

**Validating sap flux density measurement methods in *Citrus sinensis*  
(L.)**

by

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Submitted in partial fulfilment of the requirements for the degree  
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## **Declaration**

I, Mathew Banda do hereby declare that this dissertation, which I do hereby submit for the degree of Master of Science in Horticulture at University of Pretoria, is my own work and has never been submitted by myself at any other academic institution. Except where duly acknowledged, the research work reported herein is as a result of my own investigation.

Signed\_\_\_\_\_

(Mathew Banda)

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## **Abstract**

Sap flux density (SFD) measurements methods have been widely used in woody plants to accurately quantify tree water use (transpiration). Two different methods viz., heat ratio method (HR) and the compensation heat pulse (CHP) were tested against a pre-calibrated mini-weighing lysimeter (gravimetric method) that served as the control. The experiments were conducted on large potted *Citrus sinensis* ('Midknight' Valencia trees) in a glasshouse at the University of Pretoria. The main aim was to compare these methods and establish the most reliable and accurate method to be used to estimate transpiration in citrus trees. A strong positive linear relationship between the HR method and gravimetric method ( $R^2 = 0.98$ ) was obtained when a wound width of 2.0 mm (width of the widest probe) was used, with tree transpiration underestimated by an average of 1.08 % day<sup>-1</sup>. The CHP method was satisfactorily accurate as indicated by an  $R^2$  value of 0.91 and an overestimation of tree transpiration by an average of 1.23 % day<sup>-1</sup>. However, a large portion of missing data was one of the challenging factors to adopt the CHP method. The measurements also showed evidence of a time lag between sap flow measured with the CHP and HR method and transpiration measured with the gravimetric method, indicating some degree of capacitance in these potted trees.

Although validation of the methods in potted citrus against a weighing lysimeter showed that both the HR and CHP methods accurately estimated transpiration, it was important to assess the methods in commercial orchards where long term measurements were to be made. The HR and CHP methods were tested in a commercial *Citrus sinensis* ('Washington' Navels) orchard for their ability to quantify transpiration under field conditions. Field trials were conducted on Patryberg farm near Citrusdal from 10 - 14 March 2015. Micrometeorological measurements for the estimation of evapotranspiration (ET) were performed using an EC (eddy covariance) system and soil evaporation ( $E_s$ ) was determined using the microlysimeters. Transpiration ( $T_{res}$ ) was calculated as a residual of ET and  $E_s$  and compared with transpiration ( $T_{sap}$ ) determined by the CHP and HR method. In comparison to  $T_{res}$  the HR method ( $R^2 = 0.97$ ) resulted in a better agreement than the CHP method ( $R^2 = 0.80$ ) when a wound width of 2.5 mm corresponding to the width of the widest probe was used. However, both the HR and CHP methods underestimated orchard transpiration by 42 % and 36 % respectively, when compared to  $T_{res}$ . The underestimation was mainly attributed to an underestimation of the wound width and possible additive errors, which includes the scaling of whole tree water use to orchard water use and errors in the determination of soil evaporation. Even though the SFD methods underestimated transpiration, the good agreement between the SFD methods and  $T_{res}$  enabled the calibration of SFD methods. As wound width is one of the most difficult parameters to determine for the upscaling of heat pulse velocities to sap flow in the field, wound width was used to calibrate the CHP and the HR methods. For the CHP method a virtual wound width of 3.6 mm resulted in the best agreement between  $T_{res}$  and  $T_{sap}$  (1.4% overestimation of transpiration), whilst for the HR method a virtual wound width of 4.4 mm resulted in the best agreement between the two measurement methods (0.4% underestimation). At the end of the measurements the actual wound width was determined and was on average 4.7 mm for HR method probe sets. *In situ* stem staining was also performed at this time to determine the

sapwood depth and heartwood radius. Using these measurements the calculated  $T_{\text{sap}}$  underestimated  $T_{\text{res}}$  by 5 % on average per day. Provided accurate estimates of wound width and sapwood conducting area are obtained at the end measurement period the HR and CHP methods can be used with confidence in *Citrus sinensis*. However, it is advised that during the measurement period a calibration is performed against an independent measure of transpiration, as wound width and sapwood conducting area do vary between trees and are difficult to determine with a great degree of accuracy.

Additionally quantification of SFD at half hourly intervals of the trees instrumented with the HR and CHP method in a glasshouse and hourly intervals for the trees instrumented with the HR method in the field was conducted and compared with the VPD, leaf water potential (LWP) and stomatal conductance ( $g_s$ ) for selected days. For both experiments a second order polynomial relationship was observed between transpirational sap flow and VPD ( $R^2 = 0.60$  for the HR method and  $R^2 = 0.72$  for the CHP method in the glasshouse and  $R^2 = 0.72$  and  $R^2 = 0.87$  for tree 1 and 2 instrumented with the HR method in the field). Leaf conductance was predominantly controlled by VPD and LWP for the glasshouse experiment, but this was not observed in the field, as the trees were exposed to wind which can affect the  $g_s$  of the trees. Leaf to leaf variation in  $g_s$  was most pronounced in the field trees when compared to the trees in the glasshouse. The hourly rate of change in LWP showed several distinct oscillations during the day which were also observed in  $g_s$ . The variations in sap flow were better explained by the VPD for both glasshouse and in field trees than the  $g_s$ , which yielded no significant relationship with sap flow.

# Contents

Declaration.....	i
Acknowledgements.....	ii
Conference contributions .....	iii
Abstract.....	iv
Tables.....	xi
Table of figures .....	xii
List of abbreviations .....	xviii
Outline of the dissertation .....	xx
CHAPTER 1 General introduction .....	1
1.1 Hypotheses.....	5
1.2 Aim .....	6
1.3 Objectives.....	6
CHAPTER 2 Literature review.....	7
2.1 The movement of sap in plants .....	7
2.1.1 Water flow from the soil to the roots.....	9
2.1.2 Water flow through the stem (root pressure theory) .....	10
2.1.3 Water flow from the leaves to the atmosphere .....	11
2.2 Methods for measuring tree water use .....	11
2.2.1 Sap flow techniques.....	12
2.2.2 Background of heat pulse techniques .....	13
2.3 Methods for calibration and validation of sap flow techniques .....	21
2.3.1 Weighing lysimeters.....	21
2.3.2 Potometer or cut tree method .....	23
2.3.3 Stem perfusion.....	24

2.3.4	Micrometeorological methods .....	25
2.4	General conclusions .....	27
CHAPTER 3 Validating sap flux density measurement methods in a glasshouse using weighing lysimeters.....29		
3.1	Introduction.....	29
3.2	Materials and methods.....	30
3.2.1	Experimental site .....	30
3.2.2	Plant material.....	30
3.2.3	Experimental design and measurement protocol .....	32
3.2.4	Calibration of the load cell on the weighing lysimeters .....	32
3.2.5	Sap flow equipment and measurements .....	36
3.2.6	Data analysis .....	39
3.2.7	Parameters to calculate sap flux density .....	39
3.2.8	Heat ratio and compensation heat pulse method error analysis .....	43
3.3	Results and discussion .....	44
3.3.1	Weather variables.....	44
3.3.2	Testing the heat ratio method in <i>E. marginata</i> .....	45
3.3.3	Validating sap flux density measurement methods in ‘Midnight’ Valencia ( <i>Citrus sinensis</i> L. Osbeck).....	48
3.3.4	Sap flow quantification in the scion versus the rootstock for potted ‘Midnight’ Valentias in the glasshouse.....	58
3.4	Conclusions .....	60
CHAPTER 4 Infield validation of the sap flux density measurements using a micrometeorological method .....		
4.1	Introduction.....	62
4.2	Materials and methods.....	62
4.2.1	Description of the experimental orchard.....	62

4.2.2	Evapotranspiration measurements.....	64
4.2.3	Soil evaporation .....	66
4.2.4	Sap flow measurements .....	67
4.2.5	Empirical determination of wound correction coefficient.....	69
4.2.6	Infield determination of the wound correction coefficient and sapwood depth .....	70
4.3	Results and discussion .....	72
4.3.1	Weather variables.....	72
4.3.2	Evaporative water loss from the soil.....	73
4.3.3	Hourly measured sap flux densities using the heat ratio method.....	75
4.3.4	Hourly measured sap flux densities using the compensation heat pulse method .....	76
4.3.5	Comparison of daily reference evapotranspiration, evapotranspiration and $T_{sap}$ from the HR and CHP methods.....	77
4.3.6	Comparison of daily sap flow ( $T_{sap}$ ) versus residual transpiration .....	78
4.3.7	Comparison of the calibrated daily sap flow to transpiration.....	80
4.3.8	Comparison of the daily sap flow calculated using actual wound width and heartwood radius to residual transpiration.....	83
4.4	Conclusions .....	84
CHAPTER 5	Comparison of sap flux density measurements with vapour pressure deficit, stomatal conductance and leaf water potentials .....	86
5.1	Introduction .....	86
5.2	Materials and methods.....	86
5.2.1	Weather data .....	86
5.2.2	Physiological measurements .....	86
5.3	Results and discussion .....	88
5.3.1	Oscillations in sap flow for <i>Citrus sinensis</i> trees in a glasshouse and under field conditions.....	88
5.3.2	Oscillations in stomatal conductance and leaf water potential in a glasshouse and under field conditions .....	90

5.4	Conclusions .....	97
CHAPTER 6	General conclusions.....	98
	Recommendations and future research .....	103
	References .....	105
	Appendix.....	119

## Tables

<b>Table 3-1</b> Details of the <i>E. marginata</i> tree used for testing the heat pulse velocity equipment ..	30
<b>Table 3-2</b> 'Midnight' Valencia citrus trees used for testing of the HR method and CHP method sap flow equipment .....	31
<b>Table 4-1</b> Trunk circumference (mm) and probe insertion depths for trees selected for sap flow measurements using the HR method .....	68
<b>Table 4-2</b> Trunk circumference (mm) and probe insertion depths for trees selected for sap flow measurements using the CHP method.....	69
<b>Table 4-3</b> The coefficients of the regression equations ( $ax^2+bx+c$ ) between the resulting $T_{sap}$ for a specific wound width and the apparent wound width for the specific day which matched $T_{res}$ .	81
<b>Table 4-4</b> Statistical analysis of the correction factors for 12 wound sizes. ....	82

## Table of figures

**Figure 2-1** A schematic representation of water movement through the soil-plant-atmosphere continuum according to the cohesion-tension theory  
(<https://www.boundless.com/biology/textbooks/boundless-biology-textbook/plant-form-and-physiology-30/transport-of-water-and-solutes-in-plants-183/movement>) ..... 8

**Figure 2-2** Pathways for the movement of water and solutes in roots (source: [http://www.nicerweb.com/bio1152/locked/media/ch36/root\\_transport.html](http://www.nicerweb.com/bio1152/locked/media/ch36/root_transport.html))..... 10

**Figure 2-3** Configuration of the CHP method probes inserted into a stem of radius  $R$  with the heartwood boundary at distance  $h$  from the centre of the stem. Adapted from Smith and Allen (1996) ..... 15

**Figure 2-4** Transfer of the heat pulse released from the heater probe into a stem containing moving sap for the configuration shown in Figure 2-3. 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) ..... 16

**Figure 2-5** Configuration of the HR method probes inserted into a stem of radius  $R$  with the heartwood boundary at distance  $h$  from the centre of the stem. Adapted from Burgess et al. (2001) ..... 19

**Figure 2-6** Diagrammatic representation of the stem perfusion experiment using a Mariotte-based verification system (Steppe et al. 2010) ..... 25

**Figure 2-7** Comparison between residual transpiration measured by the eddy covariance system and microlysimeters and transpiration determined using the compensated heat pulse (CHP) method for three seasons in a Merlot vineyard (Poblete-Echeverría et al. 2012). ..... 27

**Figure 3-1** Cantilever weighing lysimeters for checking the calibration of sap flow sensors ..... 32

<b>Figure 3-2</b> 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.....	33
<b>Figure 3-3</b> Stability of the load cell output over a seven day period (23 – 30 July 2014) as observed from the mV readings. ....	34
<b>Figure 3-4</b> (A) Placement of the <i>E.marginata</i> trees and (B) attachment of 2 L beaker for recalibration on the weighing lysimeters and plastic covering to eliminate evaporation and drainage from the pots .....	35
<b>Figure 3-5</b> Regression analysis for the calibration of lysimeter 1 and 2 after the placement of trees on the weighing lysimeters .....	35
<b>Figure 3-6</b> Placement of the trees on the weighing lysimeters and probe installation .....	36
<b>Figure 3-7</b> Heat pulse velocity determined at 8 mm below the cambium for the HR method, bold line is the adjusted baseline and dashed line is the raw data from the logger for citrus from 08 – 19 Oct 2015. ....	38
<b>Figure 3-8</b> Heat pulse velocity determined at 8 mm below the cambium for the CHP method, bold line is the adjusted baseline and dashed line is the raw data from the logger .....	39
<b>Figure 3-9</b> Schematic outlay of the process for determining the sapwood conducting area .....	40
<b>Figure 3-10</b> Determination of the sapwood (pink stained) and the heartwood (white unstained area) using Adobe Photoshop.....	41
<b>Figure 3-11</b> Wounding response in 'Midknight' Valencias after HR probe installations (A) and for the CHP probe installations (B), measurement of the wound width with a vernier callipers (C) .	43
<b>Figure 3-12</b> Hourly values of average air temperature (°C), relative humidity (%) and average VPD (kPa) in the glasshouse from 9-15 Nov 2015 .....	44
<b>Figure 3-13</b> Hourly sap flux densities (SFD) of the HR method installed at 8 and 12 mm depth in <i>E. marginata</i> .....	45

**Figure 3-14** Diurnal trend for (A) hourly mass loss between the gravimetric method (lysimeter) and sap flow method (HR method) on *E. marginata* and (B) relationship between hourly mass loss measured with a weighing lysimeter and heat ratio (HR) method for *E. marginata*.....46

**Figure 3-15** Comparison of daily mass loss between the weighing lysimeter and the heat ratio method (HR method) in *E. marginata* dashed line is a 1:1 line .....47

**Figure 3-16** Corrected sap flux densities (SFD) measured at 8, 12 and 15 mm depths for a potted 'Midknight' Valencia tree .....48

**Figure 3-17** Hourly transpiration of 'Midknight' Valencia (A) and (C) measured with the heat ratio method (HR) and gravimetric methods. The relationship between hourly water use measured by the heat ratio and gravimetric methods is given for 'Midknight' Valencia (B) and (D).....49

**Figure 3-18** The relationship between total day time water use measured using a weighing lysimeter and the HR method for a potted 'Midknight' Valencia tree over a period of 37 days. Total day time transpiration was calculated from sum of hourly rates between 06:00 and 18:00 .....51

**Figure 3-19** Comparison of hourly water use of citrus determined with HR method and that determined gravimetrically on irrigated and non-irrigated days.....52

**Figure 3-20** Sap flux densities (SFDs) measured using the CHP method at 8, 12 and 15 mm depth for a potted 'Midknight' Valencia tree.....54

**Figure 3-21** (A) and (C) Hourly transpiration of 'Midknight' Valencia trees measured with the compensation heat pulse method and gravimetric methods. (B) and (D) The relationship between hourly water use measured by the CHP and gravimetric methods is given for the 'Midknight' Valencia trees for two respective methods. ....55

**Figure 3-22** The relationship between daytime (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.....56

<b>Figure 3-23</b> The resultant error in daily sap flow from the (A) CHP method and (B) HR method due to errors in selected variables used in the determination of the sap flow .....	58
<b>Figure 3-24</b> Diffusely arranged active xylem in the scion (A) and the evenly distributed active xylem in the rootstock of the same tree (B) .....	59
<b>Figure 3-25</b> Cross section micrograph of diffusely arranged active xylem in the scion (A) and the evenly distributed active xylem in the rootstock of the same tree (B).....	59
<b>Figure 4-1</b> Location of the 'Washington' navel orchard and the automated weather station .....	63
<b>Figure 4-2</b> Lattice mast in the 'Washington' navels orchard showing position of eddy covariance sensors .....	64
<b>Figure 4-3</b> 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 .....	66
<b>Figure 4-4</b> Diagrammatic representation for the placement of microlysimeters (double circles) for evaporation measurements in the 'Washington' Navel orchard .....	67
<b>Figure 4-5</b> Regression analysis of wound width against the resulting $T_{sap}$ for (A) the CHP method and (B) the HR method .....	70
<b>Figure 4-6</b> Wounding response in 'Washington' navels at the end of the measurements (24 months).....	71
<b>Figure 4-7</b> Schematic outlay of the process for determining the sapwood depth .....	72
<b>Figure 4-8</b> Hourly values of average air temperature ( $^{\circ}\text{C}$ ), solar radiation ( $\text{W m}^{-2} \cdot 100$ ), rainfall (mm), wind speed ( $\text{m s}^{-1}$ ) and average VPD (kPa) at Patrysberg from 10-14 March 2015.....	73
<b>Figure 4-9</b> Cumulative soil evaporation in the 'Washington' Navel orchard on 10 of March .....	74
<b>Figure 4-10</b> Total daily soil evaporation (E) ( $\text{mm day}^{-1}$ ) and evapotranspiration (ET) ( $\text{mm day}^{-1}$ ) in the 'Washington' Navel orchard from 10-14 March 2015 .....	75
<b>Figure 4-11</b> Sap flux densities (SFD) measured using the HR method with a wound correction factor of 2.5 mm at 8, 13, 22 and 30 mm depth for a 'Washington' Navel tree.....	76

**Figure 4-12** Sap flux densities (SFD) measured using the CHP method with a wound correction factor of 2.5 mm at 10, 15, 25 and 35 mm depth for ‘Washington’ navels.....77

**Figure 4-13** Comparison of daily evapotranspiration ( $\text{mm day}^{-1}$ ), transpiration estimates from HR and CHP methods using a wound width of 2.5 mm, and reference evapotranspiration from ‘Washington’ Navels from 10 - 14 Mar 2015.....78

**Figure 4-14** Daily total  $T_{\text{res}}$  and  $T_{\text{sap}}$  determined using the heat ratio (HR) method and compensation heat pulse (CHP) methods (A) and correlation of  $T_{\text{res}}$  and  $T_{\text{sap}}$  determined with the HR and the CHP methods for the ‘Washington’ Navel orchard (B). The dashed line is the 1:1 line .....79

**Figure 4-15** (A) Daily total residual transpiration and calibrated sap flow by the heat ratio (HR) and compensation heat pulse (CHP) method and (B) Regression analysis of daily  $T_{\text{res}}$  with the HR and the CHP method ‘Washington’ Navel orchard. The dashed line is a 1:1 line. ....83

**Figure 4-16** (A) Daily total residual transpiration and sap flow by the heat ratio (HR) method and (B) Regression analysis of daily  $T_{\text{res}}$  with the HR method ‘Washington’ Navel orchard. The dashed line is a 1:1 line.....84

**Figure 5-1** The diurnal trend of sap flow and VPD of ‘Midnight’ Valencia determined with A) CHP and C) HR method on a typical clear day. Each data point is an average of 30 minutes, (B) and (D) the relationship between sap flow and VPD for the ‘Midnight’ Valencia trees in a glasshouse.....89

**Figure 5-2** The diurnal trend of VPD and sap flow determined using the heat ratio (HR) method in a ‘Washington’ Navel orchard for (A) tree 1 and (C) tree 2 on a typical clear day. Each data point is an average of 1 hour. The relationship between sap flow and VPD for (B) tree 1 and (D) tree 2 for the ‘Washington’ Navel trees infield. ....90

**Figure 5-3** The diurnal trend of sunlit leaf stomatal conductance of ‘Midnight’ Valencia trees on a typical clear day in a glasshouse for (A) tree 1 and (B) tree 2. The relationship between sunlit leaf  $g_s$  and VPD is given for the ‘Midnight’ Valencia trees for (C) tree 1 and (D) tree 2 average

of two days 11 and 13 November 2015. The relationship between sunlit leaf  $g_s$  and LWP is given for the 'Midknight' Valencia trees for (E) tree 1 and (F) tree 2 for 11 and 13 November. ...91

**Figure 5-4** The diurnal trend of sunlit leaf stomatal conductance of 'Washington' navel trees on a typical clear day in the field for (A) tree 1 and (B) tree 2. The relationship between sunlit leaf  $g_s$  and VPD is given for the 'Washington' navel trees for (C) average of 4 trees on 11 March 2015 and (D) average of 4 trees on 12 March 2015. The relationship between sunlit leaf  $g_s$  and LWP is given for the 'Washington' navel trees for (E) average of 4 trees on 11 March 2015 and (F) average of 4 trees on 12 March 2015. ....93

**Figure 5-5** Diurnal trend of the LWP for Midknight' Valencia (A) tree 1 and (B) tree 2. Rate of change of leaf water potential ( $\Delta\psi_l/\Delta t$ ) on 'Midknight' Valencia (C) tree 1 and (D) tree 2 on 11 November in a glasshouse vertical bars indicate two standard deviations. The relationship between sunlit LWP and VPD is given for the 'Midknight' Valencia trees for (E) tree 1 and (F) tree 2 for 11 and 13 November .....95

**Figure 5-6** Diurnal trend of the LWP for 'Washington' navel (A) tree 1 and (B) tree 2. Rate of change of LWP ( $\Delta\psi_l/\Delta t$ ) on 'Washington' navel (C) tree 1 and (D) tree 2 on 11 March 2015 in the field vertical bars indicate two standard deviations. The relationship between sunlit LWP and VPD is given for the 'Washington' navel trees for (E) tree 1 and (F) tree 2 for 11 and 13 March 2015.....96

## List of abbreviations

<b>Abbreviation</b>	<b>Explanation</b>	<b>units</b>
CGA	Citrus Growers Association	-
CHP	Compensation Heat Pulse	-
EBC	Energy Balance Closure	-
EBr	Evaporation between the tree rows	mm
EC	Eddy Covariance	-
Ecan	Evaporation under the tree canopy	mm
Es	Soil evaporation	mm
ETo	Reference evapotranspiration	mm
es	Saturated vapour pressure	kPa
ET	Evapotranspiration	mm
G	Soil heat flux	W m <sup>-2</sup>
gs	Stomatal conductance	mmol m <sup>-2</sup> s <sup>-1</sup>
H	Sensible heat flux	W m <sup>-2</sup>
HFD	Heat Field Deformation	-
HR	Heat Ratio	-
IRGA	Infra-Red Gas Analyser	-
LE	Latent heat flux	W m <sup>-2</sup>
LWP	Leaf Water Potential	MPa
MAE	Mean Absolute Error	-
MBE	Mean Biased Error	-
Mc	Wood moisture content	g
RH	Relative Humidity	%
RMSE	Root Mean Square Error	-

SFD	Sap Flux Density	$\text{cm}^3 \text{ cm}^{-2} \text{ s}^{-1}$
Rn	Net radiation	$\text{W m}^{-2}$
SHB	Stem Heat Balance	-
Ta	Air temperature	$^{\circ}\text{C}$
TDP	Thermal Dissipation Probe	-
THB	Trunk Heat Balance	-
Tres	Transpiration determined as a residual of evapotranspiration and evaporation	mm
Tsap	Transpiration determined by sap flow	$\text{cm}^3$
VPD	Vapour Pressure Deficit	kPa
VSF	Volumetric Sap Flow	$\text{cm}^3$
Wd	Oven dried wood mass	g
Wf	Wood fresh mass	g
WRC	Water Research Commission	-

## Outline of the dissertation

**Chapter 1** provides an introduction to the dissertation and defines and categorises different sap flow methods. It also outlines the motivation for the study, hypotheses, aims and objectives of this study.

**Chapter 2** is a literature review which covers water transport in plants, describing the driving forces behind plant water uptake and the pathways water follow through the plant. Various sap flux density (SFD) measurements and the theory behind the measurement methods, such as the (compensation heat pulse) CHP and (heat ratio) HR methods, is discussed. In addition, methods of validating SFD measurement methods are described, with reference to previous work that has been done.

**Chapter 3** outlines the methodology for validating two SFD measurement methods (CHP and HR) in a glasshouse using a weighing lysimeter. Prior to the measurements in citrus, *Eucalyptus marginata* was used as a model species to test the equipment and methodology SFD measurements and the results are presented in this chapter. The results for the testing of the three SFD methods in *Citrus sinensis* are presented.

**Chapter 4** describes the methodology and the results for the infield calibration of two SFD methods (CHP and HR). Transpiration was determined as a residual of evapotranspiration measured with an eddy covariance system and evaporation from the soil determined using microlysimeters. In this chapter calibration focussed on a wound width correction and the empirically determined wound correction factors are presented.

**Chapter 5** discusses the relationship between SFD measurements and vapour pressure deficit, stomatal conductance and leaf water potential.

**Chapter 6** summarises the main findings of this Master's study and formulates their implications and challenges for on-going and future research. Questions for future research and promising areas for future work are also discussed.

## CHAPTER 1

## General introduction

Quantification of plant transpiration 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 transpiration, viz. plant physiology based (chamber system and sap flow methods), hydrological based methods (weighing lysimeters) and micrometeorological based methods (aerodynamic and eddy covariance (EC) methods) (Rana and Katerji, 2000). 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 able to 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 alternatives for estimating tree transpiration. They are also powerful tools for scaling between an individual tree to an entire plant community, and also for the measure of plant water use in a multifaceted heterogeneous terrain (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 transpiration (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 and the remaining 95 % is lost into the atmosphere by transpiration through stomata in the leaves (Schroeder et al. 2001).

Sap flow measurement techniques are thermal 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) and comprise the trunk heat balance (THB) and stem heat balance (SHB) techniques. 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 (Smith and Allen 1996). Gauges should also be installed on straight sections of the stem without 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 probe (TDP) method (Granier,1981) 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 the thermocouple downstream from the heater needle. Heat pulse techniques include the heat ratio (HR) method and the compensation heat pulse (CHP) method (Ortuño 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 and Clothier, 1988). They can be used to quantify water use without calibration in plants, which are classified as thermally homogeneous (Smith and Allen, 1996) and have been used intensively to estimate transpiration of individual trees (Dunn and Connor 1993; Barrett et al. 1995; Bauerle et al. 2002). However, it is essential 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 SFD ( $\text{cm}^3 \text{cm}^{-2} \text{h}^{-1}$ ) such as TDP, CHP and HR methods, which assesses the amount of sap flowing through a certain surface of stem per time and those measuring volumetric sap flow rate ( $\text{g h}^{-1}$ ) such as SHB and THB, that determines the total sap flow in a plant stem or stem section (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 (Vandegehuchte and Steppe, 2013). 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). Which 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 cause sap stream disruptions which can modify the thermal homogeneity of the adjacent sap-conducting wood, and ultimately leads to a systematic underestimation of measured heat pulse velocity (Fernández et al. 2001). As a result, calibration coefficients which relate the measured heat pulse velocity 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). Fernández et al. (1999), highlighted that theoretical calibration coefficients have not been widely tested and that further tests are required. In addition, 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 sap flow techniques. These include the cut tree method (Dunn and Connor 1993; Barrett et al. 1995; Dye et al. 1996), stem perfusion (Cohen et al. 1981; Green and Clothier, 1988; Marshall et al. 1997; Fernández et al. 1999;

Fernandez et al. 2001; Phillips et al. 2009; Steppe et al. 2010), micrometeorological methods (Williams et al. 2004; Poblete-Echeverria 2012), and weighing lysimeters (Dugas et al. 1992; Zreik et al. 2003; Bleby et al. 2004; Nortes et al. 2008).

In this study the accuracy of the CHP and HR methods was checked in *Citrus sinensis* using weighing lysimeters in a glasshouse, which provide reliable, accurate measurements of transpiration if properly constructed. Two assumptions were employed with the use of weighing lysimeters namely, (i) water loss only occurred through the plant, and; (ii) the plant lost no mass other than that of water (Cirelli et al. 2012). Two SFD measurement methods were employed in-field as they have low power requirements and therefore suited for measurements in remote locations. In-field validation of the HR method and the CHP method was also conducted against micrometeorological methods in a commercial orchard to assess the validity of the calibration performed under controlled conditions. *Citrus sinensis* was chosen for this study as there is a need for good estimates of citrus water use and it is widely grown in South Africa and in order to ensure accurate water use estimates, an appropriate sap flow technique needs to be calibrated for citrus. In South Africa citrus is produced in the Western Cape, Northern Cape, Eastern Cape, Limpopo, Mpumalanga and KwaZulu Natal provinces, where rainfall is seasonal. Since citrus is an evergreen tree, farmers have to supplement crop water needs with irrigation. According to the Citrus Growers Association (CGA) approximately 60 355 ha were under citrus production (CGA, 2012) and this has further increased by 4 155 ha over three years to about 64 510 ha (CGA, 2015). Of the 64 510 ha 41 110 is under *Citrus sinensis* cultivation constituting about 64 % of the total citrus production (CGA, 2015). As a result, the demand for irrigation water is gradually increasing and projections show that water shortage will become an increasingly important problem in South Africa, since 98% of the available water is already allocated (Von Bormann and Gulati, 2014). The increase in demand for irrigation water has prompted research to determine the specific water needs of citrus. Therefore, the Water Research Commission

(WRC) of South Africa solicited and funded a project on water use in citrus orchards (Project K5/2275//4). In this study heat pulse velocity techniques were chosen for calibration as the equipment is much cheaper and they are well-established methods in the research group.

## 1.1 Hypotheses

1. A positive linear relationship ( $R^2 \geq 0.7$ ) exists between the HR method and the weighing lysimeter in *Eucalyptus marginata*, a model species for sap flow measurements, which indicates that the heat pulse velocity equipment is fully functional.
2. An intercept equal to zero and slope equal to unity at 95% confidence level will be observed with a linear regression analysis between:
  - i. Transpiration determined by the weighing lysimeter and  $T_{\text{sap}}$  (transpiration determined by a sap flow method i.e. HR method and the CHP method in the glasshouse).
  - ii. Transpiration determined as a residual of evapotranspiration and evaporation and  $T_{\text{sap}}$  determined by a calibrated HR method and CHP method in the field.
3. The HR method will estimate sap flow more accurately in small potted citrus trees than the CHP method when compared to the weighing lysimeter, as the citrus canopy is small and the HR method is reported to capture low flow rates more accurately than the CHP method.
4. The CHP method will estimate sap flow more accurately in field citrus trees than the HR method when compared to the micrometeorological method, as a mature citrus canopy is large and the CHP method is reported to capture high flow rates more accurately than the HR method.
5. Variations in sap flow throughout the day in citrus can be explained by stomatal oscillations and a direct linear relationship will be observed between stomatal conductance ( $g_s$ ) and sap flow.

## 1.2 Aim

The aim of this study was to:

1. To validate and determine an appropriate SFD measurement method to quantify transpiration in *Citrus sinensis*.

## 1.3 Objectives

1. To establish the performance of CHP and HR methods in citrus and *E.marginata*
2. To determine if a constant positive linear correlation ( $R^2 \geq 0.70$ ) exists between the SFD measurement methods and the weighing lysimeter in *Citrus sinensis*.
3. To determine the calibration coefficients of a number of test trees of the same species in the glasshouse and if these coefficients would be applicable to measurements in the field.
4. To ascertain if the variations in sap flow throughout the day were due to variations in stomatal conductance.

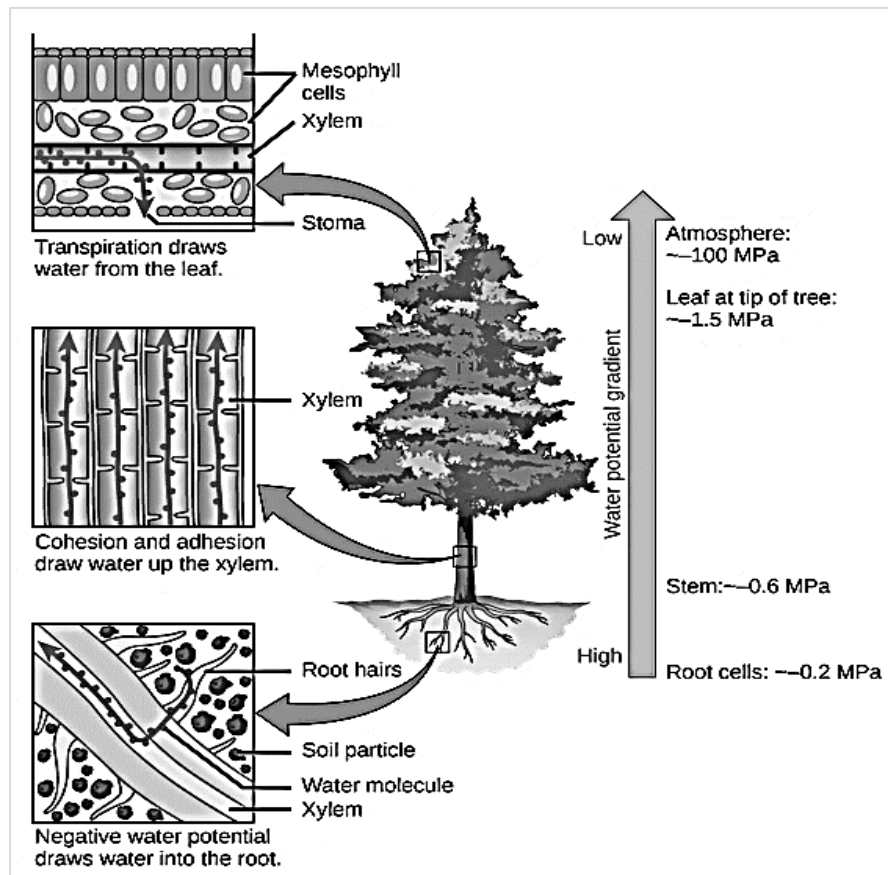
## CHAPTER 2

## Literature review

### 2.1 The movement of sap in plants

Water is essential in plants for maintenance of turgor, as a solvent and as a medium for all biochemical reactions. A large proportion of water taken up by trees is lost back to the atmosphere through transpiration (Jackson et al. 2000). For plants to survive, enough water needs to move from the roots to the leaves to replenish the water lost through transpiration (Kramer and Boyer, 1995). Water moves from the soil to plant to the atmosphere along a continuum of increasingly negative water potential, flowing 'downhill' thermodynamically but 'uphill' physically from root to shoot (Jackson et al. 2000), as shown in Figure 2-1. The cohesion-tension theory which explains the mechanism of the ascent of water in plants was suggested by Dixon and Joly (1894) around the 19th century and has not been modified for more than 100 years. The theory states that water transport in plants is mainly driven by transpirational pull. The tensional force required for the continuous running of water columns is conveyed from the transpiring surfaces of the leaves to the roots (Meinzer et al. 2001). Water loss from plant cells, mainly those in the leaves, causes a decrease in water potential, which results in the movement of water from the xylem to the cell surfaces. This lessens the pressure in the xylem sap and creates a water potential gradient or tension in the cohesive hydraulic system of the plant (Kramer and Boyer, 1995). The tension at the evaporating surface of the leaves is eventually conveyed to the roots where it causes a drop in the water potential of the roots below the water potential of the soil water, as illustrated in Figure 2-1 (Tyree, 1997). This results in the uptake of water from the roots all the way to the leaves to replenish the water that has been lost from the leaf surfaces (Tyree, 1997). Plant water loss by transpiration and evaporation is regulated by vapour pressure deficit (VPD) and stomatal conductance ( $g_s$ ) (Jackson et al. 2000). The transpiration rate is governed primarily by the continuous supply of

water, energy to vaporise the water and the resistance of the vapour pathway (Kramer and Boyer, 1995).

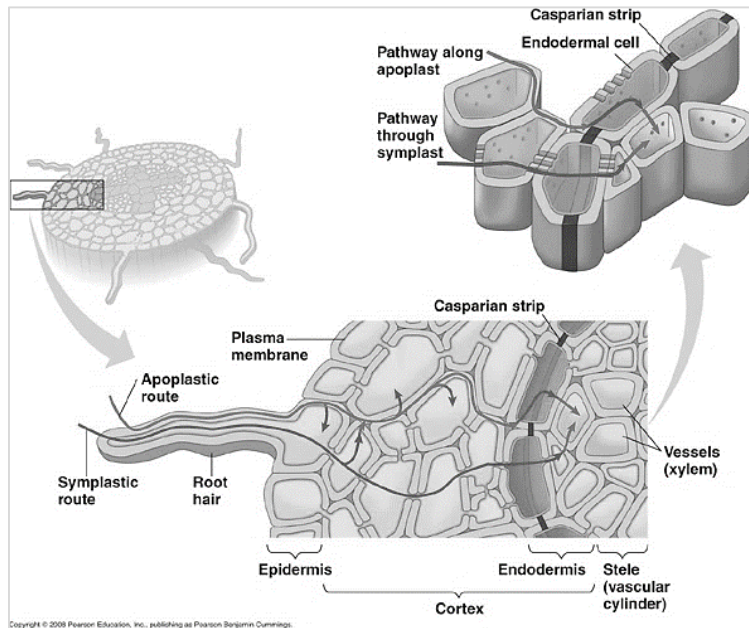


**Figure 2-1** A schematic representation of water movement through the soil-plant-atmosphere continuum according to the cohesion-tension theory (<https://www.boundless.com/biology/textbooks/boundless-biology-textbook/plant-form-and-physiology-30/transport-of-water-and-solutes-in-plants-183/movement>)

Dixon and Joly (1894) mistakenly concluded that water ascent in the trees is achieved only by tension created in the vessels due to transpiration pull. However, Zimmermann et al. (1995) was able to demonstrate using a xylem pressure probe that water ascent in plants is also facilitated tissue osmotic pressure.

### **2.1.1 Water flow from the soil to the roots**

Water moves in soil from an area with a high (less negative) to an area with lower (more negative) water potential. As the plant takes up water, the soil around the roots become drier than the bulk soil, consequently, water flows radially from the bulk soil to the roots to replace the absorbed water. Although plants can absorb water through the stem and leaf surfaces, the majority of water that is used by plants is absorbed by the roots and is considered as the primary pathway for water uptake (Jackson et al. 2000). Water is absorbed mostly in the root hair zone. The root hairs are delicate, fine epidermal cell extensions, which lack a cuticle and provide a large surface area for water uptake. Water uptake can take place *via* two mechanisms i.e. passive and active absorption. During the active absorption, water is absorbed with the aid of energy stored in plants in the form of adenosine tri-phosphate, where absorption takes place against a concentration gradient. In passive absorption, water flows from a high to a low water potential (Steudle, 2000). Rapid transpiration removes water and reduces turgor pressure in living cells of the root (Kramer and Boyer, 1995). The suction force developed is transmitted to the root xylem that pulls water from the surrounding root hair cells to replace the lost water. Water moves through the root cortex via two dominant pathways i.e. apoplast and symplast pathway as shown in Figure 2-2. In the apoplast pathway water moves rapidly, due to its low resistance (Steudle, 2000), within the continuum of cell walls and extracellular spaces without traversing any membranes. Water can only travel up to the endodermis cells where the Casparian layer obstructs further water movement. The Casparian band separates the cortex and the endodermis and is composed of a wax-like substance called suberin, which provides an extracellular diffusion barrier within the plant roots, forcing nutrients and water to pass into the cells and thus to be subjected to the action of plasma membrane transport proteins (Kramer and Boyer, 1995). Water then has to travel via the symplastic pathway through the plasmodesmata from cell to cell.



**Figure 2-2** Pathways for the movement of water and solutes in roots (source: [http://www.nicerweb.com/bio1152/locked/media/ch36/root\\_transport.html](http://www.nicerweb.com/bio1152/locked/media/ch36/root_transport.html))

### 2.1.2 Water flow through the stem (root pressure theory)

The Casparian layer allows the accumulation of solutes in the xylem during conditions of high soil water availability, which lowers the osmotic potential (Taiz and Zeiger, 2006). Consequently, water moves into the xylem through osmosis down the water potential gradient (Taiz and Zeiger, 2006). The accumulation of water in the xylem generates a higher hydrostatic pressure in the root, which propels water up the xylem (Steudle, 2000). This is clearly noticeable when whole tree or branch excision is performed and xylem sap continues to exude from the stump. The root pressure adds to the water flow up the xylem in small trees, but is inadequate to force water up a tall tree. In addition, root pressure plays a role in the rehydration of embolized xylem conduits (Meinzer et al. 2001) .

### **2.1.3 Water flow from the leaves to the atmosphere**

In the leaf, the xylem vessels branch to form a system of fine small vessels termed leaf veins (Taiz and Zeiger, 2006). Water moves down a water potential gradient from the leaf veins across adjacent cells. As in the roots, water moves along an apoplastic pathway through the cell walls and a symplastic pathway through the protoplasm. As the water potential of the atmosphere is highly negative, water vapour from the sub-stomatal cavities is drawn through the stomata, thereby lowering the water potential of the mesophyll cells (Taiz and Zeiger, 2006). Water is then transported from the xylem to the mesophyll cells, further propagating tension throughout the water conducting system in the stem and roots. Stomatal transpiration can be actively regulated by the plant through responses to internal factors, such as the internal carbon dioxide or external factors such as light, temperature, air relative humidity and soil water availability (Taiz and Zeiger, 2006).

## **2.2 Methods for measuring tree water use**

Due to the importance of transpiration in plants, several techniques have been developed to estimate transpiration, which include weighing lysimeter (Edwards and Warwick, 1984), volumetric measurements of water in soil and plants, water vapour and sap velocity measurements (Smith and Allen, 1996) and stem heat balance and heat dissipation methods (Granier, 1987). These different techniques have their own merits and demerits, for example weighing lysimeters are very sensitive to small changes in mass loss due to transpiration and evaporation. Nonetheless the roots are limited to the volume of the container in which the plants are grown. Weighing lysimeters are also costly to construct and maintain. The most appropriate technique rests on the type and size of the plant, the duration and objective of the experiment and the apparatus available (Kramer and Boyer, 1995). Sap flux density measurement techniques are practical alternatives to quantify transpiration in a heterogeneous terrain and they are a powerful tool for scaling between the individual trees and the entire plant community

(Vertessy et al. 1997). However, these techniques suffer from uncertainties as a result of empirical calibrations. When employing sap flow techniques, the stem of the tree is the most appropriate location to quantify xylem sap flow and eventually transpiration, if measured over an appropriately long period to overlook changes in stem storage and neglecting the 2-5% of the water taken up that is used for photosynthesis (Swanson, 1994).

### **2.2.1 Sap flow techniques**

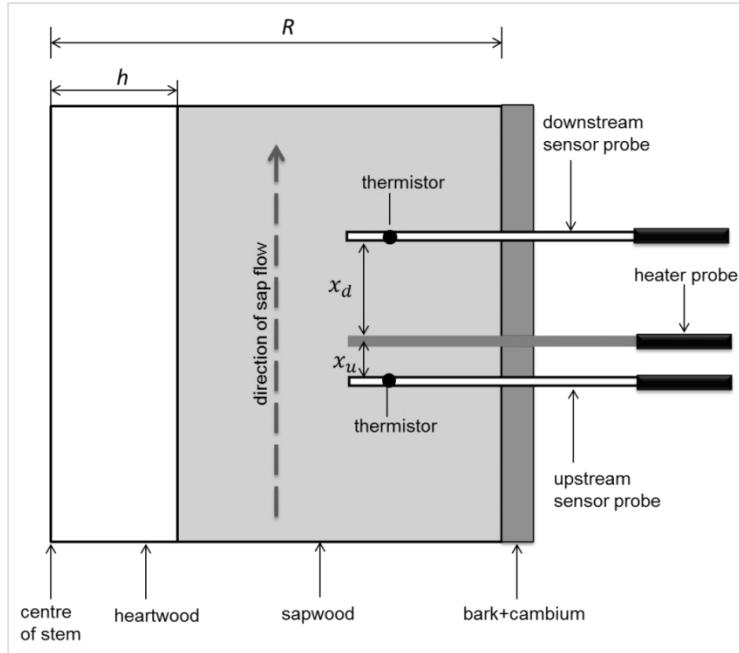
Various SFD measurement techniques have been developed to measure the rate of sap flow in plant stems, the techniques comprise of those that measure heat transported away from a controlled heat source by moving sap, those that relate heat dissipation to sap flow by an empirical relationship and those that trace the movement of a heat pulse in the sap flow (Burgess et al. 2001). Techniques that employ a heat pulse are frequently preferred since they have simple instrumentation and low power requirements. Amongst the heat pulse methods, the CHP method has been most extensively employed for high flow rates (Fernández et al. 2006), and the HR method was specifically developed for low flow rates and reverse flows (Burgess et al. 2001). When employing SFD measurement techniques precautions have to be taken due to three major methodological problems. Firstly holes have to be drilled into the tree trunk to accommodate the heater needle and the thermocouples, which causes wounding (Smith and Allen 1996) and eventually interrupts the natural stream of water in the xylem vessels. Numerical solutions have been developed to correct for wounding but these depend on accurate measurement of wound widths (Swanson and Whitfield, 1981). Secondly, precise insertion of the heater probe relative to the thermocouples is precarious, the heater probe and the thermocouples can be easily misaligned during placement into the stem leading to errors which are difficult to distinguish (Dye and Olbrich, 1993). Lastly, the heater probe that is implanted into the tree trunk can cause a temperature rise of the sapwood which can be sufficient to cause damage to the living tissue (Bauerle et al. 2002).

### **2.2.2 Background of 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 perceived by Huber (1932). In his experiments, Huber (1932) 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. 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 (1932) initially concluded that the method was only suitable for sap velocities greater than  $60 \text{ cm h}^{-1}$ , but realised later the significance of differentiating between the transport of heat by thermal conduction and the influence of convection by the moving sap. Huber and Schmidt (1937) placed thermocouples upstream and downstream from the heater to distinguish between these two effects, and this became the early form of the CHP method. 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 and Clothier, 1988). 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 sap velocities ( $< 0.01\text{-}0.02 \text{ mm s}^{-1}$ ) and were indistinguishable from zero flows with the CHP method (Becker, 1998). In such circumstances, the sensors record equivalent 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 (Becker, 1998). 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 thermocouples are placed equidistant (0.5 cm) from the heater (Burgess et al. 2001).

### 2.2.2.1 Theory of compensation heat pulse (CHP) method

The CHP method functions on the principles described by Swanson and Whitfield (1981); Smith and Allen (1996) and 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 radiata* (Teskey and Sheriff, 1996), willow (Green et al. 2003) and lemon (Alarcón et al. 2005), 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). However, the CHP method is unable to quantify reverse, low or very high sap flux densities ( $<5 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$  and  $>100 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$ ) 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 2-3), 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).

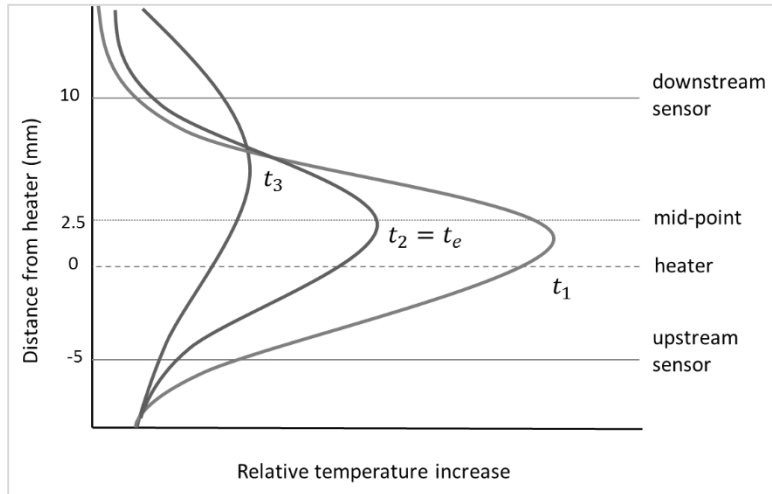


**Figure 2-3** Configuration of the CHP method probes inserted into a stem of radius  $R$  with the heartwood boundary at distance  $h$  from the centre of the stem. Adapted from Smith and Allen (1996)

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 2-4). 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 3600 converts seconds to hours.



**Figure 2-4** Transfer of the heat pulse released from the heater probe into a stem containing moving sap for the configuration shown in Figure 2-3. 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. 2000) 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), which leads to the underestimation of the heat pulse velocity. Heat pulse velocity can be corrected ( $V_r'$ ,  $\text{cm h}^{-1}$ ) for the induced effect of wounding and to account for the influence of materials used to construct the heater and sensor probes using the wound coefficients 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 ( $\text{cm h}^{-1}$ ) can be calculated using equation 6 (Barrett et al. 1995):

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

Where  $C_w$  and  $C_s$  are the heat capacities of wood and water respectively, with  $C_w = 1200 \text{ J kg}^{-1} \text{ } ^\circ\text{C}^{-1}$  at a temperature of  $20^\circ\text{C}$  (Edwards et al. 1997),  $C_s = 4182 \text{ J kg}^{-1} \text{ } ^\circ\text{C}^{-1}$  at a temperature of  $20^\circ\text{C}$ .  $M_c$  is sapwood moisture content,  $\rho_b$  is the wood density ( $\text{g cm}^{-3}$ ) and  $\rho_s$  is density of water ( $\text{g cm}^{-3}$ ).

Integration of individual sap flow velocity measurements to obtain whole stem sap flux was performed according to the method of the weighted sum of sap flux densities 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 sap flux densities at different points in the stem which are weighted according to the area of conducting sapwood for each insertion depth (Steppe et al. 2010):

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

where VSF is the volumetric sap flow  $\text{L h}^{-1}$ , which can be considered as the product of sapwood conducting area and sap velocity (Pfausch et al. 2010), 1000 represents the conversion factor

from cm<sup>3</sup> to L, n is the number of thermocouples for each heater probe and A<sub>n</sub> is different conducting sapwood areas (cm<sup>2</sup>) for each insertion depth.

### Calibration of the compensation heat pulse method in citrus

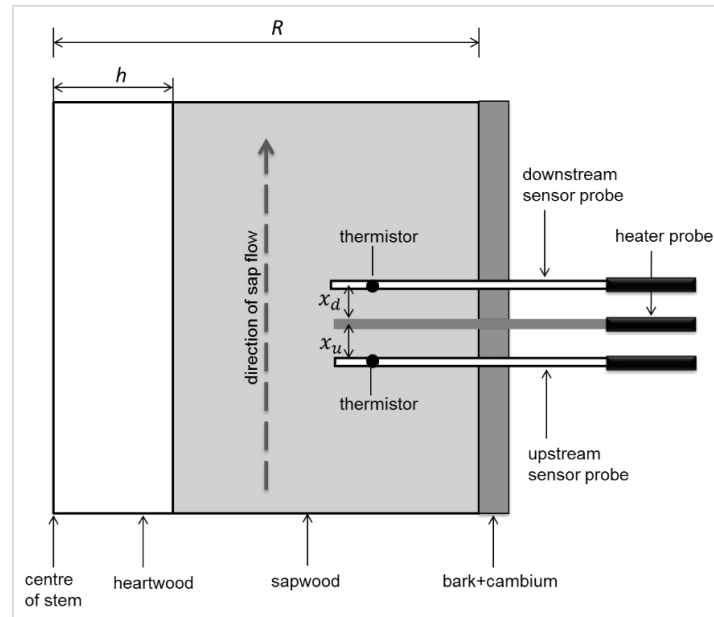
The CHP method was compared with a gravimetric method (weighing balances) to estimate transpiration in 2-year-old *Citrus limon* trees (Alarcón et al. 2005). A good correlation was found between the CHP method and the gravimetric with a coefficient of determination (R<sup>2</sup>=0.97) Fernández et al. (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 diameter 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.

#### **2.2.2.2 Theory of the heat ratio (HR) method**

Becker (1998) highlighted the limitations of the CHP method in measuring low sap flow rates. The CHP method cannot distinguish between sap flow velocities lower than 0.02 mm s<sup>-1</sup> and zero (Becker, 1998). This led to the development of the HR method, which can measure reverse and low flow rates but it is limited for high flux densities >45 cm<sup>3</sup> cm<sup>-2</sup> h<sup>-1</sup> (Vandegheuchte and Steppe, 2013; Burgess et al. 2001). The HR method works on the same principle as the CHP method, but with the difference that the thermocouples are placed equidistance from the heater (Figure 2-5). The HR method has frequently been used to compute water use in *Eucalyptus 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 heat pulse velocity,  $x$  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 positions upstream and downstream of the heater probe respectively,  $k$  is the fresh wood thermal diffusivity ( $2.5 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$ ) and 3600 converts seconds to hours



**Figure 2-5** Configuration of the HR method probes inserted into a stem of radius  $R$  with 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 result 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 Swanson's (1983) solutions did not pass through the origin and the resulting corrections yield a poor approximation of low and reverse flow rates of sap flow.

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

Where  $V_c$  the corrected heat pulse velocity,  $V_h$  is the heat pulse velocity and  $b$ ,  $c$  and  $d$  are the correction coefficients to adjust for wound width ( $x$  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 from Marshall (1958) equation, 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  $C_w = 1200 \text{ J kg}^{-1} \text{ }^\circ\text{C}^{-1}$  and  $C_s = 4182 \text{ J kg}^{-1} \text{ }^\circ\text{C}^{-1}$  are, respectively the specific heat capacities of wood and water at a temperature of  $20 \text{ }^\circ\text{C}$ ,  $M_c$  sapwood moisture content,  $\rho_b$  the wood density ( $\text{g cm}^{-3}$ ) and  $\rho_s$  the density of water ( $\text{g cm}^{-3}$ ). Volumetric sap flow is then calculated as described for the CHP method above using equation 7.

### Calibration of the heat ratio method in citrus

There has not been many studies on the calibration of sap flow techniques, specifically the heat ratio 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. (2014) to quantify transpiration. In their study, transpiration determined via sap flow measurements was scaled up to orchard transpiration 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 which would result in a 1:1 relationship between ET, measured using an EC system and transpiration measured using the HR method and not on the validity of

HR method when compared to an independent measure of transpiration and this left room for research on the accuracy of the HR method compared to the gold standard (gravimetric method) of transpiration measurement in *Citrus sinensis*.

### **2.3 Methods for calibration and validation of sap flow 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 (Cohen and Li, 1996; Fernandez et al. 1999; Steppe et al. 2010). Several independent methods are available to calibrate sap flow techniques, which relate the measured heat pulse velocities to real sap flows. These independent measures include weighing lysimetry, whole plant gas exchange chambers, and cut-tree methods, stem perfusion methods and micrometeorological methods.

#### **2.3.1 Weighing lysimeters**

For the measurement of crop evapotranspiration (ET) 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 from the soil surface using a soil cover, such as plastic (Burgess et al. 2000). Lysimeters are, however, expensive to install and 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 evapotranspiration (Tanner, 1967; Aboukhaled et al. 1982). This should, however, be considered the “gold standard” for tree

transpiration to which sap flow measurements should be compared, as it is a measure of whole plant transpiration that does not incur injury to the plant or embolisms in the xylem tissue.

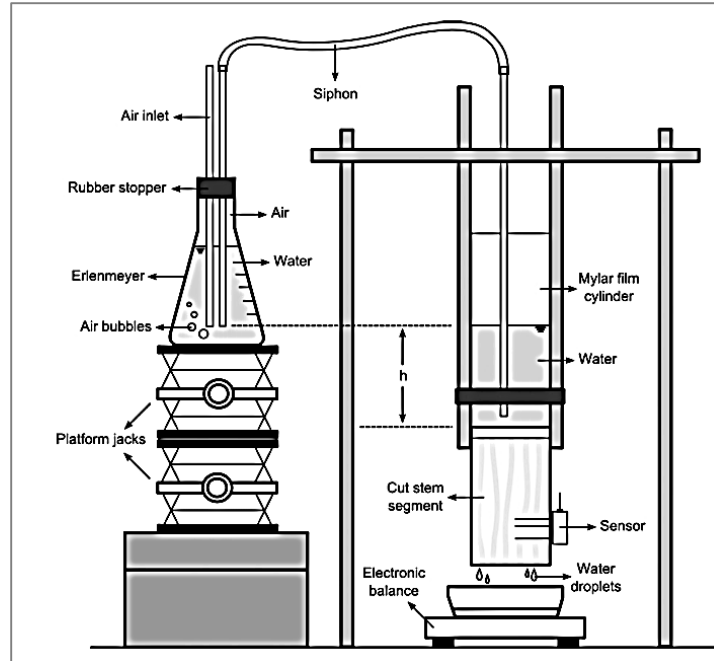
Many types of scale systems have been used in weighing lysimeter designs. Lever-load cell scales are commonly used because counterbalancing is easy and load cell signals can be recorded with high precision electronic data recorders (Howell, 1995). Weighing lysimeters are generally calibrated by adding known quantities of mass to the lysimeter (Howell, 1995) and have been used to quantify transpiration in citrus (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. (2000) in well-watered apricot trees. A strong linear relationship (regression line was within 5% of the 1:1 line) between sap flow and transpiration measured with the weighing lysimeter was found. Vellame et al. (2010) reported that the stem heat balance method proved to be reliable in estimating daily transpiration in citrus trees, but underestimated tree transpiration 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 with a coefficient of determination of 0.92, a slope close to unity and an intercept of zero. Bleby et al. (2004) validated the HR and CHP methods simultaneously in *Eucalyptus marginata* saplings using weighing lysimeters and they found an agreement of 99 % and 96 %, respectively. Slopes of one and intercepts of zero on an hourly basis were realized 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 (Nortes et al. 2008; Shackel et al. 1992), calibration is often performed in potted trees on smaller balances or specially constructed lysimeters with load cells (Alarcón et al. 2005; Burgess et al. 2000). However, potted trees with stem diameters large enough for installation of sap flow sensors are uncommon hence calibrations are also typically more done on smaller SFD's.

### 2.3.2 Potometer or cut tree method

This method of validation can be performed in-field and involves the excision of the whole tree or a branch before dawn. The tree trunk is then re-cut under water to prevent emboli formation in the xylem vessels. Heat-pulse probes are then installed and the tree is mounted in a container of water and oil is applied on the surface or plastic is used to cover the surface to eliminate any possibility of water loss by evaporation (Barrett et al. 1995). The container with water and the excised tree is mounted on an electronic balance or load cell to monitor the change in mass as the excised tree or branch transpires or water uptake can be monitored by refilling it to a fixed mark every 10-15 minutes and measuring the amount required to refill the container. Water loss via transpiration determined from the mass change of the container over time is compared with the sap flow measurements (Smith and Allen, 1996). Water is continuously added in small volumes to the container to prevent large changes in the degree of immersion of the plant stem (Barrett et al. 1995). This method of calibration has been used to calibrate CHP method in apple and kiwifruit (Green and Clothier, 1988), *E. grandis* (Olbrich, 1991), *E. maculate*, *Doryphora sassafras* and *Ceratopetalum apetalum* (Barrett et al. 1995), *E. populnea* (Hatton et al. 1995), *Pinus radiata* (Teskey and Sheriff, 1996), olives (Fernández et al. 2001), and olive, plum and orange (Fernández et al. 2006). The advantage is that the calibration is performed under field conditions in a mature tree and sap flow rates are directly related to transpiration. Another advantage of this technique is that a dye can be placed in the water that will allow the estimation of conducting wood heterogeneity with respect to xylem function (Hatton et al. 1995). However, this method causes substantial damage to the stem during excision and if care is not taken when re-cutting the stem under water, transpiration can only be able to be monitored for 12 - 24 h before the tree wilts beyond recovery. Obviously, it is also destructive and will always result in tree death.

### 2.3.3 Stem perfusion

This method allows the direct comparison of actual SFD with that obtained from a sap flow technique i.e. heat pulse velocity technique. It is a laboratory based technique and as it only requires a portion of stem or branch of sufficient diameter to implant sensors, an entire tree does not need to be destructively harvested. It also allows the calibration of a large number of different citrus species, with wood samples that can be collected from a number of different locations, in a relatively short period of time. Calibration of sap flow methods with this technique was done in *Populus alba L* and *Platanus orientalis L* (Cohen et al. 1981), in olive trees (Fernández et al. 2001), in kiwi fruit (Green and Clothier, 1988), in *E. camaldulensis* (Marshall et al. 1997) and in *Fagus grandifolia* (Steppe et al. 2010) just to mention a few. Water containing a dye is forced through a part of the stem where the heat-pulse probes have been installed and collected in a beaker. The mass is recorded by an electronic balance and compared with simultaneous measurements of sap flow rate determined by the SFD method (Figure 2-6). Many branches can be excised from a number of trees of the same species and different species in order to access the constancy of the calibration coefficients (Steppe et al. 2010). However, as with the cut tree experiments, care must be taken to avoid embolism formation in the cut segments.



**Figure 2-6** Diagrammatic representation of the stem perfusion experiment using a Mariotte-based verification system (Steppe et al. 2010)

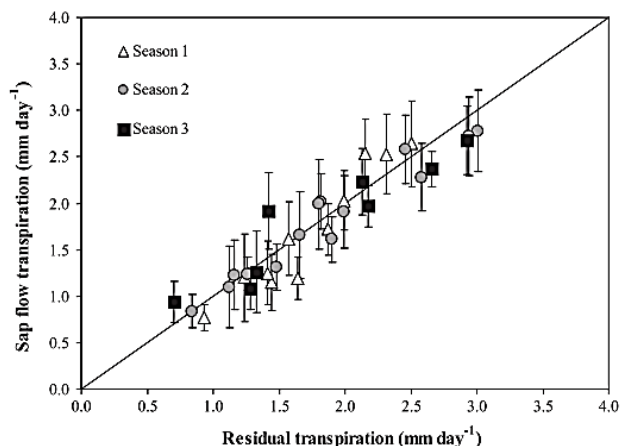
### 2.3.4 Micrometeorological methods

In-field calibration of sap flow techniques can also be performed against measurements of crop evapotranspiration ET using micrometeorological methods i.e. eddy covariance (EC) (Poblete-Echeverría et al. 2012; Williams et al. 2004; Köstner et al. 1992; Conceição and Ferreira, 2008). 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 grown on a lysimeter can be long 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 the EC are associated with some error, these errors can be evaluated and reduced through the careful analysis of data (Allen et al. 2011). Furthermore, weighing lysimeter are used to quantify water use of a single tree, whereas the EC measurements represent a number of trees and much wider 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, transpiration is calculated as the residual of evapotranspiration measurements determined through EC (ET) and soil evaporation ( $E_s$ ) determined with microlysimeters:

$$\text{Transpiration} = ET - E_s \quad (14)$$

Validation includes the linear regression analysis between transpiration determined as the residual of ET and  $E_s$  and transpiration determined with the sap flow technique (Figure 2-7). A strong linear correlation (Figure 2-7) with a coefficient of determination of 0.88 was found by Poblete-Echeverría et al. (2012) when the CHP method was calibrated by micrometeorological data and soil evaporation data using microlysimeters. 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). However, some researchers have reported drawbacks with use of microlysimeters which include inability to measure during irrigation or rain (Thompson et al. 1997) and are also limited representation of field conditions due to small sample size (Daamen et al. 1993). Micrometeorological measurements represent the crop evapotranspiration, the fetch over a large portion of the orchard, therefore calibration of sap flow systems against this method will include possible errors in the scaling up process from individual tree transpiration to stand or orchard transpiration.



**Figure 2-7** Comparison between residual transpiration measured by the eddy covariance system and microlysimeters and transpiration determined using the compensated heat pulse (CHP) method for three seasons in a Merlot vineyard (Poblete-Echeverría et al. 2012).

## 2.4 General conclusions

Numerous techniques to quantify sap flow in plants have been developed throughout the years, each with their own merits and demerits. Heat balance methods can be used to integrate sap flow in a large stem section of the plant, or the entire stem, which gives a good indication of total plant water use (Vandegehuchte, 2013). Whereas the SFD methods, give more detailed information on flow directions and spatial flow distribution (Vandegehuchte, 2013). Sap flux density measurement methods can be used to measure water use without calibration in species which are classified as thermally homogeneous. Fernández et al. (2006) argued that, calibration experiments cannot be extrapolated to species with different wood characteristics. Therefore, it is essential to calibrate the technique in plants in which the method has not previously been tested, due to possible differences in wood anatomy. Literature has also revealed that the recently developed HR method by Burgess et al. (2001) has never been calibrated in *Citrus*

*sinensis* against the weighing lysimeter. This will be important for all future infield measurements of citrus water use, where accurate measurements of transpiration are required.

## CHAPTER 3

# Validating sap flux density measurement methods in a glasshouse using weighing lysimeters

### 3.1 Introduction

Reliability, precision and accuracy are major issues with all sap flow techniques and this also applies to the CHP and HR methods. As a result much attention and care is essential to evade the errors and numerous potential problems linked with quantifying sap flow in woody plants. Therefore it is important to have comprehensive information of the margin of error accompanying any given sap-flow method when it is used to acquire quantitative measurements of plant water use (Bleby et al. 2004). A large part of the error in SFD measurement methods is that they require the insertion of thermocouples and heater needles into the sapwood, which results in wound formation and obstruction of the flow path leading to underestimations of heat pulse velocities up to 50 % or more (Swanson, 1983; Burgess et al. 2001). Consequently, it cannot be assumed that a specific SFD method can accurately quantify sap flow unless it is calibrated against a reliable alternative technique (Poblete-Echeverría et al. 2012). In this regard, Bleby et al. (2004) evaluated the CHP method and the HR method in *Eucalyptus marginata*, with transpiration measured using weighing lysimeters. Both methods performed well and yielded accurate estimates of transpiration in this species. The aim of the current study was to assess the most appropriate SFD measurement method for citrus, in order to identify the best method to use in field experiments. Experiments were performed under controlled conditions in a glasshouse using a cantilever type weighing lysimeter. The methods were first evaluated in a model species for sap flow measurements, *Eucalyptus marginata*, to ensure that the methodology and equipment was functioning properly and then in potted *Citrus sinensis* trees.

## 3.2 Materials and methods

### 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, 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 CR10X data logger (Campbell Scientific Inc., Logan, Utah, USA). Vapour pressure deficit of the air (VPD) was then determined from  $T_a$  and RH using the functions presented by Campbell and Norman (2012):

$$e_s = 0.611 * \text{Exp} \left( \frac{17.502 * T_a}{T_a + 240.97} \right) \quad (15)$$

$$VPD = e_s - \frac{e_s * RH}{100} \quad (16)$$

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 *Eucalyptus marginata* (*E. marginata*) by Burgess et al. (2001) and was shown to accurately estimate transpiration 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 weighing approximately 30 kg, are provided in Table 3-1.

**Table 3-1** 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 'Midnight' 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 3-2.

**Table 3-2** ‘Midnight’ Valencia citrus trees used for testing of the HR method and CHP method sap flow equipment

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	1.8	2.7
73.8	1.0	8.0, 12.0 and 15	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 heat pulse velocity 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 full strength Hoagland solution (Hoagland and Arnon, 1950) 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 ‘Midnight’ 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 3-1). The load cells used had a range of 0 - 500 N (0 - 51 kg) and a theoretical resolution of 4.3 g.



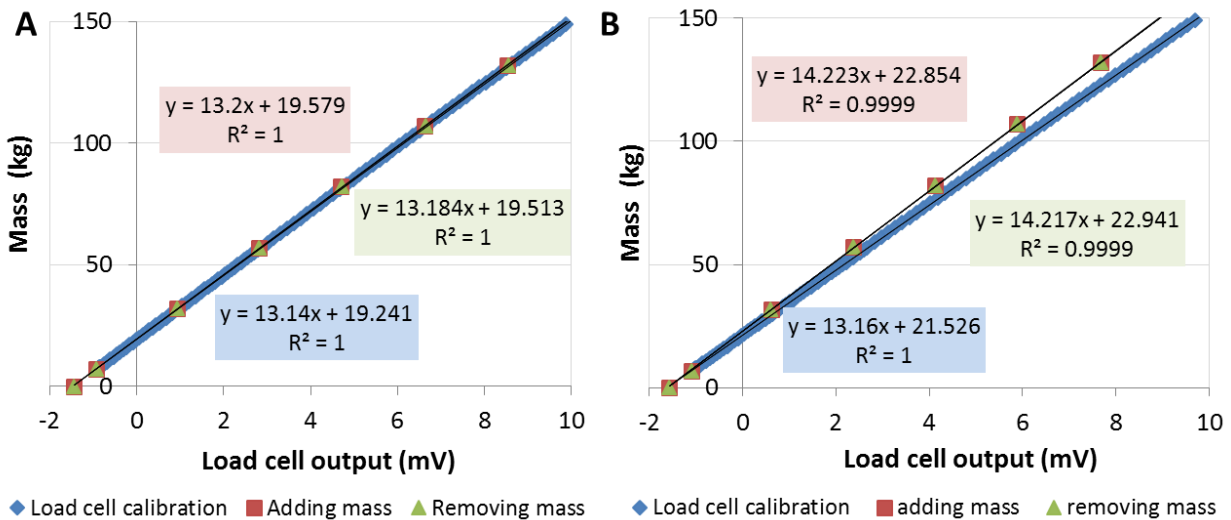
**Figure 3-1** Cantilever weighing lysimeters for checking the calibration of sap flow sensors

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 CR10X logger (Campbell Scientific Inc., Logan, Utah, USA), at one second intervals and then averaged and logged every 15 minutes.

### 3.2.4 Calibration of the load cell on the weighing lysimeters

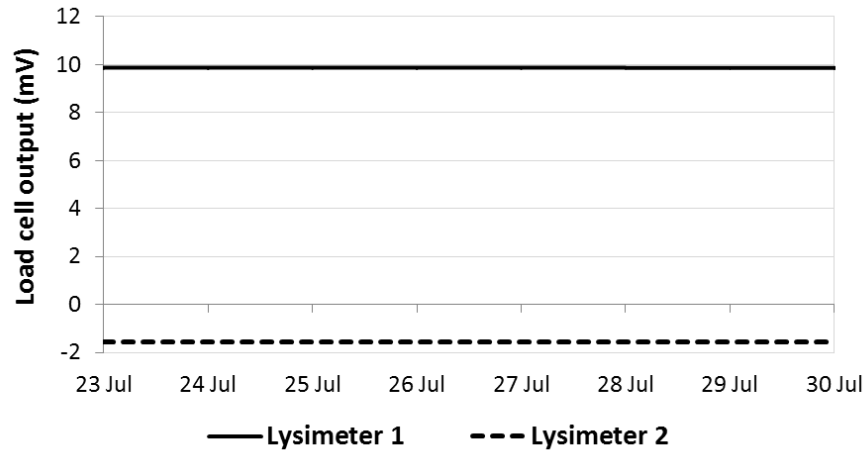
Calibration of the load cells using a known mass of water was conducted to convert the load cell measurements (mV) into mass (kg). Water was added at 100 mL intervals from 0 - 1 L, then at 1 L intervals from 1 - 50 L, 2 L intervals from 50 - 148 L and then again at 100 mL intervals from 148 - 150 L. The ambient temperature 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 3-2 A and B. The calibration results for the two lysimeters showed a perfect positive linear relationship between the load cell output (mV) and calibration mass (kg) existed ( $R^2 = 1$ ) for both lysimeters.



**Figure 3-2** 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

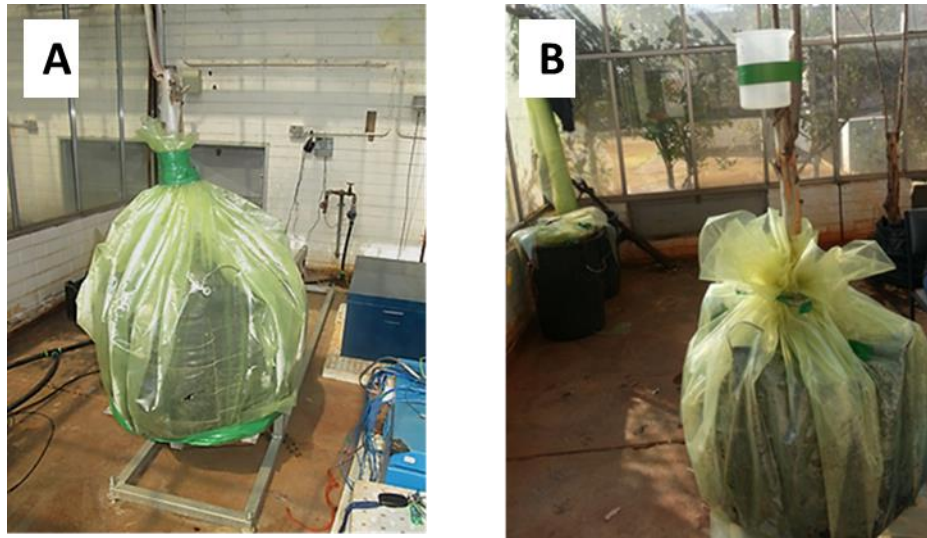
The effect of hysteresis was determined by adding mass at 25 kg interval from 0 - 150 kg as indicated by the red regression line and removing mass also at 25 kg interval from 150 - 0 kg as indicated by the green regression in Figure 3-2 A and B no hysteresis was noted. The stability of the load cells with a constant mass was also assessed, as this would be critical when calibrating the sap flow 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 evaporation. 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 3-3).



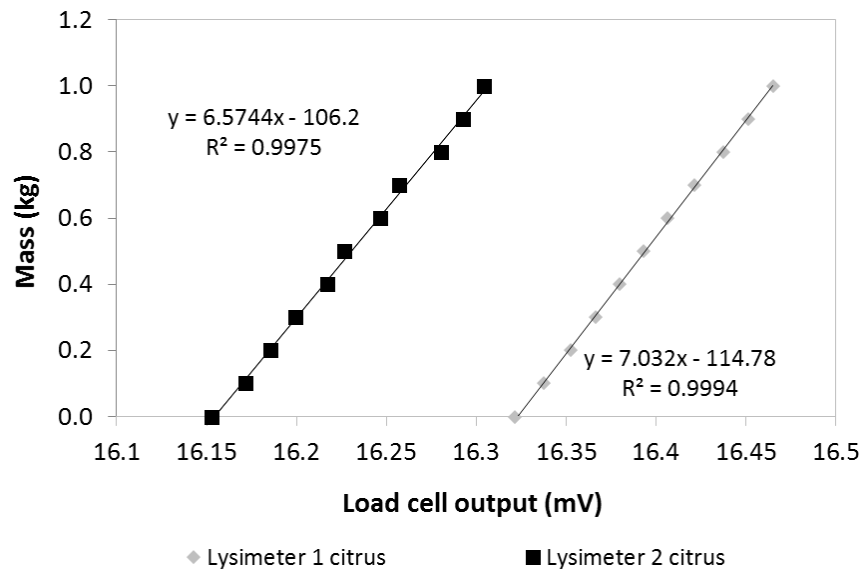
**Figure 3-3** Stability of the load cell output over a seven day period (23 – 30 July 2014) as observed from the mV readings.

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

In order to determine transpiration from the potted trees, the exposed soil surface of the pots was covered with plastic sheeting to eliminate evaporation 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) as shown in Figure 3-4. Recalibration of the lysimeter was conducted once each tree was placed on the lysimeter, using a 2 L beaker which was placed on the soil surface of the plastic bag containing the *E. marginata* tree and the dustbin containing the citrus trees. 1 L of water was then added in 100 ml intervals and the mV reading from the load cell was recorded for each addition of water and regression equations were established as shown in Figure 3-5.



**Figure 3-4** (A) Placement of the *E.marginata* trees and (B) attachment of 2 L beaker for recalibration on the weighing lysimeters and plastic covering to eliminate evaporation and drainage from the pots



**Figure 3-5** Regression analysis for the calibration of lysimeter 1 and 2 after the placement of trees on the weighing lysimeters

Water lost by the tree was calculated for 15 minute intervals as the difference in mass at the beginning and at the end of 15 minute interval, which was summed per hour to determine hourly water use.

### 3.2.5 Sap flow equipment and measurements

#### 3.2.5.1 Heat ratio method

Sap flow measurements in *E. marginata* using the HR method were conducted from 8 – 16 Aug 2014 and for 'Midnight' Valencia from 23 Sept – 20 Dec 2014 and 28 Aug – 28 Nov 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 3-6).



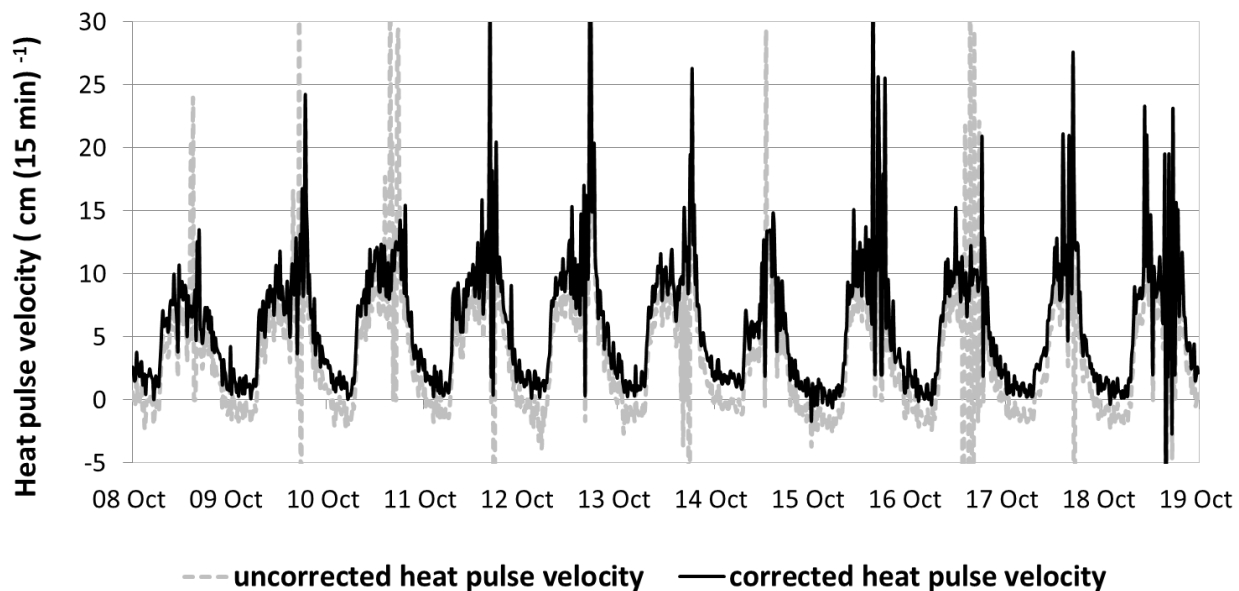
**Figure 3-6** Placement of the trees on the weighing lysimeters and probe installation

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 'Midnight' 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 outside diameter stainless steel heater probe, whilst the thermocouples consisted of Type-T copper-constantan conductors embedded in 2 mm outside diameter

polytetrafluoroethylene tubing. These probe sets were locally manufactured by Mr A Everson. 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 heat pulse velocity 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 an AM16/32B multiplexer (Campbell Scientific Inc., Logan, Utah, USA). Heat pulse velocities which were calculated as described in section 2.2.2.2 were logged at 15 min interval on a CR1000 logger (Campbell Scientific Inc., Logan, Utah, USA). Voltages of batteries powering the load cells and heat pulse velocity systems was carefully monitored to ensure it did not drop 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 3-7. High abnormal flows were also adjusted and corrected by averaging the two preceding or two following readings. The adjusted data was 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 as described in section 2.2.2.2. The area representing each probe was determined using the perfusion experiment as described later in section 3.2.7.1, following which the heat pulse velocity from each probe was multiplied by the specific area represented by the probe, which yielded the VSF per 15 minutes. This was later

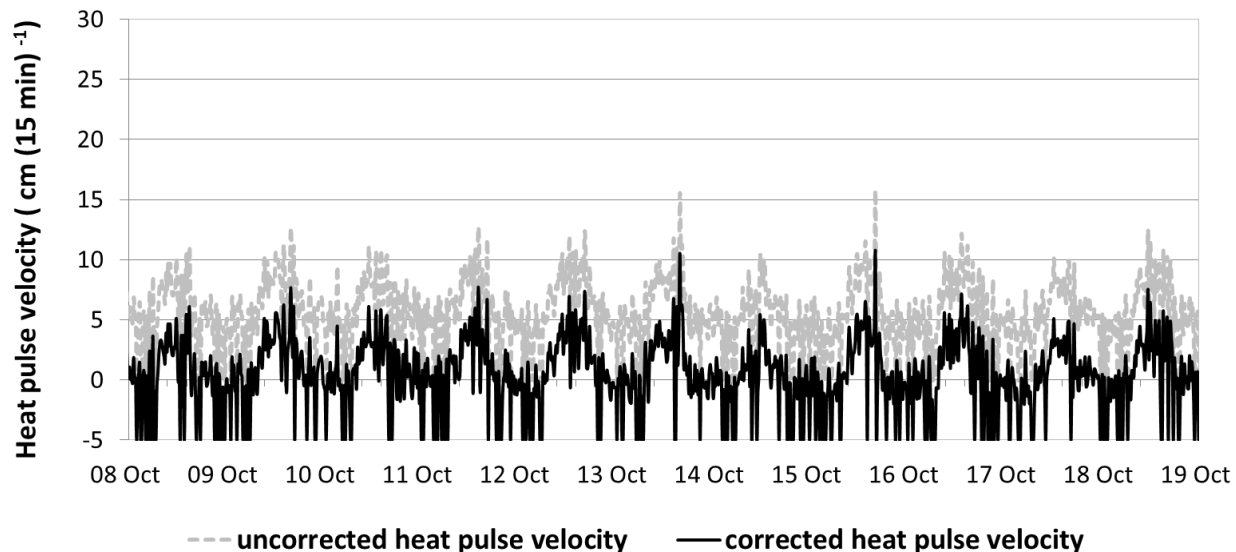
averaged to sap flow per hour ( $\text{cm}^3 \text{hr}^{-1}$ ) and compared to the weighing lysimeter at hourly intervals.



**Figure 3-7** Heat pulse velocity determined at 8 mm below the cambium for the HR method, bold line is the adjusted baseline and dashed line is the raw data from the logger for citrus from 08 – 19 Oct 2015.

### 3.2.5.2 Compensation heat pulse method

Sap flow measurements using the CHP method were only conducted in ‘Midnight’ Valencia trees for the period of 24 Apr – 24 May and 09 Sep – 29 Nov 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 was also conducted for the CHP method as shown in Figure 3-8, corrected sap velocities were calculated as described in section 2.2.2.1 and multiplied by the cross-sectional area represented by each probe to determine the VSF ( $\text{cm}^3 \text{h}^{-1}$ ).



**Figure 3-8** Heat pulse velocity determined at 8 mm below the cambium for the CHP method, bold line is the adjusted baseline and dashed line is the raw data from the logger

### 3.2.6 Data analysis

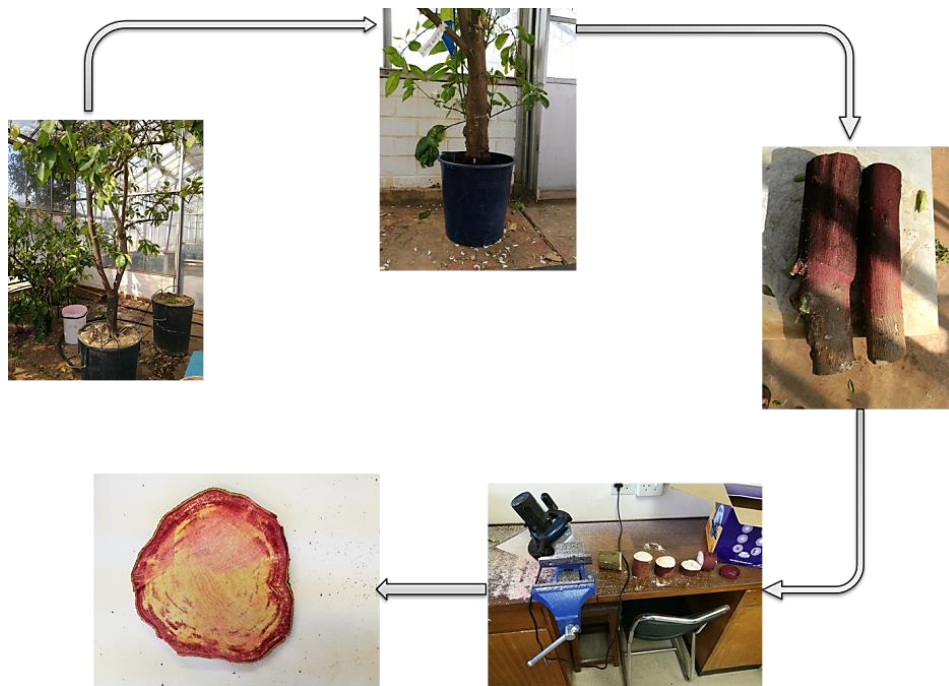
Data from the HR and CHP methods were compared to the weighing lysimeter on an hourly interval. Total daytime transpiration was calculated from the sum of hourly sap flow between 06:00 and 18:00, as during this time the plants were actively transpiring. The same was also done for the weighing lysimeter and a comparison was made between the weighing lysimeter and the HR and CHP. A regression analysis was conducted between transpiration measured from the weighing lysimeter and  $T_{\text{sap}}$  measured with the SFD measurement techniques.

### 3.2.7 Parameters to calculate sap flux density

To be able to calculate SFD additional parameters were determined, which included sapwood moisture content ( $M_c$ ), the wood density ( $\rho_b$ , g cm<sup>-3</sup>), wound width (mm) and sapwood area (cm<sup>2</sup>).

### 3.2.7.1 Sapwood area

The area of sapwood conducting tissue was determined for the 'Midnight' Valencia trees using the potometer method as described by Barrett et al. (1995) and perfusion experiment for *E. marginata* as conducted by Steppe et al. (2010). In Figure 3-9 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 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 3-9** 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 3-10). 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 lightly stained area.

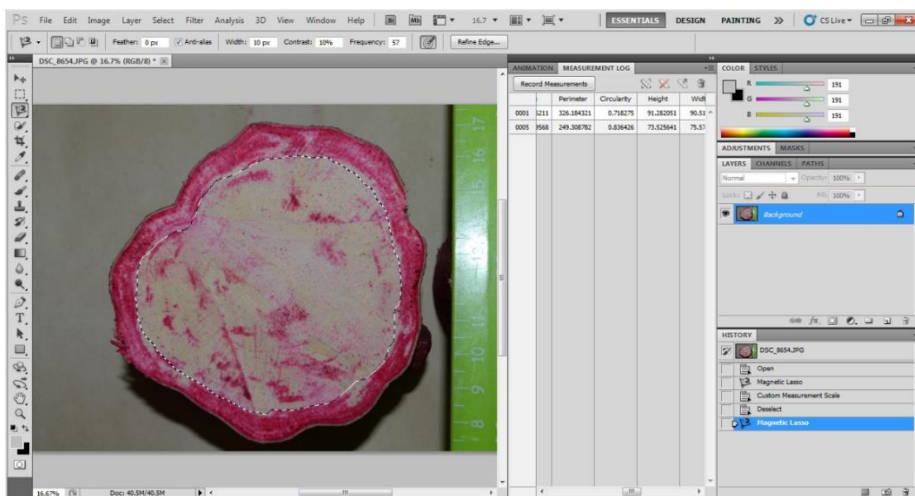
The heartwood radius was then computed from the formula for calculating the perimeter of a circle using equation 17.

$$\text{Circumference of a circle} = 2\pi r \quad (17)$$

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 3-10** Determination of the sapwood (pink stained) and the heartwood (white unstained area) using Adobe Photoshop.

### 3.2.7.2 Sapwood density and moisture content

The sapwood moisture content was determined using the method described by Steppe et al. (2010). Wood samples were collected from the potted citrus trees using an incremental borer at the end of the experiment. The wet mass was measured using a balance and the volume of the

wood extracted was determined by the water displacement theory of Archimedes. The samples were then oven-dried at 60°C until a constant mass was reached to determine the dry mass of the wood sample (Barrett et al. 1995). Sapwood wood moisture content was then calculated using equation 18:

$$M_c = \frac{W_f - W_d}{W_d} \quad (18)$$

where  $W_f$  (g) is fresh mass and  $W_d$  (g) is oven-dried mass.

And bulk wood density ( $\text{g cm}^{-3}$ ) was calculated using equation 19:

$$\rho_b = \frac{w_f}{V} \quad (19)$$

where  $V$  ( $\text{cm}^3$ ) is the volume of the sample.

### 3.2.7.3 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 3-11. Wound width of the widest probe was measured for each tree using a digital vernier callipers and an average wound size was determined. The average wound width was used for the calculation of the SFD.



**Figure 3-11** Wounding response in 'Midnight' Valencias after HR probe installations (A) and for the CHP probe installations (B), measurement of the wound width with a vernier callipers (C)

### 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 conducted by Steppe et al. (2010). Sources of possible error include parameters to calculate SFD such as sapwood moisture content, heartwood radius, bulk wood density and wound width were used in error analysis. The resultant errors in daily sap flow were expressed as percentage overestimates or underestimates of the measured value as determined by equation 20.

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

Where the true value is the measured value and the simulated value is the value when an error is made.

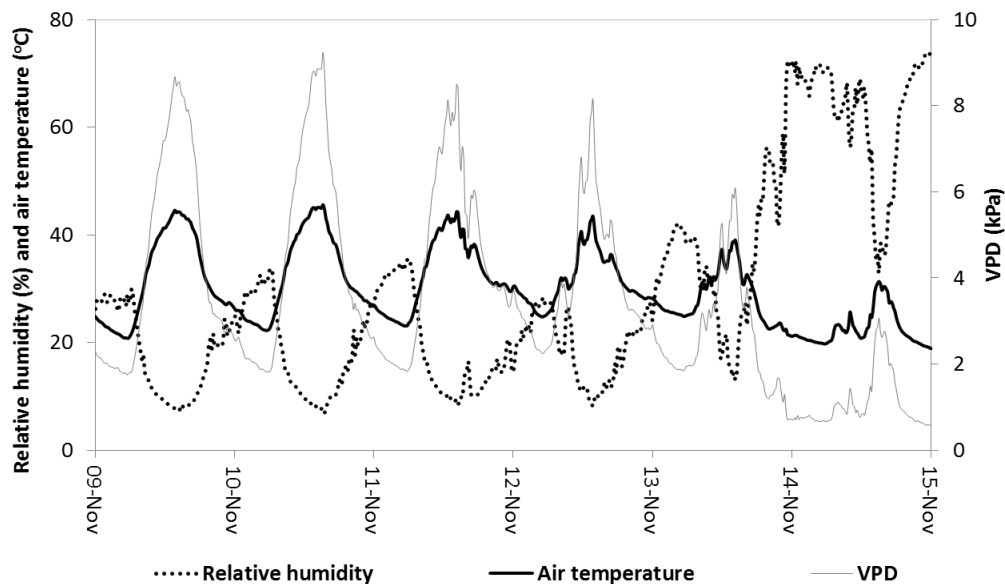
Keeping all the other variables at true measured values, separate simulations were done by increasing errors of each parameter ( $\pm 0, 10, 20, 30, 50, 70, 100\%$ ) e.g. a negative percent

increase means that the true measured value for the parameter was reduced, whilst a positive percentage indicates an increase in the true measured value by that specific percentage.

### 3.3 Results and discussion

#### 3.3.1 Weather variables

In Figure 3-12 an extract of weather data for 9-15 Nov 2015 is presented, which was generally good data. Air temperature and VPD followed a typical diurnal trend, with maximum values obtained around midday and lowest values observed at night. The fan which was used to cool the glasshouse was switched off as the wind-blown interfered with the lysimeter measurements. As a result air temperatures of up to 44 °C and very low relative humidity of up to 6% were observed and this resulted in higher VPD values of up 8 kPa. Weather variables were measured throughout the whole calibration windows.

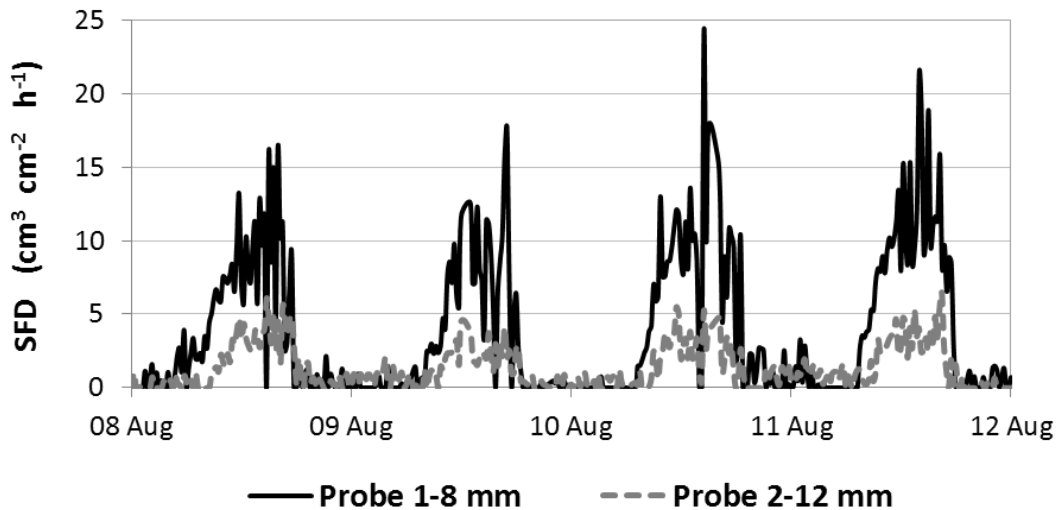


**Figure 3-12** Hourly values of average air temperature (°C), relative humidity (%) and average VPD (kPa) in the glasshouse from 9-15 Nov 2015

### 3.3.2 Testing the heat ratio method in *E. marginata*

#### 3.3.2.1 Variations of sap flux density over 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 temperature sensors of the HR method at various depths in the stem as conducted by Wullschleger and King (2000) and Poblete-Echeverría et al. (2012). Results from the two probes sets for the HR method at different depths (8 mm and 12 mm) over a 4 day period are presented in Figure 3-13.



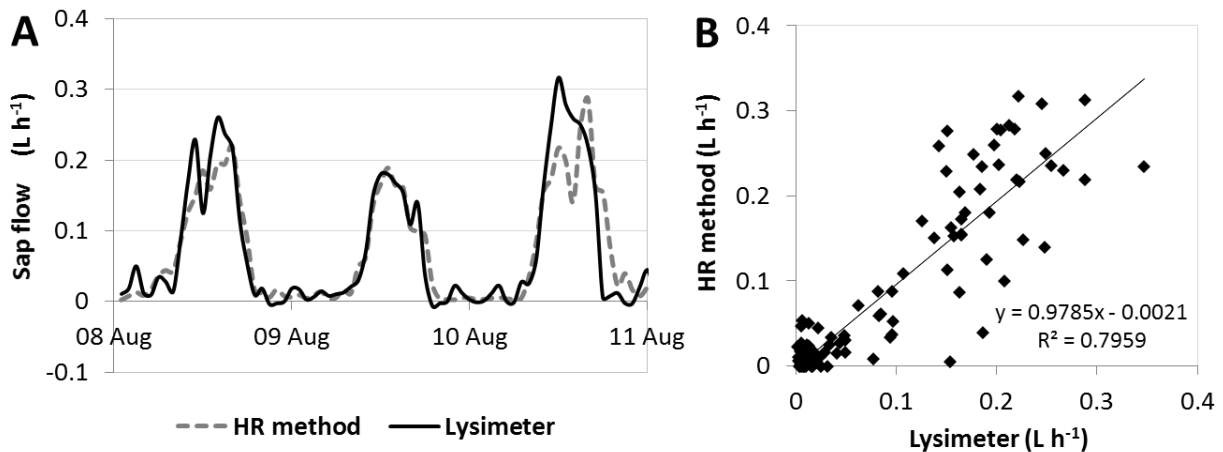
**Figure 3-13** Hourly sap flux densities (SFD) of the HR method installed at 8 and 12 mm depth in *E. marginata*

Clear diurnal trends can be observed for both probe sets, with the lower SFDs recorded by the deeper probe set (Figure 3-13). The maximum SFD determined throughout the calibration window was approximately 25 cm<sup>3</sup> cm<sup>-2</sup> h<sup>-1</sup> for the probe set installed at 8 mm depth and 5 cm<sup>3</sup> cm<sup>-2</sup> h<sup>-1</sup> for the probe set installed at 12 mm depth. The behavior depicted by the SFDs in Figure 3-13 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, as observed in grapevines by Poblete-Echeverría et al. (2012) and olive trees by Fernández et al. (2001)

### Hourly sap flow

Hourly VSF was calculated for the stem and compared with the mass loss per hour measured gravimetrically using the weighing lysimeter (Figure 3-14). A clear diurnal trend in mass loss from the weighing lysimeter and  $T_{\text{sap}}$  (HR method) was observed (Figure 3-14 A). On an hourly time scale the HR method and the gravimetric method measured 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).



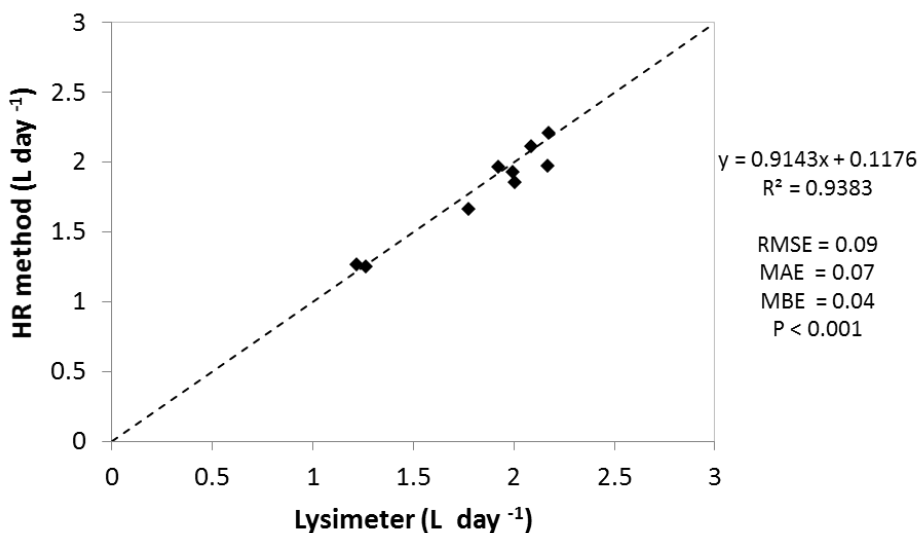
**Figure 3-14** Diurnal trend for (A) hourly mass loss between the gravimetric method (lysimeter) and sap flow method (HR method) on *E. marginata* and (B) relationship between hourly mass loss measured with a weighing lysimeter and heat ratio (HR) method for *E. marginata*

Regression analysis showed that the correlation between results from the HR method and gravimetric method was highly significant (P value < 0.001) and moderately linear ( $R^2 = 0.79$ )

(Figure 3-14 B). Also, the t-test indicated that the intercept was not significantly different from zero and the slope was not significantly different from unity.

### 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 showed that  $T_{sap}$  was less than transpiration determined with the gravimetric method with a mean biased error (MBE) of 0.04 L day<sup>-1</sup>, root mean square error (RMSE) of 0.09 L day<sup>-1</sup> and mean absolute error (MAE) of 0.04 L day<sup>-1</sup> (Figure 3-15).



**Figure 3-15** Comparison of daily mass loss between the weighing lysimeter and the heat ratio method (HR method) in *E. marginata* dashed line is a 1:1 line

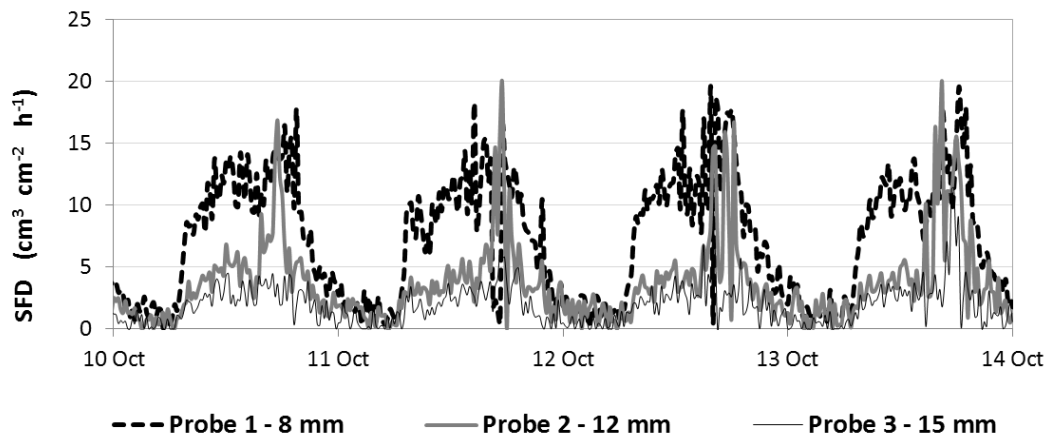
The HR method tended to underestimate tree transpiration by 1.9 % on average per day, which was a very good result. The linear regression analysis indicated that the correlation ( $R^2 = 0.94$ ) between the HR method and gravimetric estimates of daily whole-plant water use was highly significant ( $P$  value  $< 0.001$ ) and very near to 1:1 relationship. 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).

### 3.3.3 Validating sap flux density measurement methods in ‘Midnight’ Valencia (*Citrus sinensis* L. Osbeck)

#### 3.3.3.1 Heat ratio method (HR method)

Similar diurnal trends in the SFD to that of *E. marginata* were observed in ‘Midnight’ Valencia trees (Figure 3-16). Sap flux densities measured at three depths (8 mm, 12 mm and 15 mm) over a period of four days are characterised by early morning increases, midday depressions and virtually zero flows at night.

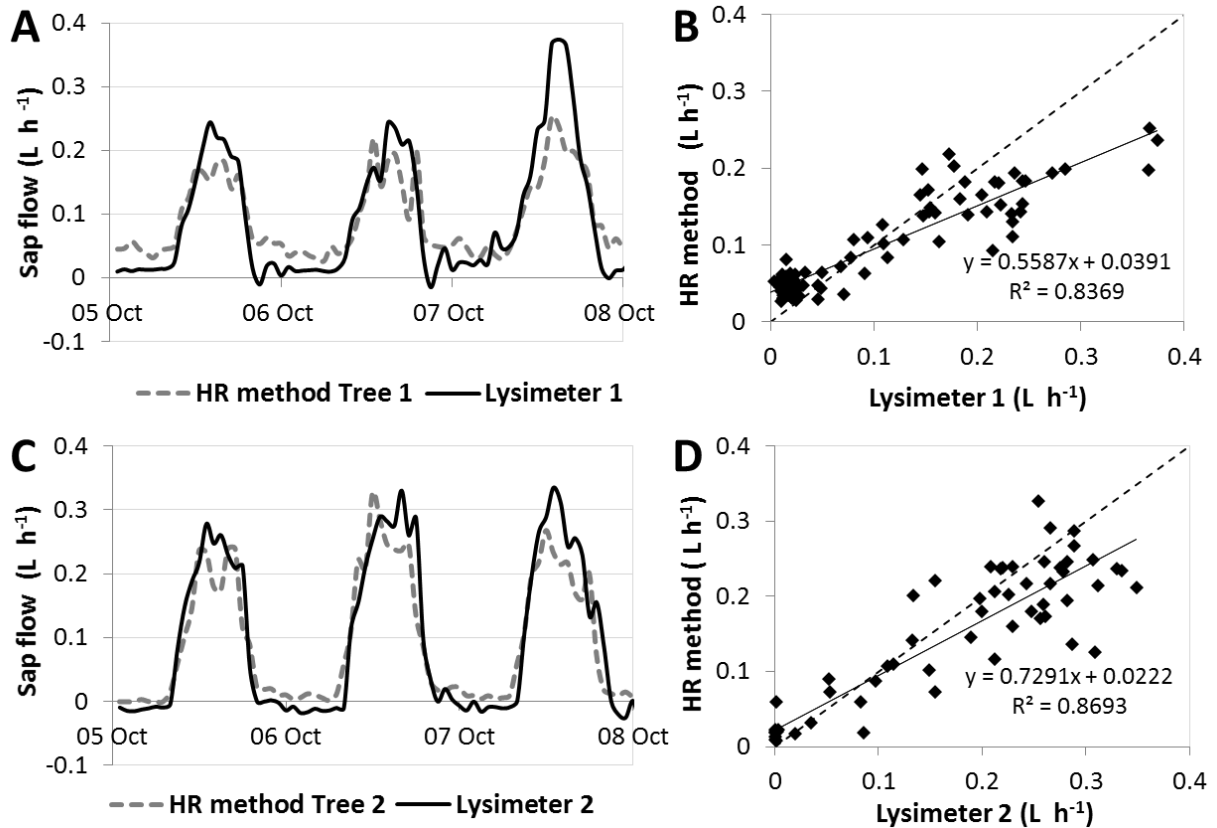


**Figure 3-16** Corrected sap flux densities (SFD) measured at 8, 12 and 15 mm depths for a potted ‘Midnight’ Valencia tree

Higher SFDs were measured by the shallower probe (8 mm) than the deeper probe (15 mm) (Figure 3-16). These values indicate that xylem conductivity decreased with depth and the exterior side of xylem was very young and active compared with the interior side (Poblete-Echeverría et al. 2012).

## Hourly sap flow

Hourly VSF was determined in the stem using the SFDs at three different points over the total sapwood conducting area and was compared to mass loss per hour from the weighing lysimeters. Both 'Midnight' Valencia trees showed a typical bell-shaped diurnal pattern as observed in Figure 3-17 A and C.



**Figure 3-17** Hourly transpiration of 'Midnight' Valencia (A) and (C) measured with the heat ratio method (HR) and gravimetric methods. The relationship between hourly water use measured by the heat ratio and gravimetric methods is given for 'Midnight' Valencia (B) and (D)

Likewise, at an hourly time scale, the HR method measured diurnal patterns of sap flow with great sensitivity. The HR method overestimated transpiration at low flows and underestimated transpiration at high flow rates for both 'Midnight' Valencia trees. These results are similar to

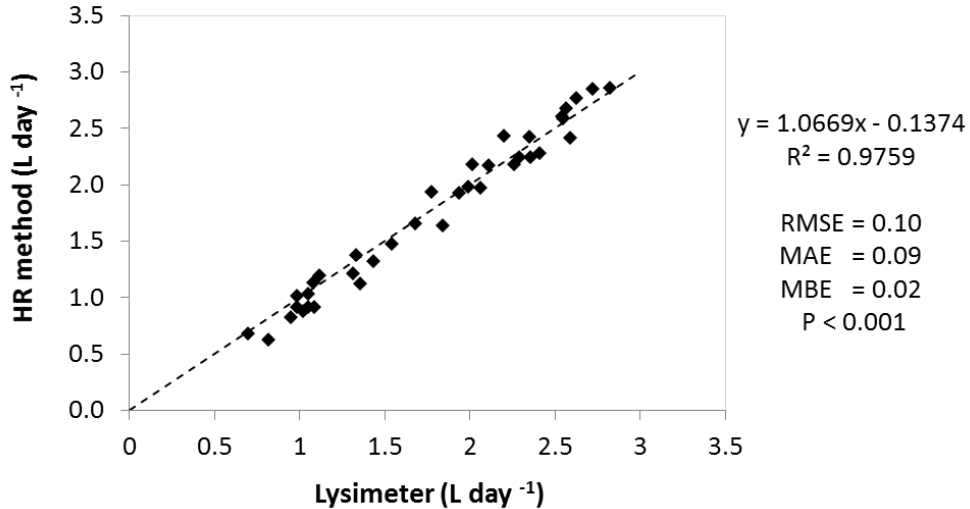
Barret et al. (1995), who observed an overestimation of sap flow at low flows and an underestimation at high flow rates when a similar validation using a potometer method was conducted in eucalyptus (Figure 3-17 B and D). Regression analysis showed that the correlation between HR method and gravimetric estimates on an hourly basis was highly significant (P value < 0.0001) for both trees and the linear relationship accounted for 84 % and 87 % of the variation in data in tree 1 and 2, respectively.

#### 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 3-17 A and C). The discrepancy between the two methods can be explained as follows: during the night time the plant refills most of the water that has been 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 tension created during the day (Fisher et al. 2007).

#### 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 transpiration obtained from the gravimetric method and that obtained by HR method ( $T_{\text{sap}}$ ) is given in Figure 3-18. Overall values of RMSE and MAE were 0.10 and 0.09 (L day<sup>-1</sup>) respectively. The statistical analysis showed that  $T_{\text{sap}}$  was less than transpiration from the weighing lysimeter with an MBE of 0.02 (L day<sup>-1</sup>). The HR method therefore tended to underestimate tree transpiration by 1.08 % on a day time water use basis. The linear regression analysis showed that the relationship between the HR method and the gravimetric estimates of daily whole-plant water use was highly significant as indicated by a P value < 0.001 and  $R^2 = 0.98$  (Figure 3-18) and very near to a 1:1 relationship. Also, the t-test indicated that the intercept was not significantly different from zero and the slope was not significantly different from unity (Appendix 2).

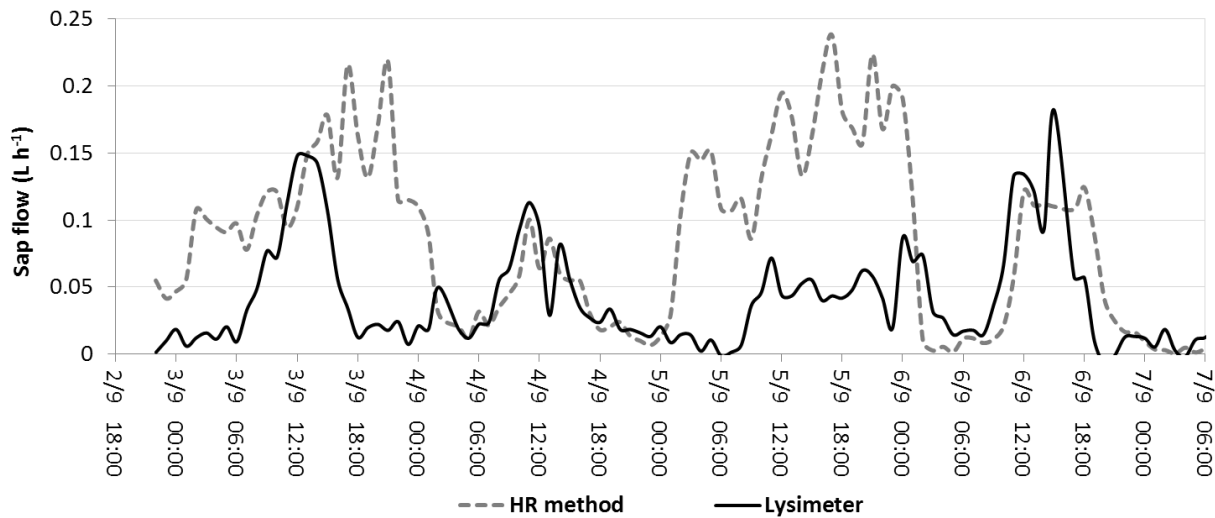


**Figure 3-18** The relationship between total day time water use measured using a weighing lysimeter and the HR method for a potted ‘Midnight’ Valencia tree over a period of 37 days. Total day time transpiration was calculated from sum of hourly rates between 06:00 and 18:00

This was an exceptional result considering that the validations are associated with a number of possible sources of error, which includes the likelihood of evaporation 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 to other similar validations involving palm frond, where Madurapperuma et al. (2009) clearly demonstrated that the HR method provided accurate measurements of transpiration in agreement ( $R^2 = 0.92$ , slope of 1.01 and intercept of 0.04) with gravimetric measurements from an electronic balance. 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).

### Limitations of the heat ratio method in water stress conditions

Irrigation was initially applied at night every second day e.g. on 2 and 4 September (Figure 3-19). However, soon after irrigation water drained from the plastic container until all gravitational water was removed. A sharp increase in sap flow was observed (for both days) at midnight to approximately  $0.15 \text{ L h}^{-1}$ , which was maintained throughout the day and increased to approximately  $0.25 \text{ L h}^{-1}$  during the night thus overestimating tree transpiration.



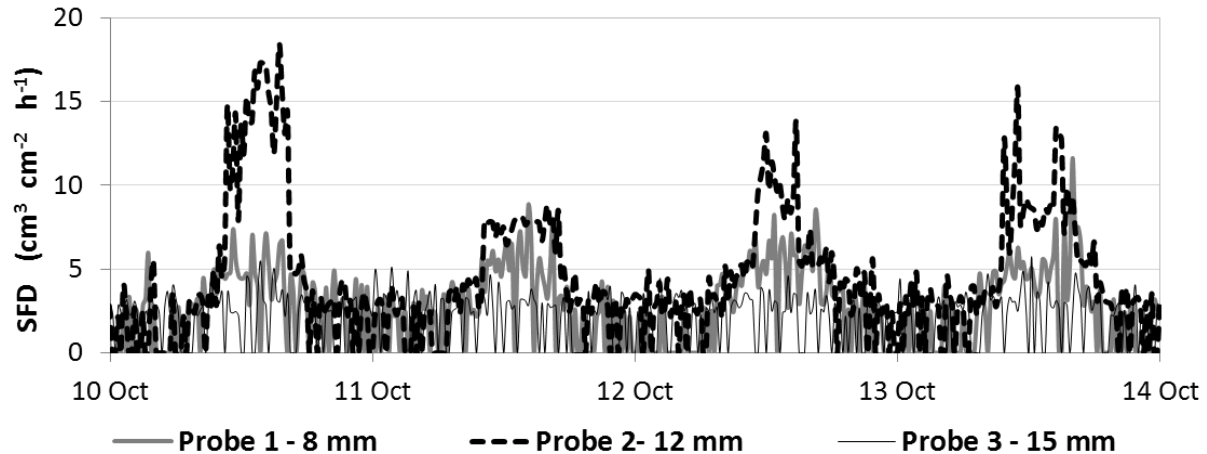
**Figure 3-19** Comparison of hourly water use of citrus determined with HR method and that determined gravimetrically on irrigated and non-irrigated days

Nonetheless an interesting phenomenon was observed the day after irrigation, when the sap flow could be correlated directly to transpiration determined from the weighing lysimeter. This behaviour continued for about two weeks until the irrigation regime was changed to irrigating twice every day. This resulted in daily sap flow rates that were directly correlated to the transpiration determined from the weighing lysimeter. The possibility therefore exists of an increase in sap flow rates following irrigation as a result of rehydration of plant tissues following water stressed conditions. The interval between irrigation events was therefore too long and by the second day following irrigation the pot was nearly dry. A large negative water potential

would have been created in the leaves and soon after irrigation this would cause a tremendous pull in the sap, thereby resulting in a surge in sap flow rates i.e. the plant is refilling (Oren et al. 1999). This is evident in Figure 3-19 as much of the water which flows at this time is stored in plant leaves and stems and nearly zero mass loss is recorded during the period following irrigation. 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). However, more research needs to be conducted to confirm these findings. Additionally, a clear lag between sap flow and transpiration was observed on 6 September. In the early morning hours, transpiration of the water stored in plant tissues occurs. At the end of the day, after the cessation of transpiration, the sap continues to flow in order to rehydrate the plant tissues (Coelho et al. 2012). From these findings, it is clear that the use of heat pulse velocity systems to determine transpiration under water stressed conditions can be misleading.

### **3.3.3.2 Compensation heat pulse method (CHP method)**

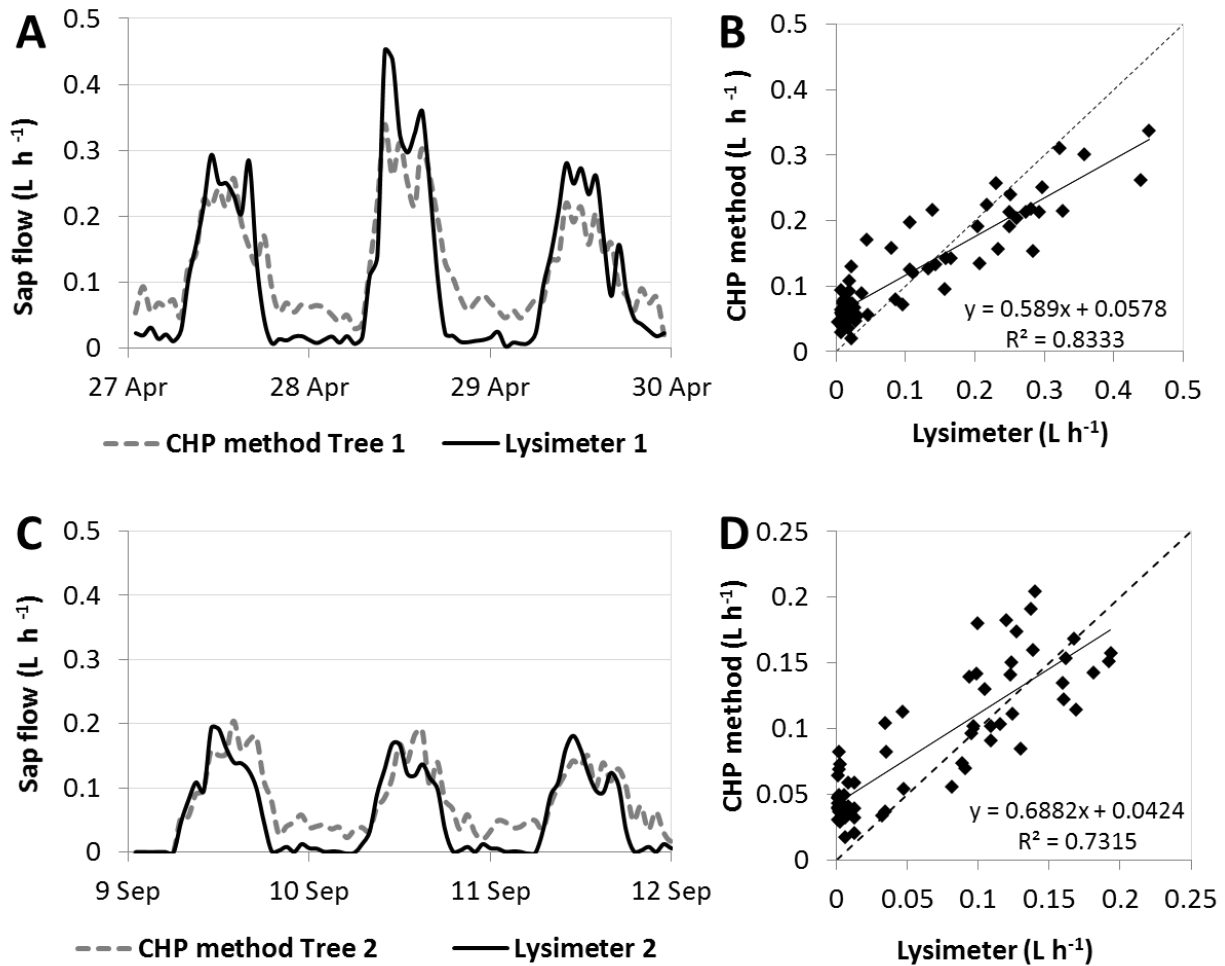
The CHP method is inherently incapable of resolving low and zero rates sap flow ( $0-5 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$ ) (Becker 1998; Burgess et al. 2001). In this study it was evident that the sensors could not resolve SFDs of approximately  $5 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$  or less. In Figure 3-20 SFDs for 4 selected days are given. As observed and explained for the HR method (section 3.3.3.1) clear diurnal trends were also observed for all measuring depths using the CHP method, with the highest SFDs of approximately  $15 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$  measured at the probe inserted 12 mm from the cambium and the lowest at the probe inserted 15 mm into the sapwood (Figure 3-20).



**Figure 3-20** Sap flux densities (SFDs) measured using the CHP method at 8, 12 and 15 mm depth for a potted 'Midnight' Valencia tree

### Hourly flows

A comparison between hourly transpiration from the CHP method and gravimetric method was conducted for two different calibration windows viz. (27 – 30 April 2015) and (09 – 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 (Figure 3-21). However, at low flow rates ( $< 0.15 \text{ L h}^{-1}$  for tree 1) transpiration was overestimated and at high flow rates ( $> 0.15 \text{ L h}^{-1}$ ) transpiration was underestimated by the CHP method. A lag between sap flow and mass loss from the weighing lysimeter was observed towards the end of the day (Figure 3-21 A and C). In the early morning hours, transpiration of the water stored in the plant tissues occurred. At the end of the day, when transpiration tends to stop, sap continues to flow in order to refill the tissue water lost through transpiration.

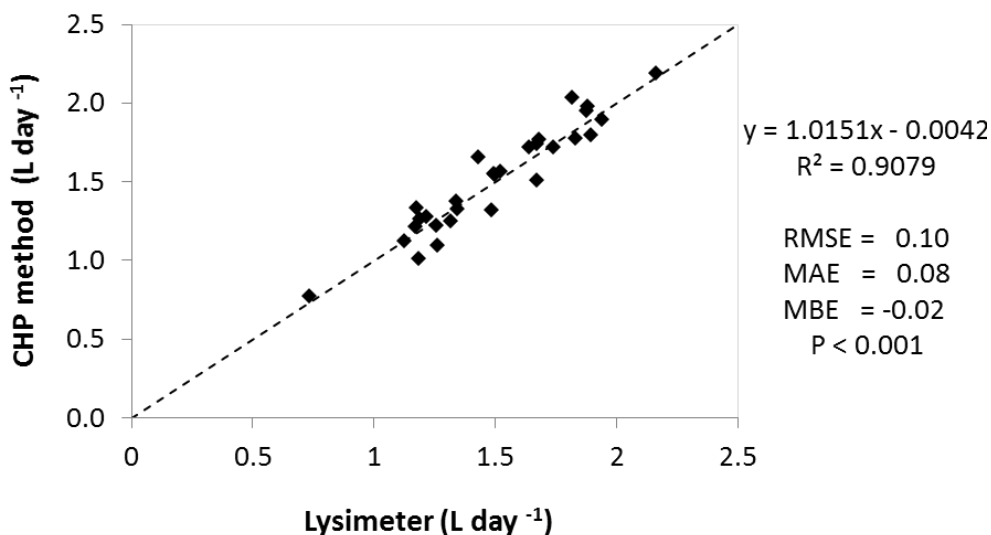


**Figure 3-21** (A) and (C) Hourly transpiration of ‘Midnight’ Valencia trees measured with the compensation heat pulse method and gravimetric methods. (B) and (D) The relationship between hourly water use measured by the CHP and gravimetric methods is given for the ‘Midnight’ Valencia trees for two respective methods.

### Day time water use

When day time water use measured with the CHP method was compared with the daily 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 3-22). Statistical analyses indicated that  $T_{sap}$  was more than transpiration determined from the weighing lysimeter with an MBE of  $-0.02 L day^{-1}$ , RMSE of  $0.1$  and MAE of  $0.08 L day^{-1}$ . Therefore CHP method

tended to slightly overestimate transpiration by  $1.23\% \text{ day}^{-1}$  which is a credible result. The slight overestimation which was observed in CHP method could be a result of the errors incurred when determining the heartwood radius (an overestimation in the heartwood radius could result in overestimation of the  $T_{\text{sap}}$ ).

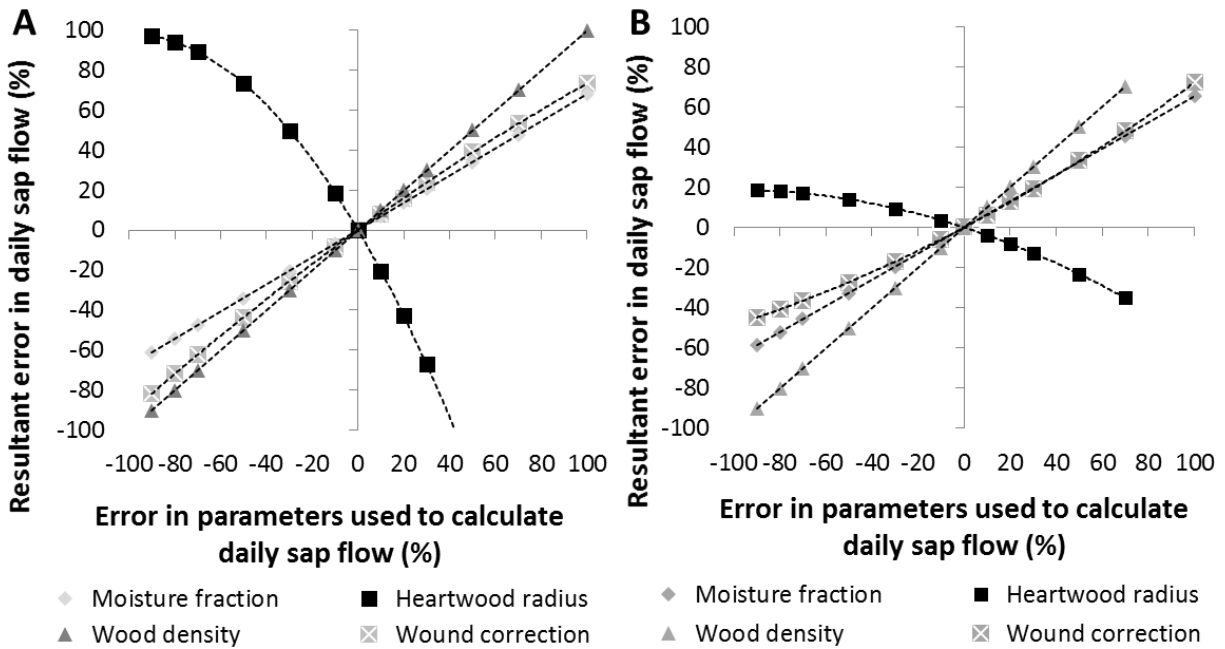


**Figure 3-22** The relationship between daytime (06:00 – 18:00) transpiration measured with a weighing lysimeter and transpiration measured with the CHP method for a ‘Midnight’ Valencia tree over a period of 30 days.

### Error analysis of heat ratio and compensation heat pulse methods

An error analyses of wood density and moisture content indicated a linear relationship between error in parameters used to calculate daily sap flow (%) in both HR method and the CHP method and the resultant error (%) in daily sap flow, whilst heartwood radius and wound correction factor, yielded a polynomial (order 2) relationship (Figure 3-23). Since these two SFD measurement techniques (HR method and CHP method) are based on the same principle, Figure 3-23 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 10 %

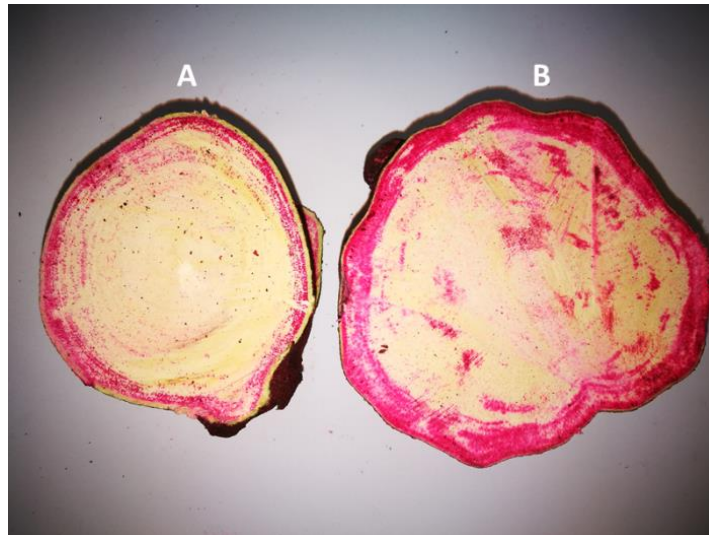
overestimate in wound correction factor resulted in 8.2 % overestimate in sap flow, whilst a 10 % underestimate of the wound correction also resulted in an 8.3 % underestimation of sap flow for both methods. In general, an overestimation or underestimation of moisture fraction and wood density by the 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*. In this study Bleby et al. (2004) observed that a 10 % overestimation in wound correction resulted in a 6.8 % overestimate of sap flow, whereas a 10 % underestimate of wound correction resulted in a 5.8 % underestimate, which was nearly of same magnitude whether underestimated or overestimated. The most sensitive parameter for both methods proved to be wood density 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, this 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. These measures can be, the use of many replications when determining a certain parameter, weighing the mass of fresh wood 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.



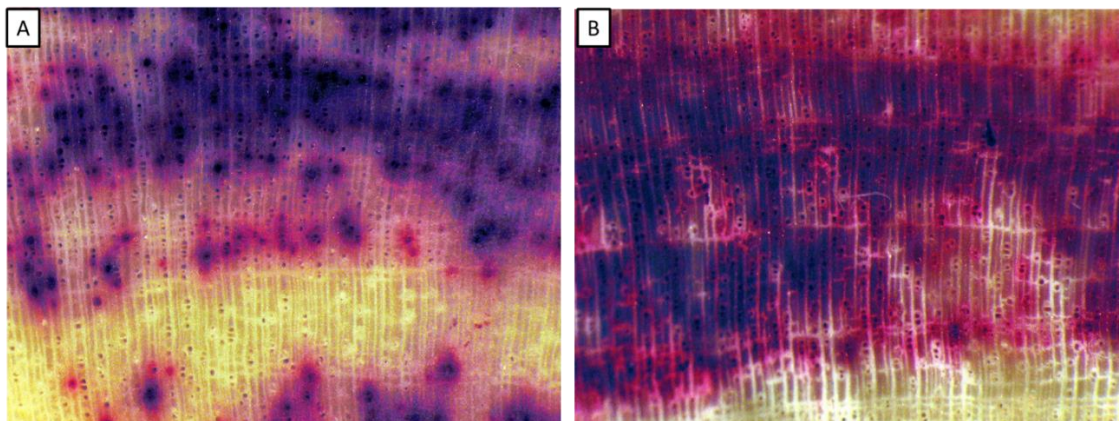
**Figure 3-23** The resultant error in daily sap flow from the (A) CHP method and (B) HR method due to errors in selected variables used in the determination of the sap flow

### 3.3.4 Sap flow quantification in the scion versus the rootstock for potted 'Midnight' Valencias in the glasshouse

In the initial experiment the probes of the various SFD techniques were installed in the scion and poor agreement was found between the SFD technique and the weighing lysimeters. The possible reasons underlying the failure of the initial attempts to calibrate SFD measurement methods, were revealed through stem staining experiments. When the probes were installed in the rootstocks very good data was collected from the SFD techniques which lead to a very good agreement with the weighing lysimeters. 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 that in many instances it was likely that only one of the sap flow sensors was recording in this band, However in the rootstock this band was a lot wider and therefore there was a greater chance of accurately capturing the correct sap velocities, as shown in Figure 3-24 A and B.



**Figure 3-24** Diffusely arranged active xylem in the scion (A) and the evenly distributed active xylem in the rootstock of the same tree (B)



**Figure 3-25** Cross section micrograph of diffusely arranged active xylem in the scion (A) and the evenly distributed active xylem in the rootstock of the same tree (B)

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 3-25 A) with a purplish colour. In the rootstock, active xylem vessels had much smaller diameter and were

more evenly distributed (Figure 3-25 B), a feature which is favourable for the heat pulse techniques. Since the heat pulse velocity 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.

### 3.4 Conclusions

Testing of the heat pulse velocity equipment in *Eucalyptus marginata* showed that, the heat pulse velocity systems were fully functional. As an  $R^2 \geq 0.7$  was achieved for the calibration of the HR method in *E. marginata* we could accept the first hypothesis that the equipment is fully functional, which allowed the continued validation of SFD measurement methods in *Citrus sinensis*. Of the three methods used to measure transpiration in *Citrus sinensis*, the HR method resulted in the most similar results to the gravimetric method used as the control, giving the most reliable and accurate results ( $R^2 = 0.98$ ) underestimating transpiration slightly by 1.08 % day<sup>-1</sup>. 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 tree transpiration by an average of 1.23 % day<sup>-1</sup>. These results suggest that the methods (CHP and HR methods) were equally accurate in estimating transpiration in a tree with a small canopy and there was no need for calibration to achieve a 1:1 relationship between the sap flow method and the weighing lysimeter. This led to the rejection of the null hypothesis which stated that, “the HR method will estimate sap flow more accurately in small potted citrus trees than the CHP method when compared to the weighing lysimeter, as the citrus canopy is small and the HR method is reported to capture low flow rates more accurately than the CHP method”. 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<sup>-1</sup> therefore posing a challenge in the quantification of tree water use for a long time period, as this will require too much “patching” of the data. In contrast, the HR method did not record any missing

data and the nearly perfect agreement between the HR method and the gravimetric method suggested a potential application for measuring transpiration rates for a long period of time.

## CHAPTER 4

# Infield validation of the sap flux density measurements using a micrometeorological method

### 4.1 Introduction

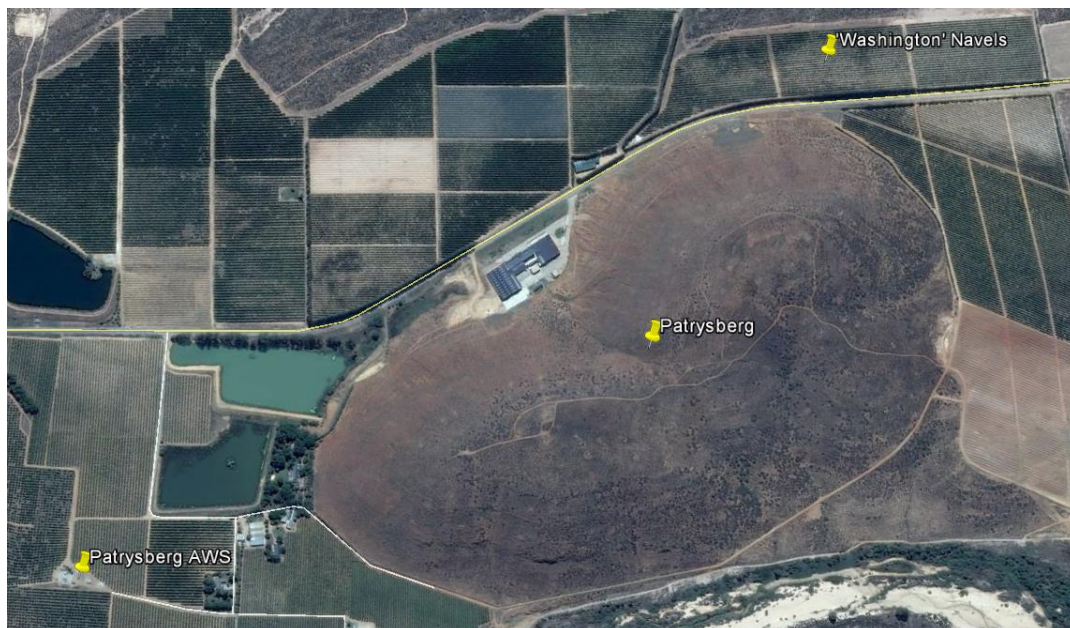
The validation of the HR and CHP methods against a weighing lysimeter in potted *Citrus sinensis* trees in a glasshouse demonstrated that these methods performed well, without the need for calibration (Chapter 3). As these methods need to be deployed in commercial orchards to estimate seasonal transpiration, it is important to demonstrate that similar accuracy can be achieved under field conditions and in larger trees. Calibration and validation of the HR and CHP method was therefore conducted against a micrometeorological method, as described by Poblete-Echeverría et al. (2012) for vineyards, Williams et al. (2004) in an olive orchard, Köstner et al. (1992) in a broad-leaved forest and Conceição and Ferreira (2008) in pear and apple orchards. Orchard transpiration was determined as the difference between evapotranspiration (ET) and soil evaporation ( $E_s$ ). The measurement of  $E_s$  was performed using microlysimeters, either for bare soil or soil covered with vegetation as conducted by Daamen et al. (1993) and ET estimates obtained using the EC technique. The EC method is preferred for the quantification of ET due to its reliability and direct field ET measurements (Wilson et al. 2001). The main objectives of this study were to i) calibrate and validate the HR and CHP methods under field conditions, and, ii) assess if performance was comparable to that under controlled conditions in a glasshouse, and, iii) to determine which method was more suitable to measurements in citrus.

### 4.2 Materials and methods

#### 4.2.1 Description of the experimental orchard

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. The trees were planted in a north-south orientation at Patryberg farm (32°27'44.30"S and 18°59'1.83"E) in the Western Cape Province near Citrusdal (Figure 4-1). Tree spacing was 2.5 x 5 m (12.5 m<sup>2</sup> tree<sup>-1</sup>) 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<sup>-1</sup>. 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 Patryberg farm (Figure 4-1). 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, air temperature and relative humidity. Measurements were stored at hourly intervals on a CR200 datalogger (Campbell Scientific Inc., Logan, Utah, USA) which was powered by a 12 V battery connected to a solar panel.



**Figure 4-1** Location of the 'Washington' navel orchard and the automated weather station

#### 4.2.2 Evapotranspiration measurements

An open path eddy covariance (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 4-2). An extended Open Path EC (OPEC) system, comprising a CSAT3 (Campbell Scientific Inc., Logan, Utah, USA) three-dimensional sonic anemometer, a fast response LI-7500 open path infrared gas (H<sub>2</sub>O and CO<sub>2</sub>) analyser (IRGA) (LI-COR Inc., Lincoln, NE, USA), was mounted at 5 m above ground (2 m above average canopy height) to determine evapotranspiration of the orchard.



**Figure 4-2** Lattice mast in the 'Washington' navels orchard showing position of eddy covariance sensors

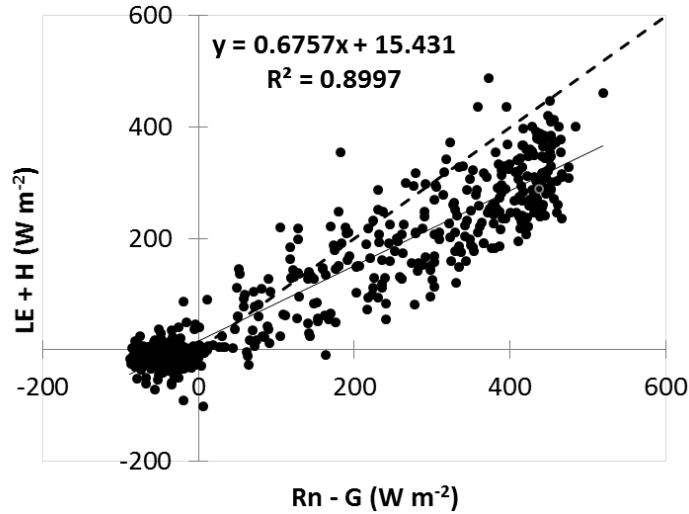
Other components of the EC system included air temperature and humidity measured using a Vaisala HMP45C temperature and humidity probe (Vaisala Oyj, Vantaa, Finland) which was mounted at 4.5 m above ground. Net radiation (R<sub>n</sub>) was measured using an NR-Lite (Kipp and Zonen, Delft, The Netherlands) net radiometer mounted at 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, TCAV-L soil temperature averaging probes (Campbell Scientific Inc., Logan, Utah, USA) were installed at 2 locations representing within-row and between-row conditions and were positioned 20 mm 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 CR5000 data logger (Campbell Scientific Inc., Logan, Utah, USA) 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):

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

Where H is sensible heat flux, LE is latent heat flux,  $R_n$  is net radiation, G is soil heat flux expressed in  $\text{MJ m}^2 \text{ day}^{-1}$  and the EBC is the energy balance closure (dimensionless) (eq.21).

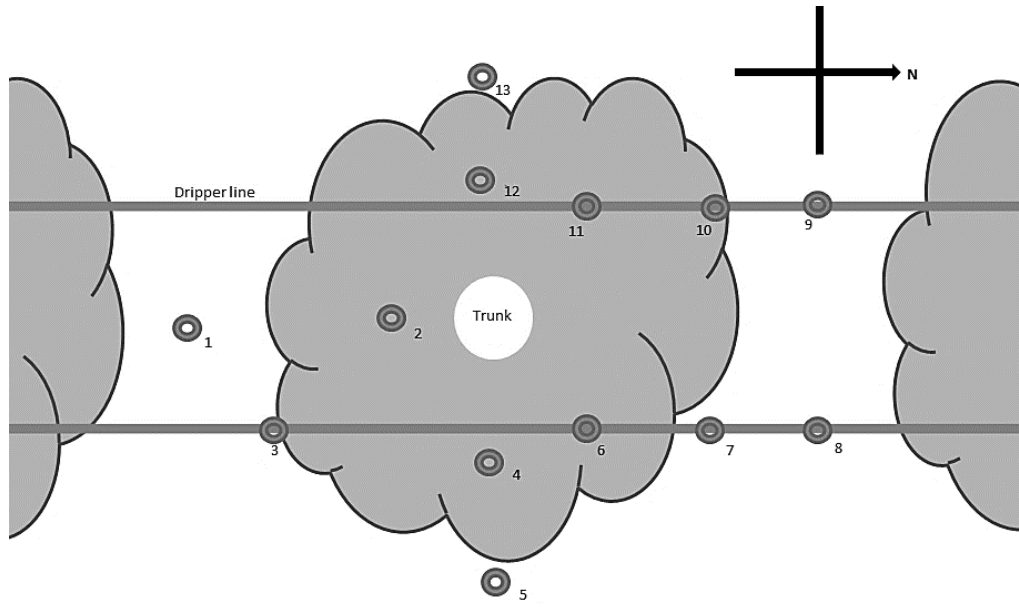
Using all valid half-hourly data, the slope between the available energy flux ( $R_n - G$ ) and the sum of sensible and latent heat fluxes ( $LE + H$ ) was 0.67, intercept of  $15.4 \text{ Wm}^{-2}$ , and the coefficient of determination ( $R^2$ ) was 0.90, as shown in Figure 4-3. The available energy ( $R_n - G$ ) 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 4-3** 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

#### 4.2.3 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 4-4) 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 (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 (2, 4 and 12), two on the canopy edge (5 and 13) and the last one in between the trees (1) (Figure 4-4). Extraction of undisturbed cores from the top soil layer was conducted as described by Daamen et al. (1993). The microlysimeter was gently hammered into the soil, following which the undisturbed soil core was extracted and a sheet metal base plate was placed on the bottom of the microlysimeter, which was secured in place with waterproof tape. These cores were then placed within the external cylinder, flush with the soil surface.



**Figure 4-4** Diagrammatic representation for the placement of microlysimeters (double circles) for evaporation measurements in the ‘Washington’ Navel orchard

One hour after an irrigation event new samples were collected from different sites, corresponding to the same positions indicated in Figure 4-4. 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):

$$E = (1 - f_c) E_{Br} + (f_c) E_{can} \quad (22)$$

Where  $f_c$  is the fractional canopy cover,  $E_{Br}$  is the soil evaporation between row  $\text{mm day}^{-1}$  and  $E_{can}$  is the soil evaporation below the canopy next to the drippers (equation 23).

#### 4.2.4 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 transpiration. 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 lowering the occurrence of corrosion.

#### 4.2.4.1 Heat ratio method

Sensors for the HR method were installed as described in Chapter 3, with a slight modification in the insertion depths as the stems were larger (Table 4-1). For each tree, 4 probes sets (heater, downstream and upstream thermocouples) were inserted to measure sap velocity at 8, 13, 22 and 30 mm depths (Table 4-1). Heat pulse velocities were logged at 1 h intervals on a CR1000 logger (Campbell Scientific Inc., Logan, Utah, USA). Baseline adjustments were conducted as previously conducted 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 sapwood density of 0.66 g cm<sup>-3</sup> and sapwood moisture content 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  $T_{\text{sap}}$  and  $T_{\text{res}}$ .  $T_{\text{res}}$  was determined as the residual of evapotranspiration and evaporation i.e.  $T_{\text{res}} = ET - E_s$ .

**Table 4-1** Trunk circumference (mm) and probe insertion depths for trees selected for sap flow measurements using the HR method

Orchard	Stem circumferences (mm)	Insertion depths of the probes for each tree (mm)
'Washington' Navel	Tree 1 = 273	Probe 1 = 8
	Tree 2 = 321	Probe 2 = 13
	Tree 3 = 295	Probe 3 = 22
	Tree 4 = 302	Probe 4 = 30

#### 4.2.4.2 Compensation heat pulse method

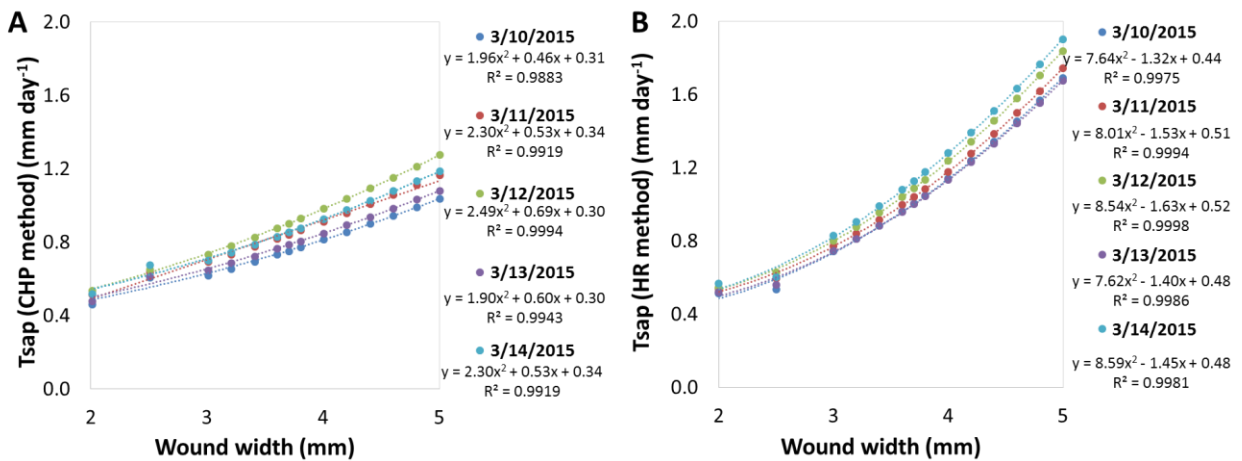
Sensors for the CHP method were installed as described in Chapter 3, also with a slight modification in the insertion depths. Four probe sets were inserted to measure sap velocity at 10, 15, 25 and 35 mm depths (Table 4-2). Heat pulse velocities were logged at 1 hour intervals on a CR1000 logger (Campbell Scientific Inc., Logan, Utah, USA) and orchard  $T_{sap}$  was calculated as described in Chapter 3.

**Table 4-2** Trunk circumference (mm) and probe insertion depths for trees selected for sap flow measurements using the CHP method

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

#### 4.2.5 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 transpiration 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 a set wound widths ranging from 2 mm to 5 mm. Regression equations were determined from 12 points as conducted by Poblete-Echeverría et al. (2012) in a vineyard (Figure 4-5). 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 5 measuring days were averaged to obtain an empirical wound width correction factor.



**Figure 4-5** Regression analysis of wound width against the resulting  $T_{sap}$  for (A) the CHP method and (B) the HR method

#### 4.2.6 Infield determination of the wound correction coefficient and sapwood depth

##### Wound correction coefficient

At the end of the experiment, sections of the tree trunk where probes were inserted were excised from 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 4-6. 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.



**Figure 4-6** Wounding response in 'Washington' navels at the end of the measurements (24 months)

#### Sapwood depth and heartwood radius

Sapwood depth was determined through staining conducting tissue with safranin dye as shown in Figure 4-7. A container filled with safranin 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 cm 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 Figure 4-7 and the width of the heavily stained area was measured using digital vernier callipers. 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.

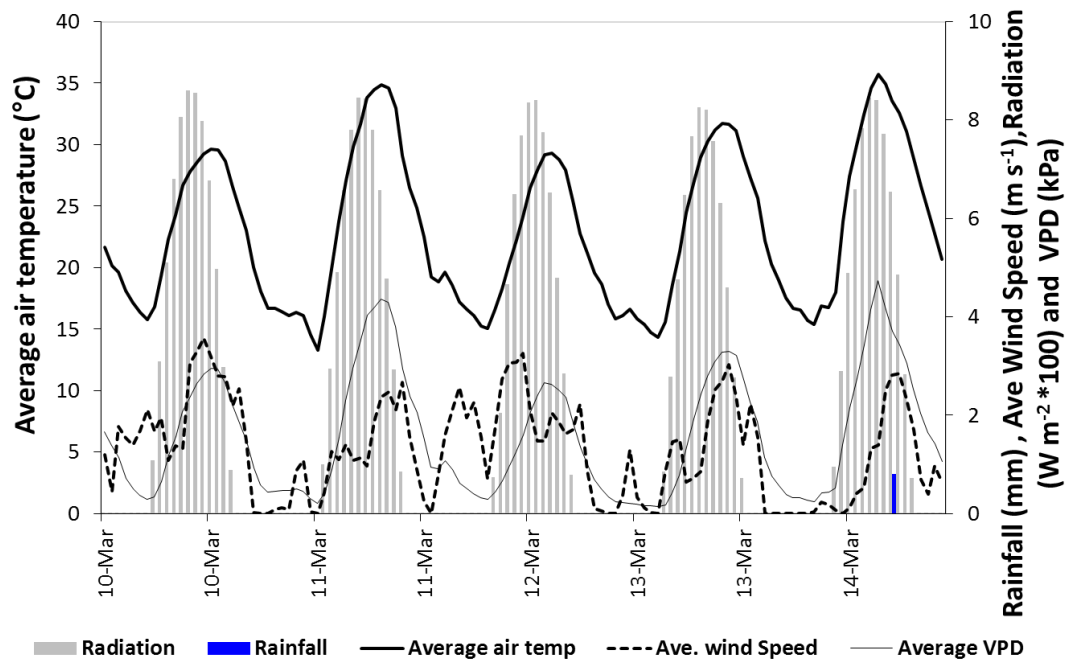


**Figure 4-7** Schematic outlay of the process for determining the sapwood depth

### 4.3 Results and discussion

#### 4.3.1 Weather variables

In Figure 4-8 weather data for the period of 10 - 14 March is presented, which was generally good data. Air temperature, 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. A total of 0.8 mm rain was recorded, which fell on 14 March. Daily VPD varied between 0.8 and 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).

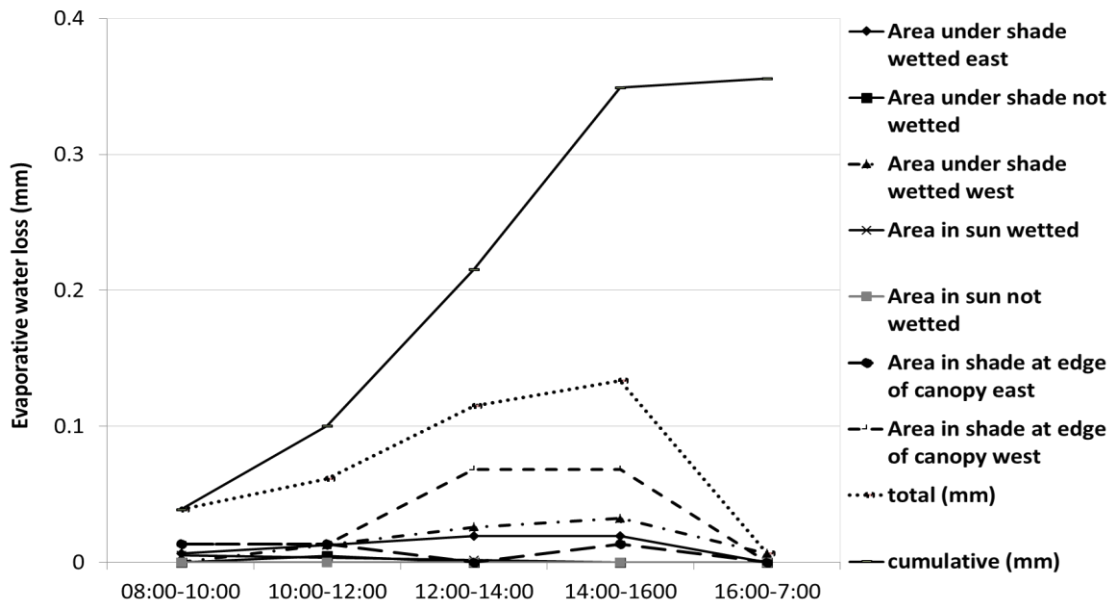


**Figure 4-8** Hourly values of average air temperature ( $^{\circ}\text{C}$ ), solar radiation ( $\text{W m}^{-2} \cdot 100$ ), rainfall (mm), wind speed ( $\text{m s}^{-1}$ ) and average VPD (kPa) at Patryberg from 10-14 March 2015

### 4.3.2 Evaporative water loss from the soil

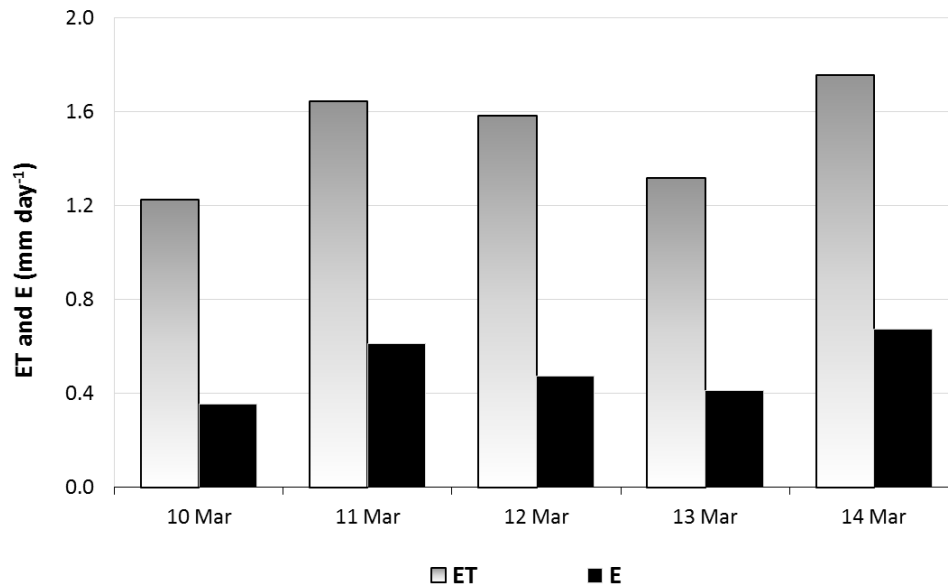
The data presented in Figure 4-9 is an example of the cumulative soil evaporation from different areas in the orchard over a day, during the period 10-14 March 2015 in a 9-year-old 'Washington' Navel orchard. Most of the soil evaporation occurred at the wetted west side of the tree, due to the fact that this side of the tree receives significant solar radiation during the hottest part of the day. Generally evaporation is a two stage process, the first phase is energy limited (Boulet et al. 2004). This is evident in Figure 4-9 on the wetted shaded and sun exposed surfaces, where evaporation increased from morning till solar noon (14:00) as the amount of energy available increased. The second phase is a water limited phase. This was evident in the wetted sun area from 14:00-16:00, as same rate of water loss (evaporation) was recorded for the wetted area in the shade and sun at 14:00 and 16:00. From 16:00 till 07:00 the following day

virtually no evaporation was observed, as there was insufficient energy during the night to drive evaporation.



**Figure 4-9** Cumulative soil evaporation in the 'Washington' Navel orchard on 10 of March

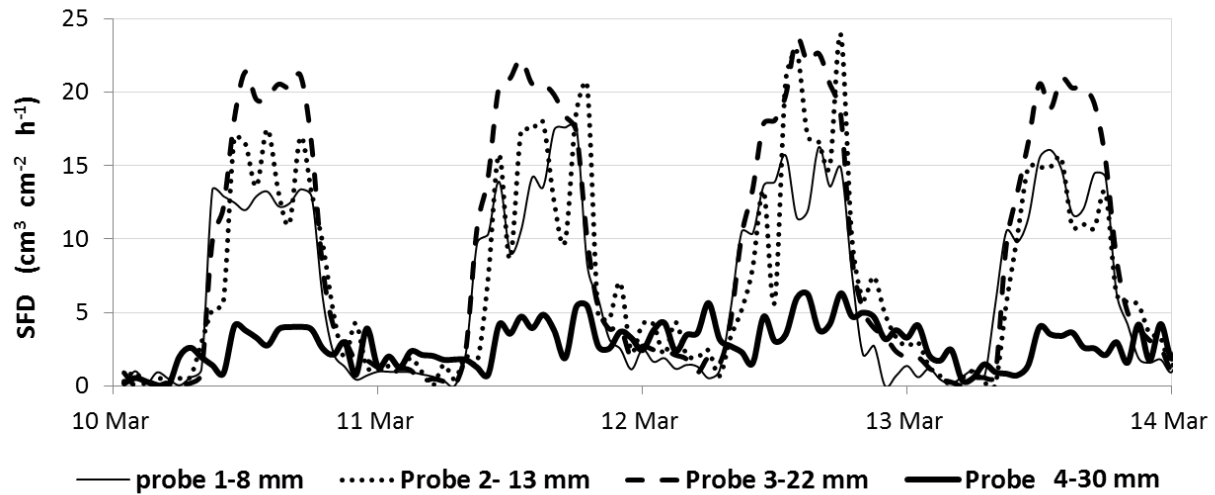
Daily total evaporation (E) and evapotranspiration (ET) for the 9-year-old 'Washington' navel orchard for the period of 10 - 14 March 2015 is presented in Figure 4-10. The average daily E over the measuring period was  $0.51 \text{ mm day}^{-1}$  and the daily average ET was  $1.51 \text{ mm day}^{-1}$ . Over the measurement period E represented approximately 34 % of ET. The maximum E ( $0.68 \text{ mm day}^{-1}$ ) and ET ( $1.75 \text{ mm day}^{-1}$ ) observed during the calibration period coincided with a day where the highest temperatures were recorded (14 March) and the lowest on 10 March when the lowest average air temperature and VPD were recorded for the measurement period.



**Figure 4-10** Total daily soil evaporation (E) (mm day<sup>-1</sup>) and evapotranspiration (ET) (mm day<sup>-1</sup>) in the 'Washington' Navel orchard from 10-14 March 2015

#### 4.3.3 Hourly measured sap flux densities using the heat ratio method

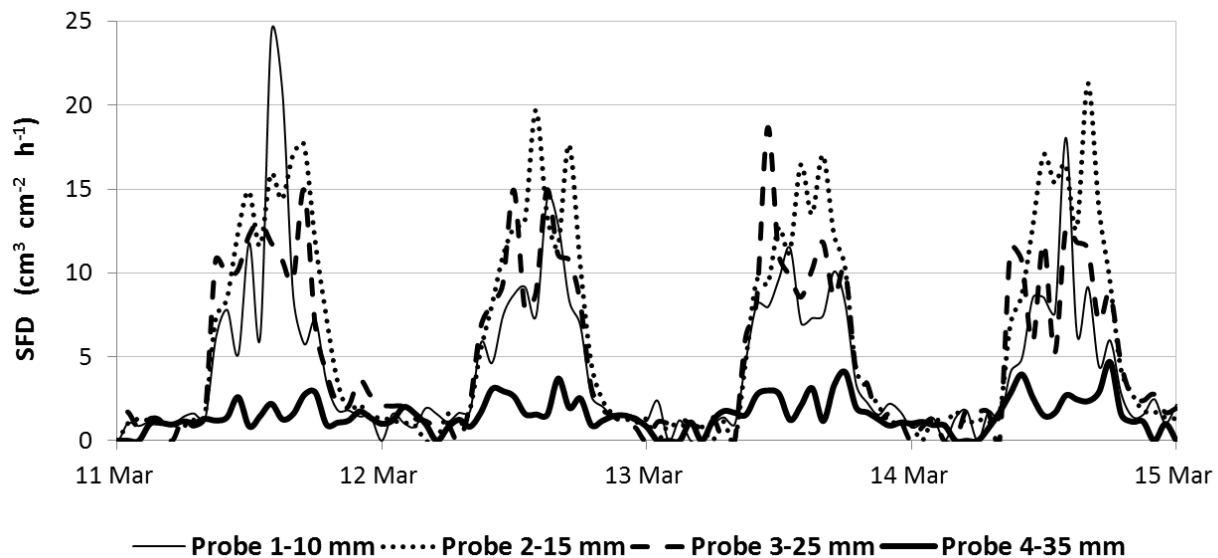
In Figure 4-11 the SFDs calculated with a wound correction factor of 2.5 mm are given for each probe. Clear diurnal trends were recorded for all probes (Figure 4-11), with SFDs characterised by an early morning increase (07:00 - 08:00), midday depressions (12:00 - 16:00), afternoon recoveries (16:00 - 18:00) and nearly zero flows at night (20:00 - 06:00). However, the variation in the four probes inserted at different depths (8, 13, 22 and 30 mm) illustrates the variability in SFD at different depths in the sapwood for the four measuring days. Sap flux densities of the same order (15 - 20 cm<sup>3</sup> cm<sup>-2</sup> h<sup>-1</sup>) 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. Probe set 4, which was installed at 30 mm, registered lower SFDs of approximately 5 cm<sup>3</sup> cm<sup>-2</sup> h<sup>-1</sup>. Sap flux density therefore decreased towards the centre of the stem, as previously noted in a number of species, including grapevine (Poblete-Echeverría et al. 2012).



**Figure 4-11** Sap flux densities (SFD) measured using the HR method with a wound correction factor of 2.5 mm at 8, 13, 22 and 30 mm depth for a ‘Washington’ Navel tree

#### 4.3.4 Hourly measured sap flux densities using the compensation heat pulse method

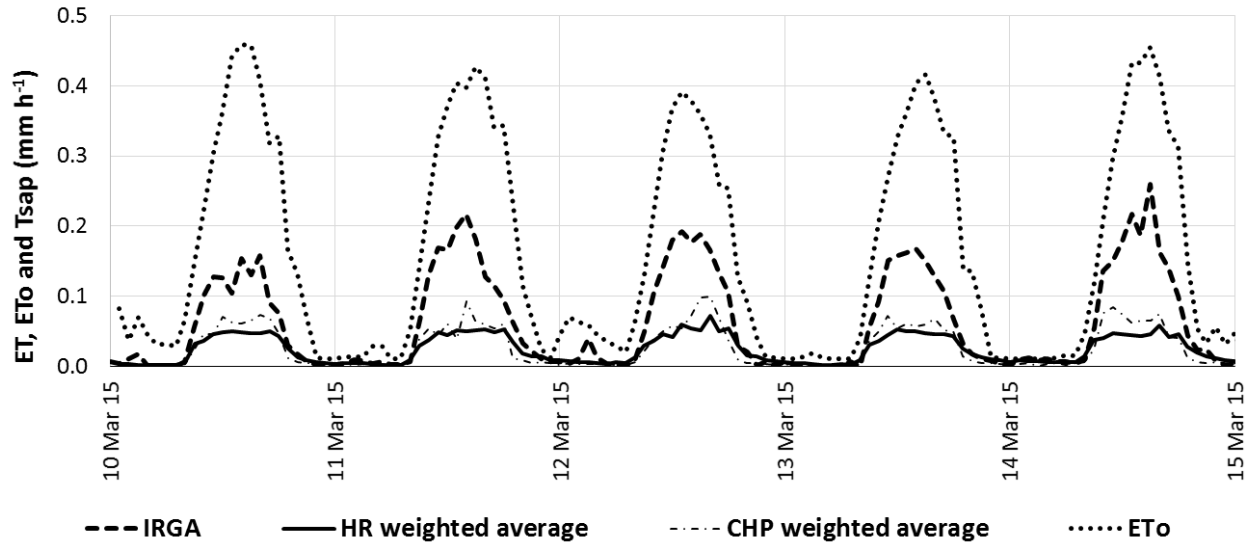
A bell-shaped diurnal trend was observed for all four probes of the CHP method (Figure 4-12). As with the HR method, SFD was characterised by an early morning increase (08:00), fluctuations in the middle of the day (10:00 - 16:00), a sharp decline in the evening (17:00 - 18:00) and nearly zero flows at night (21:00 - 06:00). On average maximum SFDs of approximately  $25 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$  were registered by the sensors at 10, 15 and 25 mm depth, also suggesting little variation in the xylem distribution across the stem section from 0 – 30 mm depth (Figure 4-12). Lowest SFDs were registered for probe 4 of less than  $5 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$ , suggesting lower conductivity of sapwood in that region possibly due to a fewer number of conducting vessels which are older and potentially blocked with gum, thereby rendering them inactive (Poblete-Echeverría et al. 2012).



**Figure 4-12** Sap flux densities (SFD) measured using the CHP method with a wound correction factor of 2.5 mm at 10, 15, 25 and 35 mm depth for ‘Washington’ navels

#### 4.3.5 Comparison of daily reference evapotranspiration, evapotranspiration and $T_{sap}$ from the HR and CHP methods

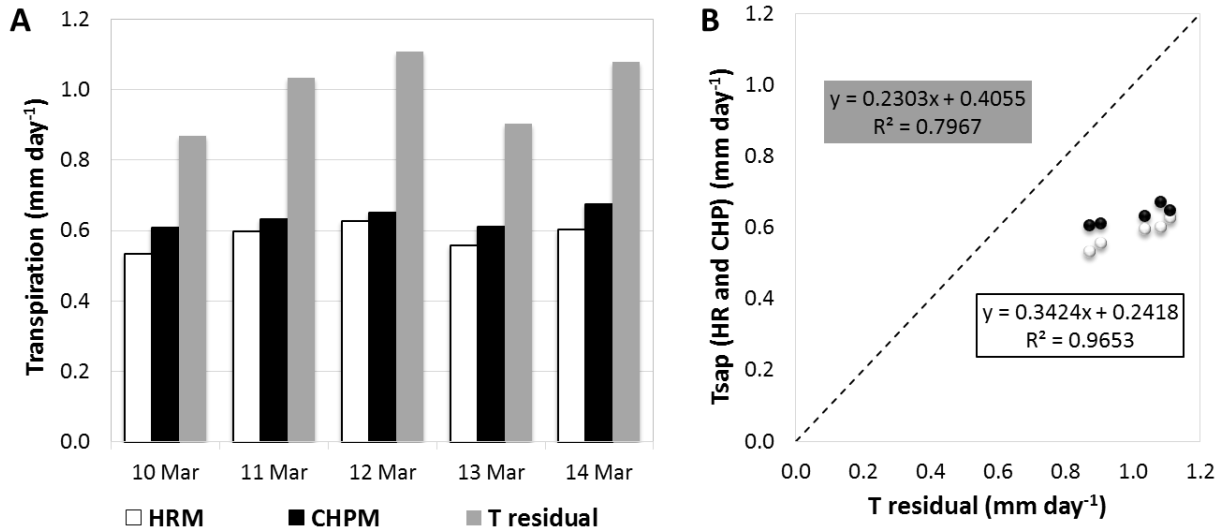
Hourly  $T_{sap}$  (HR and CHP method), evapotranspiration (ET) estimates from the EC system and reference evapotranspiration ( $ET_o$ ) are shown for the period of 10-14 March in Figure 4-13. Regardless of quantitative differences, the daily tracking of the four estimates was qualitatively similar with a bell-shaped diurnal trend. Hourly sap flow estimates from the CHP method and the HR method followed the same trend as  $ET_o$ . As observed by González-Altozano et al. (2008), sap flow rates increased exponentially from approximately 06:00 and decrease to a minimum at 21:00. The maximum sap flow rates ( $0.05 \text{ mm h}^{-1}$  for the HR method and  $0.1$  for CHP method), were recorded at 14:00, coinciding with the maximum  $ET_o$  (Figure 4-13).



**Figure 4-13** Comparison of daily evapotranspiration ( $\text{mm day}^{-1}$ ), transpiration estimates from HR and CHP methods using a wound width of 2.5 mm, and reference evapotranspiration from 'Washington' Navels from 10 - 14 Mar 2015

#### 4.3.6 Comparison of daily sap flow ( $T_{\text{sap}}$ ) versus residual transpiration

Transpirational sap flow determined with the HR and CHP methods were compared to transpiration determined as a residual of ET and E assuming negligible transpiration for cover crops (clean orchard), as shown in Figure 4-14 A and B. 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  $T_{\text{res}}$  by 42 %, whilst the CHP method was lower than the  $T_{\text{res}}$  36 % (Figure 4-14 A). When a comparison was made between the two methods of T estimation, a highly significant linear relationship between  $T_{\text{sap}}$  determined by HR method and  $T_{\text{res}}$  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 CHP method and  $T_{\text{res}}$  (P value < 0.5,  $R^2 = 0.80$ , RMSE = 0.01, MAE = 0.36 and MBE = - 0.36).



**Figure 4-14** Daily total  $T_{res}$  and  $T_{sap}$  determined using the heat ratio (HR) method and compensation heat pulse (CHP) methods (A) and correlation of  $T_{res}$  and  $T_{sap}$  determined with the HR and the CHP methods for the 'Washington' Navel orchard (B). The dashed line is the 1:1 line

The large underestimations observed were mainly attributed to an underestimation of the wound width. Actual wound width determination showed that the wound can extend up to 7.25 mm in the 'Washington' Navels when compared to a wound width of 2.5 mm which was used initially. Although there are a number of sources of potential error associated with this kind of calibration, many of those errors (upscaling evaporation measurement and EC data) can be minimised through applying the correct methodology. However, of all the parameters required to calculate SFD, i.e. wood bulk density, wood moisture content, sapwood depth and heartwood radius, the wound width is probably the most difficult parameter to determine *in situ*, because it 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 for the 'Washington' navels, with a coefficient of variation (CV) of 37 %. This was also observed in 'Midnight' Valencia (CV of 43 %) and 'Afourer'

mandarin (CV of 9 %) orchards where similar measurements were performed in 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. As a result of the variation in wound widths, it is recommended that SFD methods are calibrated against an independent measure of transpiration in the field.

#### **4.3.7 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 4-3). 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 in each method and the fact that the CHP method was installed 4 months after the HR method. The coefficients of the linear regression equations (i.e. a, b and c) used to determine virtual wound widths on a daily basis, as well as the virtual wound widths are presented in Table 4-3. Table 4-4 shows the results of the statistical analysis of the 12 wound sizes chosen in this study, as performed by Poblete-Echeverría et al. (2012). The values of daily  $T_{sap}$  ( $\text{mm day}^{-1}$ ) obtained using different wound sizes were compared with the values of  $T_{res}$  ( $\text{mm day}^{-1}$ ). The  $T_{sap}$  obtained using a wound size of 2 mm presented an underestimation of 46 %, a RMSE value of 0.01  $\text{mm day}^{-1}$  and a MAE of 0.46  $\text{mm day}^{-1}$  for the CHP method and an underestimation of 50 %, an RMSE value of 0.006  $\text{mm day}^{-1}$  and a MAE of 0.5  $\text{mm day}^{-1}$  for the HR method. On the other extreme, when  $T_{sap}$  was calculated using a wound size of 5.0 mm, it produced an overestimation of 77 % with a RMSE value of 0.06  $\text{mm day}^{-1}$  and a MAE of 0.77  $\text{mm day}^{-1}$  for the CHP method

and an underestimation of 15 % an RMSE value of 0.03 mm day<sup>-1</sup> and a MAE of 0.15 mm day<sup>-1</sup> for the HR method.

**Table 4-3** The coefficients of the regression equations ( $ax^2+bx+c$ ) between the resulting  $T_{sap}$  for a specific wound width and the apparent wound width for the specific day which matched  $T_{res}$

Date	a	b	c	R <sup>2</sup>	Apparent wound width (mm)	T <sub>res</sub>	Corrected T <sub>sap</sub>
<b>HR method</b>							
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
<b>Average</b>					<b>4.4</b>		
SD					0.01		
CV					0.2 %		
<b>CHP method</b>							
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
<b>Average</b>					<b>3.6</b>		
SD					0.02		
CV					0.6 %		

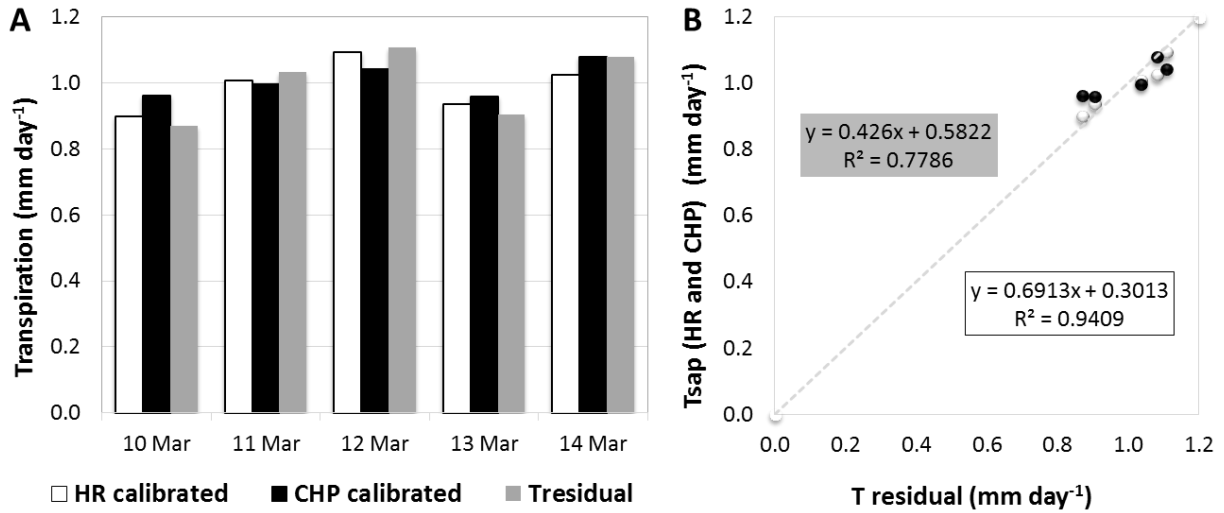
The best significant relationship (P value < 0.05) was obtained using a wound size of 3.6 mm for the CHP method and a highly significant (P value < 0.01) relationship in HR method was obtained with a wound size of 4.4 mm (Table 4-4). In this case the analysis presented an MBE of only 0.01 mm day<sup>-1</sup> an RMSE value of 0.03 mm day<sup>-1</sup> and MAE of 0.05 mm day<sup>-1</sup> for the CHP method and MBE of only -0.01 mm day<sup>-1</sup> an RMSE value of 0.02 mm day<sup>-1</sup> and MAE of 0.03 mm day<sup>-1</sup> for the HR method.

**Table 4-4** Statistical analysis of the correction factors for 12 wound sizes.

wound width (mm)	CHP method			HR method		
	RMSE*	MAE	MBE	RMSE	MAE	MBE
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 4-15 shows the results for the calibrated HR method and the CHP method, a strong linear positive correlation was observed between the  $T_{\text{sap}}$  determined by the HR method and the  $T_{\text{res}}$  ( $R^2 = 0.95$ ) and a lower ( $R^2 = 0.78$ ) was observed between the  $T_{\text{sap}}$  determined by the CHP method and the  $T_{\text{res}}$ .

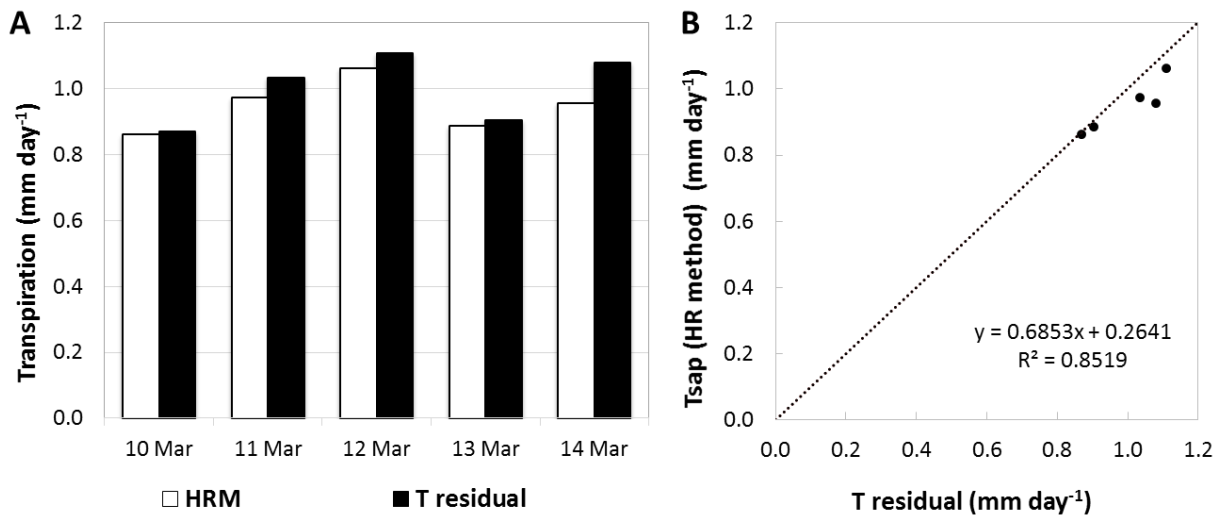


**Figure 4-15** (A) Daily total residual transpiration and calibrated sap flow by the heat ratio (HR) and compensation heat pulse (CHP) method and (B) Regression analysis of daily  $T_{res}$  with the HR and the CHP method ‘Washington’ Navel orchard. The dashed line is a 1:1 line.

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

Although the CHP and HR method both provided good estimates of transpiration once calibrated, frequent missing data points which were replaced by averaging the previous and the succeeding value and random peaks and troughs were observed with the CHP method. 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. 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  $-0.05 \text{ mm day}^{-1}$ , a RMSE of  $0.04 \text{ mm day}^{-1}$  and MAE of  $0.05 \text{ mm day}^{-1}$ . Orchard transpiration was underestimated by 5 % on average per day, which is considered reasonable. The close match of the HR method to micrometeorological method Figure 4-16 shows 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.



**Figure 4-16** (A) Daily total residual transpiration and sap flow by the heat ratio (HR) method and (B) Regression analysis of daily  $T_{res}$  with the HR method 'Washington' Navel orchard. The dashed line is a 1:1 line.

#### 4.4 Conclusions

For the in-field measurements using a wound width of 2.5 mm (width of the widest probe) led to a serious underestimation of transpiration with both the HR and CHP methods. Both these methods were therefore calibrated using an independent estimate of transpiration, calculated as a residual of ET, determined using EC system, and E, determined using microlysimeters.

Calibration was performed by determining a virtual wound width, which resulted in 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 index of agreement for the HR method (0.92) as opposed to the CHP (0.85) and this led to the rejection of the hypothesis which states that the CHP method would estimate sap flow more accurately in field citrus trees than the HR method, as a mature citrus canopy is 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 different from unity, for the calibrated HR method the slope and the intercept were not significantly different from unity and zero, respectively. 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 high possibility of determining transpiration in citrus trees using the HR method without calibration for low transpiration rates. However this should be tested for a number of other citrus varieties. The ongoing research on quantification of citrus water use in citrus orchards (soft citrus, grape fruit, and sweet oranges) will provide an opportunity to confirm these findings. 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 transpiration for each new orchard in which measurements are to be made. The hypothesis “An intercept equal to zero and slope equal to unity at 95% confidence level will be observed when a linear regression analysis between transpiration determined as a residual of evapotranspiration and evaporation and  $T_{sap}$  determined by a calibrated HR method and CHP method in the field” is true for the calibrated HR method but not for the calibrated CHP method.

## **CHAPTER 5**

# **Comparison of sap flux density measurements with vapour pressure deficit, stomatal conductance and leaf water potentials**

### **5.1 Introduction**

In the previous chapters SFD methods were validated in *Citrus sinensis* and it was observed that sap flow varied considerably throughout the day and was particularly noticeable around midday for as shown in Figure 3-17 A and C and Figure 3-21 A and C. Therefore to ascertain if the trend in sap flow observed throughout the day was real or an artefact of the measurement technique, sap flow was compared to VPD, LWP and  $g_s$ . Dziki et al. (2007) has previously reported stomatal oscillations which were reported to contribute to the simultaneous observations of oscillations in sap flow rates and LWP. Stomatal conductance measurements were therefore conducted, in addition to LWP to test the hypothesis that the variations in sap flow throughout the day in citrus can be explained by variations in (stomatal conductance)  $g_s$  and that a direct linear relationship between  $g_s$  and sap flow exist.

### **5.2 Materials and methods**

#### **5.2.1 Weather data**

Vapour pressure deficit data for the infield experiment was obtained from the weather station which was erected on Patrysberg farm. For the glasshouse VPD was calculated from data obtained from a Vaisala HMP45C temperature and humidity probe (Vaisala Oyj, Vantaa, Finland) installed in the glasshouse.

#### **5.2.2 Physiological measurements**

Physiological measurements included  $g_s$ , which was used to explain the variations in hourly sap flow observed and LWPs to assess if the trees were water stressed.

### **5.2.2.1 Stomatal conductance**

#### Glasshouse experiment

In the glasshouse gs measurements were conducted at hourly intervals from 07:00 - 10:00. Half hourly measurements were conducted from 10:30 - 14:00, to capture possible oscillations in gs, which may result in fluctuations in sap flow at mid-day. Hourly intervals from 14:00 - 16:00 and lastly at half hour interval from 16:00 - 17:00 from 11 – 13 Sept. Measurements were conducted on 9 selected leaves, 3 sunlit leaves on the east, 3 sunlit leaves on the west and 3 shaded leaves with virtually no radiation interception throughout the day. Measurements were conducted for 2 days on two different trees instrumented with the HR and CHP method. Stomatal conductance of sunlit leaves was averaged and compared to sap flow, LWP and VPD.

#### Infield experiment

For the infield measurements gs measurements were conducted with an AP4 leaf porometer (Delta-T Devices Ltd, Cambridge, UK) at hourly intervals from 07:00 to 18:00 from 10 - 14 March 2015. Measurements were conducted on nine selected leaves per tree on four trees (three east side, three west side and three shaded with virtually no radiation interception throughout the day). For the purposes of this study only the sunlit leaf gs was used for comparison as these are the leaves that contribute most to the observed sap flow patterns and the same was done for the glasshouse experiment.

### **5.2.2.2 Leaf water potential**

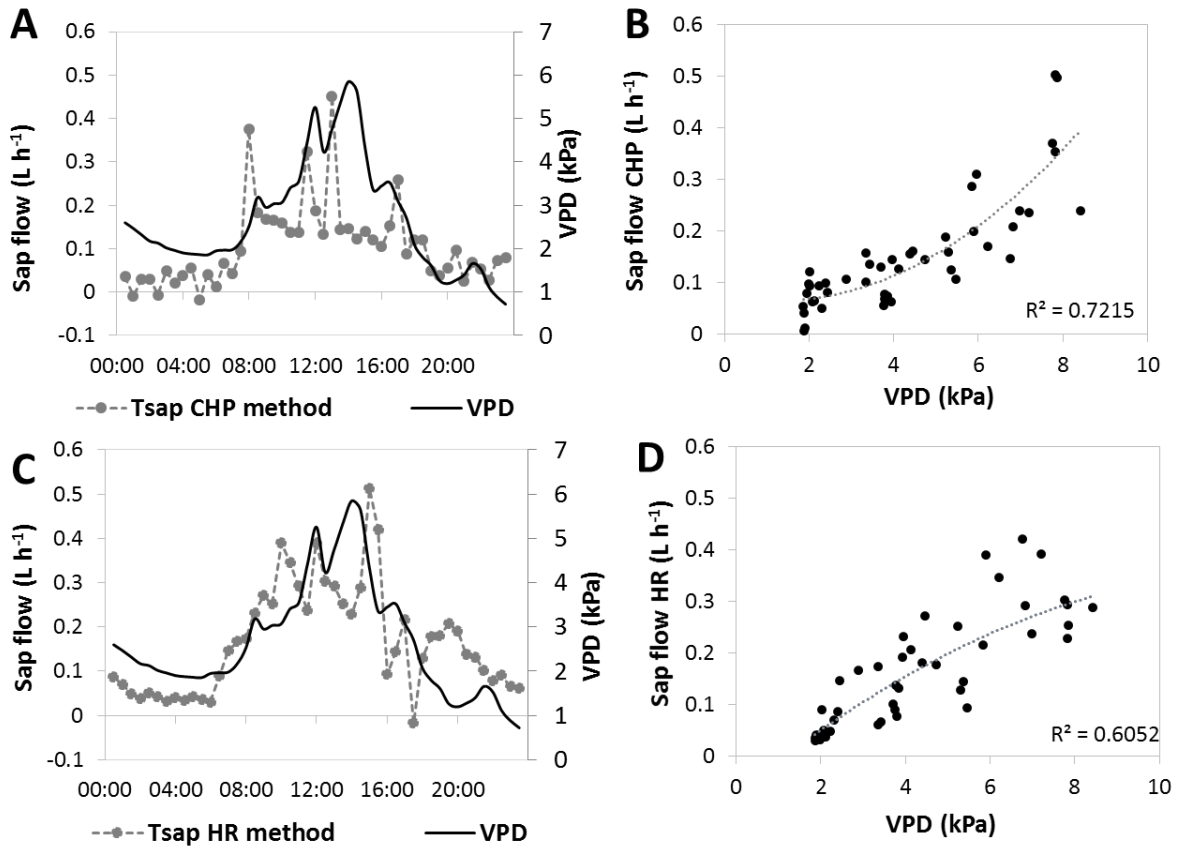
Soil water potentials were estimated from pre-dawn LWP measurements, using a Scholander Pressure Chamber (PMS3000, PMS Instruments Company, Corvallis, Oregon, USA) on selected fully expanded healthy leaves for both infield and glasshouse experiment as described by Cohen and Naor (2002). These measurements were used to assess if any soil water deficit influenced tree water use. Stem and LWP measurements were conducted hourly to determine the diurnal trends. For stem water potential, the leaves were bagged and covered with

aluminium foil for at least two hours before measurements. On average 9 leaves were measured per hour on each tree. LWP of sunlit leaves was averaged and compared to the gs, sap flow and VPD.

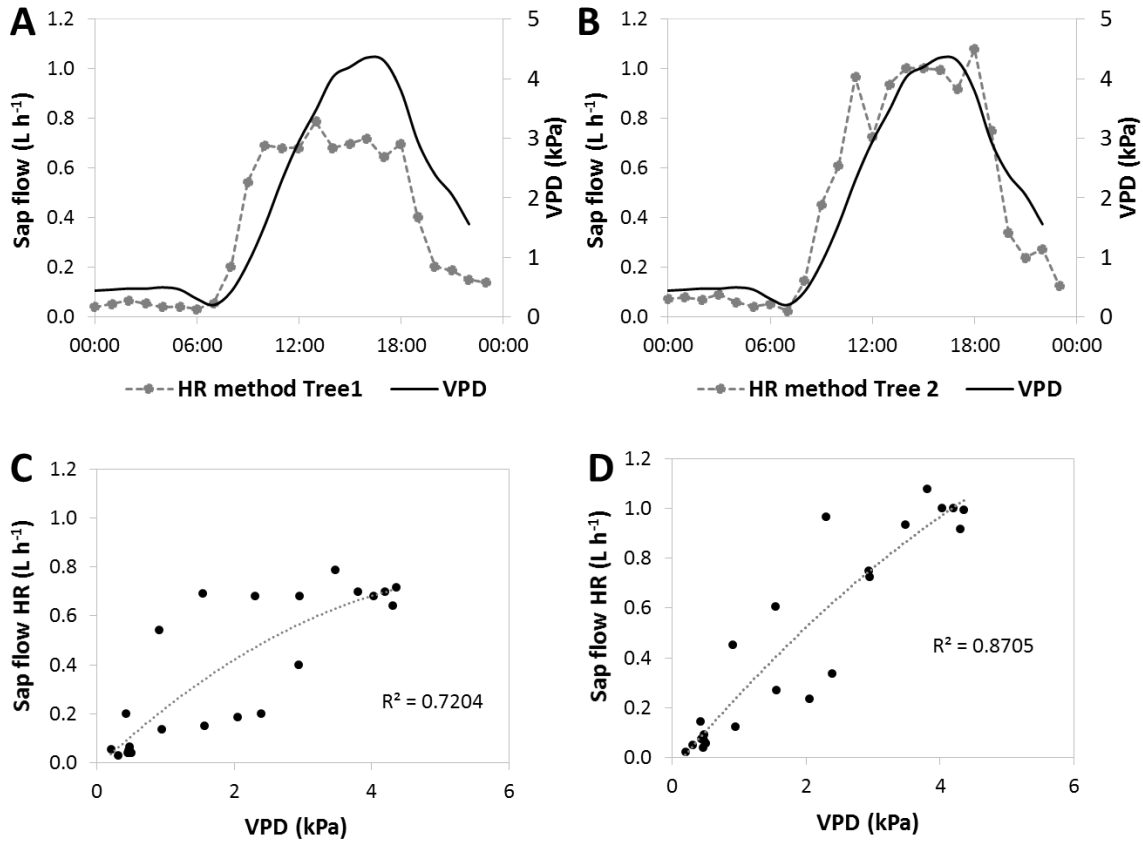
### **5.3 Results and discussion**

#### **5.3.1 Oscillations in sap flow for *Citrus sinensis* trees in a glasshouse and under field conditions**

Throughout the validation period sap flow rate increased from 07:00 due to increased solar radiation, which in turn caused an increase in VPD. Maximum sap flow rate was realised around 08:00 and this rate was maintained throughout the day until approximately 17:00 and thereafter decreased to minimal values for both glasshouse (Figure 5-1 A and C) and infield (Figure 5-2 A and C) experiments. It is evident in Figure 5-1 A and C that sap flow in 'Midnight' Valencia trees in the glasshouse did not follow a smooth curve but some form of oscillations occurred throughout the day. The oscillations were more pronounced in the glasshouse experiment than the field, since infield sap flow rates were measured at hourly intervals compared to the 30 minute intervals measurements in the glasshouse (Figure 5-2 A and C). Direct relationships were observed between sap flow and VPD and these relationships were best expressed as a second order polynomial for both the glasshouse and infield experiment (Figure 5-1 A and C). In the glasshouse VPD explained 72 % of the variation in sap flow measured using the CHP method and 61 % for the tree where sap flow was measured with HR method (Figure 5-1 B and D). For the field experiment 72 % of the variation in sap flow was explained by VPD for tree 1 and 87 % for tree 2. Oscillations in sap flow observed in the glasshouse appear to be real, as major peaks in sap flow followed closely the peaks in VPD and sap flow was well related to VPD. The relationship between sap flow and VPD observed is similar to Dzikiti et al. (2007) who observed similar, but more pronounced, oscillations in navel orange trees.



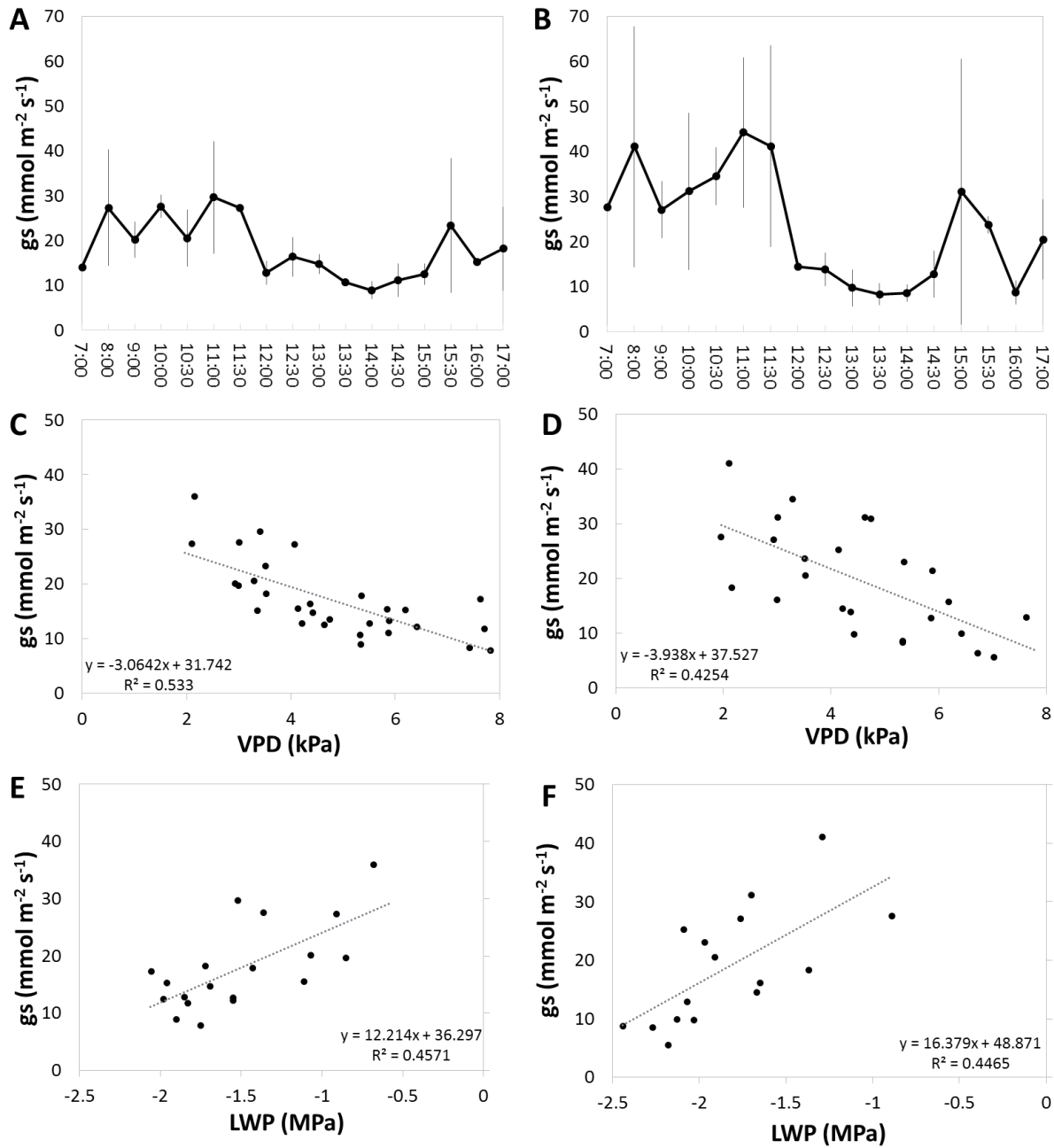
**Figure 5-1** The diurnal trend of sap flow and VPD of 'Midnight' Valencia determined with A) CHP and C) HR method on a typical clear day. Each data point is an average of 30 minutes, (B) and (D) the relationship between sap flow and VPD for the 'Midnight' Valencia trees in a glasshouse.



**Figure 5-2** The diurnal trend of VPD and sap flow determined using the heat ratio (HR) method in a ‘Washington’ Navel orchard for (A) tree 1 and (C) tree 2 on a typical clear day. Each data point is an average of 1 hour. The relationship between sap flow and VPD for (B) tree 1 and (D) tree 2 for the ‘Washington’ Navel trees infield.

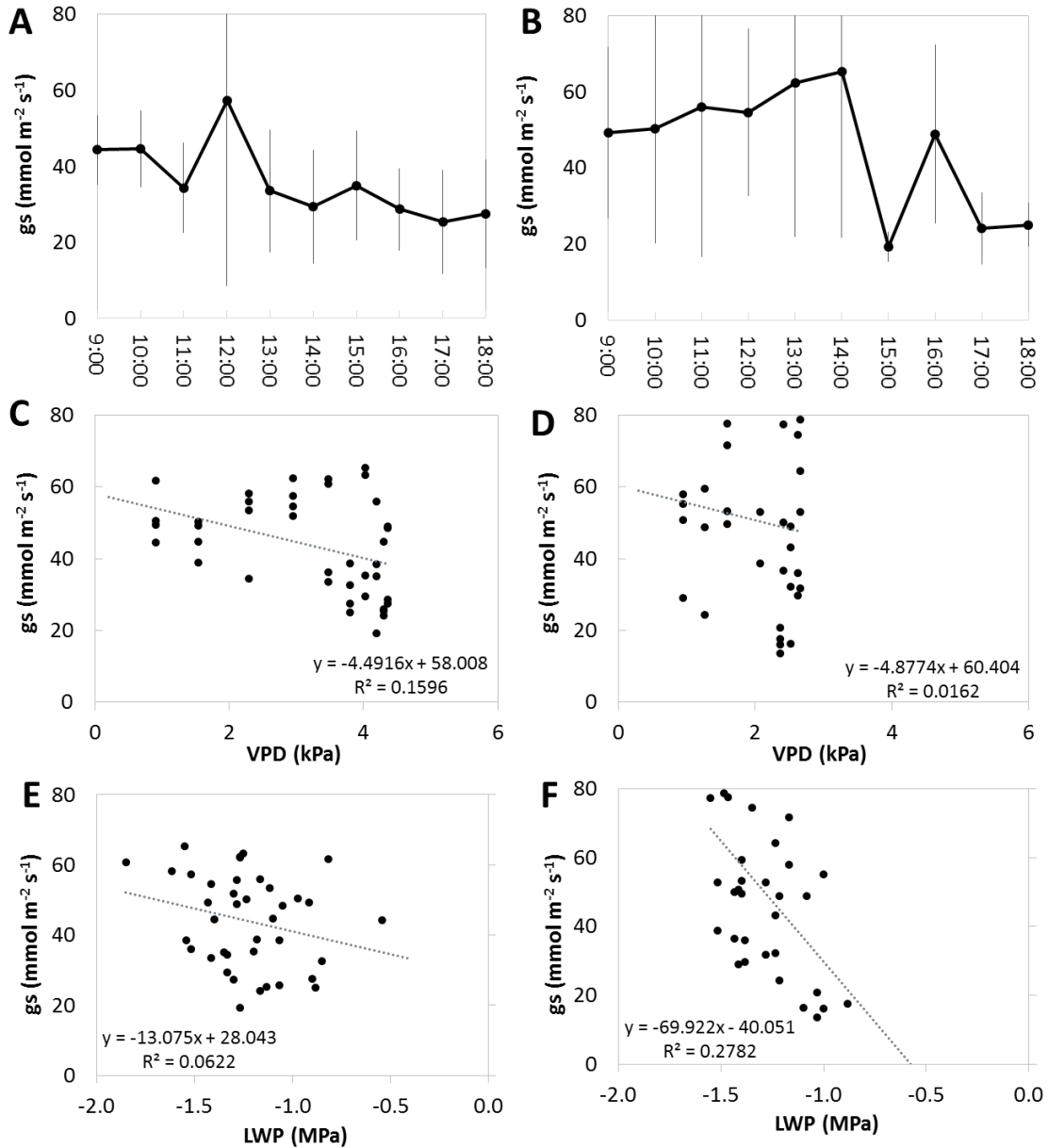
### 5.3.2 Oscillations in stomatal conductance and leaf water potential in a glasshouse and under field conditions

The daily course of mean sunlit leaf  $g_s$  in ‘Midnight’ Valencia trees in a glasshouse are presented in Figure 5-3 A and B. Sunlit leaf  $g_s$  increased in the morning, reaching its peak between 10:00 and 11:00 with high variation in leaf to leaf  $g_s$ . Stomatal conductance decreased at noon in both trees for approximately 2 hours and then increased again towards the end of the day with a peak in  $g_s$  observed between 15:00 and 16:00 on both occasions.



**Figure 5-3** The diurnal trend of sunlit leaf stomatal conductance of 'Midnight' Valencia trees on a typical clear day in a glasshouse for (A) tree 1 and (B) tree 2. The relationship between sunlit leaf  $gs$  and VPD is given for the 'Midnight' Valencia trees for (C) tree 1 and (D) tree 2 average of two days 11 and 13 November 2015. The relationship between sunlit leaf  $gs$  and LWP is given for the 'Midnight' Valencia trees for (E) tree 1 and (F) tree 2 for 11 and 13 November.

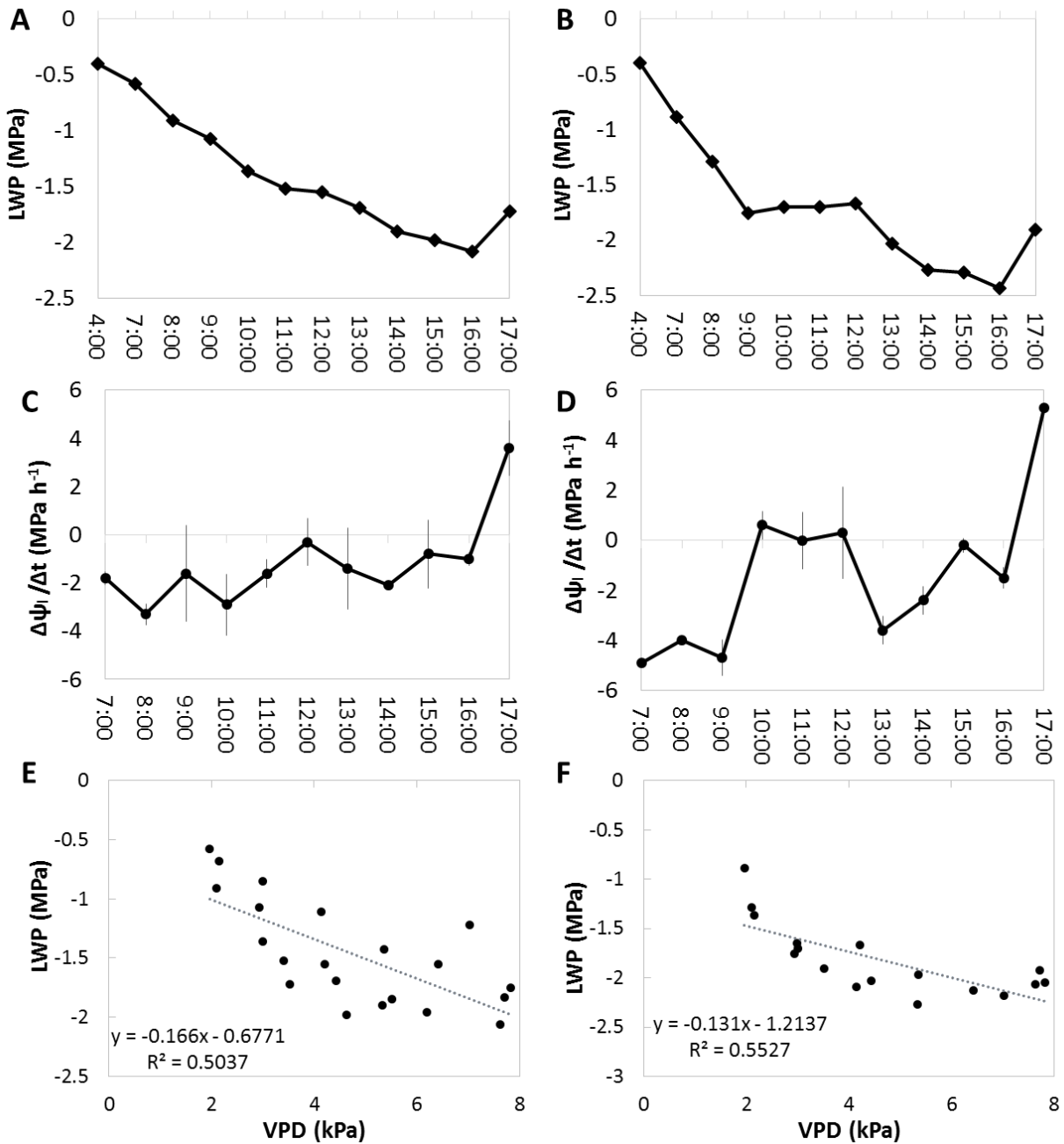
At lower  $g_s$ , i.e. between 12:00 to 15:00, there was little variation in  $g_s$  between the leaves, which again increased as the  $g_s$  increased in the afternoon (Figure 5-3 A and B). Lower  $g_s$  observed at midday is most likely a result of an increase in VPD, as stomatal closure was observed to occur around 3 kPa. This possibly indicates a feedback mechanism whereby rapid water loss is regulated in order to prevent desiccation and loss of hydraulic integrity, as previously reported by Sinclair and Allen (1982) in a citrus tree. The increase in  $g_s$  close to the end of the day can be attributed to a decrease in VPD, as a result of a decrease in temperature and solar radiation. Approximately 53 % of the variation in  $g_s$  on the tree instrumented with the CHP method and 43 % for a tree with HR method were explained by the VPD. Generally an inverse relationship between  $g_s$  and VPD was observed (Figure 5-3 C and D), whereas a decrease in water potential resulted in a decrease in  $g_s$  (Figure 5-3 E and F) as observed by Moriana et al. (2002) in olive tree and Cohen and Cohen (1983) in citrus trees. For the infield experiment the oscillations in  $g_s$  were not as apparent when compared to the glasshouse experiment, as the  $g_s$  measurements were conducted at hourly intervals throughout the day. As observed with the trees in the glasshouse,  $g_s$  in the 'Washington' navels trees also increased early in the morning, reaching maximum values between 12:00 and 14:00 and decreasing thereafter. As expected leaf to leaf  $g_s$  varied widely in field as indicated by the error bars in Figure 5-4 A and B as observed by Cohen and Cohen (1983) in citrus trees. The trees measured infield were bigger trees when compared to the glasshouse and are exposed to a natural environmental. An inverse relationship between  $g_s$  and VPD and  $g_s$  and LWP was observed for both the 'Midknight' Valencias and 'Washington' navels. However, the relationship between  $g_s$  and VPD and  $g_s$  and LWP was moderately strong in 'Midknight' Valencias ( $R^2 = 0.53$  and  $R^2 = 0.43$  for the trees instrumented with CHP and HR method respectively (Figure 5-5 E and F), whilst weak relationships ( $R^2 = 0.16$  and  $R^2 = 0$  for tree1 and 2 respectively (Figure 5-6 E and F) were observed in 'Washington' navels under field conditions.



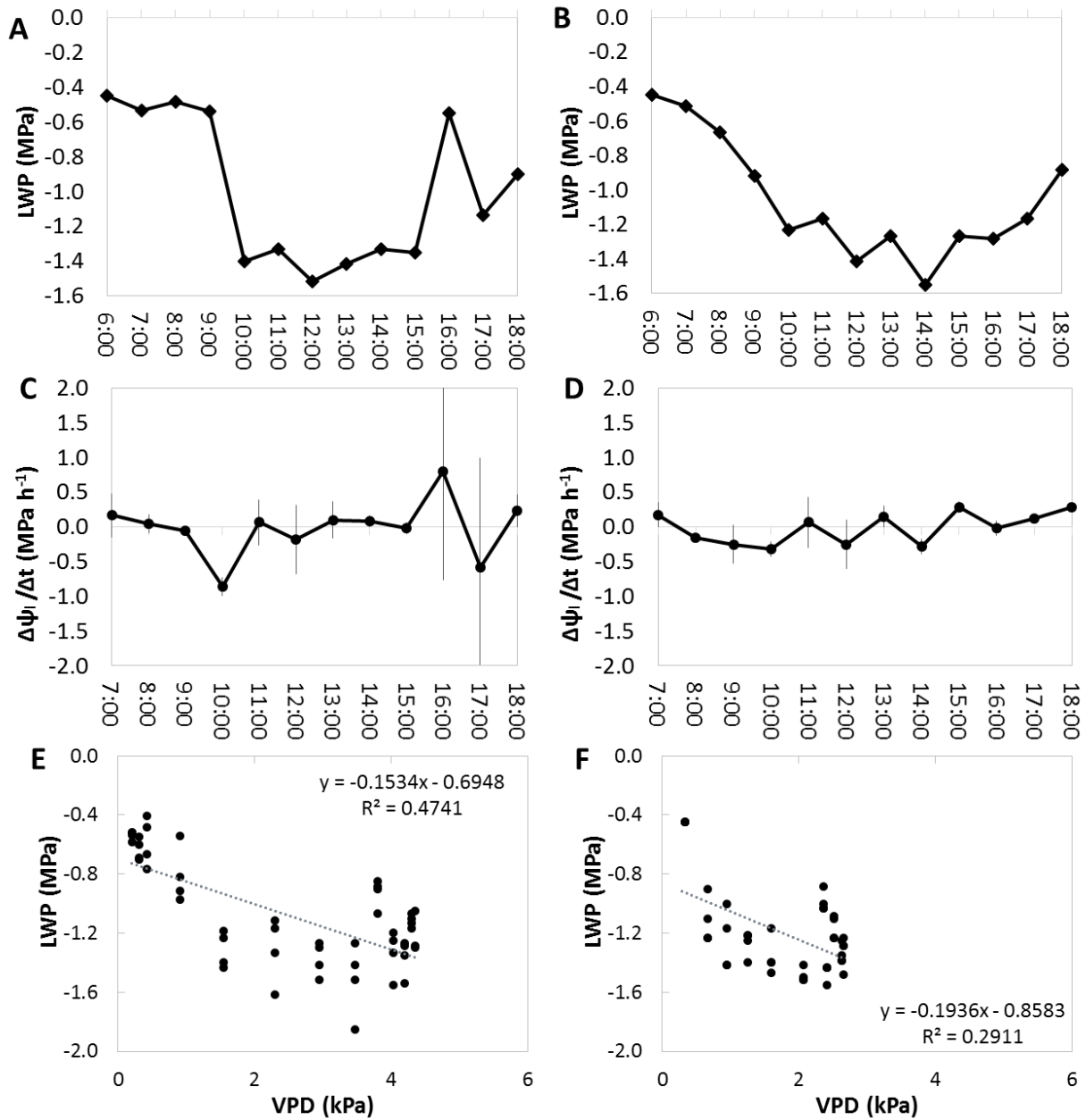
**Figure 5-4** The diurnal trend of sunlit leaf stomatal conductance of ‘Washington’ navel trees on a typical clear day in the field for (A) tree 1 and (B) tree 2. The relationship between sunlit leaf  $gs$  and VPD is given for the ‘Washington’ navel trees for (C) average of 4 trees on 11 March 2015 and (D) average of 4 trees on 12 March 2015. The relationship between sunlit leaf  $gs$  and LWP is given for the ‘Washington’ navel trees for (E) average of 4 trees on 11 March 2015 and (F) average of 4 trees on 12 March 2015.

Low  $g_s$  values measured in this study are consistent to those found in 'Bahianinha' navel trees (Dzikiti et al. 2007; Steppe et al. 2006) and Valencia orange trees (Fereres et al. 1979). Low leaf  $g_s$  in citrus is considered an important factor in restricting transpiration from citrus groves in a hot arid environment (Van Bavel et al. 1967). The daily pattern of LWP followed a typical sinusoidal curve in both the glasshouse and under field conditions (Figure 5-5 A and B and Figure 5-6 A and B). For the 'Midknight' Valencia in the glasshouse LWP dropped gradually from a high value at predawn to a low value at 16:00 and then increased again for both trees, as shown in Figure 5-5 A and B. Similar trends were also observed for the 'Washington' navel trees in the field, except that minimal values were observed at around 12:00 to 14:00 which then increased after 15:00 (Figure 5-6 A and B).

It is assumed that plant and soil come into equilibrium overnight and reaches the daily maximum level just before sunrise (Sellin, 1999). Therefore, the predawn LWP is a measure of the plant available soil moisture level and it can be used as a surrogate for root zone soil water potential (Dzikiti et al. 2010). For citrus predawn LWP values of less than -0.5 MPa typically suggest that the tree is water stressed (Kriedemann and Barrs 1981; Ribiero and Machado 2007). For this calibration window, the predawn LWP for both the glasshouse trees and infield trees was above -0.5 MPa indicating that the trees were well irrigated and unlikely to be experiencing water stress as a result of a soil water deficit. The diurnal variations in the rate of change of LWP also suggest oscillations (Figure 5-5 C and D and Figure 5-6 C and D) and the variance of the parameter is much smaller than the amplitude of the cycle as also observed by Cohen and Cohen (1983). As mentioned previously,  $g_s$  increases in response to VPD this results in water loss from the plant leaves to the atmosphere creating higher negative water potential in the leaves, this is evident in Figure 5-5 E and F and Figure 5-6 E and F as high VPD corresponded to low LWP and vice versa.



**Figure 5-5** Diurnal trend of the LWP for 'Midnight' Valencia (A) tree 1 and (B) tree 2. Rate of change of leaf water potential ( $\Delta\psi_l/\Delta t$ ) on 'Midnight' Valencia (C) tree 1 and (D) tree 2 on 11 November in a glasshouse vertical bars indicate two standard deviations. The relationship between sunlit LWP and VPD is given for the 'Midnight' Valencia trees for (E) tree 1 and (F) tree 2 for 11 and 13 November



**Figure 5-6** Diurnal trend of the LWP for ‘Washington’ navel (A) tree 1 and (B) tree 2. Rate of change of LWP ( $\Delta\psi_i/\Delta t$ ) on ‘Washington’ navel (C) tree 1 and (D) tree 2 on 11 March 2015 in the field vertical bars indicate two standard deviations. The relationship between sunlit LWP and VPD is given for the ‘Washington’ navel trees for (E) tree 1 and (F) tree 2 for 11 and 13 March 2015

## 5.4 Conclusions

The diurnal trend of the  $g_s$  was similar to that of sap flow increasing from morning due to an increase in solar radiation and thereafter decreased to low values due to increased VPD. Leaf water potential decreased from sun rise and reached lowest values around midday and thereafter increased, which was an inverse relationship to  $g_s$ . The variations in sap flow throughout the day, which were thought to be caused by stomatal oscillations, were not observed in this study. However at 30 minutes interval in the glasshouse and hourly interval in the field the variations in sap flow were better explained by the VPD. This led to the rejection of the hypothesis which stated that, variations in sap flow over the day in citrus can be explained by stomatal oscillations and a direct linear relationship will be observed between  $g_s$  and sap flow. Even though the hypothesis was rejected this chapter shows that the measurements of sap flow which were conducted infield and in the glass were real as supported by physiological measurements.

## CHAPTER 6

## General conclusions

According to the Department of Agricultural Forestry and Fisheries (DAFF) (2015), the South African citrus industry is ranked as the third largest horticultural industry after deciduous fruits and vegetables. The number of workers directly employed by the industry in orchards and pack houses exceeds 100 000 people (DAFF, 2015). Additional people are also employed throughout the supply chain services such as transport, port handling and marketing. Therefore it is estimated that, over a million households rely on the South African citrus industry for their livelihood (DAFF, 2015). During the 2013/2014 season the industry represented about 15 % of the total gross value (R 53.2 billion), contributing R 9.69 billion to total gross value of South African agricultural production (DAFF, 2015). The citrus industry therefore plays an important role in South African agriculture and its sustainability should be greatly encouraged.

A number of factors are currently threatening the sustainability of the industry, which include citrus black spot, the African fruit fly, greening disease, and the availability of water. Water is debatably the most important, as the industry relies on irrigation for production. As citrus is an evergreen crop, mainly grown in areas with erratic rainfall, the vast majority of orchards are irrigated throughout the year. According to the Citrus Growers Association (CGA) (2015) approximately 64 510 ha was under citrus production in South Africa and the industry is still expanding. Expansion of the industry places serious pressure on water resources, of which 98% of the available water has been allocated already (Von Bormann and Gulati, 2014). As a result, the industry needs to justify the volumes of water they use to guarantee future quotas. It is for these reasons that the Water Research Commission of South Africa solicited, funded and managed a project on quantification of citrus (soft citrus, grape fruit and sweet oranges) water use, together with co-funding from Citrus Research International.

There are a number of reliable methods for quantifying orchard level water use, including EC and weighing lysimeters. However, for this project evaporation and transpiration measurements needed to be conducted separately in order to assess and model these processes separately as suggested by Kool et al. (2014). Lysimetry methods, which are considered as the golden standard, 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 were sap flow techniques (Smith and Allen 1996) and there are quite a number of techniques available. Whilst, the theory behind the different methods differs, all the techniques use heat as a tracer to estimate water movement up a stem. The stem heat balance is regarded as one of the most accurate techniques and does not require any calibration, however, it was not suitable for this study, as it is limited to trees with a diameter not greater than 12.5 cm. As a result it was decided to focus on SFD measurement method, which can be used for a wide range of stem sizes and these included the HR and the CHP methods. These methods have been used extensively as non-destructive methods to estimate transpiration of woody species such as 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 radiata* (Teskey and Sheriff, 1996), willow (Green et al. 2003) and lemon (Alarcón et al. 2005), amongst many others. The major concern with these techniques is that they tend to underestimate transpiration (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). Calibration involves the determination of an empirical correction factor to account for a systematic underestimation of transpiration and this is best done via an independent measure of transpiration. As accurate estimates of transpiration were required for the citrus water use project, it was imperative to evaluate the accuracy of these techniques in citrus prior to the commencement of field measurements. The initial test of

the heat pulse velocity equipment with the HR method in a *Eucalyptus marginata* tree (model specie for HR method) gave good results indicating there were no faults with the equipment. After this test, the SFD measurement methods (HR and CHP methods) were calibrated and validated in potted 'Midnight' Valencia trees with the use of weighing lysimeters in a glasshouse, following which the transferability of the glasshouse results were evaluated in a commercial 'Washington' Navel orchard, in Citrusdal.

At the start of the study the main question was can SFD measurement methods be used to accurately quantify transpiration in citrus? The simple answer to this questions is yes, as accurate measurements of transpiration were obtained in a glasshouse and in a commercial orchard when compared against a second measure of transpiration. The two heat pulse velocity methods evaluated, the HR and CHP methods, performed equally well in the 'Midnight' Valencia trees when compared to the weighing lysimeters in the glasshouse on a day time basis. Over a period of 37 and 30 days for the HR and the CHP method, an error less than 2% was noted between the HR and CHP methods and the weighing lysimeter, when a wound width of 2.0 mm (width of the widest probe). However on an hourly basis both the HR and the CHP method overestimated transpiration at low flow rates less than about  $0.1 \text{ L h}^{-1}$  and underestimated transpiration at flow rates greater than  $0.1 \text{ L h}^{-1}$ . The measurement of sap flow and the actual process of transpiration are spatially separated on the tree, time lags between the two processes may occur (Oren et al. 1999). 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 which is most likely associated with plant refilling as reported by Alarcón et al. (2000); Caird et al. (2007); Burgess and Dawson, (2008) and Herrera et al. (2012). Even though the hourly comparisons of the  $T_{\text{sap}}$  to the weighing lysimeters did not yield a good agreement, the sap flow values observed on hourly basis were real, as variations in sap flow were well explained by the VPD for both the

glasshouse and field experiments as observed by Ortuno et al (2006), Zhang et al. (2011) and Poblete-Echeverría et al. (2012).

In order to verify the findings obtained from the 'Midnight' Valencia trees in the glasshouse, the HR and CHP methods were calibrated and validated in a commercial 'Washington' Navel orchard using a micrometeorological method 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 transpiration by approximately 42 % and 36 % when both the HR and the CHP methods 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 coefficient of variation (CV) of 37 % in 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. Therefore due to a smaller variation in wound width for glasshouse experiment and a nearly 100 % match between the  $T_{sap}$  (HR and CHP method) there was no need for calibration of the heat pulse velocity techniques. 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, as conducted in previous studies (Poblete-Echeverría et al. 2012, Taylor et al. 2015). An agreement of 94 % was observed between the HR method and  $T_{res}$ , underestimating orchard transpiration 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 well against  $T_{res}$  with a correlation coefficient of 78 %, overestimating transpiration 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 ( $<5 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$  and  $>100 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$ ) the HR method was tested within its limits, the maximum SFDs observed were around  $20 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$  which is below its upper limit of  $45 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$  (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,  $T_{\text{sap}}$  values within 5% of  $T_{\text{res}}$  were obtained for the HR method. These were good results considering the potential large sources of error associated with this validation and what it means is that, the SFD methods, specifically the HR method, can be used in citrus orchards without *in situ* calibration. Therefore accurate determination of the sapwood width, heartwood radius and the wound width is essential for correct estimates of transpiration in *Citrus sinensis*. Insertion of the probes into the conducting sapwood is also essential and this can be improved by stem staining prior to field measurements, as this can give an indication of the radius of conducting sapwood within the stem. However, a general conclusion about whether heat pulse techniques can be used in all citrus varieties and all citrus orchards cannot be made. Generally, if the parameters such as wound width, sapwood conducting area and heartwood radius can be determined accurately, the heat pulse techniques can be used to quantify transpiration in *Citrus sinensis* (Navels and Valencia trees), which should be classified under a list of forest and woodland eucalyptus for which validation studies have been reported in the literature, including: *Eucalyptus regnans* F.J. Muell. (Vertessy et al. 1997), *Eucalyptus grandis* (Dye and Olbrich 1993), and *Eucalyptus populnea* (Hatton et al. 1995). It is still advisable to conduct calibrations for specific citrus varieties to ensure accurate measurements.

## Recommendations and future research

For the glasshouse experiment the HR and CHP method performed equally well, however, for the infield experiment the HR method performed better than the CHP method. The calibrated CHP method resulted in an intercept that was not significantly different from zero but the slope was significantly different from unity. For the calibrated HR method the slope and the intercept were not significantly different from unity and zero, respectively. A lot of missing data was observed for the CHP method making data processing difficult, which was not the case with the HR method. Therefore the HR method is highly recommended for long term measurement of citrus water use. This study also reiterates the need for caution when measuring the parameters required to convert heat pulse velocity ( $\text{cm h}^{-1}$ ) to sap flow ( $\text{L h}^{-1}$ ). These parameters include bark thickness, sapwood depth, wood moisture content and wood density as indicated by sensitivity analyses.

Despite the excellent results obtained in the study, a number of challenges were encountered and should serve as a caution for future calibration studies. Firstly, very poor results were obtained when attempts were made to calibrate all the three methods at once in a single stem, which was mainly attributed to thermal interferences amongst the probes of the different methods. Secondly, far more accurate measurements were made when the probes were placed in the rootstock, as opposed to the scion, which was attributed to differences in the radius of the conducting tissue in the scion as opposed to the rootstock. Finally, there is no distinctive colour change between conducting sapwood and non-conducting heartwood in citrus and thus the use of a stain is key to determining sapwood conducting area in a stem needed to convert sap flux densities to sap volumes. Although the stems for the glasshouse study were stained by destructively cutting the stem and placing it in a staining solution, experiments in the field have shown that this can be done *in situ*, without significant damage to the trees. These kinds of

staining experiments can be done prior to insertion to determine appropriate insertion depths and after the study to assess sapwood area in the measurement trees.

Ongoing research is focusing on the quantification of citrus water use at orchard scale with the use of calibrated HR method, so this will provide an opportunity for frequent infield calibration exercises to show if the same wound correction factor holds over a long period of time for the same orchard. Installation of the heat pulse velocity sensors in the rootstocks will also be carried out in the field to ascertain if the same wound corrections are applicable to the rootstocks since they differ in wood anatomy. Practical measurements of the wound width and sapwood were conducted and this resulted in a close match between the HR method and the  $T_{res}$  infield, this can be improved by use of advanced technologies in wound width and sapwood determination. Therefore future research will focus on practical measurements and assessment of the wound widths using advanced technologies such MRI and scanning electron microscope, as these are not always easy to determine with the naked eye.

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## Appendix

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

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.96865461
R Square	0.938291754
Adjusted R Square	0.92947629
Standard Error	0.091148277
Observations	9

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	0.88427971	0.88427971	106.437	1.74115E-05
Residual	7	0.058156059	0.008308008		
Total	8	0.942435768			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
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 'Midnight' Valencia in the glasshouse

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.987901548
R Square	0.975949468
Adjusted R Square	0.97526231
Standard Error	0.109833476
Observations	37

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	17.13326518	17.13326518	1420.269246	6.33117E-30
Residual	35	0.422218733	0.012063392		
Total	36	17.55548391			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
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 'Midnight' Valencia in the glasshouse

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.952820451
R Square	0.907866812
Adjusted R Square	0.904454471
Standard Error	0.105656966
Observations	29

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	2.970065836	2.970065836	266.0540067	1.6647E-15
Residual	27	0.301411652	0.011163395		
Total	28	3.271477487			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
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 'Midnight' Valencia in the glasshouse

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.474100901
R Square	0.224771665
Adjusted R Square	0.160169303
Standard Error	0.163793462
Observations	14

ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	0.093343975	0.093343975	3.479310356	0.086769096
Residual	12	0.321939577	0.026828298		
Total	13	0.415283553			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
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

<i>Regression Statistics</i>						
Multiple R	0.983085374					
R Square	0.966456852					
Adjusted R Square	0.955275802					
Standard Error	0.006497788					
Observations	5					

<i>ANOVA</i>						
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>	
Regression	1	0.003649479	0.003649479	86.43704293	0.002634041	
Residual	3	0.000126664	4.22212E-05			
Total	4	0.003776143				

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
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

<i>Regression Statistics</i>						
Multiple R	0.9464803					
R Square	0.895824959					
Adjusted R Square	0.861099945					
Standard Error	0.049957055					
Observations	5					

<i>ANOVA</i>						
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>	
Regression	1	0.064383472	0.064383472	25.79768482	0.014743032	
Residual	3	0.007487122	0.002495707			
Total	4	0.071870594				

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
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

<i>Regression Statistics</i>						
Multiple R	0.970017534					
R Square	0.940934016					
Adjusted R Square	0.921245354					
Standard Error	0.021365134					
Observations	5					

<i>ANOVA</i>						
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>	
Regression	1	0.02181495	0.021815	47.79065441	0.006204003	
Residual	3	0.001369407	0.000456			
Total	4	0.023184357				

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>
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

<i>Regression Statistics</i>						
Multiple R	0.887257346					
R Square	0.787225598					
Adjusted R Square	0.716300798					
Standard Error	0.010974624					
Observations	5					

<i>ANOVA</i>						
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>	
Regression	1	0.001336843	0.001336843	11.09944043	0.044666507	
Residual	3	0.000361327	0.000120442			
Total	4	0.00169817				

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