and MBE = -0.36 mm day^{-1}).

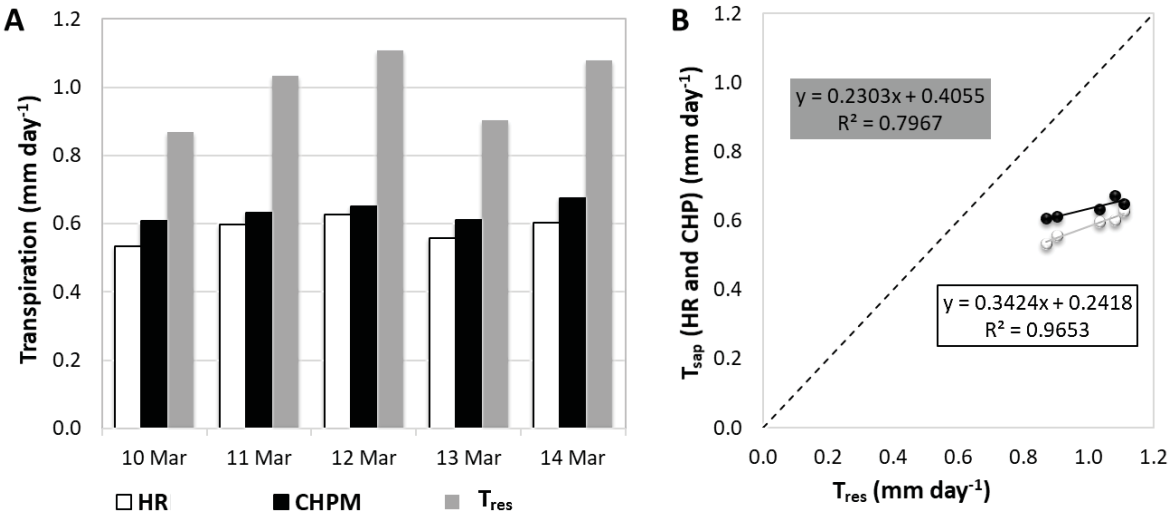

Figure 7.7 (A) Transpiration (T_{sap}) determined with the heat ratio (HR) and compensation heat pulse (CHP) method and daily transpiration (T_{res}) taken as the residual between evapotranspiration (measured with an eddy covariance system) and evaporation (E_s) measured with microlysimeters. (B) Correlation between T_{res} and T_{sap} in a 'Washington' navel orchard. The 1:1 line is given by the black dashed line

The large underestimations observed were mainly attributed to the underestimation of wound width. Actual measurements of the wound width showed that the wound can extend up to 7.25 mm in the 'Washington' navels. Although there are a number of sources of potential error associated with this kind of calibration, many of the errors (upscaling E measurements and EC data) can be minimised through applying the correct methodology. However, of all the parameters required to calculate SFD, (i.e. wound width, ρ_b, m_c, sapwood depth and heartwood radius), wound width is probably the most difficult parameter to determine *in situ*. This is because wound width measurements can only be done at the end of the experiment and appears to vary between trees and probes and along the length of the probe. The measurement of the wound width in the 'Washington' navel orchard varied between 3.18 mm and 7.25 mm (CV of 37%). This was also observed in 'Midknight' Valencia (CV of 43%) and 'Afourer' mandarin (CV of 9%) orchards where similar measurements were performed at the same location. The variation in wound width among different trees and probe sets is not easily explained, but could possibly be related to the amount of drilling required to install each probe set, as the deeper probes tended to be associated with a greater amount of wounding. Therefore, SFD methods should be calibrated against an independent measure of transpiration in the field.

7.7 Comparison of the calibrated daily sap flow to transpiration

As a result of the underestimation of T_{res} by the two SFD measurement methods, it was deemed necessary to calibrate the techniques. Calibration was performed by determining the virtual wound width. The average virtual wound width which resulted in acceptable agreement between daily T_{sap} and T_{res} measurements was 3.6 mm for the CHP method and 4.4 mm for the HR method (Table 7.1). The two different virtual wound widths obtained for the HR and the CHP method could reflect the different numerical solutions used to account for the wounding for each method and the fact that the CHP method was installed four months after the HR method. The coefficients of the linear regression equations (a, b and c) used to determine virtual wound widths daily, as well as the virtual wound widths are presented in Table 7.1. Table 7.2 shows the results of the statistical analysis of the 12 wound sizes chosen in this study, as performed by Poblete-Echeverría et al. (2012). Daily T_{sap} (mm day^{-1}) values obtained using different wound sizes were compared with the values of T_{res} (mm day^{-1}). The T_{sap} obtained using a wound width of 2 mm resulted in a 46% underestimation of T_{res}, a RMSE value of 0.01 mm day^{-1} and a MAE of 0.46 mm day^{-1} for the CHP method and a 50% underestimation of T_{res}, a RMSE value of 0.006 mm day^{-1} and a MAE of 0.5 mm day^{-1} for the HR method. On the other extreme, when T_{sap} was calculated using a wound size of 5.0 mm, it resulted in an overestimation of 77% with a RMSE value of 0.06 mm day^{-1} and a MAE of 0.77 mm day^{-1} for the CHP method and an underestimation of 15% an RMSE value of 0.03 mm day^{-1} and a MAE of 0.15 mm day^{-1} for the HR method.

Table 7.1 Regression coefficients (ax^2+bx+c) between transpiration measured with the heat ratio and compensation heat pulse methods (T_{sap}) calculated for a specific wound width and the apparent wound width for the specific day that matched transpiration, which was taken as the residual between evapotranspiration (measured with an eddy covariance system) and evaporation measured with microlysimeters (T_{res})

Date	a	b	c	R^2	Apparent wound width (mm)	T_{res}	Corrected T_{sap}
HR method							
10-Mar-15	1.96	0.46	0.31	1	4.3	0.87	0.87
11-Mar-15	2.30	0.53	0.34	1	4.4	1.03	1.03
12-Mar-15	2.49	0.69	0.30	1	4.5	1.11	1.11
13-Mar-15	1.91	0.60	0.30	1	4.3	0.90	0.90
14-Mar-15	2.30	0.53	0.34	1	4.6	1.08	1.08
Average					**4.4**		
Standard deviation					**0.01**		
Coefficient of variation					**0.2%**		
CHP method							
10-Mar-15	7.65	-1.32	0.44	1	3.4	0.87	0.87
11-Mar-15	8.01	-1.53	0.51	1	3.7	1.03	1.03
12-Mar-15	8.54	-1.63	0.52	1	3.7	1.11	1.11
13-Mar-15	7.62	-1.40	0.48	1	3.5	0.90	0.90
14-Mar-15	8.59	-1.45	0.48	1	3.6	1.08	1.08
Average					**3.6**		
Standard deviation					**0.02**		
Coefficient of variation					**0.6%**		

A significant relationship (P value < 0.05) was obtained using a wound size of 3.6 mm for the CHP method and a highly significant (P value < 0.01) relationship for the HR method was obtained with a wound width of 4.4 mm (Table 7.2). In this case the analysis presented an MBE of 0.01 mm day^{-1} an RMSE value of 0.03 mm day^{-1} and MAE of 0.05 mm day^{-1} for the CHP method and MBE of -0.01 mm day^{-1} an RMSE value of 0.02 mm day^{-1} and MAE of 0.03 mm day^{-1} for the HR method.

Table 7.2 Statistical analysis of the correction factors for 12 wound sizes

	CHP method			HR method		
Wound width (mm)	**RMSE* (mm day^{-1})**	**MAE (mm day^{-1})**	**MBE (mm day^{-1})**	**RMSE (mm day^{-1})**	**MAE (mm day^{-1})**	**MBE (mm day^{-1})**
2.0	0.01	0.46	-0.46	0.006	0.5	-0.50
3.0	0.02	0.22	-0.22	0.01	0.32	-0.32
3.2	0.02	0.15	-0.15	0.01	0.28	-0.28
3.4	0.02	0.08	-0.07	0.01	0.24	-0.24
3.6	0.03	0.05	0.01	0.01	0.2	-0.20
3.8	0.03	0.09	0.10	0.02	0.15	-0.15
4.0	0.03	0.19	0.19	0.02	0.1	-0.10
4.2	0.04	0.3	0.3	0.02	0.06	-0.06
4.4	0.04	0.41	0.41	0.02	0.03	-0.01
4.6	0.05	0.52	0.52	0.02	0.04	0.04
4.8	0.05	0.64	0.64	0.03	0.1	0.10
5.0	0.06	0.77	0.77	0.03	0.15	0.15

*RMSE is the root mean square error; MAE is the mean absolute error and MBE is the mean bias error.

Figure 7.8 shows the results for the calibrated HR method and the CHP method. A strong linear positive correlation was observed between T_{sap} determined by the HR method and T_{res} ($R^2 = 0.95$). For the CHP method a weaker linear positive correlation ($R^2 = 0.78$) was found between T_{sap} and T_{res}.

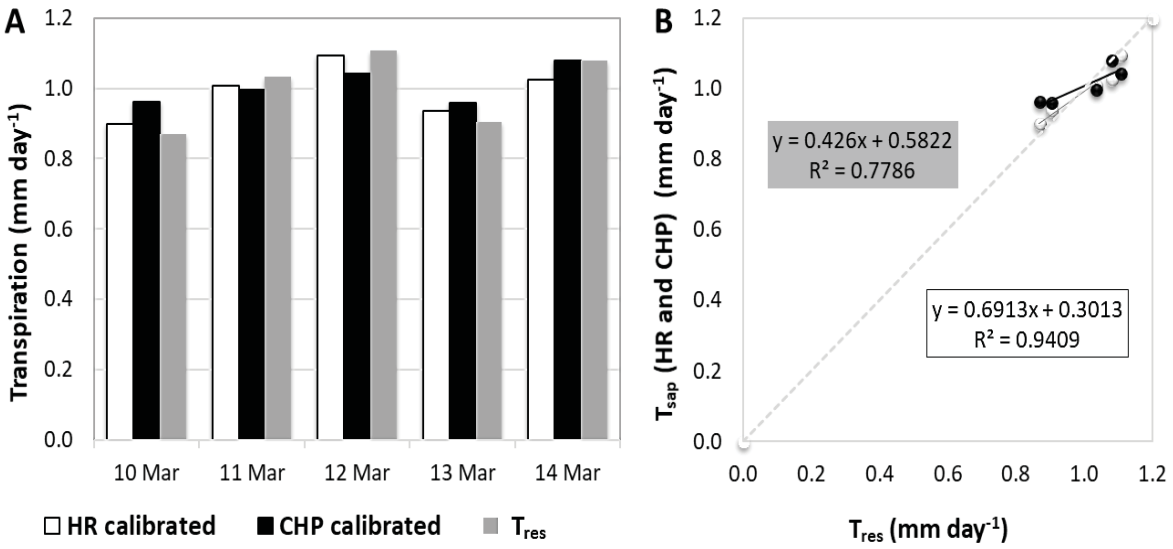

Figure 7.8 (A) The calibrated transpiration (T_{sap}) determined with the heat ratio (HR) method and compensation heat pulse (CHP) methods and daily transpiration (T_{res}) taken as the residual between evapotranspiration (measured with an eddy covariance system) and evaporation (E_s) measured with microlysimeters. (B) Correlation between T_{res} and calibrated T_{sap} in a 'Washington' navel orchard. The 1:1 line is given by the black dashed line

7.8 Comparison of the daily sap flow calculated using actual wound width and heartwood radius to residual transpiration

Although the CHP and HR methods provided good estimates of T once calibrated, frequent missing data and outlier data points were observed with the CHP method. These missing and outlier data points were replaced by averaging the previous and the succeeding value. Some CHP method probes also registered noisy data which required a significant amount of data patching and baseline adjustment to minimise the "noise". As the main aim of this study was to determine the most appropriate method for the quantification of T in citrus, the CHP method was not considered to be an ideal method for long term measurements because of the overall quality of the data obtained. In contrast, the HR method provided an almost complete record for the calibration period and when raw data quality is considered, the HR method is, therefore, a better suited technique for long term measurements. Importantly, when T_{sap} was determined with the HR method using the measured wound width (4.7 mm) and heartwood and sapwood radii there was a close match between T_{sap} and T_{res} ($R^2 = 0.85$), with a MBE of -0.05 mm day^{-1}, a RMSE of 0.04 mm day^{-1} and MAE of 0.05 mm day^{-1}. Orchard T was underestimated by 5% on average per day, which is considered reasonable. The close match of T_{sap} from the HR method to T determined as a residual of ET (eddy covariance) and E_s (microlysimeter) measurements (Figure 7.9) shows that if the parameters (wound width, sapwood depth and

heartwood radius) for determining SFD are measured accurately, accurate measurements of transpiration in *Citrus sinensis* using the HR method can be achieved.

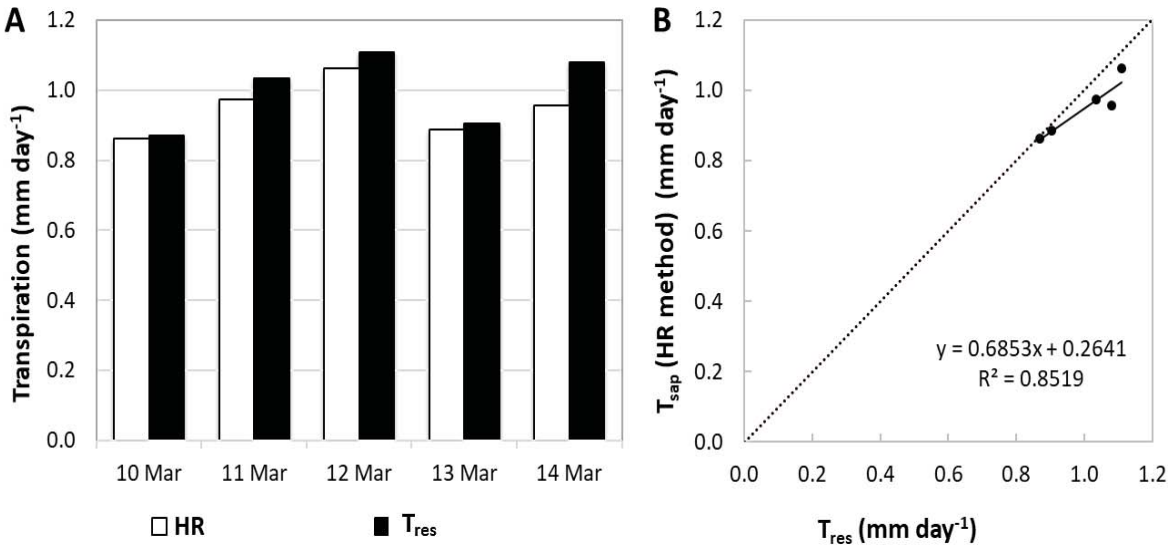

Figure 7.9 (A) The transpiration (T_{sap}) determined from the heat ratio (HR) method, calculated from a wound width of 4.7 mm and daily transpiration (T_{res}) taken as the residual between evapotranspiration (measured with an eddy covariance system) and evaporation (E_s) measured with microlysimeters. (B) Correlation between T_{res} and T_{sap} (wound width of 4.7 mm) in a 'Washington' navel orchard. The 1:1 line is given by the black dashed line

7.9 Conclusions

For the infield measurements using a wound width of 2.5 mm (width of the widest probe) led to a serious underestimation of transpiration for both the HR and CHP methods. Both these methods were therefore calibrated using an independent estimate of transpiration. Transpiration was calculated as a residual of ET, where ET was determined with an EC system and E_s, determined using microlysimeters. Calibration was performed by determining a virtual wound width, which resulted in the agreement between T_{res} and T_{sap}. The best agreement between T_{sap} and T_{res} was found by employing a virtual wound width of 4.4 mm for the HR method and 3.6 mm for the CHP method. The statistical analysis indicated that calibrated CHP method resulted in an overestimation of orchard transpiration by 1.4%, whilst the calibrated HR method resulted in almost no underestimation (0.4%). There was a higher value, for the Willmott index of agreement, for the HR method (0.92) as opposed to the CHP (0.85), although it was expected that the CHP method would estimate sap flow more accurately than the HR method. This is because, mature citrus canopies are large and the CHP method is reported to capture high flow rates more accurately than the HR method. The

calibrated CHP method resulted in an intercept that was not significantly different from zero, but the slope was significantly less than unity. For the calibrated HR method the slope and the intercept were not significantly different from unity and zero. Additionally, *in situ* measurement of the wound width and heartwood radius for the HR method at the end of the trial resulted in 5% underestimation of transpiration on average per day. This shows that, there is a good possibility of determining T in citrus trees using the HR method without calibration for low T rates. Due to large variation in wound width between trees it is still recommended that the chosen measurement technique is calibrated against an independent measure of T for each new orchard in which measurements are to be made.

8 GENERAL CONCLUSIONS

There are a number of reliable methods for quantifying orchard level water use, including EC measurements and weighing lysimeters. Lysimetry methods, which are considered to be the reference method, take years to be established before measurements are conducted (Subedi et al., 2013) and lysimeters sufficient to house large citrus trees are costly to construct and maintain. The only realistic and relatively inexpensive methods available for transpiration measurements are SFD techniques. Whilst, the theory behind the different methods differs, all the techniques use heat as a tracer to estimate water movement in a stem. It was, therefore, decided to focus on SFD measurement methods, which can be used for a wide range of stem sizes. The major concern with these techniques is that they tend to underestimate T, and as a result species-specific calibration is often required.

Results from the stem perfusion technique showed that SFD was consistently underestimated by the HR, CHP and TD methods for the citrus cultivars and species tested. Underestimation of SFD with the HR method was lower than the other two methods. Although fairly good correlations between SFD, determined with the HR, CHP and TD methods, and that determined gravimetrically were obtained, the slope of the relationships were inconsistent within a cultivar or between species. As a result a single empirical calibration factor could not be calculated to correct sap flow measurements from SFD techniques to that determined gravimetrically. However, the data showed that all three methods can potentially be used to estimate water use in citrus, provided corrections are made for thermal inhomogeneity of the sapwood and that the area of conducting sapwood is accurately determined. Our analysis of the performance of SFD techniques showed that the HR method should perhaps be considered before the CHP and TD methods, as the underestimations of SFD were less with this method. Capturing low flow rates is one of the strengths of the HR method.

The large variation in calibration factors between stems of an individual species and between species was attributed to a number of factors. Firstly, it was noted that on a number of occasions the inserted probes were placed in non-conducting tissue. Large variations in conducting sapwood were noted in some stems, which was impossible to identify prior to the insertion of probes. Secondly, the mature leaf sapwood of citrus branches in this study was not thermally homogenous and therefore deviated from the ideal HPV theory. However, the flush leaf wood adhered to the definition of a thermally homogenous medium, but was not found in all species. Thirdly, the xylem anatomy of the stem will have a large influence on measurements and this must be taken into account with the installation of the HR and CHP probe sets.

Initial tests of the HPV equipment and method against a weighing lysimeter, in a *Eucalyptus marginata* tree (model specie for HR method), gave reliable results. This gave the assurance that the local manufactured HPV systems and methods are reliable and accurate. Both the HR and CHP methods were then tested on 'Midknight' Valencia trees in the glasshouse, where they performed equally well when compared to the weighing lysimeters in a glasshouse. Over a period of 37 days for the HR and 30 days for the CHP method, an error less than 2% was found between the HR and CHP methods and the weighing lysimeter, when a wound width of 2.0 mm (width of the widest probe) was used in the SFD calculations. However, on an hourly basis both the HR and the CHP method overestimated T when flow rates were less than 0.1 L h^{-1} and underestimated T at flow rates greater than 0.1 L h^{-1}. A time lag between sap flow measurements and mass loss measured with the lysimeter was observed. The measurement of sap flow and the actual process of T are spatially separated on the tree, which may contribute to the time lags between the two measurements techniques. The lag was more pronounced at low flow rates towards the end of the day when the weighing lysimeter registered virtually no mass loss, whilst the sap flow methods continued to record sap movement. Another process that may be involved is the refilling of the tree at the end of the day. After cessation of T (closing of stomata) no mass loss is registered with the lysimeter, but sap continues to flow (recorded with HR method) in order to rehydrate the plant tissues.

The HR and CHP methods were calibrated and validated in a commercial 'Washington' navel orchard against EC measurements combined with estimates of E_s. The wound width was initially assumed to be 2.5 mm (width of the widest probe), which was valid for the glasshouse experiment. Brass collars were used in the field experiments to house the heater probes in order to protect them against corrosion. However, this resulted in a severe underestimation of T_{res} by approximately 42% with the HR method and 36% with the CHP method when compared to T_{res}. Wound width is one of the most difficult parameters to determine in the field and an attempt to measure the actual wound width resulted in a 37% coefficient of variation (CV) in the field, while for the glasshouse a CV of 1% was observed. The exact cause of the large variation in wound width in field experiments is not known, but one can expect a greater wound response in fast growing trees when compared to the potted, slower growing 13 year old trees in the glasshouse. Nonetheless, a large variation in wound width for the infield experiments led to the calibration of the techniques focusing mainly on a wound width correction factor. An agreement of 94% was observed between T_{sap} (HR method) and T_{res}, underestimating orchard T by only 0.4%, when a virtual wound width correction factor of 4.4 mm was used. On the other hand, the CHP method also performed reasonably well against T_{res} with a correlation coefficient of 78%, overestimating T by 1.4%, when a virtual wound width of 3.6 mm was used. Of the two methods, the HR method performed better than

the CHP method, though the CHP is known to perform well under high flow rates (< 5 $cm^3\,cm^{-2}\,h^{-1}$ and >100 $cm^3\,cm^{-2}\,h^{-1}$). The HR method was tested within its limits, with the maximum SFDs observed were approximately 20 $cm^3\,cm^{-2}\,h^{-1}$, which is below its upper limit of 45 $cm^3\,cm^{-2}\,h^{-1}$ (Vandegehuchte and Steppe, 2013). The wound width correction factor (CHP 3.6 mm and HR 4.4 mm) determined in this experiment exceeded the wounding expected by Barrett et al. (1995) who suggested that the wound extends 0.3 mm either side of the widest probe. However, observations of wound width at the end of the study suggest that this is a real value for citrus, as the observed wound width was on average 4.7 mm. Coupled with the results from staining of the conducting sapwood, T_{sap} values within 5% of T_{res} were obtained for the HR method. These were good results considering the potential large sources of error associated with this validation and it is concluded that SFD methods, especially the HR method, can be used in citrus orchards, if the parameters such as wound width, sapwood conducting area and heartwood radius are determined accurately.

9 RECOMMENDATIONS

When using the stem perfusion method it is suggested that a better way is found to achieve different flow rates, as sudden changes in flow are registered by the gravimetric readings but are not necessarily taken into account by the SFD techniques. Future research should also focus on improved practical infield measurements and assessment of the wound widths.

In this study a technique to measure tree water use was validated and rigorously tested. This technique can give detailed insight into tree water use and changes in tree water use due to external factors, such as changes in canopy size and irrigation management. Therefore, in the light of the recent droughts, future research should focus on quantifying tree water use when water saving practices are implemented, such as the reduction in canopy size and changes of irrigation practices and systems. The research should take into account the impact of reduced tree water use on yield, fruit quality and the time it takes to recover to previous yield levels under optimal conditions.

10 LIST OF REFERENCES

ABOUKHALED, A., ALFARO, A. and SMITH, M. (1982) Lysimeters. FAO Irrigation and Drainage Paper No. 39. FAO, Rome, Italy.

ALARCÓN, J., ORTUNO, M., NICOLÁS, E., TORRES, R. and TORRECILLAS, A. (2005) Compensation heat-pulse measurements of sap flow for estimating transpiration in young lemon trees. *Biologia Plantarum* **49** (4) 527-532.

ALARCÓN, J.J., ORTUÑO, M.F., NICOLÁS, E., NAVARRO, A. and TORRECILLAS, A. (2006) Improving water-use efficiency of young lemon trees by shading with aluminised-plastic nets. *Agricultural Water Management* **82** (3) 387-398.

ALLEN, R.G., PEREIRA, L.S., HOWELL, T.A. and JENSEN, M.E. (2011) Evapotranspiration information reporting: I. Factors governing measurement accuracy. *Agricultural Water Management* **98** (6) 899-920.

BALDOCCHI, D. (1997) Flux footprints within and over forest canopies. *Boundary-Layer Meteorology* **85** (2) 273-292.

BARRETT, D., HATTON, T., ASH, J. and BALL, M. (1995) Evaluation of the heat pulse velocity technique for measurement of sap flow in rainforest and eucalypt forest species of south-eastern Australia. *Plant, Cell and Environment* **18** (4) 463-469.

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* **110** (4) 275-284.

BECKER, P. (1998) Limitations of a compensation heat pulse velocity system at low sap flow: implications for measurements at night and in shaded trees. *Tree Physiology* **18** (3) 177-184.

BEESON, R. (2011) Weighing lysimeter systems for quantifying water use and studies of controlled water stress for crops grown in low bulk density substrates. *Agricultural Water Management* **98** (6) 967-976.

BLEBY, T.M., BURGESS, S.O. and ADAMS, M.A. (2004) A validation, comparison and error analysis of two heat-pulse methods for measuring sap flow in *Eucalyptus marginata* saplings. *Functional Plant Biology* **31** (6) 654-658.

BOULET, G., CHEHBOUNI, A., BRAUD, I., DUCHEMIN, B. and LAKHAL, A. (2004) Evaluation of a two-stage evaporation approximation for contrasting vegetation cover. *Water Resources Research* **40** (12).

BURGESS, S.O., ADAMS, M.A., TURNER, N.C., BEVERLY, C.R., ONG, C.K., KHAN, A.A.H. 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.

BURGESS, S.S. and DAWSON, T.E. (2008) Using branch and basal trunk sap flow measurements to estimate whole-plant water capacitance: a caution. *Plant and Soil* **305** (1-2) 5-13.

BURGESS, S.S., ADAMS, M.A. and BLEBY, T.M. (2000) Measurement of sap flow in roots of woody plants: a commentary. *Tree Physiology* **20** (13) 909-913.

BUSH, S.E., HULTINE, K.R., SPERRY, J.S. and EHLERINGER, J.R. (2010) Calibration of thermal dissipation sap flow probes for ring-and diffuse-porous trees. *Tree Physiology* **30** (12) 1545-1554.

CAMPBELL, G.S. and NORMAN, J.M. (1998) *An introduction to environmental biophysics*, Springer, New York.

CASPARI, H., GREEN, S. and EDWARDS, W. (1993) Transpiration of well-watered and water-stressed Asian pear trees as determined by lysimetry, heat-pulse, and estimated by a Penman-Monteith model. *Agricultural and Forest Meteorology* **67** (1-2) 13-27.

CHEN, X., MILLER, G.R., RUBIN, Y. and BALDOCCHI, D.D. (2012) A statistical method for estimating wood thermal diffusivity and probe geometry using in situ heat response curves from sap flow measurements. *Tree Physiology* **32** (12) 1458-1470.

CIRELLI, D., LIEFFERS, V.J. and TYREE, M.T. (2012) Measuring whole-plant transpiration gravimetrically: a scalable automated system built from components. *Trees* **26** (5) 1669-1676.

CLEARWATER, M.J., MEINZER, F.C., ANDRADE, J.L., GOLDSTEIN, G. and HOLBROOK, N.M. (1999) Potential errors in measurement of nonuniform sap flow using heat dissipation probes. *Tree Physiology* **19** (10) 681-687.

COELHO, R.D., VELLAME, L.M, JÚNIOR, F. and EUSÍMIO, F. (2012) Estimation of transpiration of the'Valencia'orange young plant using thermal dissipation probe method. *En.genharia Agrícola* **32** (3) 573-581.

COHEN, Y., FUCHS, M. and GREEN, G. (1981) Improvement of the heat pulse method for determining sap flow in trees. *Plant, Cell and Environment* **4** (5) 391-397.

CONCEIÇÃO, N. and FERREIRA, M. (2008) *Combination of sap flow and eddy covariance techniques to obtain long term transpiration in a pear orchard*. In Proc. VII International Workshop on Sap Flow 846, (October), 53-60.

DAAMEN, C.C., SIMMONDS, L., WALLACE, J., LARYEA, K. and SIVAKUMAR, M. (1993) Use of microlysimeters to measure evaporation from sandy soils. *Agricultural and Forest Meteorology* **65** (3-4) 159-173.

DAWSON, T.E., BURGESS, S.S., TU, K.P., OLIVEIRA, R.S., SANTIAGO, L.S., FISHER, J.B., SIMONIN, K.A. and AMBROSE, A.R. (2007) Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiology* **27** (4) 561-575.

DE JAGER, J. (1994) Accuracy of vegetation evaporation ratio formulae for estimating final wheat yield. *Water SA* **20** 307-307.

DE OLIVEIRA REIS, F., CAMPOSTRINI, E., DE SOUSA, E.F. and E SILVA, M.G. (2006) Sap flow in papaya plants: Laboratory calibrations and relationships with gas exchanges under field conditions. *Scientia Horticulturae* **110** (3) 254-259.

DUNN, G. and CONNOR, D. (1993) An analysis of sap flow in mountain ash (Eucalyptus regnans) forests of different age. *Tree Physiology* **13** (4) 321-336.

DYE, P. and OLBRICH, B. (1993) Estimating transpiration from 6-year-old Eucalyptus grandis trees: development of a canopy conductance model and comparison with independent sap flux measurements. *Plant, Cell and Environment* **16** (1) 45-53.

EDWARDS, W.R.N., BECKER, P. and ÈERMÁK, J. (1997) A unified nomenclature for sap flow measurements. *Tree Physiology* **17** (1) 65-67.

EILMANN, B. and RIGLING, A. (2012) Tree-growth analyses to estimate tree species' drought tolerance. *Tree Physiology* **32** (2) 178-187.

FERNÁNDEZ, J., DURÁN, P., PALOMO, M., DÍAZ-ESPEJO, A., CHAMORRO, V. and GIRÓN, I. (2006) Calibration of sap flow estimated by the compensation heat pulse method in olive, plum and orange trees: relationships with xylem anatomy. *Tree Physiology* **26** (6) 719-728.

FERNÁNDEZ, J., PALOMO, M., DÍAZ-ESPEJO, A., CLOTHIER, B., GREEN, S., GIRÓN, I. and MORENO, F. (2001) Heat-pulse measurements of sap flow in olives for automating irrigation: tests, root flow and diagnostics of water stress. *Agricultural Water Management* **51** (2) 99-123.

FERNÁNDEZ LUQUE, J.E., MARTÍN PALOMO, M., DÍAZ-ESPEJO, A. and GIRÓN MORENO, I.F. (1999) Calibrating the compensation heat-pulse technique for measuring sap flow in olive. In *III International Symposium on Olive Growing*, Chania, Crete, Greece.

FISHER, J.B., BALDOCCHI, D.D., MISSON, L., DAWSON, T.E. and GOLDSTEIN, A.H. (2007) What the towers don't see at night: nocturnal sap flow in trees and shrubs at two AmeriFlux sites in California. *Tree Physiology* **27** (4) 597-610.

GONZÁLEZ-ALTOZANO, P., PAVEL, E., ONCINS, J.A., DOLTRA, J., COHEN, M., PAÇO, T., MASSAI, R. and CASTEL, J. (2008) Comparative assessment of five methods of determining sap flow in peach trees. *Agricultural Water Management* **95** (5) 503-515.

GRANIER, A. (1985) A new method of sap flow measurement in tree stems. *Annales des Sciences Forestieres (France)* **42** 193-200.

GREEN, G.C. and MORESHET, S. (1979) An analysis of seasonal water use characteristics of Valencia orange trees in the Sundays River Valley. *Crop Production* **8** 179-183.

GREEN, S. and CLOTHIER, B. (1988) Water use of kiwifruit vines and apple trees by the heat-pulse technique. *Journal of Experimental Botany* **39** (1) 115-123.

GREEN, S., CLOTHIER, B. and JARDINE, B. (2003) Theory and practical application of heat pulse to measure sap flow. *Agronomy Journal* **95** (6) 1371-1379.

GREEN, S., CLOTHIER, B. and PERIE, E. (2008) *A re-analysis of heat pulse theory across a wide range of sap flows*. In Proc. VII International Workshop on Sap Flow 846, (October), 95-104.

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** (4) 219-227.

HORST, T. and WEIL, J. (1992) Footprint estimation for scalar flux measurements in the atmospheric surface layer. *Boundary-Layer Meteorology* **59** (3) 279-296.

HOWELL, T., SCHNEIDER, A., DUSEK, D., MAREK, T. and STEINER, J. (1995) Calibration and scale performance of Bushland weighing lysimeters. *Transactions of the ASAE* **38** (4) 1019-1024.

HULTINE, K., NAGLER, P., MORINO, K., BUSH, S., BURTCH, K., DENNISON, P., GLENN, E. and EHLERINGER, J. (2010) Sap flux-scaled transpiration by tamarisk (Tamarix spp.) before, during and after episodic defoliation by the saltcedar leaf beetle (Diorhabda carinulata). *Agricultural and Forest Meteorology* **150** (11) 1467-1475.

KOOL, D., AGAM, N., LAZAROVITCH, N., HEITMAN, J., SAUER, T. and BEN-GAL, A. (2014) A review of approaches for evapotranspiration partitioning. *Agricultural and Forest Meteorology* **184** 56-70.

KRAMER, P.J. and BOYER, J.S. (1995) *Water relations of plants and soils*, Academic press.

LUNDBLAD, M., LAGERGREN, F. and LINDROTH, A. (2001) Evaluation of heat balance and heat dissipation methods for sapflow measurements in pine and spruce. *Annals of Forest Science* **58** (6) 625-638.

MADURAPPERUMA, W.S., BLEBY, T.M. and BURGESS, S.S. (2009) Evaluation of sap flow methods to determine water use by cultivated palms. *Environmental and Experimental Botany* **66** (3) 372-380.

MARSHALL, D.C. (1958) Measurement of sap flow in conifers by heat transport. *Plant Physiology* **33** (6) 385-396.

NADEZHDINA, N., ČERMÁK, J. and CEULEMANS, R. (2002) Radial patterns of sap flow in woody stems of dominant and understory species: scaling errors associated with positioning of sensors. *Tree Physiology* **22** (13) 907-918.

NADEZHDINA, N., NADEZHDIN, V., FERREIRA, M.I. and PITACCO, A. (2007) Variability with xylem depth in sap flow in trunks and branches of mature olive trees. *Tree Physiology* **27** (1) 105-113.

NORTES, P., BAILLE, A., EGEA, G., GONZÁLEZ-REAL, M. and DOMINGO, R. (2008) *Comparison of almond tree transpiration determined by sap flow measurements and lysimetry*. In Proc. VII International Workshop on Sap Flow 846, (October), 359-366.

OLMSTEAD, M.A., LANG, N.S., EWERS, F.W. and OWENS, S.A. (2006) Xylem vessel anatomy of sweet cherries grafted onto dwarfing and nondwarfing rootstocks. *Journal of the American Society for Horticultural Science* **131** (5) 577-585.

OREN, R., PHILLIPS, N., EWERS, B., PATAKI, D. and MEGONIGAL, J.P. (1999) Sap-flux-scaled transpiration responses to light, vapor pressure deficit, and leaf area reduction in a flooded Taxodium distichum forest. *Tree Physiology* **19** (6) 337-347.

ORTUNO, M.F., GARCÍA-ORELLANA, Y., CONEJERO, W., RUIZ-SÁNCHEZ, M.C., ALARCÓN, J.J. and TORRECILLAS, A. (2006) Stem and leaf water potentials, gas exchange, sap flow, and trunk diameter fluctuations for detecting water stress in lemon trees. *Trees* **20** (1) 1-8.

PAUDEL, I., KANETY, T. and COHEN, S. (2013) Inactive xylem can explain differences in calibration factors for thermal dissipation probe sap flow measurements. *Tree Physiology* **33** (9) 986-1001.

PEARSALL, K.R. (2011) *Dual Heat Pulse Sensors for Determining Volumetric Water Use in Grapevines*, UNIVERSITY OF CALIFORNIA, DAVIS.

PHILLIPS, N.G., SCHOLZ, F.G., BUCCI, S.J., GOLDSTEIN, G. and MEINZER, F.C. (2009) Using branch and basal trunk sap flow measurements to estimate whole-plant water capacitance: comment on Burgess and Dawson (2008). *Plant and Soil* **315** (1-2) 315.

POBLETE-ECHEVERRÍA, C., ORTEGA-FARIAS, S., ZUÑIGA, M. and FUENTES, S. (2012) Evaluation of compensated heat-pulse velocity method to determine vine transpiration using combined measurements of eddy covariance system and microlysimeters. *Agricultural water management* **109** 11-19.

RANA, G., KATERJI, N. and DE LORENZI, F. (2005) Measurement and modelling of evapotranspiration of irrigated citrus orchard under Mediterranean conditions. *Agricultural and Forest Meteorology* **128** (3) 199-209.

SCHROEDER, J.I., ALLEN, G.J., HUGOUVIEUX, V., KWAK, J.M. and WANER, D. (2001) Guard cell signal transduction. *Annual Review of Plant Biology* **52** (1) 627-658.

SHACKEL, K.A., JOHNSON, R.S., MEDAWAR, C.K. and PHENE, C.J. (1992) Substantial errors in estimates of sap flow using the heat balance technique on woody stems under field conditions. *Journal of the American Society for Horticultural Science* **117** (2) 351-356.

SMITH, D. and ALLEN, S. (1996) Measurement of sap flow in plant stems. *Journal of Experimental Botany* **47** (12) 1833-1844.

SORENSEN, R., JONES, T., CAMPBELL, G. and MONTES-HELU, M. (1999) Heat pulse needles to measure pecan tree transpiration. *Applied Engineering in Agriculture* **15** (6) 651-658.

STEPPE, K., DE PAUW, D.J., DOODY, T.M. and TESKEY, R.O. (2010) A comparison of sap flux density using thermal dissipation, heat pulse velocity and heat field deformation methods. *Agricultural and Forest Meteorology* **150** (7) 1046-1056.

STEPPE, K. and LEMEUR, R. (2007) Effects of ring-porous and diffuse-porous stem wood anatomy on the hydraulic parameters used in a water flow and storage model. *Tree physiology* **27** (1) 43-52.

SUBEDI, A., CHÁVEZ, J.L. and ANDALES, A.A. (2013) Preliminary performance evaluation of the Penman-Monteith evapotranspiration equation in southeastern Colorado. *Hydrology Days* **970** 84-90.

SUN, H., AUBREY, D.P. and TESKEY, R.O. (2012) A simple calibration improved the accuracy of the thermal dissipation technique for sap flow measurements in juvenile trees of six species. *Trees* **26** (2) 631-640.

SWANSON, R.H. (1994) Significant historical developments in thermal methods for measuring sap flow in trees. *Agricultural and Forest Meteorology* **72** (1) 113-132.

SWANSON, R.H. and WHITFIELD, D.W.A. (1981) A numerical analysis of heat pulse velocity theory and practice. *Journal of Experimental Botany* **32** (1) 221-239.

TANEDA, H. and SPERRY, J.S. (2008) A case-study of water transport in co-occurring ring-versus diffuse-porous trees: contrasts in water-status, conducting capacity, cavitation and vessel refilling. *Tree Physiology* **28** (11) 1641-1651.

TANNER, C.B. and SINCLAIR, T.R. (1983) Efficient water use in crop production: Research or re-search? In Taylor HM, WR Jordan and TR Sinclairs (eds), *Limitations to efficient water use in crop production,* Am. Soc. of Agron., Crop Sci. Soc. of Am., and Soil Sci. Soc. of Am., Madison, Wisconsin.

TAYLOR, N., MAHOHOMA, W., VAHRMEIJER, J., GUSH, M.B., ALLEN, R.G. and ANNANDALE, J.G. (2015) Crop coefficient approaches based on fixed estimates of leaf resistance are not appropriate for estimating water use of citrus. *Irrigation science* **33** (2) 153-166.

TESKEY, R. and SHERIFF, D. (1996) Water use by Pinus radiata trees in a plantation. *Tree Physiology* **16** (1-2) 273-279.

THOMPSON, A.L., MARTIN, D.L., NORMAN, J.M., TOLK, J.A., HOWELL, T., GILLEY, J. and SCHNEIDER, A. (1997) Testing of a water loss distribution model for moving sprinkler systems. *Transactions of the ASAE* **40** (1) 81-88.

VANDEGEHUCHTE, M.W. and STEPPE, K. (2013) Corrigendum to: Sap-flux density measurement methods: working principles and applicability. *Functional Plant Biology* **40** (10) 1088-1088.

VELLAME, L.M., COELHO FILHO, M.A., PAZ, V.P. and COELHO, E.F. (2010) Stem heat balance method to estimate transpiration of young orange and mango plants. *Revista Brasileira de Engenharia Agrícola e Ambiental* **14** (6) 594-599.

VERTESSY, R., HATTON, T., REECE, P., O'SULLIVAN, S. and BENYON, R. (1997) Estimating stand water use of large mountain ash trees and validation of the sap flow measurement technique. *Tree Physiology* **17** (12) 747-756.

VIEWEG, G. and ZIEGLER, H. (1960) Thermoelektrische registrierung der geschwindigkeit des transpirationsstromes. *Ber. dtsch. bot. Ges* **73** (22) l-226.

VILLALOBOS, F.J., TESTI, L., ORGAZ, F., GARCÍA-TEJERA, O., LOPEZ-BERNAL, A., GONZÁLEZ-DUGO, M.V., BALLESTER-LURBE, C., CASTEL, J.R., ALARCÓN-CABAÑERO, J.J. and NICOLÁS-NICOLÁS, E. (2013) Modelling canopy conductance and transpiration of fruit trees in Mediterranean areas: a simplified approach. *Agricultural and Forest Meteorology* **171** 93-103.

WILLIAMS, D., CABLE, W., HULTINE, K., HOEDJES, J., YEPEZ, E., SIMONNEAUX, V., ER-RAKI, S., BOULET, G., DE BRUIN, H. and CHEHBOUNI, A. (2004) Evapotranspiration components determined by stable isotope, sap flow and eddy covariance techniques. *Agricultural and Forest Meteorology* **125** (3-4) 241-258.

WILLMOTT, C.J., ROBESON, S.M. and MATSUURA, K. (2012) A refined index of model performance. *International Journal of Climatology* **32** (13) 2088-2094.

WILSON, K.B., HANSON, P.J., MULHOLLAND, P.J., BALDOCCHI, D.D. and WULLSCHLEGER, S.D. (2001) A comparison of methods for determining forest evapotranspiration and its components: sap-flow, soil water budget, eddy covariance and catchment water balance. *Agricultural and Forest Meteorology* **106** (2) 153-168.

WULLSCHLEGER, S.D. and KING, A.W. (2000) Radial variation in sap velocity as a function of stem diameter and sapwood thickness in yellow-poplar trees. *Tree Physiology* **20** (8) 511-518.

ZHANG, Z., TIAN, F., HU, H. and YANG, P. (2014) A comparison of methods for determining field evapotranspiration: photosynthesis system, sap flow, and eddy covariance. *Hydrology and Earth System Sciences* **18** (3) 1053-1072.

ZREIK, C., GONZÁLEZ-ALTOZANO, P. and CASTEL, J. (2000) *Sap flow determination in plum and mandarin trees by the compensated heat pulse method*. In Proc. 5th International Workshop on Plant Water Relations and Sap Flux Measurements, Tognetti R and R Raschis (eds), Firenze Italy (9-10 November), 93-108.

ZWIENIECKI, M.A. and HOLBROOK, N.M. (2009) Confronting Maxwell's demon: biophysics of xylem embolism repair. *Trends in Plant Science* **14** (10) 530-534.

11 APPENDIXES

Appendix 1: Linear regression analysis between the heat ratio method and the weighing lysimeter for daytime water use in *E. marginata* in the glasshouse

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.96865461
R Square	0.938291754
Adjusted R Square	0.92947629
Standard Error	0.091148277
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.88427971	0.88427971	106.437	1.74115E-05
Residual	7	0.058156059	0.008308008		
Total	8	0.942435768			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.117552028	0.166280742	0.706949144	0.502446	-0.275639446	0.510743502
LYS	0.914322909	0.088624385	10.31683221	1.74E-05	0.70475954	1.123886279

Appendix 2: Linear regression analysis between the heat ratio method and the weighing lysimeter for daytime water use in 'Midknight' Valencia in the glasshouse

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.987901548
R Square	0.975949468
Adjusted R Square	0.97526231
Standard Error	0.109833476
Observations	37

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	17.13326518	17.13326518	1420.269246	6.33117E-30
Residual	35	0.422218733	0.012063392		
Total	36	17.55548391			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.137413843	0.053225774	-2.581716183	0.014177369	-0.245467909	-0.029359776
LYS 2 kg day-1	1.066850303	0.028308584	37.68645972	6.33117E-30	1.009380823	1.124319783

Appendix 3: Linear regression analysis between the compensation heat pulse method and the weighing lysimeter for daytime water use in 'Midknight' Valencia in the glasshouse

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.952820451
R Square	0.907866812
Adjusted R Square	0.904454471
Standard Error	0.105656966
Observations	29

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.970065836	2.970065836	266.0540067	1.6647E-15
Residual	27	0.301411652	0.011163395		
Total	28	3.271477487			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.004220726	0.09541425	-0.044235804	0.965041982	-0.199994595	0.191553143
LYS 1 kg day-1	1.015083555	0.062232449	16.31116203	1.6647E-15	0.887393117	1.142773992

Appendix 4: Linear regression analysis between the thermal dissipation probe method and the weighing lysimeter for daytime water use in 'Midknight' Valencia in the glasshouse

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.474100901
R Square	0.224771665
Adjusted R Square	0.160169303
Standard Error	0.163793462
Observations	14

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.093343975	0.093343975	3.479310356	0.086769096
Residual	12	0.321939577	0.026828298		
Total	13	0.415283553			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.743029902	0.47477635	5.777520097	8.77651E-05	1.7085811	3.777478704
X Variable 1	-0.21312372	0.114257628	-1.865290957	0.086769096	-0.462069704	0.035822265

Appendix 5: Linear regression analysis between the heat ratio method and the weighing lysimeter for daily water use in 'Washington' navel in the field

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.983085374
R Square	0.966456852
Adjusted R Square	0.955275802
Standard Error	0.006497788
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.003649479	0.003649479	86.43704293	0.002634041
Residual	3	0.000126664	4.22212E-05		
Total	4	0.003776143			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.217688237	0.030524429	7.13160715	0.005675334	0.120545881	0.314830594
T residual	0.282769302	0.030414614	9.29715241	0.002634041	0.185976426	0.379562178

Appendix 6: Linear regression analysis between the compensation heat pulse method and the weighing lysimeter for daily water use in 'Washington' navel in the field

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9464803
R Square	0.895824959
Adjusted R Square	0.861099945
Standard Error	0.049957055
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.064383472	0.064383472	25.79768482	0.014743032
Residual	3	0.007487122	0.002495707		
Total	4	0.071870594			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.344331337	0.168930771	-2.0382985	0.134266363	-0.881944445	0.193281771
ET	0.56502939	0.111245044	5.079142134	0.014743032	0.210998011	0.919060769

Appendix 7: Linear regression analysis between the calibrated heat ratio method and the weighing lysimeter for daily water use in 'Washington' navel in the field

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.970017534
R Square	0.940934016
Adjusted R Square	0.921245354
Standard Error	0.021365134
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.02181495	0.021815	47.79065441	0.006204003
Residual	3	0.001369407	0.000456		
Total	4	0.023184357			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.301267756	0.100366242	3.001684	0.057591574	-0.018142419	0.620677932
T residual	0.691343539	0.100005162	6.913079	0.006204003	0.373082479	1.009604599

Appendix 8: Linear regression analysis between the calibrated compensation heat pulse method and the weighing lysimeter for daytime water use in 'Washington' navel in the field

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.887257346
R Square	0.787225598
Adjusted R Square	0.716300798
Standard Error	0.010974624
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.001336843	0.001336843	11.09944043	0.044666507
Residual	3	0.000361327	0.000120442		
Total	4	0.00169817			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*
Intercept	0.365705423	0.051555106	7.093485976	0.005763161	0.201634066
T residual	0.171142151	0.051369631	3.331582271	0.044666507	0.007661059