



Monitoring the simultaneous response of various water
status indicators for use in the irrigation scheduling and
drought stress detection of a greenhouse tomato crop

by

Sebastian Nicholas Grey

Supervisor: Mr. E. Mashonjowa

Department of Physics

Faculty of Science

University of Zimbabwe

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For
Albert Grey
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Abstract

An experiment was conducted in a greenhouse at the Department of Biological Sciences, University of Zimbabwe to test the use of various plant water status indicators for use in the irrigation scheduling and water stress detection of a greenhouse tomato crop. These indicators included midday leaf water potential (LWP_{midday}), leaf temperature, stem diameter, fruit diameter, stomatal resistance and sap flow. Two replicated treatments were used for the experiment namely a well watered treatment in which plants were always given sufficient water ('sufficient' being based on calculations of water requirements made using the FAO-Penman-Monteith equation), and a drought stressed treatment in which plants were periodically subjected to drought stress by turning off their water supply valves for a number of days. The procedure was to monitor the variation of these indicators with time for the 60 days of the experiment in both treatments (and their replicates) and correlate these indicators to plant water requirements calculated using the FAO-Penman-Monteith equation. Also twice during the 60 day period of the experiment the drought stressed treatment (and its replicate) were subjected to the drought stress mentioned earlier and the physiological responses noted.

In terms of determining irrigation quantity, sap flow and daily mean leaf temperature showed the highest correlations with ET_o ($R^2 = 0.64$), these were followed by daily maximum leaf temperature and maximum daily stem shrinkage (MDS) with R^2 values of 0.454 and 0.401, respectively. Daily fruit growth was a distant fifth ($R^2 = 0.221$) with stomatal resistance and leaf water potential being found to have no value in determining irrigation quantity of greenhouse grown tomatoes in this case. In terms of stress detection and irrigation timing, LWP_{midday} showed the best and quickest response (≈ 2 days) with sap flow (≈ 3 days) and leaf to air temperature difference (≈ 4 days) being second and third, respectively. For LWP_{midday} it was possible to define a threshold of $\leq -1600\text{kPa}$ while for leaf temperature a threshold for leaf-air temperature difference between the time of 3:00 pm and 4:45 pm of $\geq -2^\circ\text{C}$ was determined at which point plants require

water urgently. Stem diameter fluctuations and fruit growth showed some value in terms of monitoring growth rates in a commercial setting. However, they had little value in drought stress detection mainly because of the difficulty in coming up with defined thresholds at which water stress is said to be occurring. Stomatal resistance showed good response to drought stress but suffers due to its reliance on other environmental parameters. However, it was found that stomatal resistance is higher in water stressed plants, and in severely stressed plants will increase with increasing solar radiation; possibly as a defense mechanism to reduce transpiration. LWP_{midday} showed promise for future research on irrigation scheduling and stress detection. However, the need for commercially operational and possibly automated systems points to the use of sap flow, leaf temperature and MDS in irrigation scheduling and stress detection of greenhouse crops. Future experiments or operational tests should include an experimental phase for data collection followed by a validation phase in which the indicators and the results obtained from them are used in operational automated systems and compared against each other.

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Table of Contents

Abstract.....	ii
Acknowledgements	iv
Table of Contents	v
List of Tables	ix
Table of Figures.....	x
Abbreviations and Symbols	xiii
Chapter 1: Introduction	1
1.1 Background and Justification.....	1
1.2 Aims and Objectives	3
1.3 Potential Benefits of the Study	4
1.4 Thesis Layout.....	5
Chapter 2: Literature Review.....	6
2.1 Introduction.....	6
2.2 The Importance and Functions of Water to Greenhouse Plants	7
2.3 The Movement of Water within Plants.....	9
2.4 Tomato Characteristics and Water Requirements.....	11
2.5 Greenhouse Crops and Water Stress.....	12
2.5.1 What is Water Stress?	12
2.5.2 The Effects of Water Stress on Plants.....	12
2.5.3 Detecting the Water Status and Water Needs of Greenhouse Plants.....	15
2.5.3.1 Empirical Formula	15
2.5.3.2 Substrate Based Methods.....	17
2.5.3.3 Pan Evaporation Methods.....	19
2.5.3.4 Radiation Methods.....	20
2.5.3.5 Plant Based Methods.....	21
2.5.3.5.1 Visible Symptoms.....	23
2.5.3.5.2 Measurement of Plant Water Content or Energy	23
2.5.3.5.3 Fruit and Stem Diameter Changes	26
2.5.3.5.4 Leaf Thickness	27
2.5.3.5.5 Leaf Temperature.....	28

2.5.3.5.6 Sap Flow Measurements	30
2.5.3.5.7 Remote Sensing (RS).....	31
2.5.3.5.8 Stomatal Resistance	32
2.5.3.6 Other Methods	33
2.6 Summary	34
Chapter 3: Materials and Methods	36
3.1 Introduction.....	36
3.2 Experimental Location.....	36
3.3 The Greenhouse	38
3.4 The Planting and Care of Tomato	39
3.5 The Experimental Setup.....	41
3.6 The Calculation of Irrigation Amounts.....	43
3.7 Meteorological Measurements.....	44
3.7.1 Temperature and Humidity Measurements.....	44
3.7.2 Radiation Measurements.....	45
3.7.2.1 Solar Radiation.....	45
3.7.2.2 Net Radiation	47
3.7.2.3 Photosynthetically Active Radiation (PAR)	48
3.7.3 Wind Speed Measurements.....	48
3.8 Physiological Measurements	50
3.8.1 Measurement of Leaf Water Potential	50
3.8.2 Measurement of Stomatal Resistance	52
3.8.3 Measurement of Stem and Leaf Temperature.....	52
3.8.4 Measurement of Stem and Fruit Diameter.....	54
3.8.5 Measurement of Sap Flow	56
3.9 Instrument Calibration	58
3.9.1 Thermocouples.....	58
3.9.2 Radiation Thermometers.....	59
3.9.3 Solar Radiation Sensors	60
3.9.3.1 Tube Solarimeter.....	60
3.9.3.2 Quantum Sensors	61
3.9.3.3 Net Radiometer	61
3.9.3.4 Matrix Radiation Sensor	61

3.9.4 Temperature and Humidity Sensors.....	62
3.10 Data Logging and Data Collection	63
Chapter 4: Results and Discussion	65
4.1 Introduction.....	65
4.2 Instrument Calibration	65
4.2.1 Thermocouples.....	65
4.2.2 Temperature and Humidity Probes	66
4.2.3 Radiation Sensors.....	68
4.2.3.1 TSL Tube Solarimeter.....	68
4.2.3.2 LI-190SZ Quantum Sensors (LI-COR Inc, Nebraska, USA)	69
4.2.3.3 Q7.1 Net Radiometer (REBS, Washington, USA)	69
4.2.3.4 Mk-1-G Radiation Sensor (Matrix Solar Services, Arizona, USA).....	70
4.2.4 Radiation Thermometers.....	71
4.3. Microclimate Inside and Outside the Greenhouse	72
4.3.1 Air Temperature.....	74
4.3.2 Vapour Pressure Deficit.....	75
4.3.3 Photosynthetically Active Radiation (PAR)	76
4.3.4 Solar Radiation.....	77
4.4 Water requirement calculations	78
4.4.1 ET _o Calculations Using the FAO-Penman-Monteith Equation	78
4.5 Physiological measurements	82
4.5.1 Leaf Temperature.....	82
4.5.1.1 Comparison between Stem and Leaf Temperature	84
4.5.1.2 The Use of Leaf Temperature in Irrigation Scheduling.....	87
4.5.1.3 Response of Leaf Temperature to Drought Stress	91
4.5.2 Stem Diameter	93
4.5.2.1 Stem Diameter Derived Indices	93
4.5.2.2 The Use of Stem Diameter in Stress Detection	97
4.5.3 Fruit Diameter	98
4.5.4 Stomatal Resistance	101
4.5.5 Leaf Water Potential	104
4.5.6 Sap Flow	107
4.5.6.1 The Use of Sap Flow to Calculate Daily Irrigation Quantity	107

4.5.6.1.1 The Main Variables Driving Sap Flow	111
4.5.6.2 The Response of Sap Flow to Water Stress	117
4.6 Summary	119
Chapter 5: Conclusions and Recommendations	123
References	125
Appendix A: Significance Tests	131

List of Tables

Table 2.1: Approximate magnitudes of water potentials in the soil-plant-atmosphere continuum.	11
Table 3.1: The fertiliser schedule followed in the growing of the tomato plants	40
Table 3.2: The main tomato diseases and the chemicals used for their treatment.....	41
Table 3.3: Days on which plants in treatments B and D were subjected to drought stress	43
Table 3.4: Summary of the meteorological parameters measured and the instruments used.	50
Table 3.5: Summary of the physiological parameters measured and the sensors used. ...	57
Table 4.1: Results of thermocouple calibrations done on 4 th September 2009.....	66
Table 4.2: Results of infrared thermocouple calibrations done on 4 th September 2009 ...	72
Table 4.3: Summary of the microclimate outside the greenhouse for the entire period of the experiment from 1 January 2010 to 1 March 2010	73
Table 4.4: The usefulness of the investigated plant indicators in determining water requirements (quantity) for a greenhouse tomato crop	120
Table 4.5: The usefulness of the investigated plant indicators in the detection of drought stress and irrigation timing.....	121

Table of Figures

Figure 2.1: The pathway of water movement in the soil plant atmosphere continuum showing resistances, capacitances and water potentials (Challand and Bakker, 1995)....	10
Figure 3.1: A satellite image of the University of Zimbabwe showing the location of the greenhouse used at the Department of Biological Sciences, as well as the location of the Department of Physics New Wing and its roof top (Image courtesy of Google Earth/ Europa Technologies, 2009).	37
Figure 3.2: The experimental greenhouse at the Department of Biological Sciences, University of Zimbabwe.	39
Figure 3.3: Floor plan of the greenhouse showing the setup used for the purpose of this project.	43
Figure 3.4: The louvered radiation shield containing the HMP45C temperature and humidity sensors a) inside the greenhouse and b) outside the greenhouse.....	45
Figure 3.5: A tube solarimeter measuring radiation above the canopy of the tomato crop	46
Figure 3.6: Images of the Mk-1-G Sol-A-Meter (Matrix Solar Services, Arizona, USA) showing a) a close up image and b) its location at the greenhouse (circled in red).....	47
Figure 3.7: The Q7.1 net radiometer (REBS, Washington, USA) used in the greenhouse	47
Figure 3.8: The LI-190SZ Quantum Sensor (LI-COR Inc, Nebraska, USA) used in the greenhouse	48
Figure 3.9: Image showing a) the use of a heated bead anemometer to sample wind speeds within the greenhouse and b) the heated bead anemometer used.	49
Figure 3.10: The Wescor Thermocouple Psychrometer kit with sample chambers, switchbox, microvoltmeter and the four airtight plastic chambers that were used to collect the samples.....	51
Figure 3.11: The measurement of stomatal resistance of the leaves of tomato plants using the AP4 porometer (Delta-T Devices, Cambridge, UK).....	52
Figure 3.12: A thermocouple clipped onto the underside of a leaf so as to measure leaf temperature.	53
Figure 3.13: A thermocouple inserted into a stem (circled in red) to measure stem temperature.	54
Figure 3.14: The measurement of stem diameter using a) a micrometer screw gauge and b) a DEX 70 dendrometer (Dynamax, USA).....	55
Figure 3.15: The measurement of fruit diameter using a) a vernier caliper and b) a DEX 100 dendrometer (Dynamax, USA).	55
Figure 3.16: The sap flow gauge a) before installation and b) after installation covered with a reflective heat shield to reduce errors.	56
Figure 3.17: The two different types of data loggers used in the experiment namely (a) the CR10X and (b) one of the two CR23X data loggers.	63
Figure 3.18: PC208W screenshot with the Z10 data logger highlighted preceding downloading of data.....	64
Figure 4.1: Calibration results of the temperature sensor inside the HMP45C probe	67

Figure 4.2: Calibration results of the RH sensor inside the HMP45C probe.....	67
Figure 4.3: The results of the calibration of the tube solarimeter done on 23 August 2009	68
Figure 4.4: The inter-comparison results of the two Quantum Sensors done on the 9 th of March 2009	69
Figure 4.5: Results of the calibration of the Q7.1 net radiometer (REBS, Washington, USA) done on the 19 th and 20 th of May 2009	70
Figure 4.6: Results of the calibration of the Mk-1-G Radiation Sensor done on the 9 th of March 2009	71
Figure 4.7: Daily mean temperature inside and outside the greenhouse for the period of the experiment (1 January = DOY 1)	74
Figure 4.8: Daily mean vapour pressure deficit inside and outside the greenhouse for the period of the experiment	75
Figure 4.9: Daily mean PAR inside and outside the greenhouse for the period of the experiment.....	76
Figure 4.10: Daily mean solar radiation inside and outside the greenhouse for the period of the experiment	77
Figure 4.11: The variation of daily ET_o with time throughout the course of the experiment	78
Figure 4.12: The correlation between daily total net radiation and ET_o for the 60 days of the experiment.....	79
Figure 4.13: The correlation between daily mean air temperature and daily ET_o calculated using the FAO-Penman-Monteith Equation for the 60 days of the project	80
Figure 4.14: The correlation between daily average vapour pressure deficit and daily ET_o calculated using the FAO-Penman-Monteith Equation for the 60 days of the project	81
Figure 4.15: The typical (average) leaf to air temperature difference at each time of day for the entire 60 days of the experiment	83
Figure 4.16: Daily mean leaf temperature for each treatment compared with daily mean air temperature inside the greenhouse.....	84
Figure 4.17: Comparison between leaf temperature, stem temperature and air temperature on a typical cloudless hot day (DOY 18; 18 January 2010).	85
Figure 4.18: Comparison between leaf temperature, stem temperature and air temperature on a relatively cool cloudy day (DOY 31; 31 January 2010).	86
Figure 4.19: The correlation between stem and leaf temperature on a) a relatively hot day (DOY 18) and b) a relatively cool day (DOY 31)	87
Figure 4.20: The correlation between daily average leaf temperature and daily ET_o calculated using the FAO-Penman-Monteith Equation for the 60 days of the project	88
Figure 4.21: The correlation between daily maximum leaf temperature and daily ET_o calculated using the FAO-Penman-Monteith Equation for the 60 days of the project	89
Figure 4.22: The typical values of leaf temperature over the course of the experiment (for example in this graph a value of 21.9 at 8am is the average 8am value over the entire course of the experiment).....	90
Figure 4.23: Leaf temperatures and air temperature on DOY 51 and 52 showing high leaf temperatures (approaching air temperature) at certain times of day in the two treatments subjected to water stress.....	91

Figure 4.24: Leaf to Air temperature difference in stressed and non-water stressed plants on DOY 51 and 52	92
Figure 4.25: Stem diameter derived indices of a non water stressed tomato plant over three typical days (DOY 45 – 47)	94
Figure 4.26: The variation of daily MDS and ET_o over the course of the experiment.....	95
Figure 4.27: The correlation between maximum daily stem shrinkage (MDS) of a non water stressed tomato plant and ET_o for 16 days (DOY 44 to DOY 60).....	96
Figure 4.28: Comparison of the MDS of a stressed (MDS B) and non-stressed (MDS A) tomato plant for DOY 44 – 60 (stressing started DOY 47)	97
Figure 4.29: The diurnal course of fruit diameter of a medium sized, healthy, non water stressed, growing fruit over two typical days (DOY 46 – 47)	99
Figure 4.30: The correlation between the daily fruit growth (DG) of a non water stressed tomato plant and ET_o for 16 days (DOY 44 to DOY 60)	100
Figure 4.31: Daily equations of fruit growth rates for a water stressed tomato plant for nine days (DOY 46 – DOY 54)	101
Figure 4.32: The variation of stomatal resistance with PAR of leaves in a healthy non-water stressed tomato plant on DOY 52	102
Figure 4.33: The variation of stomatal resistance with PAR of leaves in a water stressed tomato plant on DOY 52	103
Figure 4.34: The variation of midday leaf water potential from DOY 20 to DOY 58 ...	105
Figure 4.35: The correlation between midday leaf water potential and ET_o of a non water stressed tomato plant from DOY 20 to DOY 58.....	106
Figure 4.36: The total daily sap flow and daily ET_c for each of the 60 days of the project.	107
Figure 4.37: The correlation between daily total sap flow and ET_o calculated using the FAO-Penman-Monteith Equation for the 60 days in which the experiment was done. .	109
Figure 4.38: The diurnal course of sap flow and ET_c on a typical clear sunny day (DOY 38)	110
Figure 4.39: The relationship between sap flow and ET_o on a typical clear sunny day (DOY 38)	111
Figure 4.40: The diurnal course of sap flow (blue line) and solar radiation (red line) on a typical, cloudless sunny day (DOY 38)	112
Figure 4.41: The diurnal course of sap flow (blue line) and leaf temperature (red line) on a typical, cloudless sunny day (DOY 38)	113
Figure 4.42: The diurnal course of sap flow (blue line) and vapour pressure deficit (red line) on a typical, cloudless sunny day (DOY 38)	114
Figure 4.43: The correlation between sap flow and solar radiation on a typical sunny day (DOY 38) showing a medium dependence of sap flow on solar radiation.....	115
Figure 4.44: The relationship between leaf temperature and sap flow on a typical sunny day (DOY 38) showing a high medium correlation between the two variables.....	116
Figure 4.45: The relationship between vapour pressure deficit and sap flow on a typical sunny day (DOY 38) showing a medium to high correlation between the two variables.	117
Figure 4.46: Sap flow in treatment A and D showing the number of days for sap flow to reduce from the day that stressing began (DOY 47).....	118

Figure 4.47: Sap flow in treatment A and D on a) DOY 47 and b) DOY 52 showing that sap flow was similar in the two treatments on DOY 47 but very different on DOY 52. 119

Abbreviations and Symbols

LWP _{midday}	Midday leaf water potential
LWP _{predawn}	Predawn leaf water potential
ET _o	Reference crop evapotranspiration
ET _c	Crop evapotranspiration for a particular crop at a particular growth stage
K _c	Crop coefficient
VPD	Vapour pressure deficit
MDS	Maximum daily shrinkage (usually of a stem)
NDVI	Normalized difference vegetation index
FAO	Food and Agricultural Organisation of the United Nations
PAR	Photosynthetically active radiation (in the range 400 to 700 μm)
pH	Hydrogen potential
R _n	Net radiation at the crop surface ($\text{MJ m}^{-2} \text{ day}^{-1}$),
G	Ground heat flux density ($\text{MJ m}^{-2} \text{ day}^{-1}$),
T	Mean daily air temperature at 2 m height ($^{\circ}\text{C}$),
u ₂	Wind speed at 2 m height (m s^{-1}),
e _s	Saturation vapour pressure (kPa),
e _a	Actual vapour pressure (kPa),
Δ	Slope of the vapour pressure temperature curve ($\text{kPa } ^{\circ}\text{C}^{-1}$),
γ	Psychrometric constant ($\text{kPa } ^{\circ}\text{C}^{-1}$).
MnFD	Minimum fruit diameter
MnSD	Minimum stem diameter
MxFD	Maximum fruit diameter
MxSD	Maximum stem diameter
DG	Daily growth (of a fruit or stem)
DR	Daily recovery (of a stem)
RH	Relative Humidity

Chapter 1: Introduction

1.1 Background and Justification

In commercial greenhouses where irrigation scheduling is based on micrometeorological parameters, water stress conditions are not always avoidable even under controlled conditions such as those found in greenhouses. The traditional methods of calculating irrigation quantity based on environmental variables can be difficult to use and are imprecise at best. Unexpected heat waves, mistakenly high ventilation and faults in the irrigation system can all result in water stress which may not yet be visibly detected but still be serious enough to cause a reduction in the quantity and/ or quality of yield. Given this the major problem with growing of crops in greenhouses (and in open fields for that matter) can be said to be the difficulty in determining the correct irrigation amount while at the same time being able to quickly uncover the healthiness or unhealthiness of these crops before serious adverse effects begin to take place. This is difficult since most stress conditions may have already persisted for a number of days before they are visibly detected by the grower and even when detected fairly quickly may already have occurred for long enough to have adversely affected yields.

The calculation of irrigation quantity and the detection of drought stress can be done by monitoring either the plant's environment or the plant itself. The traditional method of monitoring the environment is indirect and often imprecise, and cannot really tell us whether the plant itself is at an optimum water status. Thus it has been suggested that direct crop monitoring is essential to detect sub-optimal growth conditions at a very early stage, so that both productivity and quality can still be guaranteed. This approach, known as the "speaking plant" approach (Udink ten Cate *et al.*, 1978), can be used for supporting decision-making processes for climate and irrigation control, and other management strategies. Possible indicators include the use of measurements of stem diameter fluctuations (dendrometry), fruit diameter changes, leaf to air temperature difference, stomatal resistance, stem water potential and leaf water potential. These are monitored because they have a direct relationship to various aspects of plant health and growth. For

Introduction

example, stem diameter fluctuations are a result of water content of the plant, while leaf to air temperature difference is related to water content since water is needed to cool the plant through transpiration, and fruit diameter increase is also related to water content and plant health since water is required to provide turgor pressure for growing plant cells. There is also a relationship between water content and stomatal resistance which can be investigated since this has an effect on photosynthesis through the regulation of CO₂ uptake. In fact leaf water content has been considered the primary factor regulating stomatal resistance (Jones and Tardieu, 1998). Leaf water potential can also be an important indicator of plant water status or water needs and in particular Misra *et al* (2005) state that predawn and midday values of water potential can be good indicators of plant water status in many horticultural crops. However, many techniques of measuring plant water potential are destructive and require the removal of leaves, while the use of non-destructive (or in situ) methods requires expensive equipment with skilled installation and interpretation.

With that in mind, Sato *et al* (2006) state that the timing of irrigation and the determination of plant health should be based on actual plant water status rather than other traditional methods such as soil based methods or empirical formulae. Plant physiological responses provide the most direct measure of identifying the plant demand for water. However, it should be noted that while plant water status indicators provide a direct measure of when water is required, they often do not provide a direct volumetric measure of the volume of water required to be applied (Misra *et al*, 2005). There is thus a need to develop methods which can be used to detect plant water status based on direct plant measurements and use this to determine plant water requirements. These methods should be able to determine plant water requirements or detect plant water status and possibly compare this status to a critical threshold value for the determination of both the timing and quantity of irrigation.

There is also a need to determine which plant based indicators show the best and quickest responses to drought stress and water status so that growers with less financial resources

can direct their resources to monitoring these. These indicators should also be sensitive, precise and easy to use on a daily basis to calculate water requirements and monitor water status of greenhouse crops. With this in mind the leaf water potential, the leaf temperature, stomatal resistance, stem diameter and fruit diameter are all factors which can be monitored to better and quicker calculate water requirements and monitor the water status of plants. There is a need however, to investigate the effectiveness and sensitivity of these indicators and the possibility of using them in an operational environment.

1.2 Aims and Objectives

The main aim of the project is to explore the use of different plant based indicators for use in the early detection of water stress and irrigation scheduling for a greenhouse crop.

The specific objectives of the project are to:

- monitor the variations of leaf temperature, stem temperature, leaf water potential, leaf stomatal resistance, stem diameter, fruit diameter and sap flow of a greenhouse tomato crop under normal water conditions and drought stress conditions;
- investigate the behaviour of leaf temperature, stem temperature, leaf water potential, leaf stomatal resistance, stem diameter, fruit diameter and sap flow with climatic parameters, such as air temperature, vapour pressure deficit (VPD) and solar radiation for use in irrigation scheduling and detection of drought stress in a greenhouse tomato crop;
- propose appropriate mathematical models of plant water requirements based on leaf temperature, leaf water potential, stem diameter, fruit diameter, leaf stomatal resistance and sap flow under greenhouse conditions for possible application in irrigation scheduling in commercial greenhouses;

- propose possible thresholds of plant water stress based on the plant indicators mentioned above for use in irrigation timing and drought stress detection in commercial greenhouses.

1.3 Potential Benefits of the Study

The first benefit of the study comes from the determination of mathematical models which can be used to determine plant water requirements or detect stress based on easily measured direct plant indicators. The second benefit derived comes from the testing of the sensitivity of various plant responses to water stress. This will allow growers to choose which plant indicators to monitor without spending unnecessary resources. For example, a grower with few resources will be able to monitor just one or two plant indicators which have good and quick response to water stress. There is also the possibility of potential water savings when using plant indicators in determining irrigation quantity and timing since these indicators are a direct measure of plant water status whereas other methods such as the Penman-Monteith Method are indirect. The mathematical models for determining irrigation quantity as well as the threshold values for stress may also be developed into computer software which can be used to automate irrigation amount and timing based on direct plant measurements rather than microclimatic measurements.

Another important benefit will be that if one can accurately simulate the variation of the water status of a plant organ using a mathematical model based on plant indicators rather than climatic variables, then this model can possibly be incorporated into crop growth simulation models so as to better simulate crop growth and yield of crops grown in protected environments. Models which rely on a soil water balance approach can be adjusted to use actual plant water status derived from direct indicators to more accurately determine the effects of different irrigation or rainfall regimes on growth and yield.

Introduction

The use of direct plant measurements may also aid in development of real time irrigation systems which determine plant water needs at intervals of as little as 15 minutes. Methods such as the Penman-Monteith method require intervals of a day or more and still rely on many assumptions such as ground heat flux being zero over 24 hours. Using that formula at shorter intervals requires tedious and expensive measurements.

Another benefit would be to tomato growers who would have more options for managing their crop better as well as gaining an understanding of the crops different responses to water deficit so as to be able to better and quicker diagnose drought stress. They will also have a wider range of options for calculating irrigation quantity and determining irrigation timing. The development of thresholds of what is regarded as normal water status based on the various plant indicators will also help growers to better understand their crops and quicker diagnose drought stress before visible signs are seen.

1.4 Thesis Layout

This thesis is composed of five chapters. Chapter 1 is the introduction and outlines the problem, objectives of the study and benefits of the study. Chapter 2 is a review of some literature on the topic including results from similar studies as well as a review of current methods of measurement of plant water status and current methods used for the determination of plant water needs, along with their strengths and weaknesses. Chapter 3 goes into the details of the materials used and the methods by which the study was done. Chapter 4 presents the results and findings of the study as well as a general discussion. In Chapter 5 the conclusions and recommendations are made.

Chapter 2: Literature Review

2.1 Introduction

The growing of horticultural and other crops comes with many problems. Among these is the determination of plant water requirements as well as detecting plant water status when environmental and physiological conditions are sub-optimum. While calculating plant water requirements has largely been based on climatic conditions it is possible that these are not a true reflection of actual plant water status. Often plants may suffer due to water stress (caused by both water deficit and excess), temperature stress (both hot and cold) or disease long before any of these conditions is observed by the grower. Of these, stress caused by water deficits is the most common. According to Misra *et al* (2005), plants experience water stress when the deficit within their tissues and cells reaches a critical level, affecting various physiological processes including leaf extension and growth. If stress situations can be detected quickly, the greenhouse climate can be adjusted in order to reduce the stress before it affects growth and yield. It is for this reason that agriculturalists and scientists have sought early indicators of plant water status which can be used to detect water stress, diagnose water availability and determine irrigation quantity before any harm is done to the plant (Misra *et al*, 2005). These indicators include stem diameter, fruit diameter, xylem cavitations, leaf water potential, sap flow, leaf temperature, stomatal resistance and leaf fluorescence among others which can all be used in determining irrigation quantity, irrigation timing or both.

Optimum water supply, particularly at peak needs of the plants, is thus an important issue for greenhouse and field vegetable production. Water should be given in proper amounts and at accurate times. For this reason Harmanto *et al* (2004) state that water management is key to avoiding plant moisture stress during the various crop growth stages. In a greenhouse on a sunny day, evaporation and transpiration (evapotranspiration) can occur so rapidly that water loss can cause plant damage before wilting symptoms are visible if water supply is not adequate. Even at lower temperatures the restricted rooting in greenhouses leads frequently to plant water deficiency. Thus, no matter how slight,

drought stress can result in a significant reduction in growth and, in turn, of harvest and yield or their quality (Zimmerman D *et al*, 2008).

2.2 The Importance and Functions of Water to Greenhouse Plants

As with all living things the maintenance of an adequate water supply is crucial for obtaining the maximum productivity of horticultural crops. This is primarily because water is fundamental to the maintenance of normal physiological activity and membrane transport processes (Jones and Tardieu, 1998). Water is an essential plant component and is a major constituent of plant cells. About 70-90% of the mass of growing plants consists of water (although water content does vary between plants as well as between organs of the same plant). Seeds may consist of just 5-15% water while succulent fruit can consist of between 90-95% water (Milthorpe and Moorby, 1979). It is the most vital plant component and is important for germination, growth, development, photosynthesis, cooling and chemical reactions within plant cells. Water is one of two main physical requirements for photosynthesis the other being carbon dioxide. Water also acts as a solvent, transporting dissolved salts, nutrients and minerals from the soil to where they are needed in the plant as well as transporting growth regulatory compounds from plant cell to plant cell. At cellular level water is especially efficient in the transport of metabolites through and between plant cells. This is because water is a highly polar structure and thus readily dissolves large quantities of ions and polar organic metabolites. At whole plant level water is a medium for the transport of carbohydrates, nutrients and phyto-hormones from the roots to growth organs.

Some aspects of plant growth and development absolutely depend on the use of water as a solvent. For example, the nitrates and phosphates found in fertilizer can only be absorbed by plants once dissolved in water (Jones, 1990a). This is especially true in greenhouses where some form of hydroponics is practiced (i.e. plants are grown in a soilless medium). Water also helps keep turgor in plant tissue. This is important because

plants require this turgor for vegetative growth and development. This is because water provides a positive turgor pressure against cell walls which enables them to grow and expand. For example, leaf extension and cell growth both require water. When plants lose turgor they first stop growing and soon wilt.

Jones (1990a) also states that water is important for maintaining cell turgidity. This is important for both photosynthesis and transpiration since stomatal opening are governed by the turgidity of guard cells which regulate their movement. When guard cells are turgid then stomata are open thus allowing the diffusion of oxygen (O₂), carbon dioxide (CO₂) and water vapour. Turgidity of the stem and leaves also helps the plant to stand up and without this internal force the plants would bend against their own weight.

In horticultural crops there is a further reason for the importance of water; that is because much horticultural produce is sold by weight, and since water is the major component of most fresh horticultural commodities there is often a marked premium in ensuring that water content is maximised, whilst ensuring that produce quality does not suffer. The quality and texture of horticultural produce is also reliant on water since it is determined by a combination of tissue structure, cell wall properties and turgor pressure. According to Milthorpe and Moorby (1979), the amount of water transpired on a hot day may be several times the plant's fresh weight and of the total water absorbed by plants. This is because up to 98% of the water absorbed can be transpired in order to dissipate energy from the sun as latent heat energy through evaporation and transpiration from the leaves. Water is a cooling agent and transpiration is essential for dissipating solar energy from the plant. Without latent heat consumption plants would die from overheating. Thus water is essential for plant cooling and without it plants would die from overheating. In fact only about 1% of the water taken up by plants is kept in plant cells while the other 99% is transpired to keep the plant cool. This is especially true in greenhouses where ambient temperatures are high and ventilation rates are low.

Thus water is important for greenhouse crops for a number of reasons and should be adequately supplied so as to avoid any negative impacts which may occur due to its scarcity. It should also be noted that the availability of water is the most restricting factor for crop production and hence its availability and supply should be well considered. In greenhouse grown crops shortage of water is not usually an issue but can occur unexpectedly as a result of some unexpected incident.

2.3 The Movement of Water within Plants

In simple form, water moves from wet places to dry places or from high places to low places. In scientific terms however, water moves from areas of high water potential to areas of low water potential along what is called a water potential gradient. Berrie *et al* (1987) state that water in cells and tissues moves in response to differences in water potential on either side of a partitioning membrane or membranes. They go on to say that the rate of movement is proportional to the magnitude of the water potential difference. Water potential itself is defined as the potential energy of the water in a system relative to that of pure free water in reference conditions i.e. standard temperature and pressure. It is a thermodynamic expression of the energy status of water with units of kJ kg^{-1} or kPa. The term water potential is used because any movement (including that of water) needs energy (Berrie *et al*, 1987). Usually the roots penetrate a relatively wet soil, while the stem and leaves project into a comparatively dry atmosphere. Water from the soil moves into the air spaces within the leaves and other plant cells and then evaporates into the atmosphere in response to the vapour pressure gradient. Incoming solar radiation provides the energy required for evaporation so as to assist in the movement of water from soil to atmosphere. When the airspaces in plant cells lose water then the plant cells next to these then also lose water to the air spaces since they have a greater potential. This reduces their potential thus encouraging movement of water from soil to the cells. Thus there is a system of continuous movement as shown in Figure 2.1.

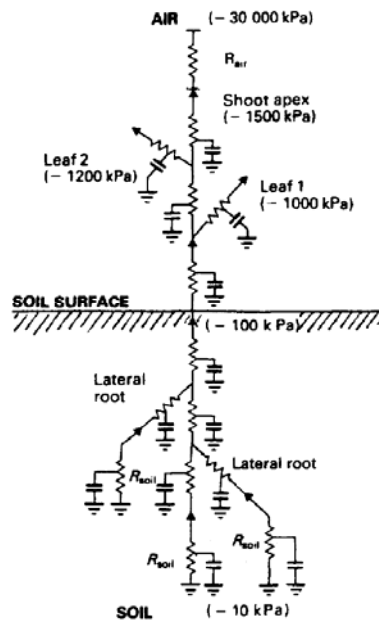


Figure 2.1: The pathway of water movement in the soil plant atmosphere continuum showing resistances, capacitances and water potentials (Challand and Bakker, 1995).

Analogous to electrical circuits', plant water flow is regarded as a network of potentials, resistances and capacitances. As seen the water potential increases from about -10 kPa in the root zone to about -30000 kPa in the atmosphere. Table 2.1 shows the magnitudes of water potential in the soil plant atmosphere continuum for a turgid and wilting plant. As can be seen for a wilting plant there is lower water potential in the root zone than for a turgid plant since this the soil is the source of water and is the main determinant of plant water status. This means that at low water in the soil then the plant struggles to absorb water from the soil even though atmospheric demand is the same. Also the water potential in the leaves of wilting plants is higher than that of those in turgid plants since the leaves have very little water within their cells.

Table 2.1: Approximate magnitudes of water potentials in the soil-plant-atmosphere continuum.

	Turgid Plant (kPa)	Wilting Plant (kPa)
Soil	-10 to -1000	-1000 to -2000
Leaves	-200 to -1500	-1500 to -3000
Atmosphere	-10000 to -200000	-10000 to -200000

After Milthorpe, F. L. and Moorby, J. (1979)

In general the water potential differences shown in Figure 2.1 and Table 2.1 encourage movement from the root zone (the source of water supply) into the plant stem then into the leaves (the site of water loss) and finally into the atmosphere. It is important to note that growing plant parts such as leaves and shoot apices have an extremely low water potential thus drawing water towards these parts so as to encourage growth and that their rate of growth and extension is more rapid than that of other plant parts even though most of the water is still used for cooling through transpiration. Also as can be seen in Figure 2.1 plant parts higher up the plant stem have a lower water potential which again aids water movement to where it is needed most.

2.4 Tomato Characteristics and Water Requirements

The Nemo Netta tomato variety were used which are an adaptable indeterminate hybrid. The plants typically reach 1 – 3 m in height and have weak woody stems with leaves that grow to between 10 to 25 cm in length (Rehm and Espig, 1991). The variety has high yield, high fruit quality and good fruit set under a wide range of climates and can be grown in both open fields or greenhouses. The fruits grow to a maximum diameter of between 50 to 60 mm and a weight of 150 to 160 grams. The first harvest is approximately 80 days after planting and will extend for a number of weeks. According

to the FAO total water requirements after transplanting, of a tomato crop grown in the field for 90 to 120 days, are 400 to 600 mm, depending on the climate. Water requirements can be related to reference crop evapotranspiration (ET_o) in mm by the crop factor (K_c) for the different crop development stages (namely, initial stage 0.4 - 0.5, development stage 0.7 - 0.8, mid - season stage 1.05 - 1.25, late-season stage 0.8 - 0.9, and harvest 0.6 - 0.65). In this case the experiments were conducted in the late season and harvest stage.

2.5 Greenhouse Crops and Water Stress

2.5.1 What is Water Stress?

According to Ghulam *et al* (2008), water stress is a physiological state of vegetation caused by a lack of water in the leaves that restricts transpiration and is expressed as reduced growth, low water potential, high leaf temperature and high stomatal resistance. The term drought stress refers to a reduction of the water content of the organs of a plant below the maximum to a point where growth and other processes start to be inhibited (Vermeulen *et al*, 2007). Factors which influence crop water stress include; moisture in the root zone, ambient temperature, evapotranspiration rate (ET), leaf water content and leaf water potential (lwp). Root zone moisture is the dominant factor controlling crop growth and therefore yields, and the primary effect of root zone moisture deficit on plant condition is exerted through the plant water potential, which in turn affects cell turgor and relative water content of the living cells of the plant (Berrie *et al*, 1987). However, factors such as ambient temperature, solar radiation, wind speed and vapour pressure deficit can also play an important role in crop growth and yields by exacerbating the effects of a root zone moisture deficit.

2.5.2 The Effects of Water Stress on Plants

Different plants respond to drought stress in different ways and to varying degrees. However, there are some responses which are common to all plant types. Experiments

done by Vermeulen *et al* (2007) showed that when subjected to drought stress plants responded in a number of interrelated ways. Firstly, there was a notable reduction in transpiration rate followed by shrinkage in stem diameter. At leaf level they found that leaf temperature increased indicating that stomata were closing and reducing the amount of cooling possible through transpiration (a hypothesis they confirmed by measuring stomatal resistance which they found to be increasing). They went on to find that the lack of cooling caused permanent damage to the leaves resulting in a permanent reduction in sap flow rate. Anderson and Peterson (2007) concurred that when water in the root zone reaches critical level (i.e. at or near the permanent wilting point) then stomata will close almost completely leading to an increase in leaf temperature to critical levels causing permanent damage. They also noted that many of the responses of plants to water stress were detected long before any visible symptoms of drought stress were observed. Water stress also results in a reduction of transpiration rate even when stomata are open if the water stress is a result of a limited availability of water in the root zone as apposed to a large atmospheric demand for water since the plant roots will not be able to draw water from the soil due to a high cohesion of the water particles to the soil matrix.

According to Challand and Baker (1995) expansive growth and especially growth of the aerial part of the plant, is one of the processes most sensitive to water deficit while in terms of fruit production, the flowering phase is most sensitive to drought stress. For these reasons Kramer (1983) states that the most obvious and general effects of drought stress are reduction in plant size, leaf area and crop yield. These effects however can take a long period of time to become apparent and thus he goes on to say that the most important and immediate effects are on the physiological processes. In terms of biophysical processes he also states that vegetative growth and in particular leaf expansion is severely affected even by only moderate water stress. While, both cell division and cell enlargement are also reduced by water deficit and these are directly related to leaf expansion and growth. However, some experiments have shown mild water stress to result in increased leaf area due to an increased number of epidermal cells.

Water stress also affects root to shoot ratios by changing the way in which a plant partitions carbohydrates away from the norm (Griffiths and Parry, 2002). In general shoot growth is reduced while roots receive more carbohydrates so as to expand and possibly reach water. This is important as new shoots are needed for fruit growth as well as for leaf growth. Consequently if leaf growth is prohibited then the plant will not reach its full potential in terms of photosynthetic capacity, and hence yields are reduced. Water deficits can reduce photosynthesis by a reduction in leaf area, an increase in stomatal resistance and a decrease in the efficiency of the carbon fixation process. Reduction in leaf area is extremely important because it is often not temporary and persists even after the stress has been relieved.

Water deficits also affect some of the enzyme mediated steps of the dark reaction of photosynthesis. This is because according to Griffiths and Parry (2002), the activity of such important enzymes as ribulose biphosphate, carboxylase, ribulose phosphate kinase and phosphoenolpyruvate carboxylase are all reduced by water stress. Low water potentials also retard the development of chlorophyll and thus again reducing photosynthesis.

What is important to note according to Van de Sanden (1995), is that it is not solely the manifestation of water stress (e.g. visible wilting) that affects crop growth and productivity. Within the range from full hydration to water stress different physiological processes have their own threshold and sensitivity to changing plant water status (Bradford & Hsiao, 1982) and therefore display a water status dependent contribution to the output of good quality produce. Given all of the above effects of water stress it is then possible to use these indicators to determine both irrigation quantity and timing as well as to detect, quantify and eliminate water stress at an early stage before any adverse effects occur.

2.5.3 Detecting the Water Status and Water Needs of Greenhouse Plants

Detecting the water status or water needs of greenhouse plants is critical for ensuring the health and productivity of the crops being grown. It can be divided into two main methods the first being those that estimate plant water use or needs in a given period of time based on environmental conditions and use this to determine the amount of water required by the plant. The second being those that measure certain aspects of plant physiological responses to determine irrigation quantity or detect the water status of the plant and hence know when this is at a critical level requiring intervention. According to Kramer (1983), there has been considerable uncertainty concerning what to measure, where to measure and how to measure it. A satisfactory method of monitoring plant water status and needs should have the following characteristics:

1. There should be good correlation between rates of physiological processes and the degree of water stress
2. Should require little or no plant material for measurement
3. The method should be simple, quick and inexpensive.
4. The units of measurement should be applicable to plant material, soil and solutions.
5. The degree of water stress should have similar physiological significance in a wide range of plant materials

Given all of the above a number of methods of monitoring water status and drought stress in plants can be reviewed as follows.

2.5.3.1 Empirical Formula

Some empirical formulae already in use for the calculation of plant water requirements are based on calculation of daily evapotranspiration on the basis that this is the amount of water which would be needed to be input into the soil system. Some examples of these

formulae include the Penman-Monteith formula which uses a combination of atmospheric parameters and plant factors in order to determine daily evapotranspiration loss from a cropped stand. According to Allen *et al* (1998) the Penman-Monteith method is based on calculating what is called the crop water requirement for a given period of time usually a day or more. This crop water requirement is the amount of water required to compensate the evapotranspiration loss from the crop. The formula has been revised many times but a standardized form called the FAO-Penman-Monteith equation is commonly used to calculate what is called reference evapotranspiration (ET_o). This reference evapotranspiration expresses the evaporating power of the atmosphere at a specific location and time of the year and does not consider the crop characteristics and soil factors. Once reference crop evapotranspiration is found then crop coefficients are used to determine actual evapotranspiration for a given crop, at a particular stage of development and under particular management practices (ET_c). The FAO-Penman-Monteith formula is given as follows:

$$ET_o = \frac{0.408\Delta R_n + \gamma \left(\frac{900}{T + 273} \right) u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \quad (2.1)$$

Where ET_o = reference crop evapotranspiration (mm day^{-1}),
 R_n = net radiation at the crop surface ($\text{MJ m}^{-2} \text{day}^{-1}$),
 G = ground heat flux density ($\text{MJ m}^{-2} \text{day}^{-1}$),
 T = mean daily air temperature at 2 m height ($^{\circ}\text{C}$),
 u_2 = wind speed at 2 m height (m s^{-1}),
 e_s = saturation vapour pressure (kPa),
 e_a = actual vapour pressure (kPa),
 Δ = slope of the vapour pressure temperature curve ($\text{kPa } ^{\circ}\text{C}^{-1}$),
 γ = psychrometric constant ($\text{kPa } ^{\circ}\text{C}^{-1}$).

Other empirical methods include the Bowen Ratio Energy Balance Method (BREB), the Blaney-Criddle Method and the Thornwaite Method. All these rely on the measurement of one or more environmental variables for use in an empirical formula to calculate

evapotranspiration above a cropped surface. The Blaney-Criddle Method for example estimates monthly evapotranspiration and relies on the formula

$$ET_o = Tp \quad (2.2)$$

Where ET_o = reference crop evapotranspiration

T = mean monthly temperature ($^{\circ}C$)

p = percentage of daytime hours that month makes up for the year (%)

A crop coefficient (k) can then be incorporated to calculate evapotranspiration for a particular crop at a particular stage of growth. The method is however subject to inaccuracies and the timescale does not allow for stress detection or irrigation scheduling based on real time data.

Empirical formulae in general, rely on a lot of data which may not always be available. They also can be inaccurate, while not being able to adjust quickly enough to real time changes in climatic conditions. For example, an unexpectedly hot day can result in crop stress which will only be detected or accounted for by these formulae when the next calculations are done, which may be a day later or even a month later depending on the data requirements of the formula. However, one method, the FAO-penman-Monteith formula has been thoroughly tested and is now recognized as a standard in the calculation of crop water requirements (Allen *et al*, 1998).

2.5.3.2 Substrate Based Methods

Substrate based methods of determining water status of plants can be used for both the timing and quantity of irrigation water application. These methods are described as the conventional method and are based on determination of the water status in the substrate (be it soil, vermiculite or any other compound) with the presumption that if the water in the substrate is at an optimal for plant needs then plant water status itself will also be at an optimal (Dzikiti, 2007). The substrate water status can be quantified as either a water

content expressed in terms of mass or volume, or as a water potential expressed as a suction or pressure with which water molecules are held to the substrate. There are a wide range of approaches and instruments for direct and indirect measurement of substrate moisture content and these include gravimetric methods, neutron probes, capacitance probes, time-domain reflectometry probes and tensiometers. In addition the substrate moisture content can be indirectly estimated by calculation using a water balance approach. In fact for field crops, a water balance approach is recommended for the determination of plant water needs and hence for irrigation scheduling (Allen *et al*, 1998). In this case changes in water content of the substrate (ΔW_r) are estimated based on the idea that storage is equal to gains minus losses which in turn can be expressed using a water balance equation given by:

$$\Delta W_r = (\text{irrigation}) - (\text{drainage} + \text{evaporation} + \text{transpiration}) \quad (2.3)$$

i.e. Storage = gains – losses

The absence of a rainfall term from equation 2.3 is because in a greenhouse water inputs are solely a result of irrigation. Hence, the change in soil water content (ΔW_r) over a specific period is given as the difference between the inputs (irrigation) and the losses (drainage plus evapotranspiration). The soil based methods rely on some predetermined thresholds namely total available water, field capacity, permanent wilting point and readily available water. Field capacity is the maximum water holding capacity of the soil and permanent wilting point is the lowest soil water content allowed before irreversible plant wilting occurs, while total available water is the difference between the two and readily available water is the water content at which plants begin to experience difficulty in extracting water from the soil.

The theory works on the premise that while water content in the soil is above the threshold of readily available water and below that of field capacity then plant water status is likely to be at an optimum. The ET component of the water balance equation still has to be calculated using empirical formula combined with knowledge of crop water

requirements such as the FAO-Penman-Monteith Equation (Allen *et al.*, 1998). In general the water balance approach is not very accurate but is robust and useful in a wide range of conditions (Jones, 2004b). The main disadvantage of this approach is that the errors are cumulative over time and thus requires periodic recalibration of calculated water balance using other methods or actual measurements. One major advantage is that soil based methods are not easily affected by environmental conditions (Intrigliolo and Castel, 2006) and thus its information can be easily used with few or no corrections for prevailing atmospheric conditions.

In general however, substrate based methods suffer from the problem that many aspects of a plants physiology respond directly to changes in the water status in the plant tissues rather than to changes in the bulk soil water status (Intriglio and Castel, 2004). Drought stress and yield declines have been observed before the soil water depletion reached the threshold values. Furthermore, the soil water suction readings have been reported to be more variable than values of midday stem water potential for example (Naor, 2000). Thus getting representative and accurate measurements is difficult and plant based stress indicators are preferable. Another problem according to Anderson and Petersen (2007) is that in greenhouses where crops are grown in mediums other than soil such as peat or vermiculite it is difficult to measure soil water status especially at low water contents. It is also important to note that plants can wilt even when the soil is moist if transpiration rates are excessively high (Ghulam *et al*, 2008). The measurement of soil water potential using tensiometers is also flawed since the ability of soil to give up water also depends on its hydraulic conductivity which is itself a function of water potential and soil type.

2.5.3.3 Pan Evaporation Methods

Evapotranspiration can also be estimated based on pan evaporation rates. Pans have proved their practical value and have been used successfully to estimate ET_o by observing the water loss from the pan and using empirical coefficients to relate pan evaporation to actual evaporation from the crops. According to Erteka *et al* (2006), pan methods are popular due to their simple and easy application. They go on to say that there

is a close relationship between plant water consumption and evaporation from a pan and this relationship is given by predetermined plant coefficients which relate evaporation from the pan to actual evapotranspiration from a cropped field (or greenhouse in this case). Baille (1996) states that evaporation pans provide for the integrated effect of radiation, wind, temperature and humidity on evaporation from an open water surface of which the most widely adopted type is the standardized U.S. Class A" pan.

In general evaporation pans are relatively inexpensive and are easy to install, maintain and monitor. They do, however, have several important shortcomings. Allen *et al* (1998), state that one of these is the fact that they are susceptible to the microclimatic conditions under which the pans are operating, and the thoroughness of the maintenance of the pans. They go on to say that their performance proves erratic. Within greenhouses space considerations are a concern. The method is also hazardous to use in a greenhouse, because of the strong heterogeneity of inside solar radiation and the high possibility of shading by nearby vegetation and other components of the greenhouse structure. The effect of pan location inside the greenhouse may affect significantly the estimation of ET_0 . However, when no other methods are applicable because of the lack of climatic data, this can be a first step for rough estimation of water requirements by means of an inexpensive and simple system (Erteka *et al*, 2006). Overall special precautions and management must be applied when working in greenhouses and coefficients must be carefully used since they are often very area specific.

2.5.3.4 Radiation Methods

Radiation methods have been long used in areas where there is a limited availability of meteorological measurements. According to Allen *et al* (1998) the radiation method was suggested for areas where available climatic data include measured air temperature and sunshine, cloudiness or radiation, but not measured wind speed and air humidity. They go on to say that the radiation methods show good results in humid climates where the aerodynamic term is relatively small, but that performance in arid conditions is erratic and tends to underestimate evapotranspiration. While Baille (1996) states that there is a strong relationship between daily evapotranspiration and solar irradiance. He goes on to

say that the relationship between the two variables can be given by the following formula:

$$ET_o = \frac{kR_s}{2.5} \quad (2.4)$$

Where

ET_o = reference evapotranspiration (mm day^{-1}),

k = empirical coefficient between 0.6 and 0.7

R_s = outside global solar radiation ($\text{MJ m}^{-2} \text{day}^{-1}$)

The formula can be adjusted for greenhouse conditions by incorporating a transmission coefficient (τ) or by measuring solar radiation inside the greenhouse rather than outside. Furthermore a collection of crop coefficients for some commonly grown greenhouse crops is available in literature. In the absence of these coefficients there is a possibility of doing an in situ calibration based on lysimetry. Radiation sensors are also relatively affordable and there are a number of methods which can be used to estimate radiation in the absence of its measurement (Baille, 1996) making this method an easily applied one for growers. The method is thus fairly commonly used in greenhouses and is relatively reliable compared to other methods.

2.5.3.5 Plant Based Methods

In almost all greenhouses climate control is based solely on environmental factors. However, in view of the above methods and their weaknesses, it can be said that the only reliable measure of the water status of plants is measurements made on the plants themselves. Spomer (1985) suggested that the determination of plant water status and needs should be done “at the site of the process of interest”. For example, those scientists concerned with the study of photosynthesis or stomatal opening should concentrate on measurements on the leaves themselves, while those concerned with the growth of fruit should concentrate on measurements on the fruit themselves (Jones, 2007). According to Helmer *et al* (2005), this physiological data could provide important information, especially if integrated into control systems or computer models in the so-called speaking

plant approach. For example, rather than calculating the transpiration rate from microclimate data, transpiration could be measured directly using some type of instrument located on the plant itself. For these reasons it is important to consider plant based methods rather than soil based methods when attempting to determine plant water requirements, status or health. Ehret *et al.* (2001), state that a range of physiological parameters such as growth, photosynthesis, transpiration, and leaf temperature may be monitored with a variety of instruments, either remotely or through physical contact. Plant based methods can be grouped into the category of direct plant monitoring or phytomonitoring which is discussed in brief in the next paragraph.

Phytomonitoring is a term coined by Phytech Ltd, Israel who define it as the automated direct monitoring of plants for use in decision making in crop production. It is a technique aimed at the early, objective detection of crop problems and on well-timed disclosing of crop response to management practices (e.g. irrigation practice) and changes in the plants environment, which a grower may undertake for improving crop production (Ton, 1997). While Ton and Kleiman (1989) state that phytomonitoring can be used for improving irrigation timing and quantity as well as management. The phytomonitoring technique addresses the limitations of irrigation scheduling based on traditional soil water balance methods or empirical methods (such as the FAO-Penman-Monteith Method). The phytomonitoring system collects physiological and environmental data directly from the plant using enhanced sensors and this data is then analysed either by the user or automatically by a computer. It is important to note that phytomonitoring is not just about the instruments or the monitoring of plant indicators but is a technique which uses a combination of state-of-the-art sensors and innovative software for collecting and analyzing data which is then used to improve the plant management. There are many methods of direct crop monitoring including the monitoring of leaf temperature, leaf thickness, fruit diameter, stem diameter, and sap flow among others and these are discussed in the next sections.

2.5.3.5.1 Visible Symptoms

This is probably the simplest means of detecting plant water status and needs. According to Jones (2007) visible symptoms range from the visible expression of increasing plant water deficit such as wilting and through to morphometric changes such as reductions in leaf expansion and growth rate and on to shrinkage of stems, leaves, or fruit. Dzikiti (2007) in his study of citrus trees stated that some of the earliest and most obvious signs of visible water deficit included the curling of immature leaves on elongated succulent shoots. He went on to say that there may also be a cessation of shoot elongation. With further soil drying he observed that young leaves showed severe distortion and old leaves began to drop.

However, the method of visible detection of water stress is uneconomic and impractical since by the time wilting is visible and apparent, a substantial proportion of the potential yield may already have been lost. Chaerle and Van de Straeten (2000) concur with this saying that by the time a plant displays visible signs of stress, it may already have suffered a decrease in growth and eventual yield. Critically the method does not help in determining how much water the plants require.

2.5.3.5.2 Measurement of Plant Water Content or Energy

Another method both of irrigation scheduling and water status detection is that of measuring plant water content or energy. According to Jones (2004b) a rigorous and sensitive measure of the plant water content should be used, for example, the bulk leaf water potential. Analysis has already shown that leaf water potential (ψ_l) is a good indicator of crop water status (Brisson and Casals, 2005). However while leaves are most commonly sampled, experiments on the use of the water potential of roots and stem have been conducted with varying results (Kramer, 1983). By definition leaf water potential is a measure of the thermodynamic energy status of the water in plant tissue and is measured in units of kPa (Baker and Bland, 1994). Many physiological processes have

been found to be responsive in some way to changes in water potential in the plant root zone but also in the plant tissues themselves.

However, the fact that plant water status and, especially the leaf water potential, is usually controlled to some extent by means of stomatal movements is problematic in that other factors (for example root to shoot chemical signaling) have a strong effect on stomatal movements (Mingo and Davies, 2001). A further problem relating the use of the leaf water potential as a measure for irrigation scheduling are the rapid fluctuations which occur in this variable, for example, due to sudden changes in the environmental conditions caused by passing clouds (Jones 1990b). This makes the use of the ψ_l as a stress indicator for irrigation needs unsatisfactory.

Given these concerns some researchers have proposed the use of a more stable variable, the xylem water potential or stem water potential (Jones, 2004b). For fruit trees, this is estimated either by measuring the water status of leaves that are under deep canopy shade or that have been enclosed in darkened plastic bags for about 6 hours before measurements are taken. These methods are thought to be preferable because they approach closely the stem/ xylem water potential which is more stable than the leaf water potential. Another possible measure is the pre-dawn leaf water potential which is approximately equal to the soil water potential. Unfortunately, this has often been found to be insensitive to variations in soil moisture content and frequent measurements are difficult to obtain. Lastly, all these variables are not suitable for automation and they are destructive and thus other stress indicators have to be considered as well. Consistency of measurements is also important and the use of similar leaves of similar age, health and exposure is imperative for the reduction of errors (Kramer, 1983).

In terms of instruments Jones (2007) says that the measurement of plant water potential is primarily by means of either pressure chamber methods or psychrometric methods. The pressure chamber method involves the excision of a healthy fully extended leaf and the subsequent exposure of the leaf to positive pressure generated by placing it in an air tight chamber whose pressure can be slowly adjusted. Specifically the leaf is placed in a vessel with only the petiole protruding through a small tight septum. The vessel is pressurized by turning a screw until the xylem sap is seen coming out of the surface of the cut petiole. This pressure is taken to be equivalent to the leaf water potential. Turner (1987) states that caution must be exercised so as to minimise errors when using pressure chambers and that the time between excision and taking of measurement should be kept small. It is also advised to wrap the leaf in aluminium foil or plastic during the removal process so as to avoid errors due to desiccation.

Psychrometric methods involve tissue equilibration with air in an enclosed chamber and the estimation of the vapour pressure using wet and dry thermocouples. Watts (1977) states that the thermocouple psychrometer is widely regarded as the most accurate instrument for the measurement of leaf water potential. According to Baker and Bland (1994) this instrument measures the humidity status of an enclosed volume of air which is assumed to be in equilibrium with the plant tissue. The measurement is achieved by measuring the change in temperature of a thermocouple enclosed along with a sample of plant tissue inside a sealed chamber. The theory is that the change in temperature of the thermocouple junction is proportional to the difference in water potential of a water droplet condensed on the thermocouple junction and the plant tissue. However, its use in the field is limited because of the very fine temperature control required as well as the tediousness associated with the use of the instrument and its measurements.

Another method of measuring plant water status is that of relative water content. It involves the collecting and weighing of a leaf sample before drying in an oven and

reweighing (Baker and Bland, 1994). The relative water content is then given by the fresh weight minus the dry weight divided by the fresh weight. The method is however, destructive and time consuming, and on the whole not feasible for use in the determination of plant water status for irrigation scheduling.

2.5.3.5.3 Fruit and Stem Diameter Changes

According to Cohen *et al* (2001), the continuous and accurate measurement of the stems and fruit is reputed to be suitable for assessing the plant water status of trees. This is because according to Dzikiti (2007), the fruit and stem diameters of plants fluctuate diurnally in response to changes in the water content of their organs. The diurnal dynamics of changes in diameter, especially of stems have been used to provide sensitive indicators of water status, where the magnitude of the daily shrinkage (i.e. maximum daily shrinkage, MDS) has been used to indicate water status (Jones, 2004b) and calculate irrigation quantity. In addition comparison of diameters on successive days gives a measure of growth rate and the trend in growth can also be used as an indicator for water stress. Ortuno *et al* (2005) argue that stem and fruit diameter fluctuation measurements are simple to make and easy to interpret making them a suitable indicator of water status and plant water requirements in many plants.

Although changes in fruit growth rate provide a particularly useful measure of plant drought stress and plant water requirements on a daily basis, such measurements are not particularly useful for the control of high frequency irrigation systems. Moreover, the uncertainty about the representiveness of the fruit that are selected for size measurements pose another difficulty. However the maximum daily shrinkage (MDS) in fruit trees has been reported to be a more sensitive indicator than other plant water status indicators such as midday stem water potential for example (Cohen *et al.*, 2001). At the same time, the maximum daily shrinkage (MDS) is more variable than midday stem water potential and other measures of plant water status. Intriglio and Castel (2006) noted in their studies of mature plum trees that reduction in stem diameter growth was only detected long after

other water status indicators had detected stress in that treatment. This contradicted with their study of young plum trees in which stem diameter shrinkage was one of the earliest signs of plant water stress. Thus there is uncertainty in the use of stem diameter as a water stress indicator for mature and young trees.

According to Naor (2008), both the sensitivity and variability of drought stress indicators need to be taken into consideration in selecting the optimal drought stress indicator (Naor, 2008) and it is for this reason that stem diameter cannot be used for early detection of crop water stress. This is because stem diameter already has high variability and has to be interpreted over a number of days and often visible indicators are already present by the time a diagnosis has been made using stem diameter. Thus Vermeulen *et al* (2007) suggest that stem diameter be used in combination with other methods if for the purpose of early detection of crop water stress. However, in terms of irrigation scheduling MDS provides a reliable measure of plant water requirements on a daily basis and can be used in irrigation scheduling.

2.5.3.5.4 Leaf Thickness

According to Dongsheng *et al* (2006) leaves are the most important plant organs and changes in their geometries can reflect the growth and water status of plants. They went on to say that changes in their thickness are periodic and regular and can thus be monitored using modern day control theory in order to optimize growth. White and Montes (2005) also noted a relationship between the variation in leaf thickness and yield of soya bean plants. Thus according to Zimmerman *et al* (2008), leaf thickness is also sometimes used as an indicator for water stress.

Leaf thickness monitoring devices are commercially available. They are non-invasive and suitable for online measurements, but have the disadvantage that changes in water status

are frequently not reflected sensitively in changes of leaf thickness. However, Dongsheng *et al* (2006) go on to say that leaf thickness is problematic in that leaves are soft and can usually only be monitored using non-contact methods, while these non-contact methods are not accurate enough to detect the sub-micrometer sized changes in leaf thickness which occur diurnally. In their studies they found that leaf thickness changes were of the order $0.1\mu\text{m}$ (i.e. $0.1 \times 10^{-6}\text{m}$). More research needs to be done on the topic to determine the usefulness of leaf thickness fluctuations in determining irrigation quantity.

2.5.3.5.5 Leaf Temperature

According to Vermeulen *et al* (2007) leaf temperature can be used to detect drought stress long before visual symptoms are observed. The leaf temperature (T_{leaf}) also provides an efficient method for the rapid, non-destructive monitoring of whole plant response to water stress (Idso *et al.*, 1981; Jackson *et al.*, 1981). While Blonquist and Bugbee (2007) state that decreased water uptake closes stomata, which then reduces transpiration and increases leaf temperature. For this reason the leaf or canopy temperature can thus be used to quantify plant water stress. The question still remains however, on how exactly to quantify water stress using leaf temperature. Jackson *et al* (1977) proposed the normalization of leaf temperature by subtracting air temperature (i.e. the leaf-air temperature difference) as an index of drought stress and hence developed the crop water stress index (CWSI). The theory behind the CWSI is the assumption that in normal water conditions, as the crop transpires the evaporated water cools the leaves to a temperature well below that of the ambient air temperature. For many plants when that plant is transpiring fully the leaf temperature is $1 - 4^{\circ}\text{C}$ below air temperature. Conversely, as the crop undergoes water stress, transpiration decreases and there is thus less cooling and leaf temperature increases to a point when it approaches air temperature. One disadvantage with this method is that the CWSI is not universal as the non-water stressed baseline may be different from crop to crop and region to region and thus needs to be determined before the CWSI can be used. Jackson *et al* (1977) also stated that the behavior of leaf temperature both under stress and non-stress conditions provided clues

for crop water status and yield performance. In terms of irrigation quantity leaf temperature has a direct influence on plant water use and transpiration and hence can possibly be used to determine irrigation quantity. The possibility of using daily mean temperature or daily maximum temperature in particular to determine irrigation quantity is possible.

Instrumentally, according to Baker and Bland (1994), leaf temperature (or foliar temperature as they call it) can be measured using either contact or non contact methods. Contact methods involve the use of fine wire thermocouples (with wire diameter less than $5\mu\text{m}$) which are either inserted into the leaves or clipped on the surface of leaves. Into the stems of growing plants so as to measure stem temperature rather than leaf temperature. With both of these there may be problems of the representatives of point measurements to the whole canopy as well as problems associated with tissue invasion if thermocouples are inserted into the leaves or stem (Baker and Bland, 1994). Non contact methods or infra red methods are based on the Stefan-Boltzmann law which states that black bodies emit radiation in proportion to the fourth power of their absolute temperature. Using this law an instrument which measures emitted radiation can be calibrated to measure thermal radiation and interpret this into a temperature, as is the case with an infrared thermometer. Baker and Bland (1994) say that an important consideration when choosing and siting infrared thermometers for specific uses is the instruments field of view (FOV), since the apparent temperature measured will be an average of the temperature of all objects within this field of view.

In terms of the practicality of their use, Dzikiti (2007) states that both contact and non-contact instruments are non-invasive and can be connected to data loggers and computers. However, leaf temperature measurements are highly susceptible to microclimatic conditions surrounding the leaf and can thus change unexpectedly even when leaf is under no stress. Also leaf temperature measurements cannot be considered alone but should be interpreted in relation to air temperature (i.e. leaf-air temperature difference), stem diameter, sap flow rate and stomatal resistance.

2.5.3.5.6 Sap Flow Measurements

According to Dzikiti (2007), for drip irrigated horticultural crops and fruit trees, the water requirements arise primarily from the water loss through transpiration. Irrigation scheduling in this case seeks to replace the water lost by transpiration. Also according to Ortuno *et al* (2005) the health of plants can thus be determined based on whether the transpiration rate is at a maximum for any given atmospheric conditions. For example, if transpiration rate is lower than the potential then this points to a physiological problem most likely caused by a deficit of soil moisture (however, it can also be due to other factors such as disease or even just cloudiness). Thus sap flow measurements can be used for determining both the timing and quantity of irrigation.

The technical problem with the transpiration measurement approach has been the lack of a suitable method to monitor transpiration continuously under field conditions. However, the advent of the use of heat balance sap flow gauges as well as heat pulse sap flow gauges has helped solve this problem. According to Ortuno *et al* (2005), sap flow measurement by the heat-pulse technique is a suitable plant-based method for estimating the daily transpiration for plants and is hence a good indicator of water use, water status and water stress. The question of representatives of gauges is still an issue however and so is the question of up scaling of measurements from a single plant or branch to the entire crop. Vermeulen *et al* (2007) state that despite the relatively fast response of sap flow rate to changes in soil water availability, sap flow measurements should be used in a dynamic model which takes into account the variability of the greenhouse microclimate conditions. For example, sap flow can change rapidly due to passing of clouds since sap flow is in part controlled by stomatal resistance which themselves are very light sensitive (Gollan *et al*, 1985). In general the use of sap flow gauges requires more research before operational application in irrigation scheduling.

2.5.3.5.7 Remote Sensing (RS)

Remote sensing is defined by Aggarwal (2004) as methods of obtaining information about an object or area without being in direct contact with that object or area. It is a group of techniques for collecting information about an object and its surroundings without being in physical contact with that object. Many methods have attempted to detect plant water status and calculate plant water requirements using remotely sensed images of plant leaves, crop canopies and land surfaces at spatial scales differing from a single leaf to an entire geographical region and temporal scales from a day to a year. Satellite monitoring of vegetation water status is of particular interest in precision agriculture and can be used to detect plant health or estimate evapotranspiration usually on the scale of a field or greater. These images can be ground based or satellite based, and are particularly useful because they can give a better spatial representation of plant water status compared to other measurements.

In terms of satellite remote sensing the normalized difference vegetation index (NDVI) can be used to detect the condition of field crops (Jones, 2004a). Kacira *et al* (2002) looked at the possibility of using visible images captured with cameras mounted just above a crop canopy in order to monitor the canopy cover. Their hypothesis was that canopy cover would reduce or cease to increase if the crop was under water stress since leaves and shoots would begin to wilt. In terms of ground based measurements there are a number of possibilities. The measurement of chlorophyll fluorescence as a means of detecting water status has been experimented with. While, thermal images can be used to provide rapid and non-invasive collection of data from both satellite based and ground based sensors. These thermal images can detect stress due to water, disease, and lack of or excess of nutrients. Grant *et al* (2007) found that thermal imagery of canopies can be used to detect between irrigated and non-irrigated plants. However, they go on to say that the technique is not so useful when used on single leaves and is more effective on whole canopies. Leaf angles can also affect results and in general thermal imagery must be taken in the context of other meteorological variables. Dziki *et al* (2010) state that other

indices which can be used for the detection of drought stress indices include the Normalized Difference Water Index (NDWI), Maximum Difference Water Index (MDWI), Water Index (WI) and the red edge inflexion point (REIP).

2.5.3.5.8 Stomatal Resistance

According to Kramer (1983), stomatal resistance is a relatively sensitive indicator of developing water stress because the guard cells that control stomatal opening and closing are very responsive to changes of turgor pressure. Arkebauer (1994) states that stomata are pressure operated valves and that the stomata are open when the guard cells are turgid and are closed when the guard cells are flaccid. Hence, leaf and plant water status are an important factor in determining stomatal resistance which can equally be interpreted that stomatal resistance is an important indicator of leaf and plant water status. Blonquist *et al* (2009) go on to say that stomatal resistance is directly related to plant water status, and thus to plant growth and crop yield. Furthermore the stomata are a passage for diffusion of water vapour out of the plant as well as for CO₂ into the plant and their resistance can thus be an indicator of photosynthetic rate as well as transpiration rate. In terms of transpiration rate stomatal resistance can thus be useful in calculating water requirements but only in the context of other meteorological parameters. Arkebauer (1994), states that stomatal resistance is high when RH and plant water status are low. This is hypothesised to be a result of a loss of turgor by guard cells which can be associated with a deficit of water within the leaf. Gollan *et al* (1985) goes on to say that in terms of water stress there is a strong correlation between stomatal resistance and root zone water content.

Several methods of measuring stomatal resistance are available and consist mostly of a group of techniques known as porometry. According to Baker and Bland (1994) porometers are instruments which are used for measuring stomatal resistance and that there are at least three fundamentally different approaches that they use to do this. The three methods are: mass flow porometry, transient porometry and steady state porometry.

However, Watts (1977) states that the measurement of stomatal resistance has been made easier by the development of several types of diffusion porometers. He states that these instruments provide a measure of the resistance to water vapour movement through the stomata. These instruments measure the rate of diffusion of water vapour from leaves and are calibrated in such a manner that readings can be converted into diffusion resistance of the leaf in s m^{-1} (or its reciprocal conductance in m s^{-1}).

However, according to Jones (1999), while it is feasible to measure stomatal resistance directly by means of leaf porometers, the problems of calibration and of adequately sampling the population of leaves in a crop has restricted the use of porometers for such purposes as water status detection for irrigation scheduling. For example, calibration must be performed at temperatures at or near those of the environment in which the porometer will be used in, thus meaning that any changes in that environmental variable may result in errors (Baker and Bland, 1994). Davies *et al* (2000) also say that stomatal closure is sensitive to environmental variables such as root zone water status and radiation, and thus stomatal oscillations are known to occur even in the absence of any change in plant water status especially due to root to shoot chemical signaling. In general measurement of stomatal resistance can be time-consuming and labour-intensive and still may not give meaningful results of plant water status.

2.5.3.6 Other Methods

There are many other methods which can be used to detect water stress in horticultural crops. Most are variations or combinations of the methods mentioned above and all are subject to more research to determine which can be most easily and effectively used. Many of the methods mentioned have a great possibility for operational use and thus should be further studied and tested so that they can be made usable by the everyday horticultural grower.

2.6 Summary

It has been seen that there are many methods of detecting the water status of plants. However, as already mentioned, a technique to measure plant water status should provide non-destructive, rapid, and reliable estimates of plant water status. It has also been shown that some of these methods are difficult to use regularly while others are tedious and time consuming. There is a need to investigate the use of these indicators to determine which are best for use by the average grower. The “best” indicator would have a combination of characteristics which include quick response, ease of measurement, low cost and possibly be non-destructive and non-invasive. The need for a means of simulating plant water status and determining plant water requirements based on one or more of these indicators is evident and requires the creation of simple or multiple regressions or even more complicated models which can help to predict plant water status based on regularly measured physiological variables such as leaf temperature, stem diameter, sap flow, stomatal resistance and leaf water potential among others.

It has also been seen that data derived from direct crop monitoring could be used to compare crops over time and space as with the use of dendrometers and to supplement the regularly monitored environmental information used in greenhouses. In a study by Shelford *et al.* (2004) it was shown that plant-based water status monitoring for the irrigation of tomatoes compared favorably with more conventional irrigation methods such as the calculation of water requirements using the FAO-Penman-Monteith method.

The use of a feedback system for management of horticultural crops (i.e. phytomonitoring) has also been discussed and gives an indication of how plant sensors combined with appropriate software and data interpretation can help improve horticultural production. This direct crop monitoring has many advantages and also provides a means of validating models derived purely from climate information. Again the example of the FAO-Penman-Monteith model can be used. The direct monitoring of transpiration using sap flow gauges can be used to validate this model in any given area, while the monitoring of leaf water potential, leaf stomatal resistance and leaf temperature

can validate models which have been developed to determine water status based on these variables. Lastly it should be said that the use of plant indicators is still a relatively untapped resource and its practical application in a commercial greenhouse needs to be explored and promoted for the benefit of both science and production technology.

Chapter 3: Materials and Methods

3.1 Introduction

The project comprised of four parts namely, the calibration and setting up of equipment, the experiment itself along with the taking of measurements, the analysis of results and finally the formulation of conclusions. Calibration was done in the Department of Physics New Wing and on the open air laboratory on its rooftop at the University of Zimbabwe, while the experiment was done at an experimental greenhouse in the Department of Biological Sciences, University of Zimbabwe and finally analysis was done in the Agro-meteorology computer lab in the Department of Physics New Wing.

3.2 Experimental Location

The experiments were carried out in Harare, Zimbabwe at the University of Zimbabwe Mount Pleasant campus (shown in Figure 3.1). This site is situated at approximately 31.1 °E and 17.8 °S and an altitude of approximately 1483 m. The site is in Natural Region IIa (Vincent and Thomas, 1960), which receives annual rainfall of approximately 850 mm per annum, and with mean annual temperature of 19 °C. The temperatures are relatively low for production of field grown tomatoes and can restrict their growth. There is also the possibility of winter frosts due to the altitude. Rainfall, while adequate for year round production, is concentrated in only a few months of the year from November to March thus limiting the growing period and even in those months can be poorly distributed. The use of a greenhouse with irrigation is thus justified and necessary for year round production of tomatoes.



Figure 3.1: A satellite image of the University of Zimbabwe showing the location of the greenhouse used at the Department of Biological Sciences, as well as the location of the Department of Physics New Wing and its roof top (Image courtesy of Google Earth/ Europa Technologies, 2009).

Thus a greenhouse at the Department of Biological Sciences was used for the planting and growing of the tomato plants on which the experiments were carried out. Instrument calibration was carried out either in the Department of Physics Laboratory, on the Department of Physics rooftop and within this greenhouse.

3.3 The Greenhouse

The greenhouse at the Department of Biological Sciences was a single span, stand alone, Venlo-type greenhouse. The floor was concrete and the structure was made up of brick half walls and an aluminium shell with a cladding of 6mm single glazed clear glass, having a radiation transmission coefficient of 65.9 % (i.e. $\tau = 0.659$). The greenhouse was oriented in a north-south direction and was divided into two compartments each measuring 6 m $\times$ 10 m (60 m²), of which only one was used for experiments. The compartment used was equipped with two air conditioners, an electric fan, and electric heating system. Natural ventilation was controlled manually using side air vents connected to a hand driven mechanical wheel and cog which was used to open and close air vents as and when required. The method of irrigation was drip irrigation using drip lines with an average emitter application rate of 2 litres per hour (as specified by the manufacturer). The north facing entrance of the experimental greenhouse used is shown in Figure 3.2.



Figure 3.2: The experimental greenhouse at the Department of Biological Sciences, University of Zimbabwe.

3.4 The Planting and Care of Tomato

The experiment was carried out on 128 healthy, mature, fully grown tomato plants of the variety Nemo Netta. These had been planted as seedlings on the 14th of September 2009 in 128 uniformly spaced plastic flower pots filled with vermiculite, which is a solid mineral compound commonly used for the hydroponic growing of greenhouse crops. Vermiculite was used because it has a number of advantages which include good water holding capacity compared to other horticultural substrates, it is clean and does not turn moldy, it is sterile and holds no soil borne diseases, it is long lasting and can be reused, it is light and easy to transport and lastly it has a near neutral pH of 7.0 – 9.5. However it does have the disadvantages of having a low root volume and low natural nutrient content.

The pots were supported on metal stands so that they were positioned at 20 cm above the concrete floor of the greenhouse. The seedlings were planted into pots which had been fully saturated with water by leaving the irrigation system on overnight the previous day so as to ensure the pots were wet. The irrigation schedule from then on was to irrigate once a day according to calculations made by the FAO-Penman-Monteith method. Care was taken to stick to irrigation according the FAO-Penman-Monteith equation so as to minimize leaching as well as keep relative humidity low so as to minimize diseases.

A combination of different fertilizers was applied at intervals and in amounts as per the recommended grower practice shown in Table 3.1. An initial dose of MaizeFert was applied in the first week of transplanting to provide the vermiculite substrate with a small amount of nutrients to facilitate root development. The main fertilizers used included Ammonium Nitrate, Quick Start, Quick Grow, and Best Bloom. Fertiliser was applied by hand and inserted directly into the rooting zone of the crop for ease of uptake.

Table 3.1: The fertiliser schedule followed in the growing of the tomato plants

Week	Fertiliser Type and Quantity (weekly dosage)					
	Quick Start (g)	Quick Grow (g)	Best Bloom (g)	Calcium Nitrate (g)	AN (g)	NUTRIFOL NO 1 (ml/L)
3 – 6	0.144	0.16	0	0.032	0	4
7 – 10	0.056	0	0.272	0.4	0.064	0
11 – 14	0	0.64	0.16	0.752	0.064	4
15 – 25	0	0	1.28	0.752	0.08	4
26 - 30	0	0.64	0.16	0.16	0.08	4

Care was also taken to observe the plants every day from transplanting and throughout their growth to check for any diseases and/ or pests which may appear. If pests or

diseases were seen they were identified and an appropriate pesticide or fungicide was sprayed. Spraying was done using a 14 litre capacity knapsack sprayer. The main pests and diseases encountered included Aphids, Red Spider Mite, Leaf Eaters and Powdery Mildew. They were kept under control using a combination of chemicals listed in table 3.2.

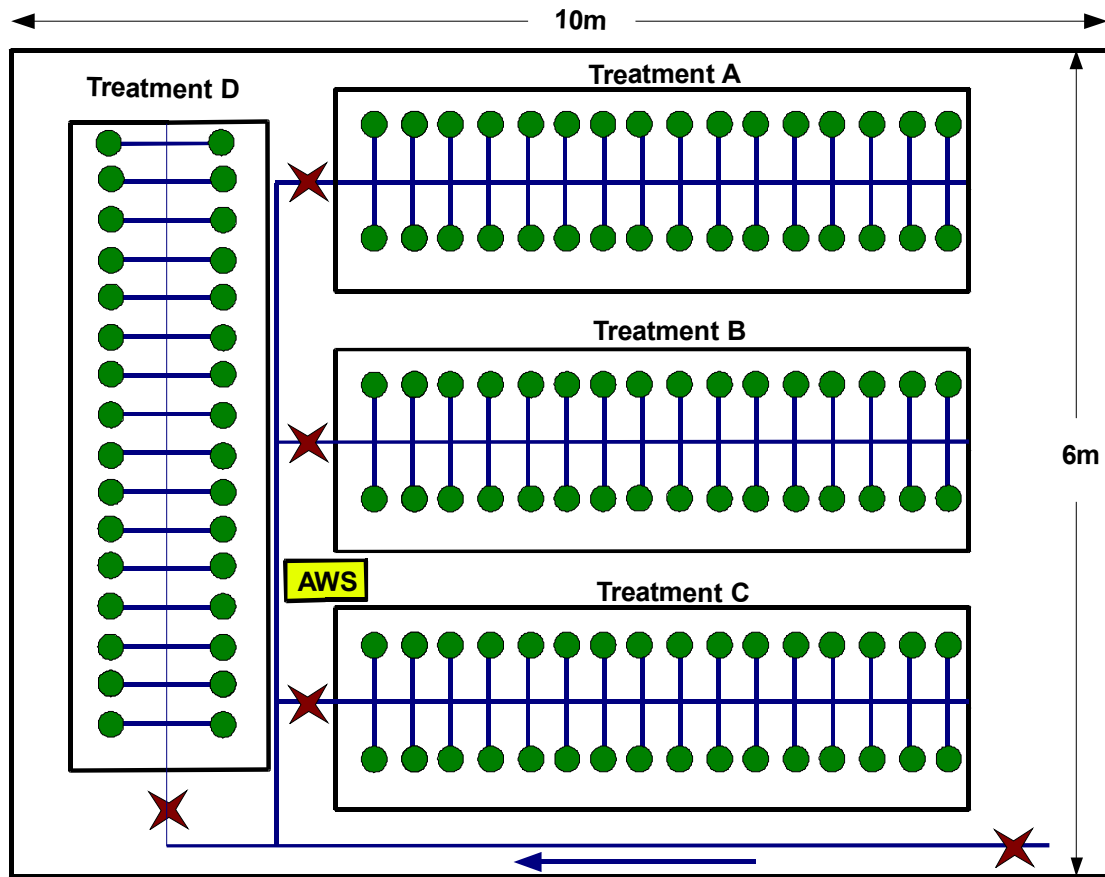
Table 3.2: The main tomato diseases and the chemicals used for their treatment

Disease/ Pest	Chemicals
Powdery Mildew	Wettable sulphur
Cut worms, Bollworms and Leaf eaters	Karate / Thionex
White flies/ Aphids	Confidor/ Metomex
Red Spider Mite	Dynamec/ Trigard

Any weeding was done manually by simply pulling the weeds out of the pot with one's hands whenever they appeared. In terms of irrigation a localized drip irrigation system was used to supply water directly to the tomato plants rooting zone. The application rate was approximately two litres per hour as specified by the manufacturer.

3.5 The Experimental Setup

For the experiment two replicated treatments with 32 plants each were used in the greenhouse. The treatments were labelled A, B, C and D with Treatment A (and its replicant Treatment C) being the well watered treatments and Treatment B (and its replicant Treatment D) being the drought stress treatment. The treatments were arranged in a set up shown in Figure 3.3 which shows the floor plan of the greenhouse used. Initially, both treatments were well watered and were irrigated according to estimation of reference crop evapotranspiration (ET_o) using the FAO-Penman-Monteith method (see equation 2.1 in Chapter Two).



✕ represents the valves used to control water to each treatment.

→ represents the direction of water flow.

— represents water pipes.

● represents a tomato plant

Figure 3.3: Floor plan of the greenhouse showing the setup used for the purpose of this project.

However, on certain dates and for certain durations the drought stress treatments (i.e. Treatment B and its replicant, Treatment D) were completely deprived of water by turning off the valve which supplied it with water thus subjecting it to drought stress. Irrigation in the drought stressed treatments was only allowed to return to the normal irrigation schedule once visible and detrimental signs of water stress were seen such as leaf curling, loss of turgidity and wilting. The plants were then allowed to recover fully by irrigating according to calculation of ET_o using the FAO-Penman-Monteith Equation and the responses noted. The dates on which they were deprived of water for this experiment are shown in Table 3.3.

Table 3.3: Days on which plants in treatments B and D were subjected to drought stress

Stressing Period	Dates
1	DOY 36 – DOY 39
2	DOY 47 – DOY 53

(The Julian calendar is used here and in the rest of the project, where DOY 1 = 1 January)

Observations were made every day during the drought stress periods as well as at least one week before and one week after so as to observe the differences between healthy and stressed plants and to determine which indicators responded quickest to the drought stress.

3.6 The Calculation of Irrigation Amounts

The calculated irrigation amounts for the treatments throughout the project was done based on the FAO-Penman-Monteith Equation described in Chapter Two (equation 2.1). This relied on a number of meteorological measurements namely net radiation, air

temperature, leaf temperature, wind speed and relative humidity to calculate what is called reference crop evapotranspiration. From then crop coefficients were used to convert reference crop evapotranspiration so as to determine actual crop water requirements for the tomato crop at that stage of its growth. In this case the experiments were carried out during the middle and late stage of growth and the crop coefficient (k_c) used was 0.8 according to recommendations made by Allen *et al* (1998).

3.7 Meteorological Measurements

Meteorological measurements were taken both inside and outside the greenhouse and were recorded continuously by data loggers throughout the course of the study. The measurements which were taken both inside and outside the greenhouse included, air temperature, relative humidity measured at a height of 1.5 m and photosynthetically active radiation (PAR) measured at a height of 2 m. Additionally inside the greenhouse net radiation and solar radiation were measured just above the crop canopy.

3.7.1 Temperature and Humidity Measurements

The temperature and humidity probes used for measuring air temperature and humidity inside and outside the greenhouse were Campbell Scientific HMP45C temperature and humidity sensors. The one inside the greenhouse (serial # 601) as well as the one outside the greenhouse (serial # 392) were installed and mounted within louvered radiation shields at 1.5m height in conformity with standard meteorological practices. These probes consist of a Platinum Resistance Temperature probe and a Vaisala HUMICAP® 180 capacitance relative humidity sensor. Figure 3.4 shows the louvered radiation shields containing the HMP45C temperature and humidity sensors both inside and outside the greenhouse.

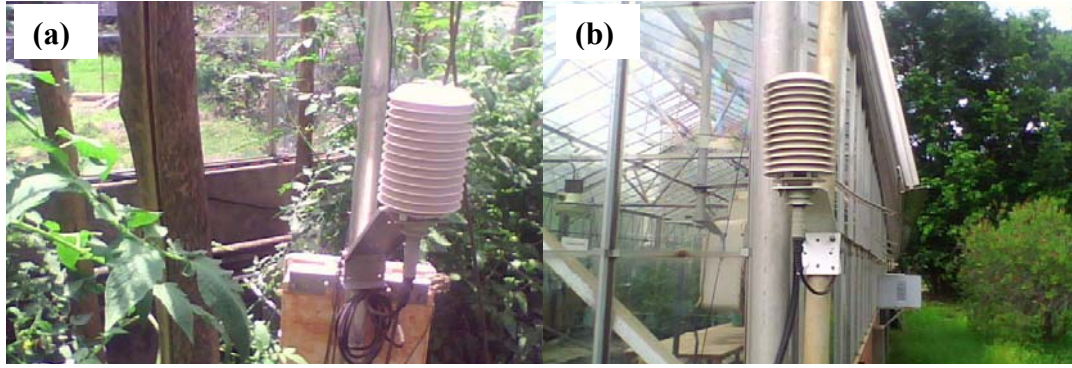


Figure 3.4: The louvered radiation shield containing the HMP45C temperature and humidity sensors a) inside the greenhouse and b) outside the greenhouse.

The temperature sensor has a measurement range of $-40\text{ }^{\circ}\text{C}$ to $+60\text{ }^{\circ}\text{C}$ with accuracy at manufacture of $\pm 0.2\text{ }^{\circ}\text{C}$ at standard room temperature of $20\text{ }^{\circ}\text{C}$. The HUMICAP[®] 180 sensor has a measurement range of 0 to 100% with accuracy at manufacture of $\pm 2\%$ at a temperature of $20\text{ }^{\circ}\text{C}$. The temperature dependence of the relative humidity measurement is $\pm 0.05\%/^{\circ}\text{C}$.

3.7.2 Radiation Measurements

There were a number of radiation measurements made both inside and outside the greenhouse. These included solar radiation, net radiation and photosynthetically active radiation (PAR) which were done using a tube solarimeter, a net radiometer and a quantum sensor, respectively. The instruments used and the details of their installations are as follows:

3.7.2.1 Solar Radiation

Measurement of incoming solar radiation in the greenhouse was done using a tube solarimeter (model TSL, Campbell Scientific, USA, Serial # 2912) mounted at a height just above the crop canopy as shown in figure 3.5.



Figure 3.5: A tube solarimeter measuring radiation above the canopy of the tomato crop

The instrument was leveled using its in built spirit level so that it faced directly upwards and its orientation was generally north to south. The instruments height was also periodically adjusted as the crop height increased so that it was always just above the crop canopy.

For the measurement of solar radiation outside the greenhouse one Mk-1-G Sol-A-Meter (Matrix Solar Services, Arizona, USA) was installed on a 3m high mast on the outside of the greenhouse. This instrument measures total incoming solar radiation from the entire electromagnetic spectrum in units of W m^{-2} . The instrument and its location outside the greenhouse are shown in figure 3.6.

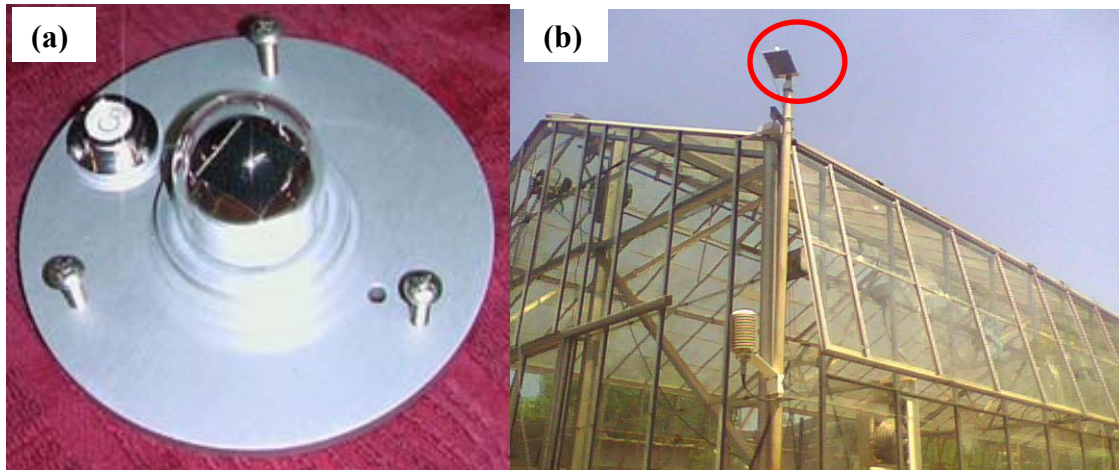


Figure 3.6: Images of the Mk-1-G Sol-A-Meter (Matrix Solar Services, Arizona, USA) showing a) a close up image and b) its location at the greenhouse (circled in red)

3.7.2.2 Net Radiation

A Q7.1 net radiometer (Radiation and Energy Balance Systems, Washington, USA) was installed inside the greenhouse to measure net radiation above the crop canopy as shown in Figure 3.7. The instrument calculates net radiation by measuring shortwave and longwave radiation incident on the top and bottom of the instrument and calculating the difference between the four components.



Figure 3.7: The Q7.1 net radiometer (REBS, Washington, USA) used in the greenhouse

3.7.2.3 Photosynthetically Active Radiation (PAR)

Two LI-190SZ Quantum Sensors (LI-COR Inc, Nebraska, USA) were installed for the measurement of PAR in the project. One was placed inside the greenhouse and one outside the greenhouse. The quantum sensor located inside the greenhouse is shown in Figure 3.8.



Figure 3.8: The LI-190SZ Quantum Sensor (LI-COR Inc, Nebraska, USA) used in the greenhouse

These instruments measure radiation in the range of $400 \mu\text{m} - 700 \mu\text{m}$, also known as photosynthetically active radiation (PAR), in units of $\mu\text{mol m}^{-2} \text{s}^{-1}$. This is the actual energy used by plants in photosynthesis.

All of these radiation sensors were connected to one of two different data logger models namely CR10X or CR23X (Campbell Scientific, Logan, USA) which were both programmed to sample measurements at 5 second intervals and store the average of these values every 15 minutes.

3.7.3 Wind Speed Measurements

A Testo 425 heated bead anemometer (Serial # 373) was used to measure wind speed within the greenhouse on selected days so as to obtain average wind speed within the greenhouse. The instrument and its use are shown in Figure 3.9.

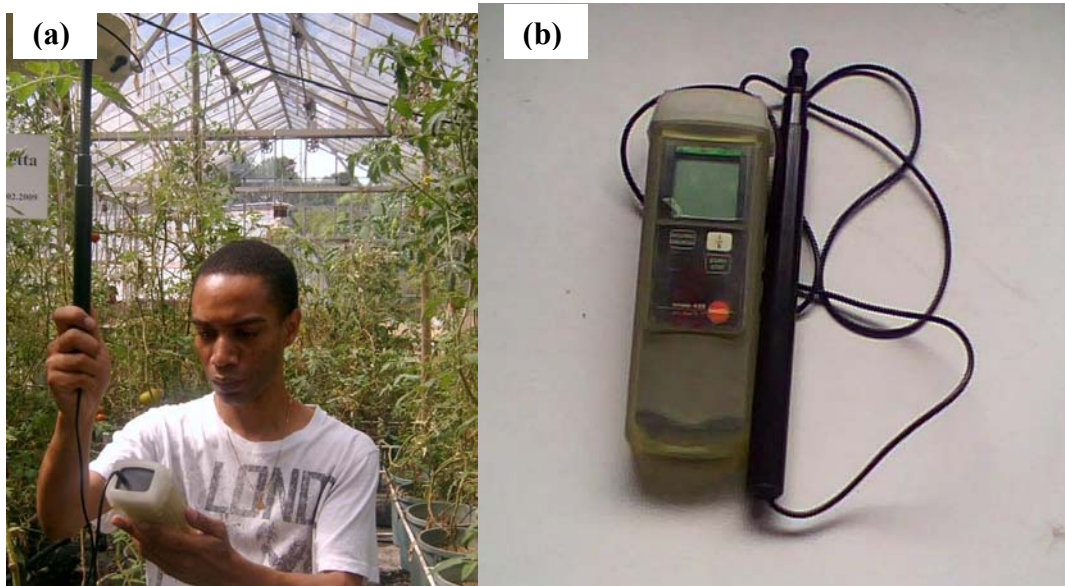


Figure 3.9: Image showing a) the use of a heated bead anemometer to sample wind speeds within the greenhouse and b) the heated bead anemometer used.

The instrument was used because it has a very fine resolution of 0.01 m s^{-1} making it suitable for measurement of low wind speeds such as those in greenhouses. The instrument has a measurement range of between 0 to 10 m s^{-1} and can be used in environments with temperatures between 0 to $50 \text{ }^{\circ}\text{C}$. The instrument however, is handheld and cannot be automated or connected to a data logger, hence it was only used on three selected days to sample wind speed and get an average. The procedure was to monitor wind speed in different parts of the greenhouse at different times of the day and take the average of these readings. The instrument was held vertically above the head at approximately 2m height and the measurements read off the monitor.

All these meteorological measurements and the instruments used are shown in Table 3.4 which summarises the meteorological parameters measured along with the details of the instrument used to measure each parameter.

Table 3.4: Summary of the meteorological parameters measured and the instruments used.

Parameter Measured	Symbol	Units	Instrument Used	Type	Accuracy	Manufacturer
PAR	PAR	$\mu\text{mol}/\text{m}^2$	Quantum Sensor	LI-190SZ Quantum Sensor	$\pm 5\%$	Li-cor Inc, USA
Net Radiation	R_n	W/m^2	Net Radiometer	Q-7.1		REBS, WA, USA
Solar Radiation	R_s	W/m^2	Tube Solarimeter	TSL	$\pm 10\%$	Delta-T Devices, UK
Solar Radiation	R_s	W/m^2	Pyranometer	Mk-1-G	$\pm 5\%$	Matrix Solar Services, USA
Relative Humidity	RH	%	Temperature and RH Probe	HMP45C	$\pm 2\%$	Campbell Scientific Inc. UK
Air Temperature	T_{air}	$^{\circ}\text{C}$	Temperature and RH Probe	HMP45C	$\pm 0.2\%$	Campbell Scientific Inc. UK
Wind Speed	U_2	m/s	Heated Bead Anemometer	Testo 425	$\pm 0.01\text{m}/\text{s}$	GmbH & Co. Germany

3.8 Physiological Measurements

A number of physiological measurements were made on the tomato plants these included leaf temperature, stem temperature, stomatal resistance, leaf water potential, fruit diameter, stem diameter and sap flow. These measurements and the instruments used for each are summarised in Table 3.5 while the details of each measurement and the procedure used follows.

3.8.1 Measurement of Leaf Water Potential

Leaf water potential was measured using a Thermocouple Psychrometer (shown in Figure 3.10) which comprised of four C-52 Sample Chambers (Wescor Inc, Logan, USA) connected to a HR33T Dew Point Micro-voltmeter (Wescor Inc, Logan, USA) through a PS-10 Psychrometer Switchbox (Wescor Inc, Logan, USA).



Figure 3.10: The Wescor Thermocouple Psychrometer kit with sample chambers, switchbox, microvoltmeter and the four airtight plastic chambers that were used to collect the samples.

The measurement of leaf water potential were done on selected days during the experiment at solar noon (between 12am and 1pm) on one leaf per treatment so as to obtain midday leaf water potential. The diurnal variation of leaf water potential was also recorded by measuring leaf water potential on one leaf per treatment every one hour on selected days between 9am and 6pm both before and after stressing. Leaf water potential was measured on the upper, most fully expanded leaves (usually 4th to 5th node from the top) which had maximum exposure to direct sunlight. The procedure was to choose a leaf and punch out a 6 mm diameter sample into an airtight plastic container which was immediately sealed and put in a shaded cool white data logger enclosure box outside the greenhouse while the other samples were taken. All samples from the four treatments were taken as quickly as possible and then taken to the laboratory for measurement of leaf water potential. The time between punching out the first sample and sealing of the last sample into the thermocouple psychrometer chamber was kept to below 10 minutes. It was believed that once samples were sealed in the sample chambers they were considered to be stable thus enabling leaf water potential to be determined for each sample one at a time by simply using the switchbox to change between samples.

3.8.2 Measurement of Stomatal Resistance

Stomatal resistance in units of s m^{-1} was measured on two healthy, mature, fully expanded leaves per treatment on selected days using a Delta-T AP4 Diffusion Porometer (Delta-T Devices, Cambridge, UK). On these days stomatal resistance was measured every hour from 9am till 6pm. For each leaf, the resistance of both the top and bottom of the leaf was measured and recorded manually along with the corresponding reading of leaf temperature and PAR which were also measured by the AP4 sensor head. The measurement of stomatal resistance was done in the vicinity and in some cases on the same plant of the measurements of leaf water potential so as to enable possible correlations to be made between the two physiological parameters.



Figure 3.11: The measurement of stomatal resistance of the leaves of tomato plants using the AP4 porometer (Delta-T Devices, Cambridge, UK)

3.8.3 Measurement of Stem and Leaf Temperature

Plant temperatures were recorded using a total of 12 type T (copper-constantan) fine wire thermocouples with a diameter of 122 μm . These were connected to two CR23x (Campbell Scientific, Logan USA) data loggers located in the greenhouse and

Materials and Methods

programmed to read temperature every 5 seconds and store the averages every 15 minutes. Each of the four treatments was assigned three fine wire thermocouples of which two were attached to the undersides of two randomly chosen healthy growing leaves while one was inserted into a healthy growing succulent stem in that treatment. For the leaves the thermocouples were attached to the underside of the chosen leaves with a plastic clip as shown in Figure 3.12



Figure 3.12: A thermocouple clipped onto the underside of a leaf so as to measure leaf temperature.

For the stems, the measurement end of the thermocouple was inserted firmly into a succulent growing stem of a randomly chosen healthy plant so that the thermocouple remained inserted on its own as shown in Figure 3.13. The data logger programme ensured that readings produced were in °C and required no conversion.



Figure 3.13: A thermocouple inserted into a stem (circled in red) to measure stem temperature.

3.8.4 Measurement of Stem and Fruit Diameter

The measurements of stem and fruit diameter were done physically using a micrometer screw gauge (Mitutoyo, Japan) and vernier caliper (Helios, Germany), respectively as well as automatically using DEX 70 and DEX 100 dendrometers (Dynamax, USA). For stem diameter (shown in Figure 3.14), the manual measurements were made on a healthy growing stem on three healthy growing plants per treatment which were chosen and marked with a permanent marker to indicate where exactly the measurements were made so as to ensure consistency of measurements. Different sized stems were chosen so as to ensure that growth or lack of was not a result of age or size of the stem. The automated measurements were made on one healthy young growing stem in each treatment so as to ascertain the typical diurnal variation in stem diameter of a tomato plant.

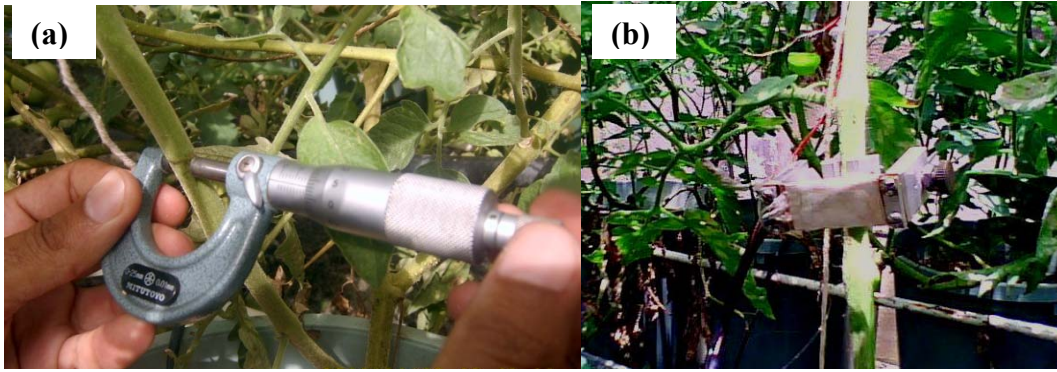


Figure 3.14: The measurement of stem diameter using a) a micrometer screw gauge and b) a DEX 70 dendrometer (Dynamax, USA).

For fruit diameter (shown in Figure 3.15), measurements were made on three healthy growing tomato fruit on three healthy growing plants per treatment. The fruit were again marked with a permanent marker to show where exactly measurements were to be made so as to ensure consistency. Different sized fruit were chosen so as to ensure that growth (or lack of) was not a result of age or size of the fruit. The automated measurements were made on one healthy young growing fruit in each treatment so as to ascertain the typical diurnal variation in fruit diameter.

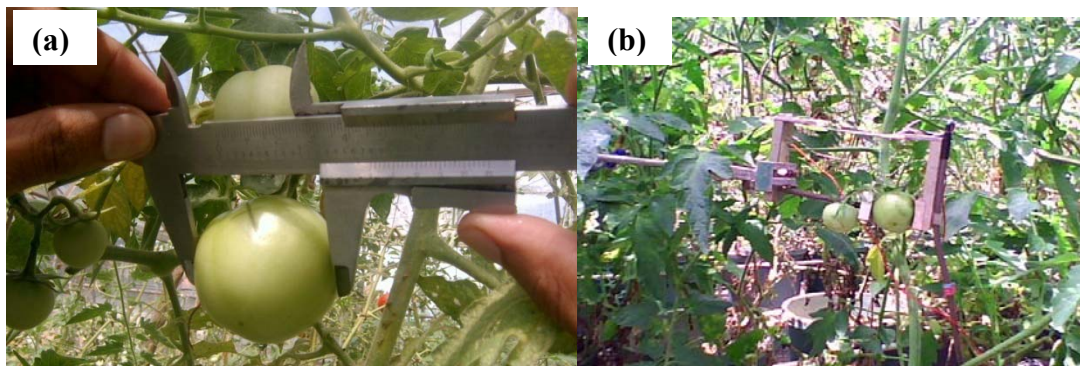


Figure 3.15: The measurement of fruit diameter using a) a vernier caliper and b) a DEX 100 dendrometer (Dynamax, USA).

The manual measurements of both stem and fruit diameter were taken at 10am every two days during the experiment and recorded with pen and paper before being input into a spreadsheet in Microsoft Excel for processing. However, these manual measurements were simply a backup for the automated dendrometers measurements.

3.8.5 Measurement of Sap Flow

Sap flow was measured using a stem heat balance sap flow gauge (SGB-10, Dynamax, USA) on one plant in treatment A and one plant in Treatment B. Due to the limitation of equipment availability sap flow measurements could not be replicated as there were only two stem heat balance sap flow gauges of the appropriate size for tomato stems available. The gauge itself and the installed product are shown in Figure 3.16. The procedure for the installation and use of the sap flow gauges followed six basic steps. Firstly, the choosing of an appropriate stem, which was a fairly smooth, straight stem with, in this case, a diameter of approximately 10 mm. This stem was cleaned with a cloth and then lubricated with an electrical insulation compounding.

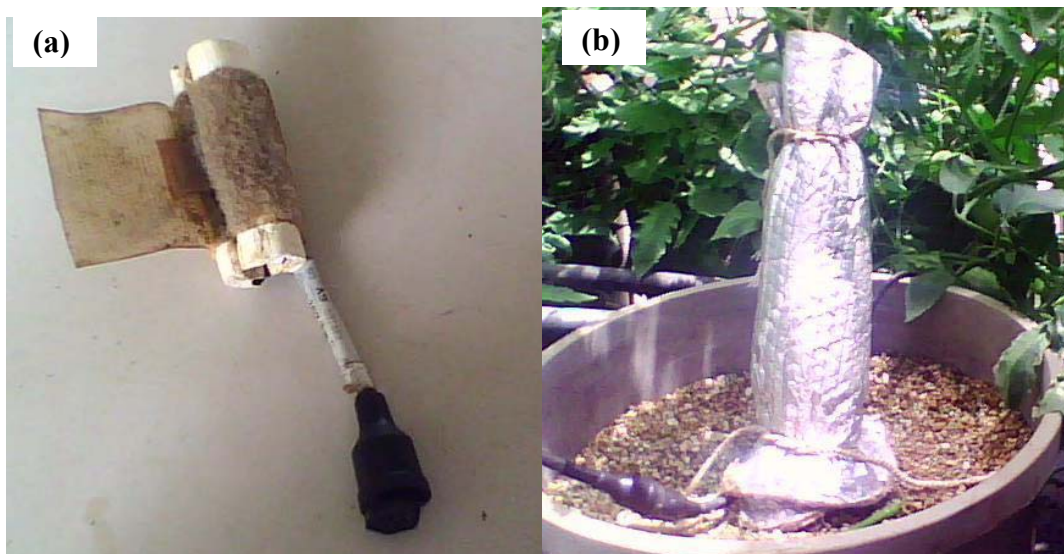


Figure 3.16: The sap flow gauge a) before installation and b) after installation covered with a reflective heat shield to reduce errors.

The gauge was then placed around the stem ensuring that the heater was wrapped around the entire circumference of the stem. Insulative o-rings were then placed at the top and bottom of the gauge before it was securely wrapped with the insulative heat shield. To account for errors in whole plant transpiration rates in the mornings and afternoons due to heat storage in the stem segment caused by temperature gradients between the stem and soil, the method proposed by Steppe *et al* (2005) was used. This involved measuring stem temperature near the sensor using a thermocouple inserted into the stem so as to correct for the energy stored in the heated stem section by accounting for it in the stem heat balance equation.

All the physiological measurements mentioned in this section and the instruments used are shown in Table 3.5 which summarises the physiological parameters measured along with the details of the instrument used to measure each parameter.

Table 3.5: Summary of the physiological parameters measured and the sensors used.

Parameter Measured	Symbol	Units	Instrument Used	Type	Error	Manufacturer
Leaf Temperature	T_l	$^{\circ}\text{C}$	Fine wire thermocouple	Type T	$\pm 0.1^{\circ}\text{C}$	UZ, Physics Department
Stem Temperature	T_s	$^{\circ}\text{C}$	Fine wire thermocouple	Type T	$\pm 0.1^{\circ}\text{C}$	UZ, Physics Department
Stomatal resistance	r_s	s m^{-1}	Porometer	AP4		Delta-T Devices, USA
Leaf water potential	ψ_l	kPa	Thermocouple Psychrometer	Wescor Psychrometer Kit		Wescor, Utah USA
Fruit Diameter	-	mm	Dendrometer	DEX 70 & DEX 100		Dynamax Inc. USA
Fruit Diameter	-	mm	Vernier Caliper		$\pm 0.1 \text{ mm}$	Helios, Germany
Stem Diameter	-	mm	Dendrometer	DEX 70 & DEX 100		Dynamax Inc. USA

Stem	-	mm	Micrometer	0-25 mm	0.01 mm	Mitutoyo Inc.,
Diameter			Screw Gauge			Japan
Sap Flow	-	mm hr ⁻¹	Stem Heat	SGB	±10%	Dynamax
			Balance Sap			Inc.USA
			Flow Gauge			

3.9 Instrument Calibration

Both before use as well as at regular intervals during their use some of the instruments used in the experiment had to be calibrated to ensure that the readings obtained were consistent and accurate enough for the purpose of the study. This calibration was meant to standardize the instruments with each other as well as with a universally accepted standard. Calibration is usually done against an in-house standard sensor instrument and ensures that the deviations from the standard are within accuracy limits advertised by the manufacturers of the sensors. Calibration is thus used to reduce instrument and systematic errors by choosing the best instruments and also by knowing what correction if any is required to be applied to measurements so as to make the results more accurate. The instruments which were calibrated and the procedures are documented next.

3.9.1 Thermocouples

The thermocouples were calibrated in the department of Physics on the 4th of September 2009. Twelve copper-constantan (Type T) 122 µm diameter fine wire thermocouples were calibrated against a platinum resistance thermometer using the Grant LTD6G water bath located in the Agro-meteorology laboratory. The thermocouples were first connected to a Campbell Scientific CR23X data logger (Z14) which had been appropriately programmed for that setup and those instruments. The data logger was programmed to sample temperature every 5 seconds and store the average of these every minute. The measurement ends of the thermocouples were tied together with a platinum resistance thermometer (taken as the standard) using an elastic band before placing the measurement ends in the fully filled water bath. The temperature of the water bath was

then set to different temperatures at 5 °C intervals between the ranges of 0 °C to 50 °C. For each temperature the water bath was given time to reach that temperature and equilibrate for 5 minutes before the time was recorded so that a comparison could be made between the standard using the results recorded by the data logger. The water bath was consistently and automatically stirred by an inbuilt rotor so that the water within it was of a homogenous temperature so as to obtain the most accurate results. Thus readings of actual water bath temperature and those of temperature recorded by the thermocouples were compared against each other in order to calibrate the thermocouples. Graphs of recorded versus actual temperature were plotted and the gradient and intercept taken as the new multiplier and offset, respectively.

3.9.2 Radiation Thermometers

Six copper-constantan (Type T) infrared thermocouples model IRT/cTM (Exergen Corporation, Massachusetts USA) were calibrated in the Department of Physics Agro-meteorology laboratory on the 5th of August 2009 using the in-house standard, a BB701 Blackbody Calibrator (OMEGA Engineering Inc, Connecticut, USA). This was done by firstly connecting the thermometers to a data logger with an appropriate programme being uploaded to the data logger via a laptop and a communication cable. The data logger was programmed to sample temperature every 5 seconds and average the results every 1 minute. Each infrared thermometer was attached to a tripod with the thermometers receiver facing and placed at a distance of 5cm from the Blackbody Calibrators target plate. The BB701 Calibrator's temperature was then adjusted in 5 °C intervals from 0 °C to 50 °C while manually recording the time at which the target plate reached each set temperature. Graphs of the standard versus the recorded temperature were plotted with the equation of the linear relationship between the two being used as the new calibration equation for each infrared thermometer. The gradient was used as the new calibration coefficient/ multiplier and the y-intercept used as the offset when writing programmes for the data logger on which these radiation thermometers were connected.

3.9.3 Solar Radiation Sensors

A number of different radiation sensors were calibrated for use in the project. The sensors that were calibrated included two quantum sensors, a matric sensor, a net radiometer and a tube solarimeter. In general the instruments were calibrated by comparing the output of the test sensor in mV to the output of the standard in W m^{-2} . The ratio of the two (known as the mean ratio) was analysed by means of linear graphs and if this deviated from 1.00 by more than 5%, a new calibration constant (or multiplier) was determined by taking the gradient of that graph as the new calibration constant for the sensor with the y-intercept being taken as the offset.

3.9.3.1 Tube Solarimeter

A TSL tube solarimeter (Campbell Scientific Inc, Logan, USA) was calibrated against the in-house standard which was the Kipp and Zonen CM11 Pyranometer (Delft, Netherlands) with serial number 997082. The calibration was done on the Physics department roof between the dates of 21st August 2009 and 24th August 2009. The method was first to place the two sensors adjacent to each other on a completely horizontal surface so that both faced directly upwards. The inbuilt spirit levels on the tube solarimeter to be calibrated as well as the one on the CM11 were used to ensure that the instruments were completely horizontal again to ensure most accurate results were obtained. The instruments were placed well away from any obstructions or objects that could harm the accuracy of results. The tube solarimeter was oriented north-south in line with recommendations by Monteith (1973). The two sensors were connected to a CR10X (Z4) data logger (Campbell Scientific Inc, Logan, USA) and the readings sampled over five seconds then averaged and recorded every 15 minutes. The results were compared with those of the standard by means of a linear graph of test sensor in mV m^{-2} versus standard in W m^{-2} . The gradient of the graph was taken to be the new multiplier or calibration constant and the y-intercept as the offset.

3.9.3.2 Quantum Sensors

The LI-190SZ Quantum Sensors (LI-COR Inc, Nebraska, USA) were calibrated between the 6th of March 2009 and the 9th of March 2009 on the Department of Physics, Agro-meteorology rooftop. The same procedure was used as that for the tube solarimeter. The Quantum Sensors were leveled with the inbuilt spirit levels so that they faced directly upwards and were placed far away from any obstructions which might compromise the results. They were also connected to a CR10X data logger (Campbell Scientific Inc, Logan, USA) which was programmed to sample readings every 5 seconds and record the average every 15 minutes. However, there was no standard available to calibrate them against and neither could be considered a standard compared to the other. Thus they were calibrated against each other by plotting their output in $\mu\text{mol m}^{-2} \text{s}^{-1}$ over the calibration period against each other. The expected output was a straight line with a one to one correlation between the two sensors.

3.9.3.3 Net Radiometer

The Q7.1 net radiometer (Radiation and Energy Balance Systems, Washington, USA) was last calibrated on the 19th and 20th of May 2009 at Mazoe Citrus Estates and was deemed to be usable based on that calibration. It was calibrated against the standard which was the CNR 1 net radiometer (Kipp & Zonen, Delft, Netherlands). Both instruments were placed facing north at a height of 2 m above a surface of short grass so that they away from any obstructions and were leveled using their inbuilt spirit levels so that they faced directly upwards. They were connected to a CR23X data logger (Campbell Scientific Inc, Logan, USA) programmed to sample readings every 5 seconds and recorded the average every 15 minutes. The results were analysed by means of a linear graph of test sensor versus standard in W m^{-2} .

3.9.3.4 Matrix Radiation Sensor

The MK-1-G Sol-a-meter (Matrix Solar Services, Arizona, USA) was calibrated on the 9th of March 2009 on the Department of Physics rooftop. It was calibrated against the in-

house standard which was a CM11 Pyranometer (Kipp and Zonnen, Delft, Netherlands) with serial number 997082. The method was first to place the two sensors adjacent to each other on a completely horizontal surface so that both faced directly upwards (the inbuilt spirit levels on both instruments were used to ensure this). The instruments were placed well away from any obstructions or objects that could harm the accuracy of results. The two sensors were connected to a CR10X data logger (Campbell Scientific Inc, Logan, USA) and the readings sampled over five seconds then averaged and recorded every 15 minutes. The results were compared with those of the standard by means of a linear graph of test sensor in mV m^{-2} versus the standard in W m^{-2} . The gradient of the graph was taken to be the new multiplier or calibration constant and the y-intercept as the offset.

3.9.4 Temperature and Humidity Sensors

Two HMP45C temperature and humidity sensors were calibrated on the 3rd and 4th of September 2009 for use in the project. The temperature and humidity sensors were calibrated against the in-house standard which was the Waltz TS-2 Dew Point Mirror Measuring System (Heinz Walz GmbH, Effeltrich, Germany) and the Platinum Resistance Thermometer (PRT). Firstly, the instruments were connected to a CR23X data logger (Campbell Scientific Inc, Logan, USA) and an appropriate programme written and uploaded using a laptop with PC208 software and a communication cable. The instruments were then placed into a well insulated cylindrical flow chamber jacket was connected to the Grant LTD6G water bath (Grant Instruments Ltd., Cambridge, UK). This setup ensured that the temperature within the jacket could be controlled by setting the LTD6G water bath temperature and circulating this water through the flow chamber jacket. Relative humidity within the flow chamber was controlled using the portable dew point generator (model LI-610). By doing this the temperature probe in the HMP45C could be calibrated as well as using the dew point mirror system to calibrate the humidity probe. The assumption was that within the jacket at any given temperature the dew point temperature could be measured. Using this dew point the relative humidity within the

sleeve could be calculated and compared with the relative humidity measured by the HMP45C probes. A platinum resistance thermometer was taken as the standard for temperature measurement within the flow chamber jacket. Regressions of the test sensor readings versus the Walz readings were plotted in order to determine the new calibration coefficients for the sensors.

3.10 Data Logging and Data Collection

Two CR23X (Campbell Scientific, Logan USA) and one CR10X (Campbell Scientific, Logan USA) data loggers were simultaneously used in the greenhouse for this experiment. The two CR23X data loggers were used for measurements inside the greenhouse while the CR10X was used for measurements taken outside the greenhouse. The data loggers were programmed to sample all instruments readings every 5 seconds and store the average of these results every 15 minutes. The two different types of data loggers are shown in Figure 3.17.

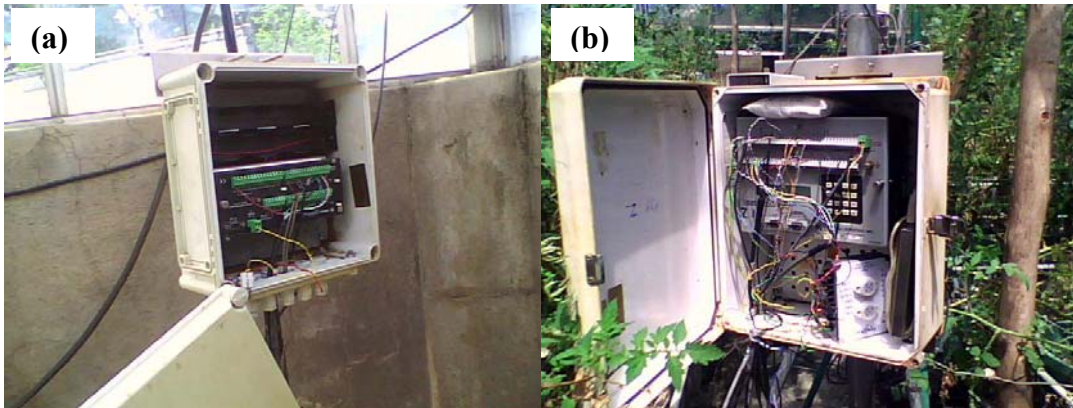


Figure 3.17: The two different types of data loggers used in the experiment namely (a) the CR10X and (b) one of the two CR23X data loggers.

The data was downloaded at the beginning of each day at approximately 9am using an HP Compaq mini laptop loaded with PC208W software for windows (shown in Figure 3.18).

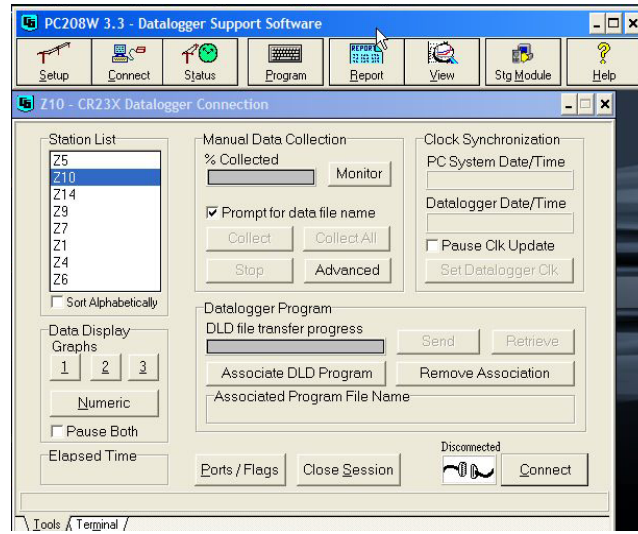


Figure 3.18: PC208W screenshot with the Z10 data logger highlighted preceding downloading of data

This data was then imported into Microsoft Excel for processing (labeling, sorting etc.) before analysis was done. The processing included labeling of columns as well as sorting of the data so that it could now be easily worked on. Selective use of data was performed by choosing days and times which suited the needs of the relationships to be investigated. For example, in some cases only sunny days were chosen while in other cases both cloudy and sunny ones were taken so as to investigate the relationships under different environmental conditions. The data from the data loggers in combination with that recorded manually was then graphically analysed using both Microsoft Excel and Sigma Plot.

Chapter 4: Results and Discussion

4.1 Introduction

This chapter presents the results of the experiment as well as discussions on their value in answering the questions posed in the objectives section of chapter one. In general the experiments went well and despite a two day power cut on DOY 29 to 30 and another one on DOY 6 all readings were taken successfully. The results were successfully analysed and are presented in the following sections.

4.2 Instrument Calibration

4.2.1 Thermocouples

The thermocouples initially gave extremely erroneous results and were thought to be useless. However, it was found that there were some faults along the wires which caused these results. The thermocouples were cut at half meter lengths at alternating ends and tested after each cutting to determine if the fault had been removed. In the end twelve working Type T thermocouples were obtained which could be used in the greenhouse for measuring leaf and stem temperature. The results of the calibration are shown in table 4.1. All the twelve thermocouples were fairly accurate and all had errors of less than the maximum allowable 5 % error required to necessitate the changing of the multiplier and offset. They were all thus used with no correction factors for measurements.

Table 4.1: Results of thermocouple calibrations done on 4th September 2009

Thermocouple Type	Serial No.	Calibration Coefficient	Calibration Offset	R-Squared Value
T	TCT0001	0.99	-0.03	0.999
T	TCT0002	1	0.03	0.999
T	TCT0003	1	0.15	0.999
T	TCT0004	0.99	0	0.999
T	TCT0005	0.99	0.2	0.999
T	TCT0006	1	-0.13	0.999
T	TCT0007	0.99	0.18	0.999
T	TCT0008	0.99	0.13	0.999
T	TCT0009	0.99	-0.167	0.999
T	TCT0010	0.99	0.2	0.998
T	TCT0011	1	0.05	0.999
T	TCT0012	0.99	0.12	0.999

4.2.2 Temperature and Humidity Probes

The two HMP45C probes were both successfully calibrated on the 3rd and 4th of September 2009 and the results are shown in Figure 4.1 (temperature sensor) and 4.2 (humidity sensor). Similar to the calibration of the thermocouples their calibration equations (gradient and intercept) were less than the maximum allowable 5% and thus were used with the multipliers and offsets specified by the manufacturer. For the temperature sensors there were high correlations ($R^2 = 0.9998$ for both). For the RH sensors as expected they were not as accurate as the temperature sensors but they still had high correlations ($R^2 = 0.9186$ and $R^2 = 0.9848$ for the 601 and 392, respectively).

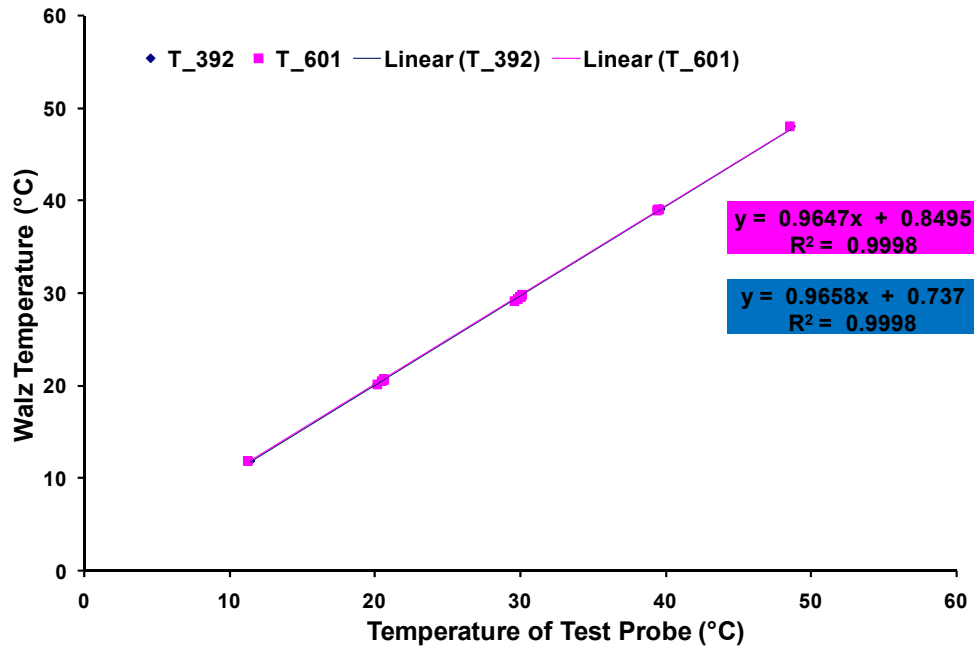


Figure 4.1: Calibration results of the temperature sensor inside the HMP45C probe

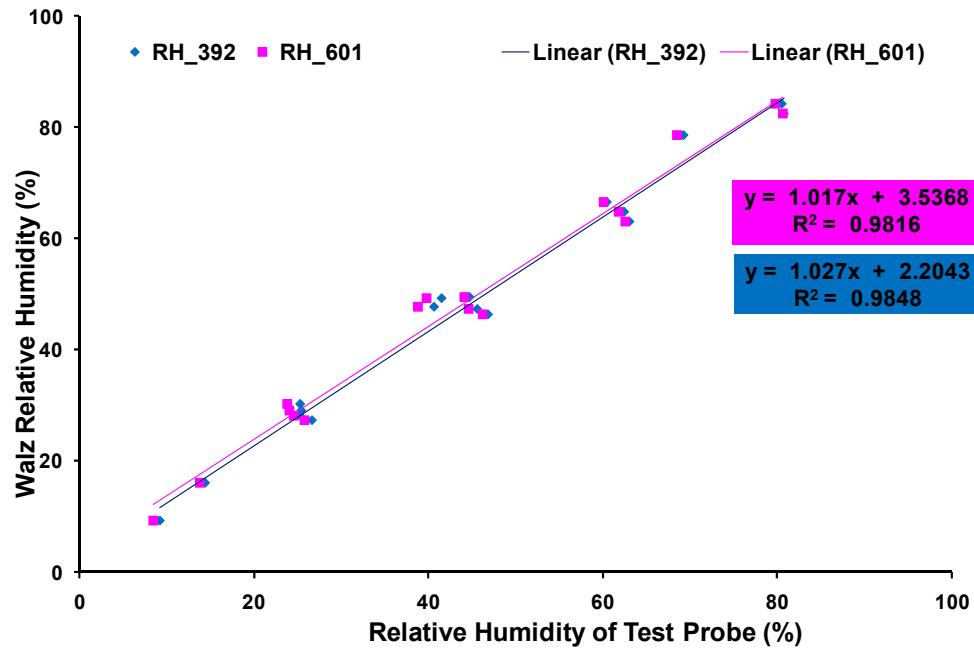


Figure 4.2: Calibration results of the RH sensor inside the HMP45C probe

4.2.3 Radiation Sensors

4.2.3.1 TSL Tube Solarimeter

The TSL tube solarimeter (Campbell Scientific Inc, Logan, USA) was successfully calibrated and the multiplier and offset changed accordingly. Figure 4.3 shows the results which give the calibration equation with the new multiplier of $72.31 \text{ W m}^{-2} \text{ mV}^{-1}$ and new offset of -3.176 W m^{-2} . The correlation between the two sensors was also high ($R^2 = 0.997$).

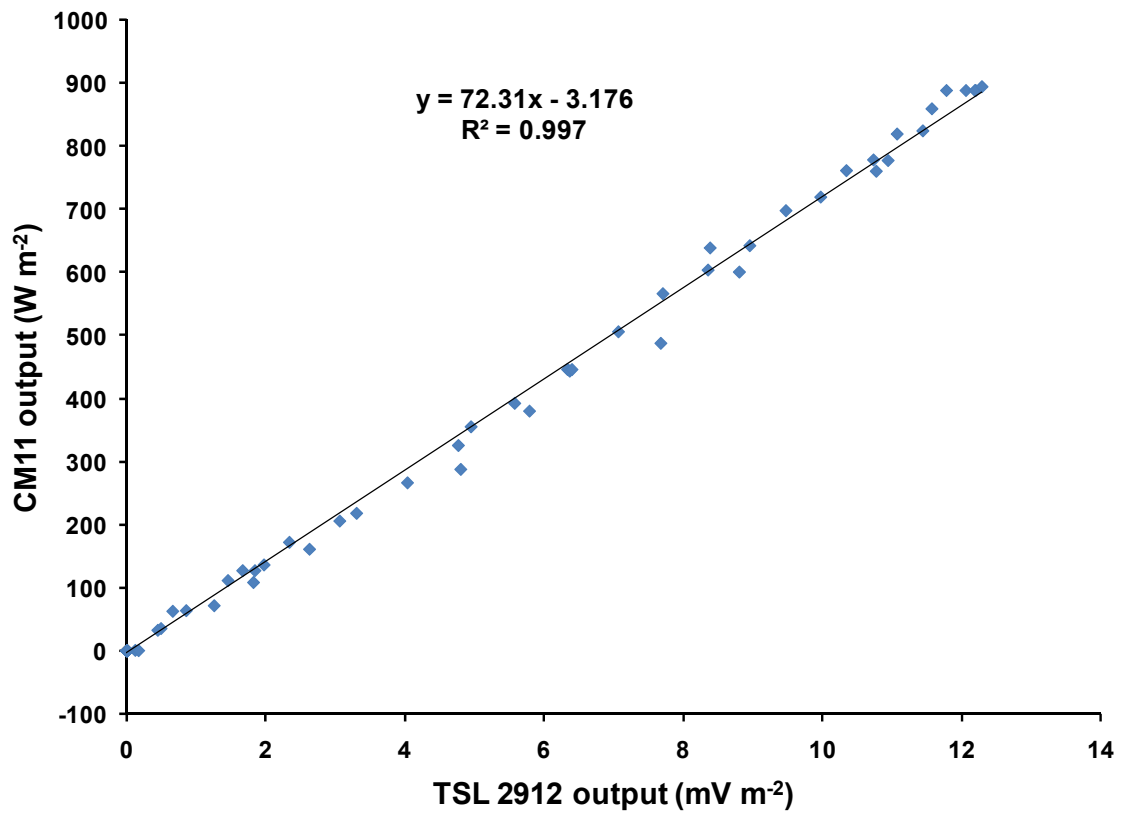


Figure 4.3: The results of the calibration of the tube solarimeter done on 23 August 2009

4.2.3.2 LI-190SZ Quantum Sensors (LI-COR Inc, Nebraska, USA)

The quantum sensors showed good correlations with each other ($R^2 = 0.993$) and had almost a one to one relationship. While this is not the ideal way to calibrate them it showed that their readings were fairly similar and thus could be used for comparison between the inside and outside of the greenhouse. Also given that their output was fairly similar it was unlikely that both were erroneous and hence it was assumed that they are both working well. The results are shown in Figure 4.4.

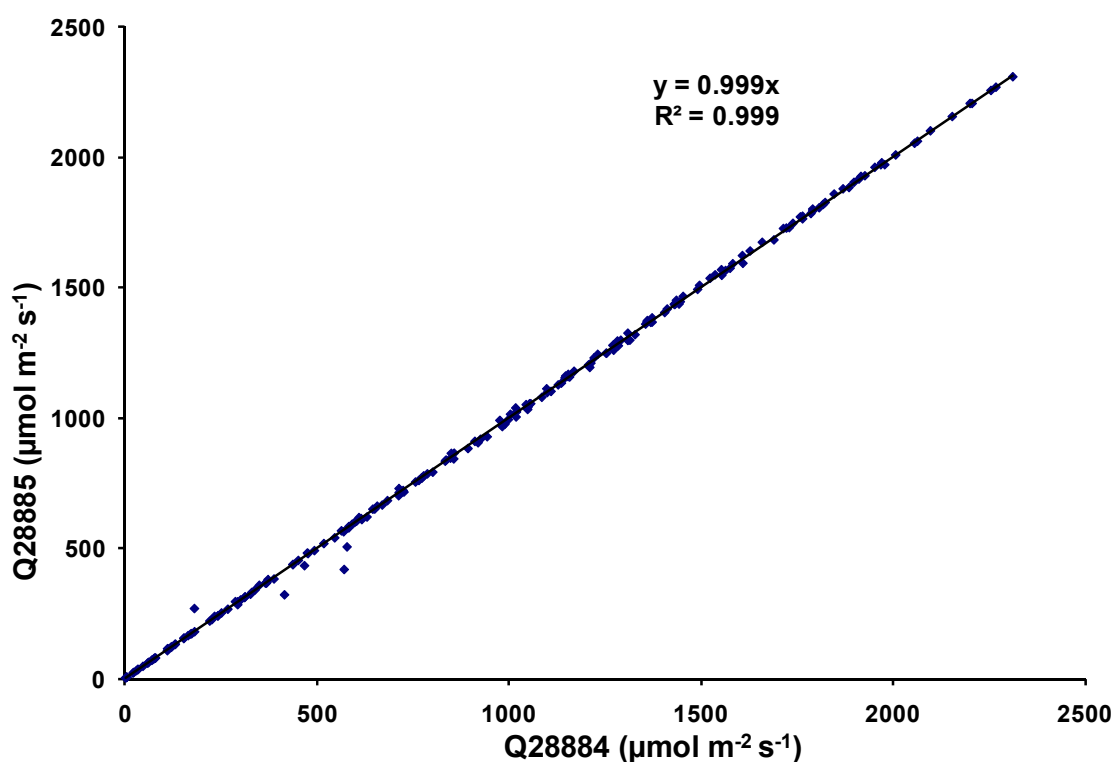


Figure 4.4: The inter-comparison results of the two Quantum Sensors done on the 9th of March 2009

4.2.3.3 Q7.1 Net Radiometer (REBS, Washington, USA)

The net radiometer calibration had satisfactory results and high correlation ($R^2 = 0.9898$) between the test sensor and standard. The instrument had less than the maximum

allowable 5% error and the results are shown in Figure 4.5. The instrument was thus used with the multiplier and offset specified by the manufacturer.

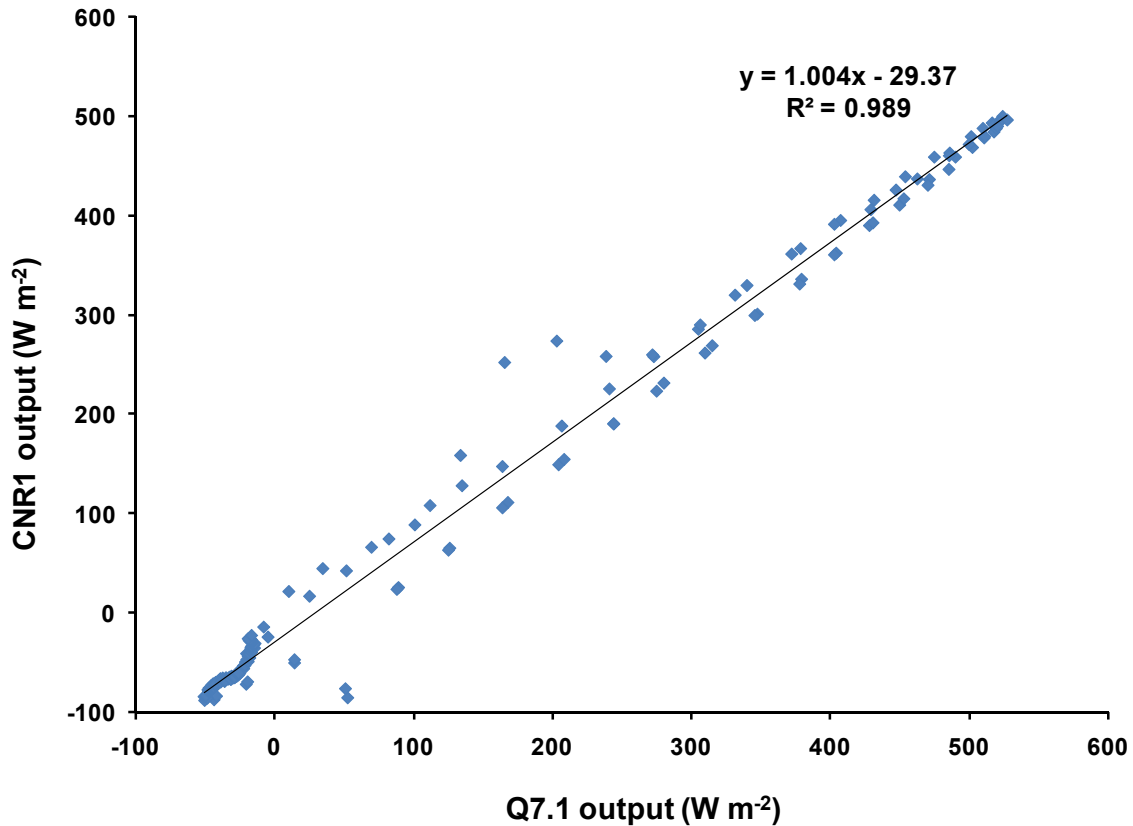


Figure 4.5: Results of the calibration of the Q7.1 net radiometer (REBS, Washington, USA) done on the 19th and 20th of May 2009

4.2.3.4 Mk-1-G Radiation Sensor (Matrix Solar Services, Arizona, USA)

The Mk-1-G Radiation Sensor (Matrix Solar Services, Arizona, USA) was successfully calibrated and showed good correlation ($R^2 = 0.993$) with the standard. The multiplier and offset as shown in the graph in Figure 4.6 were included in the data logger programme.

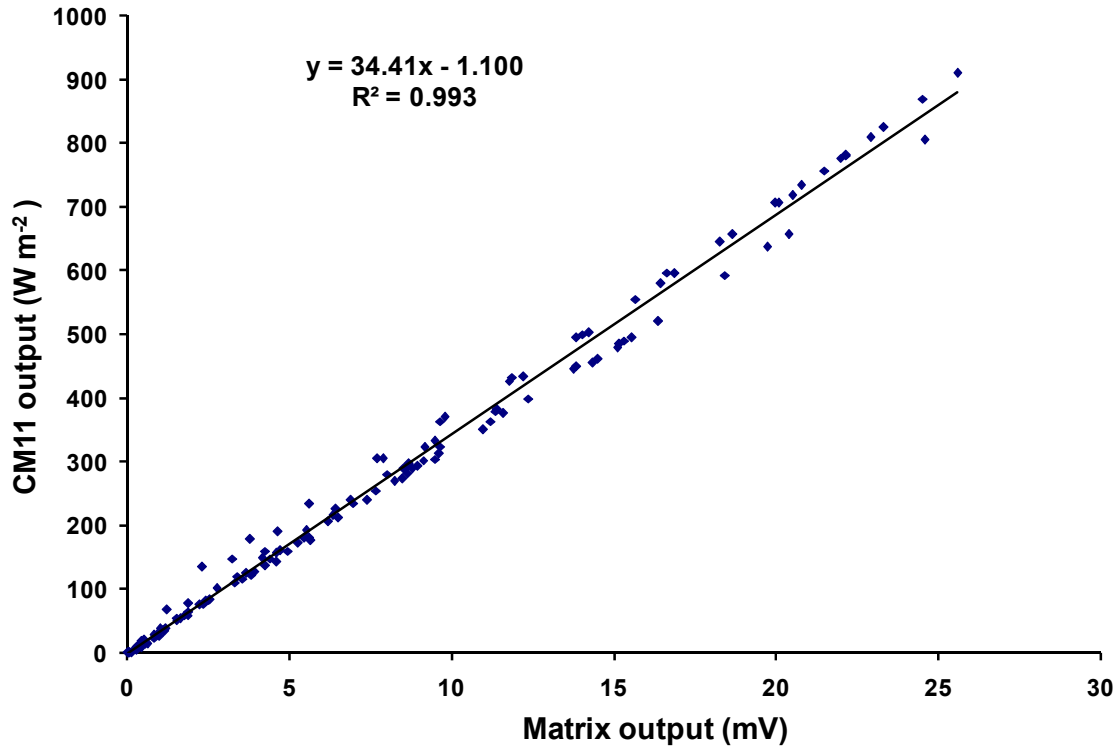


Figure 4.6: Results of the calibration of the Mk-1-G Radiation Sensor done on the 9th of March 2009

4.2.4 Radiation Thermometers

The results of the calibration of the radiation thermometers were not satisfactory. While some of them showed good correlations with actual temperatures it was found that they were damaged and could not be reliably used for the measurement of leaf temperatures within the greenhouse. The reason being their optical sensors (lens) were scratched or covered with rust and could not be cleaned enough to give consistent results. These instruments were thus not used in the experiment. The results of the calibrations are still however shown in Table 4.2 so as to justify their exclusion from the experiment.

Table 4.2: Results of infrared thermocouple calibrations done on 4th September 2009

<i>Model</i>	<i>Type</i>	<i>Manufacturer</i>	<i>Serial No.</i>	<i>Calibration Coefficient</i>	<i>Calibration Offset</i>	<i>R- Squared Value</i>
IRT/c	T	Exergen Corporation, USA	IRT0153	2.767	-41.9	0.996
IRT/c	T	Exergen Corporation, USA	IRT0135	6.143	-125.2	0.982
IRT/c	T	Exergen Corporation, USA	IRT0167	3.847	-67.4	0.991
IRT/c	T	Exergen Corporation, USA	IRT0110	2.977	-45.97	0.595
IRT/c	T	Exergen Corporation, USA	IRT0194	1.246	-9.017	0.996
IRT/c	T	Exergen Corporation, USA	IRT0187	1.718	-18.53	0.996

4.3. Microclimate Inside and Outside the Greenhouse

The microclimate outside the greenhouse for the period of the experiment (1 January 2010 to 1 March 2010) is broken down into two parts (namely 1st January – 31st January and 1st February – 1st March) and is summarized in Table 4.3. The table shows that in general January was hotter and less humid than February, while at the same time having greater total daily radiation amounts (both solar radiation and PAR).

Specifically the table shows that January was generally warmer than February with the mean temperature for the two periods being 21.6 °C and 21.2 °C, respectively. However, despite February being cooler the highest temperature recorded outside the greenhouse for the entire period occurred in that month on DOY 44 at 2:00pm and was 31.8 °C. The lowest temperature recorded outside was 14.8 °C and occurred on DOY 39 at 6:30am.

Results and Discussion

The coolest day was DOY 7 with daily average temperature of 19.33 °C while the hottest day was DOY 44 with daily average temperature of 23.87 °C.

The average relative humidity for January was 71.8 % while that for February was 75.3 % with that for the entire period being 73.5 % thus February was a relatively more humid month possibly due to the fact that it was also a cooler month. Outside the greenhouse the lowest RH recorded was 28.3 % on DOY 39 at 2:30pm while the highest recorded was 95.1 % on DOY 53 at 7:00am. The least humid day was DOY 39 with daily average RH of 58.08 % while the most humid day was DOY 31 with daily average RH of 84.81 %.

Table 4.3: Summary of the microclimate outside the greenhouse for the entire period of the experiment from 1 January 2010 to 1 March 2010

	Air Temperature (°C)			Relative Humidity (%)			Solar Radiation (MJ m ⁻² day ⁻¹)			PAR (×10 ⁶ μmol m ⁻² day ⁻¹)		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
January 1st – January 31st	14.9	21.6	30.2	30.8	71.8	94.8	14.2	18.8	25.7	20.7	29.6	43.3
February 1st – March 1st	14.8	21.2	31.8	28.3	75.3	95.1	13.1	18.2	25.8	18.2	27.9	41.9
Entire Period	14.8	21.4	31.8	28.3	73.5	95.1	13.1	18.5	25.8	18.2	28.8	43.3

In terms of total daily Solar Radiation the lowest amount of total daily solar radiation was 13.14 MJ m⁻² day⁻¹ occurring on DOY 34, while the highest figure was 25.8 MJ m⁻² day⁻¹ occurring on DOY 40. In terms of means January had a mean total daily solar radiation value of 18.8 MJ m⁻² day⁻¹ while February had a value of 18.2 MJ m⁻² day⁻¹ with the mean for the entire period being 18.5 MJ m⁻² day⁻¹. This means that February generally had less sunshine hours than January possibly due to cloudiness or shorter days in February.

For PAR the lowest daily total PAR value was 18.2 mol m⁻² day⁻¹ occurring on DOY 53 while the highest value was 43.4 mol m⁻² day⁻¹ occurring on DOY 21. Comparing January and February we find that their means are 29.6 mol m⁻² day⁻¹ and 27.9 mol m⁻²

day⁻¹, respectively with the mean for the entire period being 28.8 mol m⁻² day⁻¹. Thus, as with solar radiation it would seem that the average amount of energy received on a daily basis was greater in January than it was in February. Given the above climatic conditions being experienced outside the greenhouse it was then imperative to analyse their effects on the microclimate inside the greenhouse. This was done for each of the main meteorological parameters already mentioned namely air temperature, relative humidity, PAR and solar radiation and this is discussed in the next few sections.

4.3.1 Air Temperature

Figure 4.7 shows the course of daily mean air temperature inside and outside the greenhouse throughout the experiment.

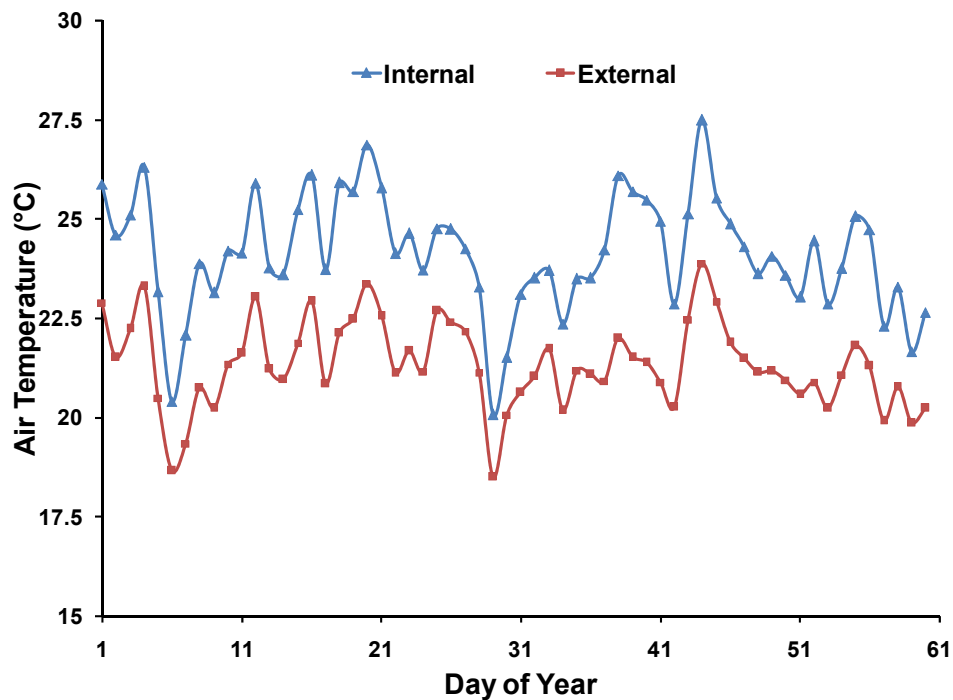


Figure 4.7: Daily mean temperature inside and outside the greenhouse for the period of the experiment (1 January = DOY 1)

As per expectations temperatures within the greenhouse were higher than those outside. Mean temperatures inside the greenhouse were on average 13% higher than those outside due to trapping of longwave radiation within the greenhouse. The internal air temperatures were fairly high but still below the thresholds regarded as damaging to crop growth and yield.

4.3.2 Vapour Pressure Deficit

Figure 4.8 shows the variation of the daily mean vapour pressure deficit with time inside and outside the greenhouse.

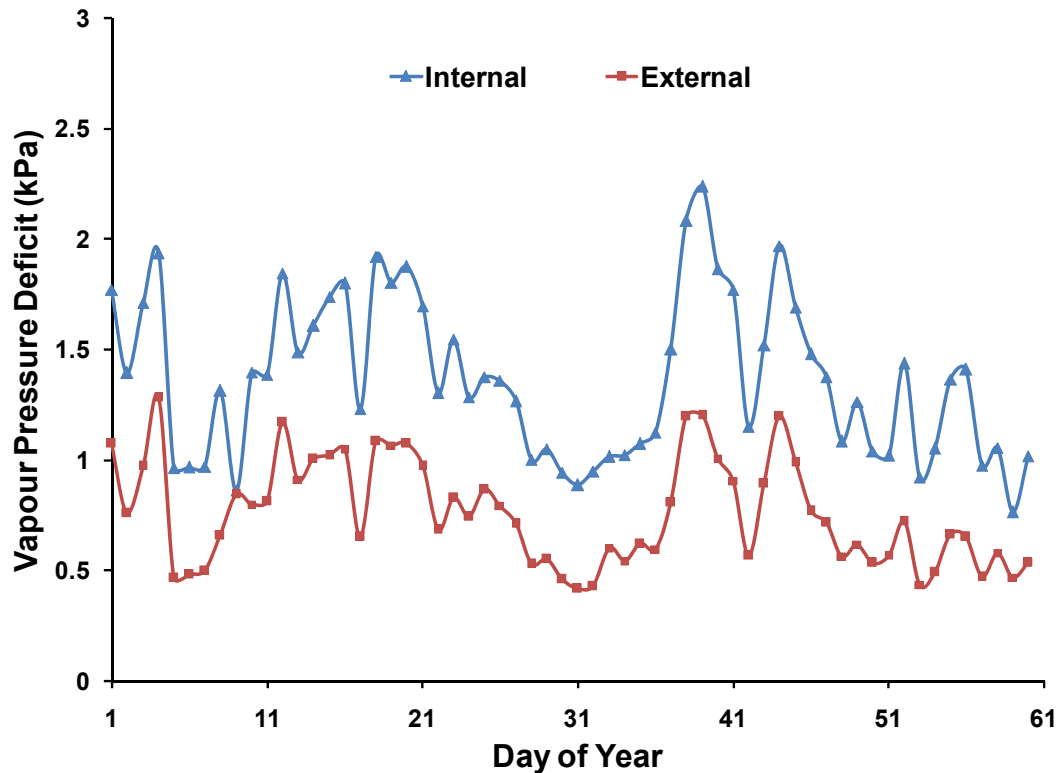


Figure 4.8: Daily mean vapour pressure deficit inside and outside the greenhouse for the period of the experiment

Vapour pressure deficit within the greenhouse was higher than that outside. In general inside vapour pressure deficit was on average 80% higher than that measured outside. This means that water demand inside the greenhouse was greater than that outside. This

may have been due to the fact that temperatures inside the greenhouse were higher than those outside while relative humidity inside the greenhouse was lower than that outside. Air temperatures in the greenhouse being higher than those outside would lead to higher saturation vapour pressure and while a low relative humidity would mean low actual vapour pressure inside the greenhouse thus leading to a high vapour pressure deficit. The measurement period also coincided with part of the rainy season hence resulting in higher RH outside than inside.

4.3.3 Photosynthetically Active Radiation (PAR)

Figure 4.9 shows the daily average PAR inside and outside the greenhouse throughout the experiment.

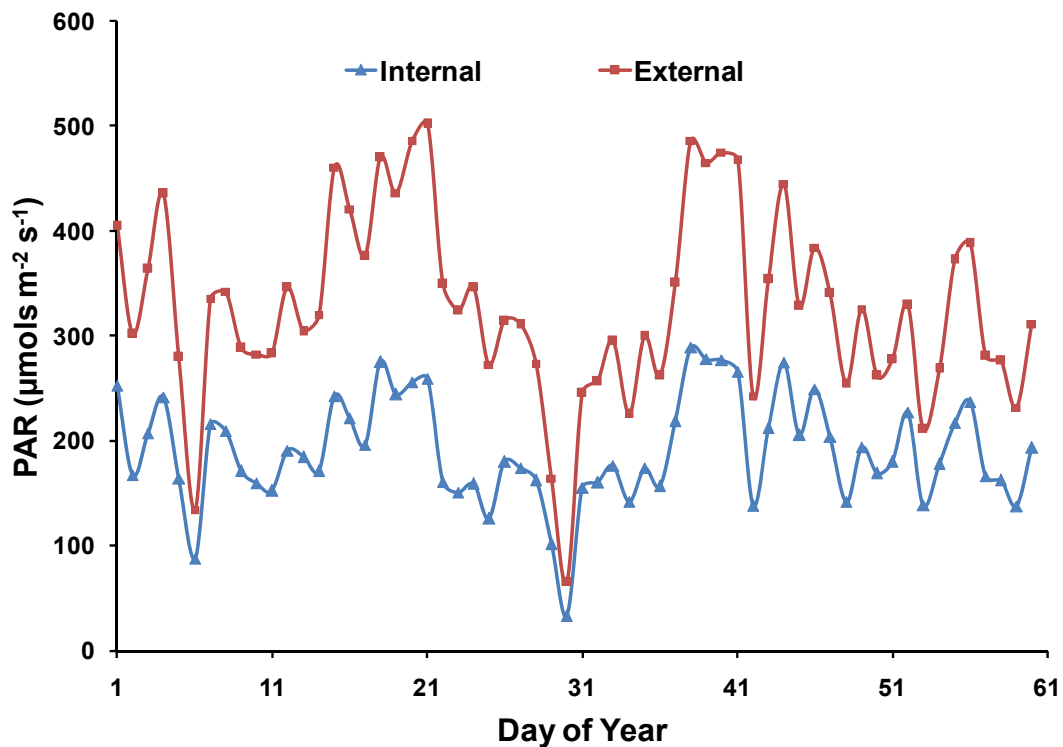


Figure 4.9: Daily mean PAR inside and outside the greenhouse for the period of the experiment

As expected PAR was lower inside the greenhouse than it was outside. This was due to the filtering of radiation by the greenhouse covering. On average PAR inside the greenhouse was 58.9 % of that measured outside. This fairly low value was also due to shading of the internal sensor at some times of the day by surrounding objects.

4.3.4 Solar Radiation

Figure 4.10 shows the course of daily mean solar radiation throughout the course of the experiment. It shows that solar radiation inside the greenhouse is consistently lower than that measured outside. This is due to the filtering of radiation by the glass panels.

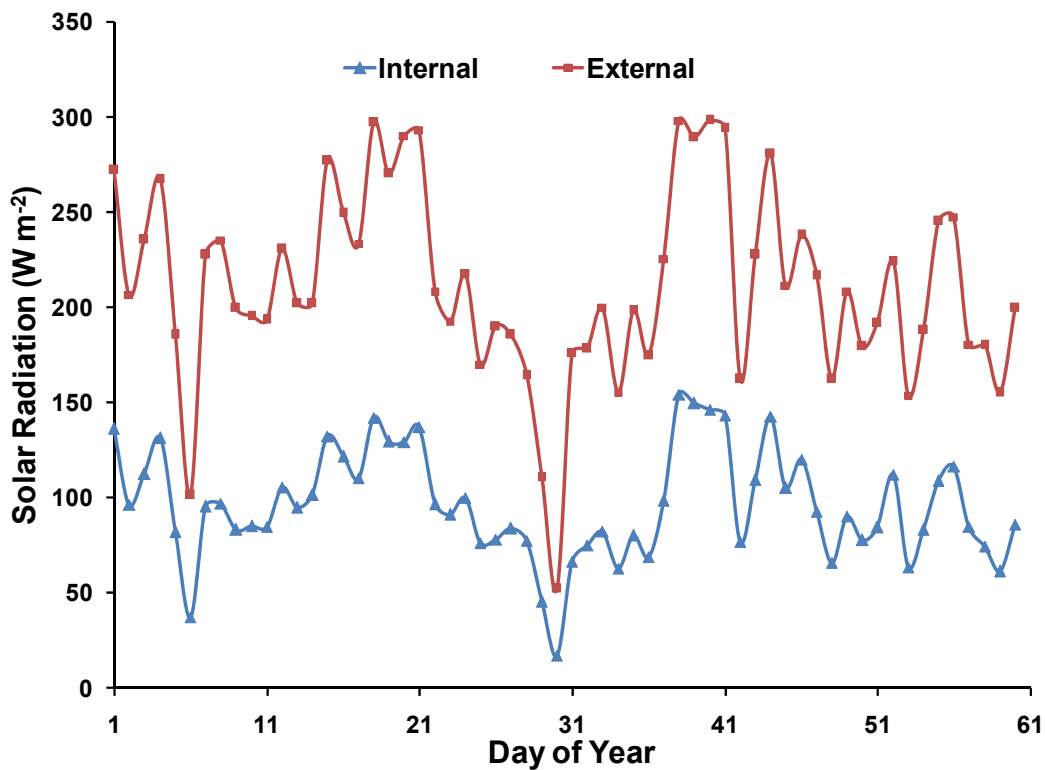


Figure 4.10: Daily mean solar radiation inside and outside the greenhouse for the period of the experiment

Specifically solar radiation as measured by the Matrix Mk-1-G sensor and TSL Tube solarimeter showed that solar radiation was approximately 44.8% of that measured outside the greenhouse. This low value is probably due to the shading of the tube

solarimeter at certain times of the day by trees surrounding the greenhouse, whereas the Mk-1-G sensor was not surrounded by any obstructions to solar radiation.

4.4 Water requirement calculations

Water requirement calculations for purposes of irrigating the plants throughout the experiment were calculated using the FAO-Penman-Monteith Equation. The results of these calculations and the justification of the use of this formula in regression analysis of the main variables are discussed in the next section.

4.4.1 ET_o Calculations Using the FAO-Penman-Monteith Equation

The values of ET_o calculated using the FAO-Penman-Monteith equation varied from a minimum of 1.27 mm day^{-1} on DOY 6 to a maximum of $4.456 \text{ mm day}^{-1}$ on DOY 21. The results for the entire period are shown in Figure 4.11.

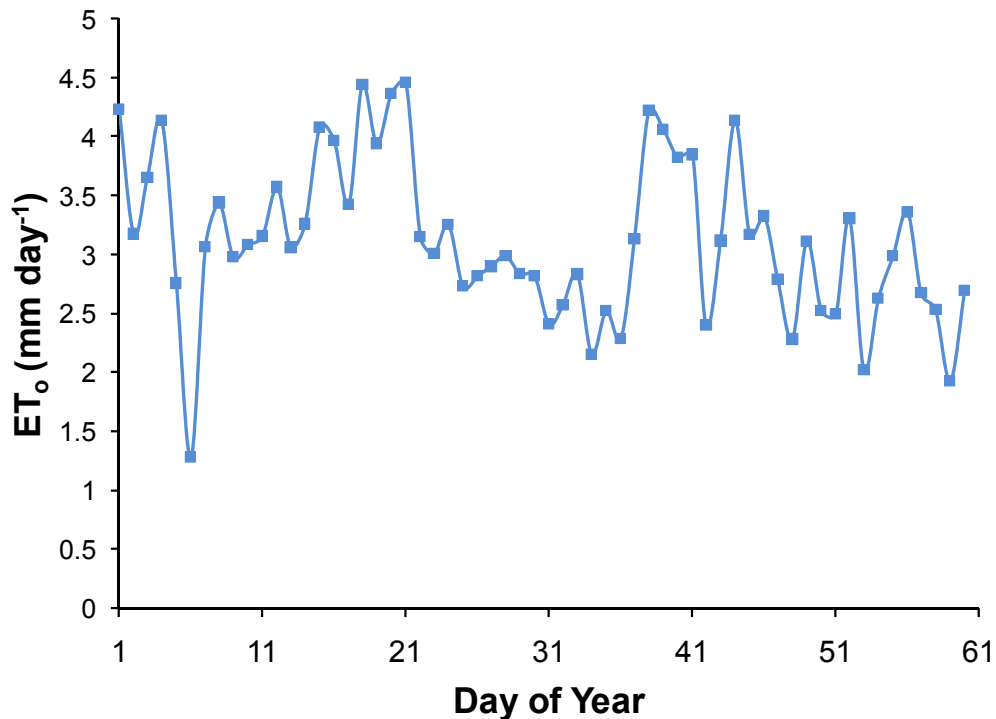


Figure 4.11: The variation of daily ET_o with time throughout the course of the experiment

The average calculated value of ET_o for the period was 3.12 mm day^{-1} . The main driving variable of ET_o calculated using the FAO-Penman-Monteith equation was net radiation as shown in Figure 4.12. This shows that there is a high correlation ($R^2 = 0.982$) between ET_o and net radiation. This justifies unconditionally the use of net radiation in calculating ET_o .

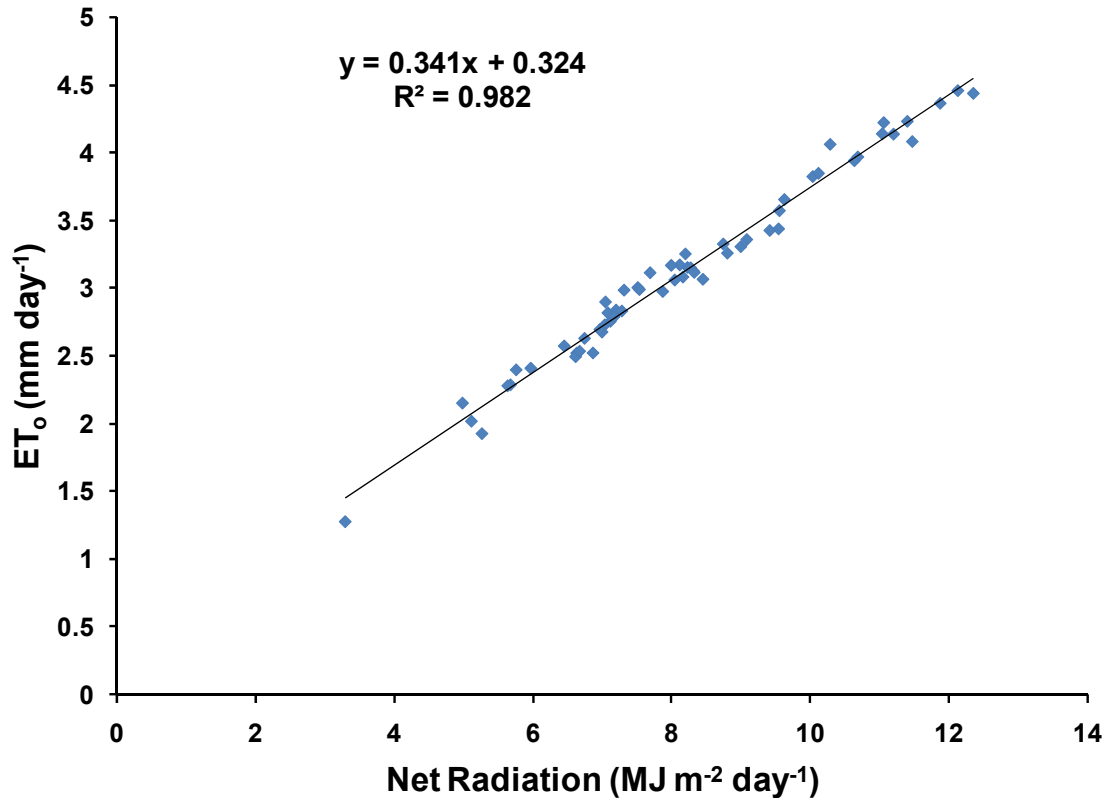


Figure 4.12: The correlation between daily total net radiation and ET_o for the 60 days of the experiment

The FAO-Penman-Monteith Equation also requires the use of daily mean air temperature and thus a regression of daily mean air temperature and ET_o is shown in Figure 4.13. This shows that there is a high correlation between daily mean air temperature and ET_o ($R^2 = 0.726$).

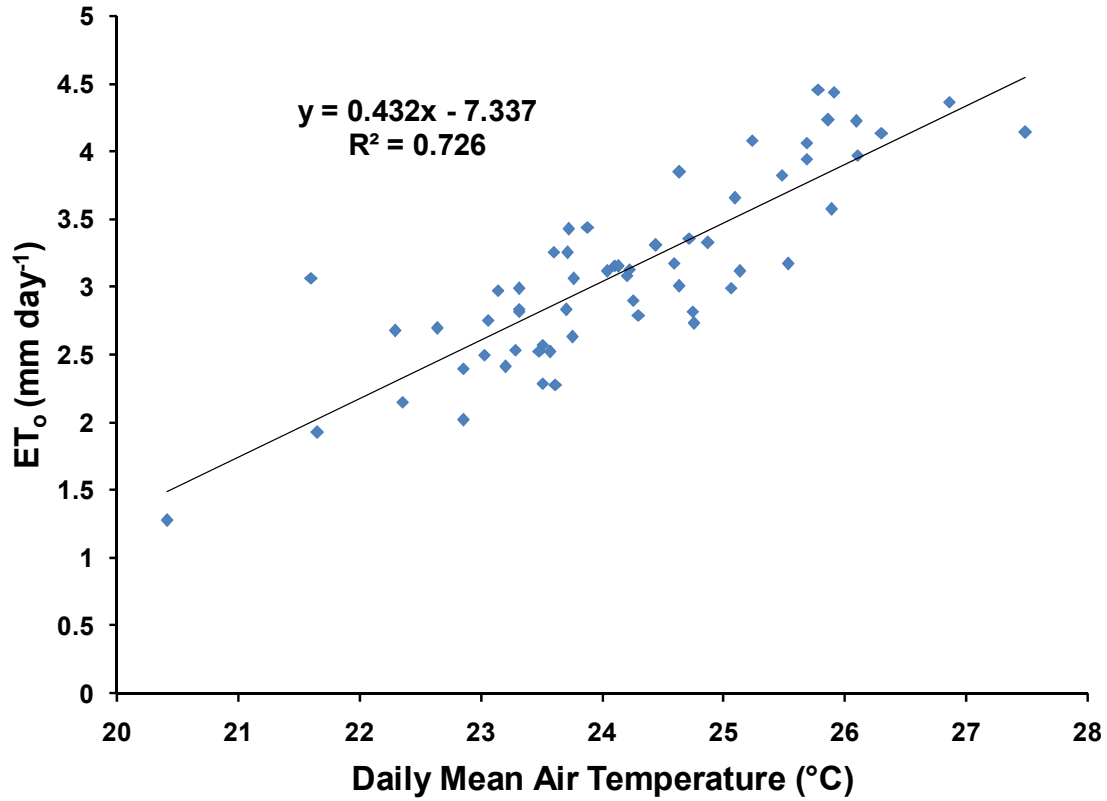


Figure 4.13: The correlation between daily mean air temperature and daily ET_0 calculated using the FAO-Penman-Monteith Equation for the 60 days of the project

Vapour pressure deficit was another of the main components of the FAO-Penman-Monteith Equation and its regression with ET_0 is shown in Figure 4.14. Vapour pressure deficit was found to have a high correlation with ET_0 ($R^2 = 0.719$) and to have almost equal importance as daily mean air temperature has in determining ET_0 .

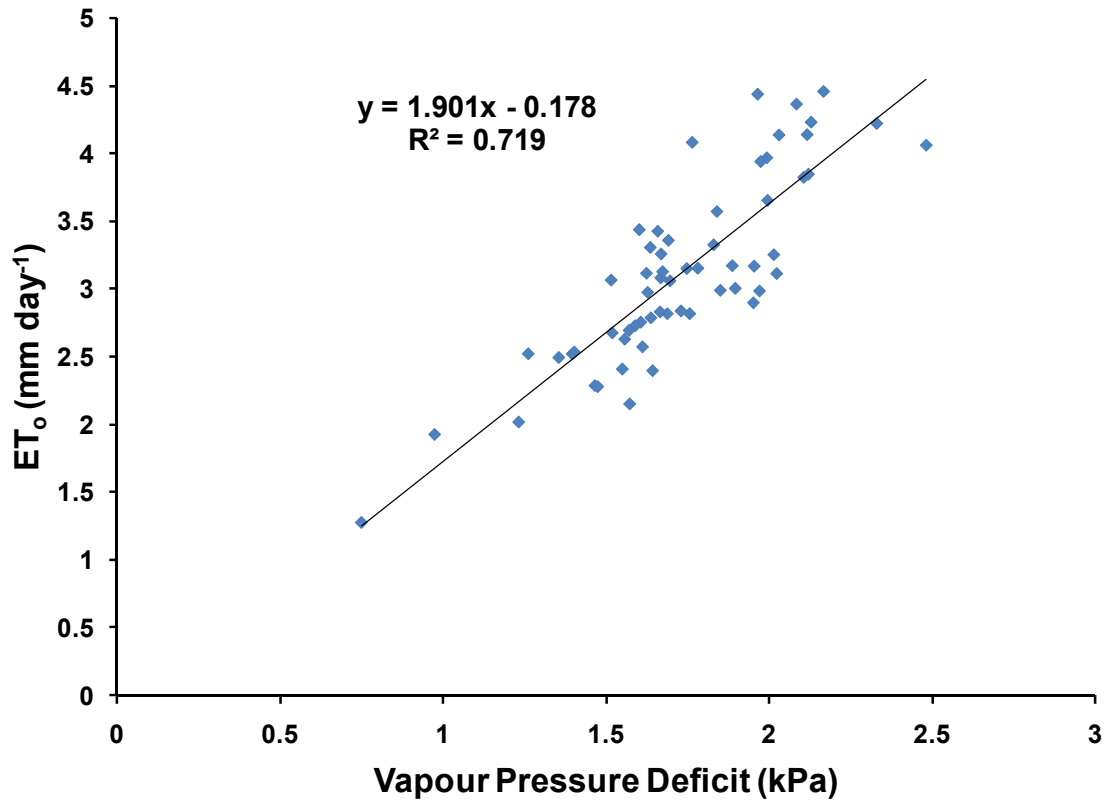


Figure 4.14: The correlation between daily average vapour pressure deficit and daily ET_0 calculated using the FAO-Penman-Monteith Equation for the 60 days of the project

The graphs in Figures 4.12, 4.13 and 4.14 show that total daily net radiation has the greatest influence on water requirement calculated using the FAO-Penman-Monteith equation. However, other parameters also play a large role such as air temperature, vapour pressure deficit and wind speed. Wind speed was not considered here since its daily average was taken to be constant within the greenhouse. All of the graphs in Figures 4.12, 4.13 and 4.14 justify the use of the FAO-Penman-Monteith equation in calculating crop water requirements since all of the parameters used in its calculation have a large effect on water use. For estimation purposes in the absence of all of the above measurements, since net radiation has to be measured with expensive instruments or calculated with difficult formula, one can use the mean daily air temperature and the regression equation in Figure 4.13 to calculate ET_0 . The advantage of using mean daily air temperature is that it can itself be estimated by assuming that the temperature at

approximately 8:15am can be regarded as the mean for the day thus allowing growers to irrigate for that day without having to wait the entire day for measurements to be collected. In other cases the calculation of ET_o using net radiation can be done using methods of estimating radiation using the number of daylight hours. In general all of the above parameters are important in calculating ET_o and can be considered as the main factors driving plant water requirements. At least one of these parameters should always be considered when attempting to estimate plant water requirements in the absence of all of the above measurements (i.e. where no automatic weather station is available).

4.5 Physiological measurements

4.5.1 Leaf Temperature

On a typical day leaf temperature is higher than air temperature at night and lower than air temperature during the day. This results in a leaf to air temperature difference which is normally negative from 8:00am to 9:00pm each day and positive at other times as shown in Figure 4.15. This graph shows the average of the leaf-air temperature difference at each time of day over the entire 60 days of the experiment and can thus be taken as the typical leaf-air temperature difference. On average leaf temperature was found to be lower than air temperature by 2.5 °C between 8:00 am and 9:00 pm. Deviations from this curve may occur depending on the prevailing weather conditions on that day however in general the graph shown in Figure 4.15 can be taken as the normal leaf to air temperature difference of a greenhouse grown tomato plant. The “abnormality” circled in red is normal and may be a result of some plant physiological reactions which begin to take place at sunrise and result in an increase in leaf temperature. These results were different to those found by Dziki (2007) in his study of Navel orange trees in which leaf temperatures were higher than air temperature during the day. This can be attributed to a high transpiration rate of tomato plants.

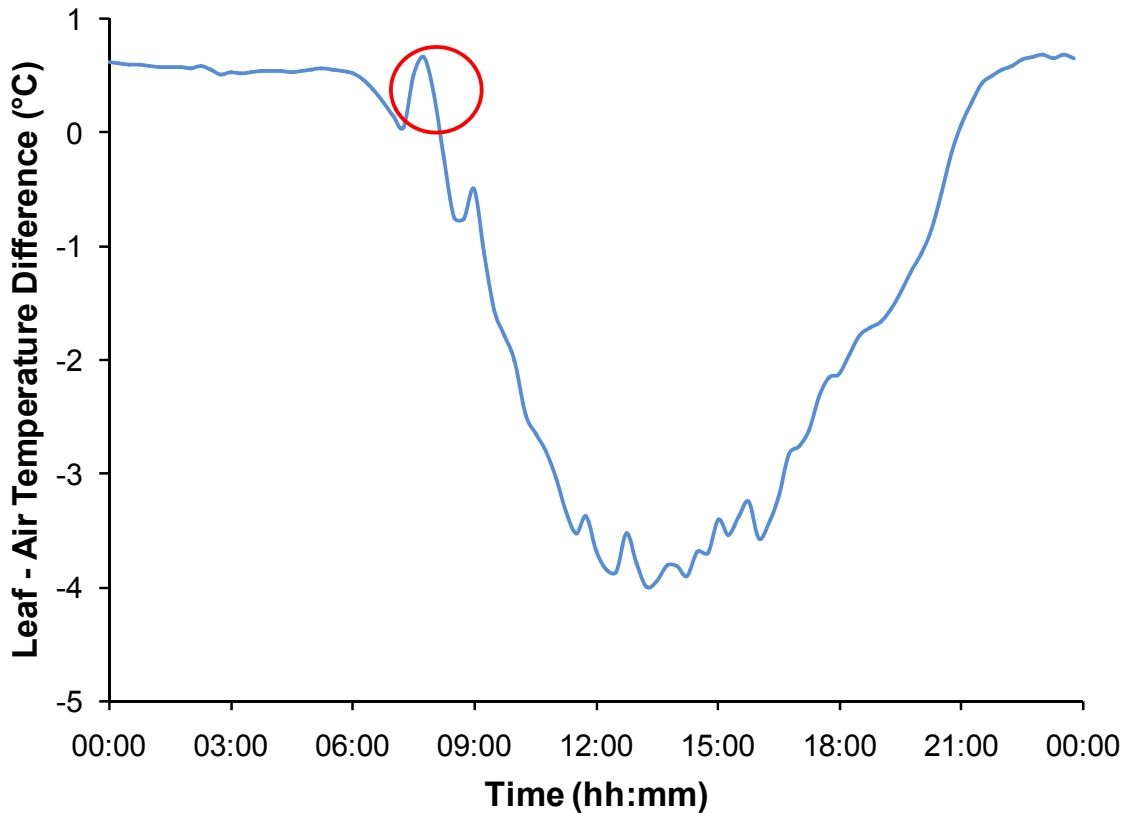


Figure 4.15: The typical (average) leaf to air temperature difference at each time of day for the entire 60 days of the experiment

For the entire course of the experiment the daily mean leaf temperatures were also lower than the daily mean air temperature and this is shown in Figure 4.16. The circled area shows the two treatments which had been subjected to water stress on those days having a higher average leaf temperature than the two treatments given normal water quantities. This shows that when a plant is stressed the leaf temperature approaches air temperature.

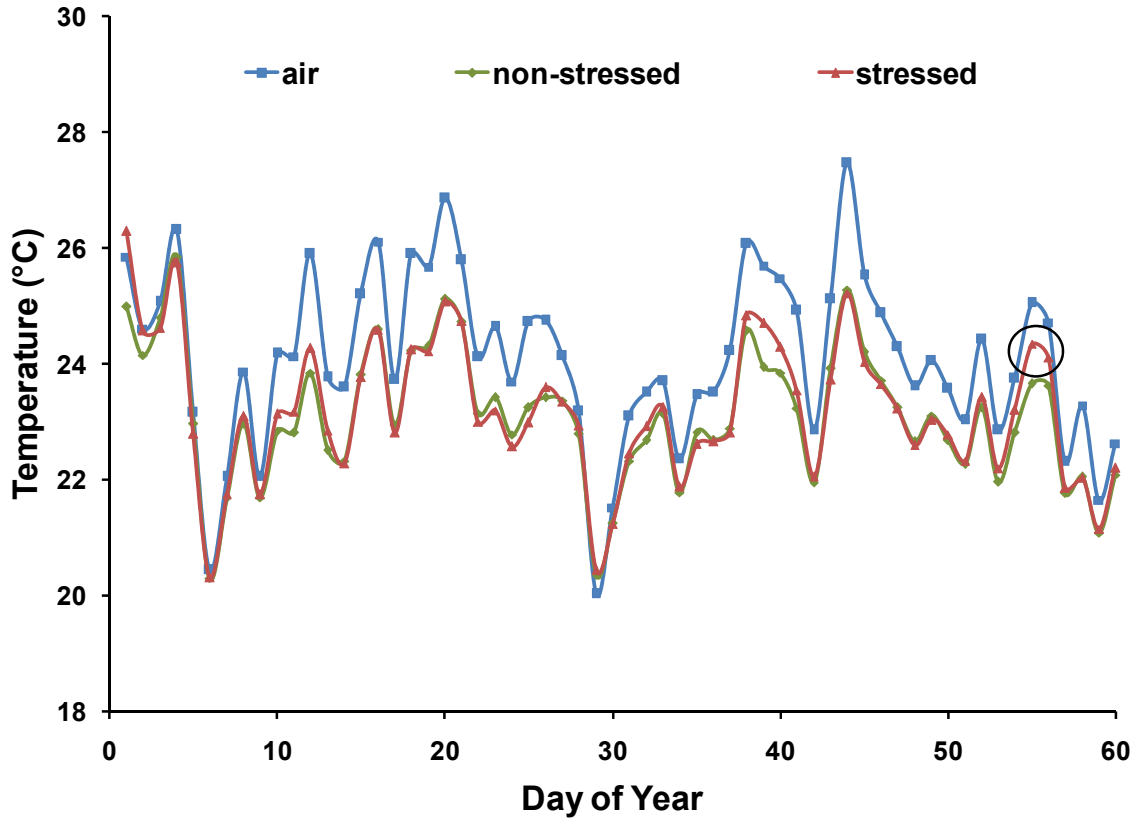


Figure 4.16: Daily mean leaf temperature for each treatment compared with daily mean air temperature inside the greenhouse

4.5.1.1 Comparison between Stem and Leaf Temperature

From the thermocouple measurements made on plant leaves and stems in each treatment it was found that leaf temperature and stem temperature were approximately equal. However, there were slight differences in their variation with time as shown in Figure 4.17 These differences appeared between the times of 11:00 am and 6:00 pm. A number of reasons could be hypothesized for these differences. These included the effect of wind since thermocouples clipped to the underside of leaves are exposed to wind more than those inserted into the stem. Shading by the leaves may also play a part in making leaf temperature lower than stem temperature. The effect of transpiration from leaf surfaces may also cool thermocouples attached to the underside of leaves more than those inserted into stems.

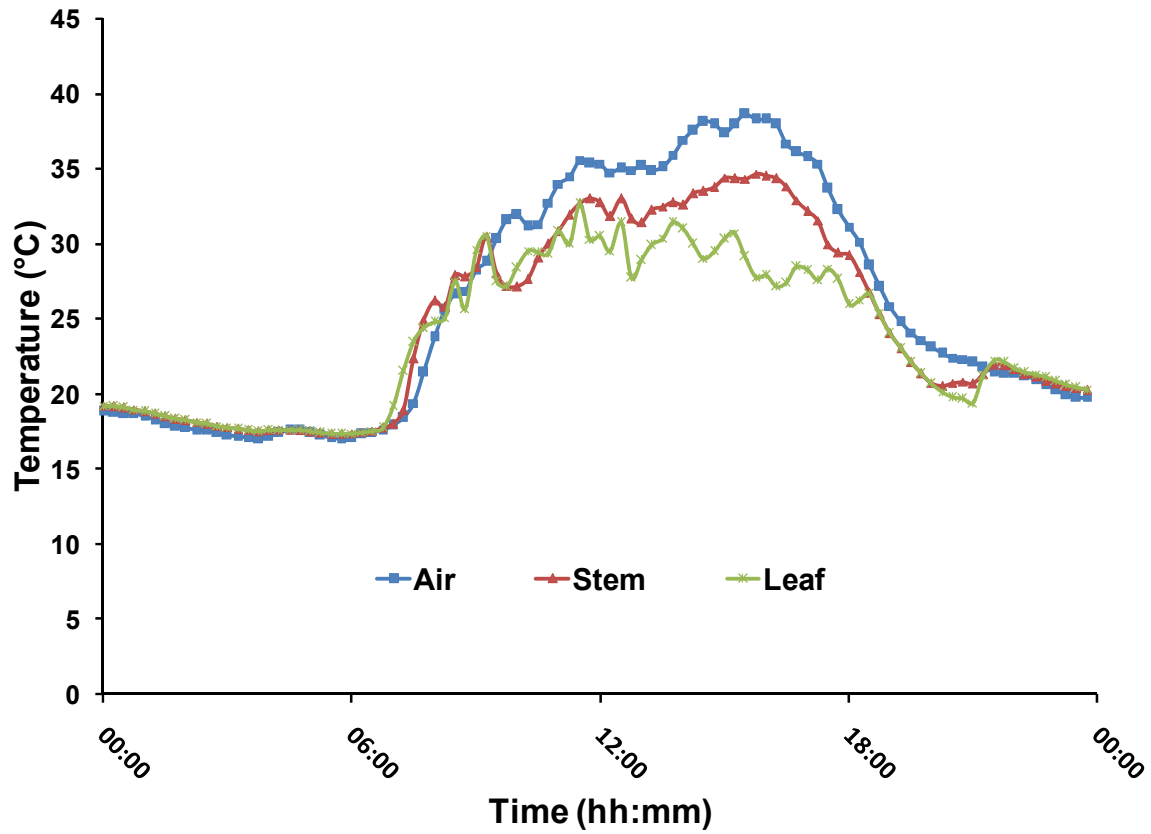


Figure 4.17: Comparison between leaf temperature, stem temperature and air temperature on a typical cloudless hot day (DOY 18; 18 January 2010).

To test these hypotheses another day was sampled as shown in Figure 4.18. This was a relatively cooler day and in this case leaf temperature and stem temperature had no significant difference throughout the course of the day.

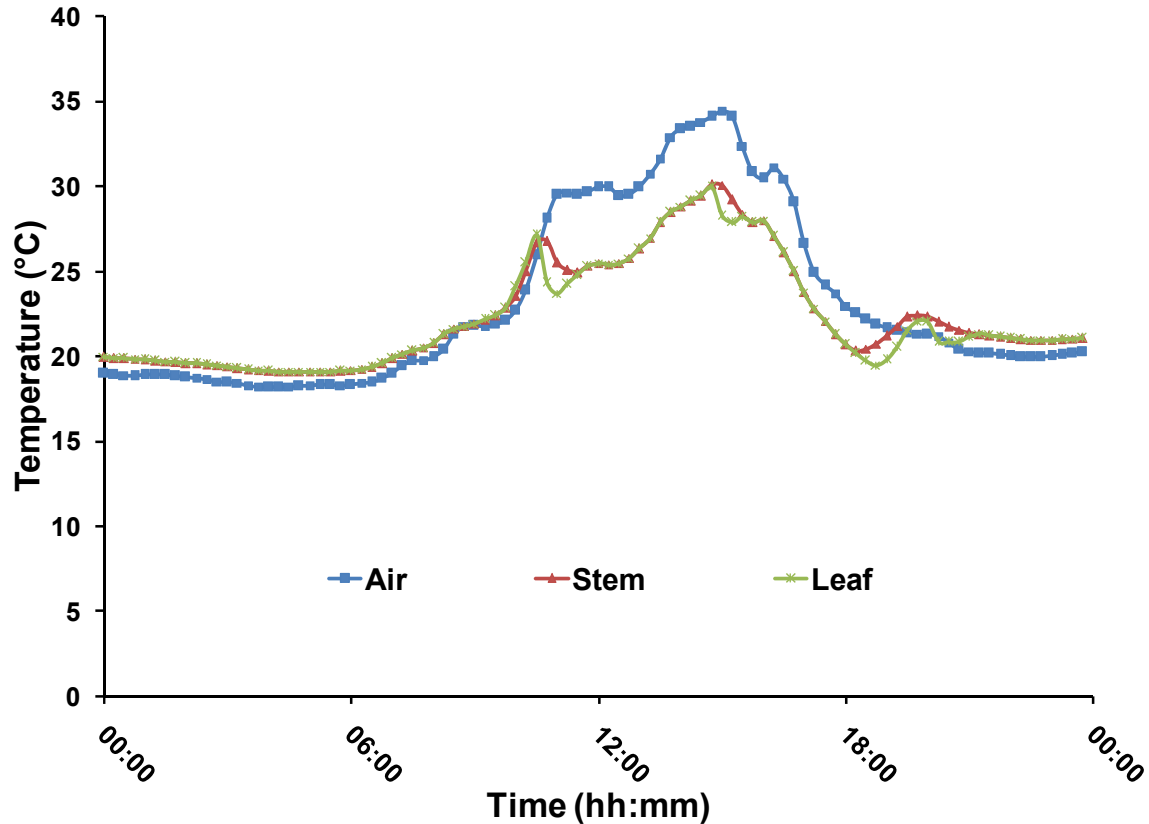


Figure 4.18: Comparison between leaf temperature, stem temperature and air temperature on a relatively cool cloudy day (DOY 31; 31 January 2010).

It remains to be seen which between stem temperature and leaf temperature can be more accurately used as a plant indicator of stress or which can be more accurately used in irrigation scheduling. Figure 4.19 however, shows that the two are similar and highly correlated on both hot and cool days ($R^2 = 0.929$ and 0.977 , respectively) and can be used interchangeably. However, the relationship is higher on cool days thus pointing to some outside factor affecting the measurements of stem and leaf temperature using fine wire thermocouples. It is possible that wind, shade or radiation affect leaf temperature measurements taken with thermocouples more than they affect stem temperature measurements taken with the same instrument. Despite the slight differences significance testing found the two to be statistically similar ($P = 0.05$) on both hot and cool days (see Table A-1 and A-2 in Appendix A for significance tests).

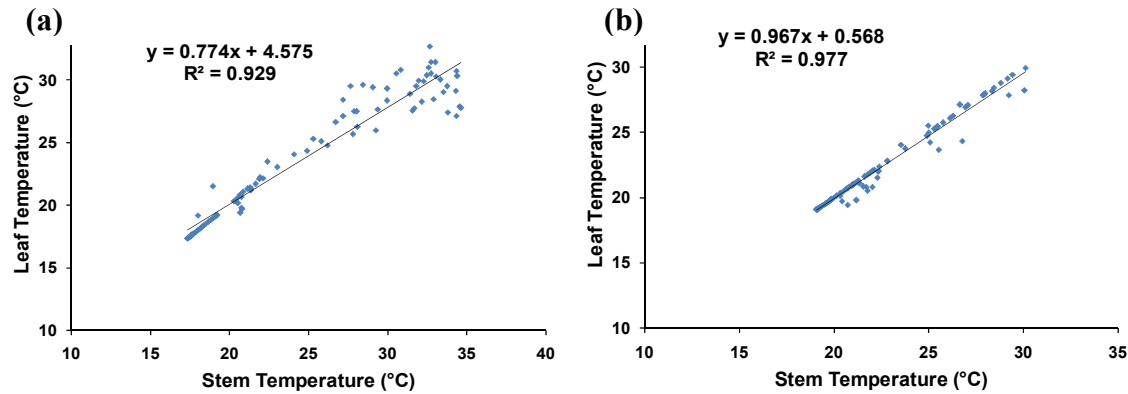


Figure 4.19: The correlation between stem and leaf temperature on a) a relatively hot day (DOY 18) and b) a relatively cool day (DOY 31)

In terms of long term measurements it is easier to insert a thermocouple into a stem than to attach it to a leaf. Also contrary to expectations a thermocouple inserted into a succulent stem does not cause any significant damage to the plant even if left in place for weeks. Physiologically both measurements are important as both the leaves and stems of plants participate in photosynthesis and other plant reactions necessary for growth and both require certain temperatures for these to occur at an optimum (Jones, 1992). Still there is usually little difference between stem and leaf temperature and thus either of the two can be used with the accuracy required for stress detection, irrigation scheduling or any other purpose.

4.5.1.2 The Use of Leaf Temperature in Irrigation Scheduling

It was suspected that certain leaf temperature derived indices (namely the daily mean and daily maximum leaf temperature) may be correlated to plant water requirements and as such could be used to determine plant water requirements on a daily basis. Of these leaf temperature derived indices it was found that mean leaf temperature for each day had the best correlation to ET_o ($R^2 = 0.648$) and could best be used in calculating plant water requirements on a daily basis as shown in Figure 4.20. This correlation was medium and tending to strong meaning that given a horticulturist knowing the mean leaf temperature for the day it is possible to calculate ET_o based on the regression equation in Figure 4.20.

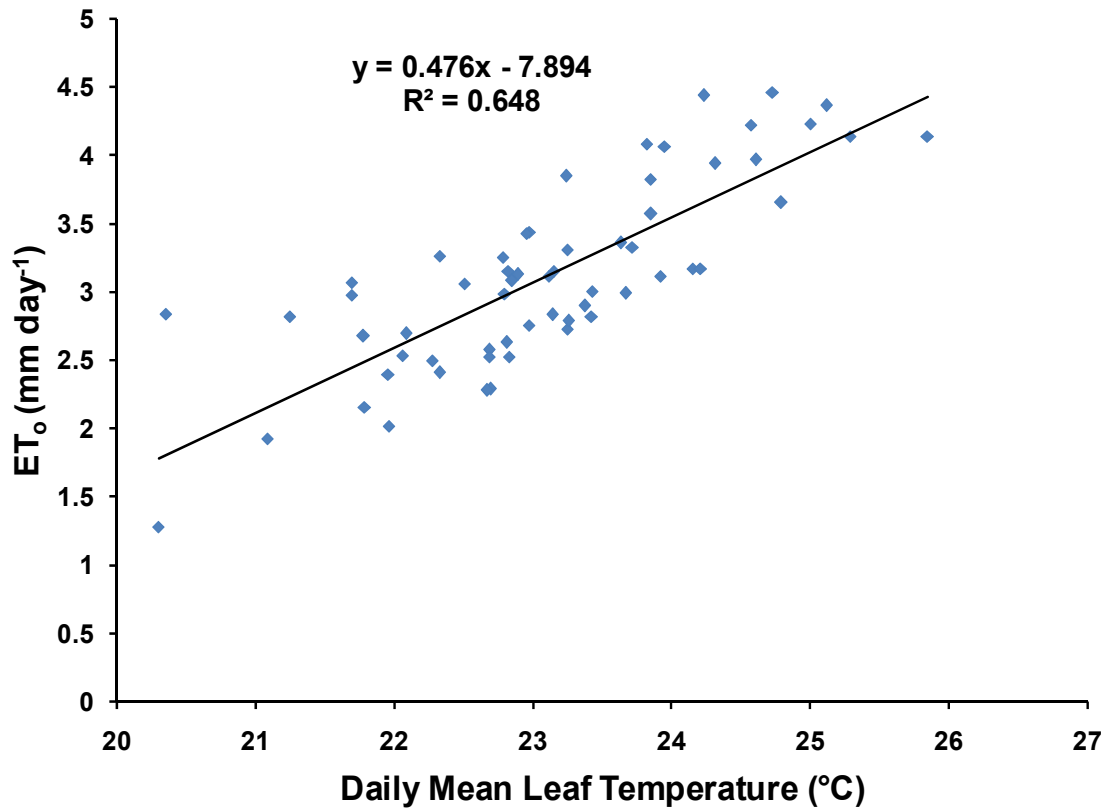


Figure 4.20: The correlation between daily average leaf temperature and daily ET_0 calculated using the FAO-Penman-Monteith Equation for the 60 days of the project

Another parameter related to plant water requirements was the daily maximum leaf temperature. It was found to have a medium strength correlation to ET_0 ($R^2 = 0.454$) as shown in Figure 4.21. While it would be possible to use the daily maximum leaf temperature in irrigation scheduling in the absence of other measurements it would be better to use the daily mean which gives a better representation of the ambient conditions throughout the day.

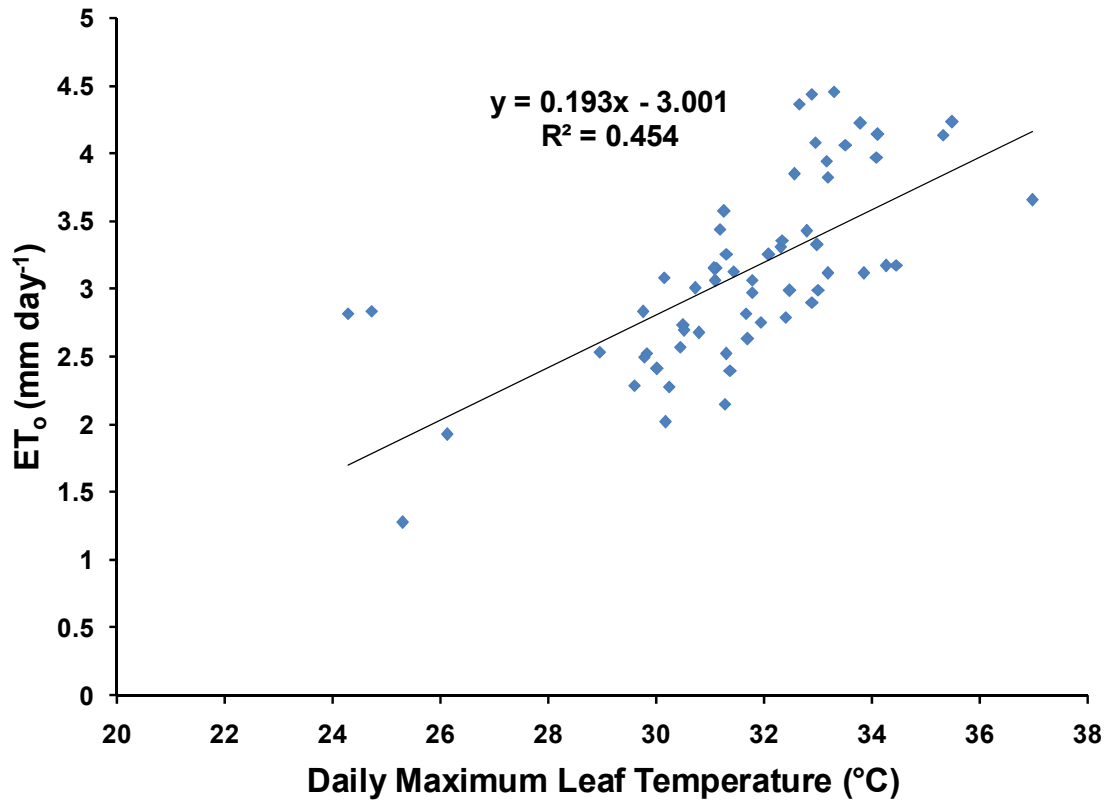


Figure 4.21: The correlation between daily maximum leaf temperature and daily ET_o calculated using the FAO-Penman-Monteith Equation for the 60 days of the project

From Figure 4.20 and 4.21 the problem is that when using mean or maximum leaf temperature for irrigation scheduling one has to take measurements over the entire day, then calculate the mean or maximum and then probably irrigate the following day. This can be avoided by determining at which times of day the mean or maximum temperature is likely to occur and thus measuring leaf temperature at this time and calculating irrigation based on this measurement. Figure 4.22 is the graph of the average leaf temperature at each time for the entire period of the experiments so as to come up with the true typical fluctuation of leaf temperature with time. This graph is useful in that in terms of irrigation scheduling based on daily minimum, maximum or mean leaf temperature one can measure leaf temperature at the times specified and take those temperatures to be the minimum, maximum and mean, respectively.

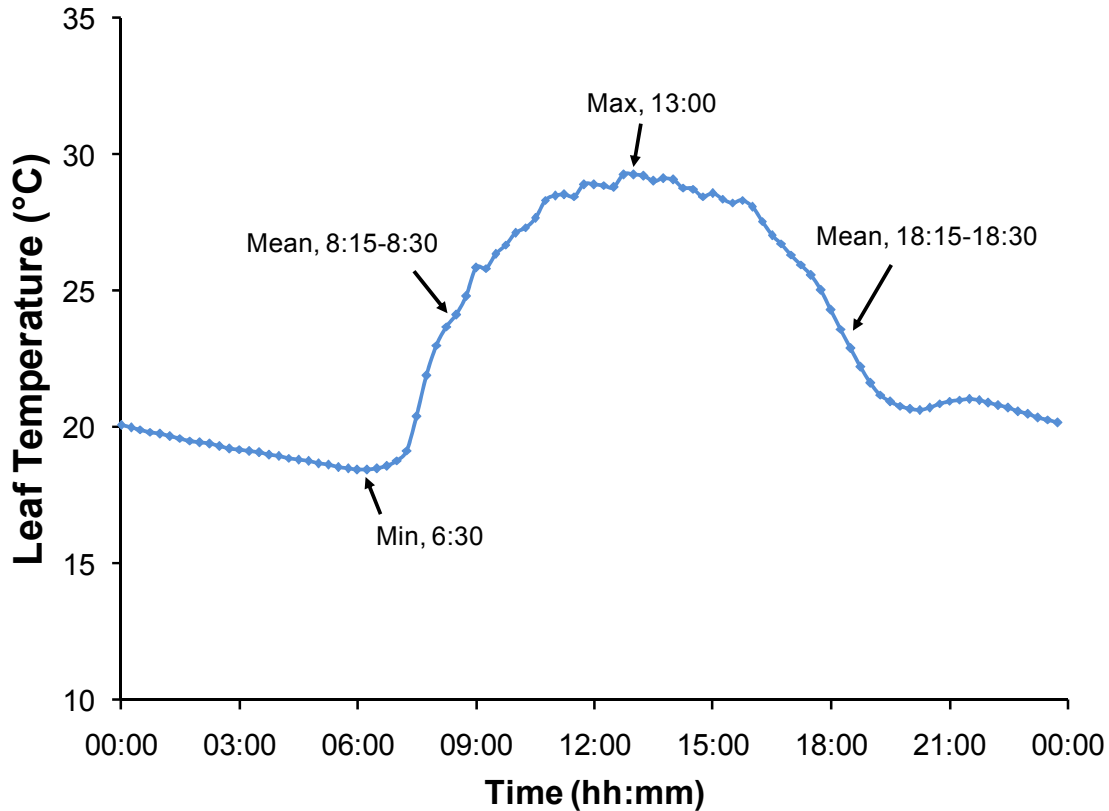


Figure 4.22: The typical values of leaf temperature over the course of the experiment (for example in this graph a value of 21.9 at 8am is the average 8am value over the entire course of the experiment).

Thus from the graph it is shown that the minimum leaf temperature occurs around 6:30 am, while the maximum occurs at around 1:00 pm while the mean can be taken as the value occurring either between 8:00 – 8:15 am or 8:15 – 8:30 pm. Thus for a greenhouse tomato crop one can measure leaf temperature with an infrared thermometer for example at 8:00 am and assume that this will be the mean leaf temperature for the day and hence determine irrigation quantities for that day based on the regression equation shown in Figure 4.20. While not perfect the method can serve as a good approximation of plant water requirements on a daily basis.

4.5.1.3 Response of Leaf Temperature to Drought Stress

In terms of stress detection, leaf temperature showed a fairly good response to drought stress. However, it took approximately six days from the start of stressing (DOY 47) for leaf temperatures in the stressed plants to show significant signs of drought stress. It should be noted however that the period of stressing occurred on relatively cool days. Figure 4.23 shows leaf and air temperatures on DOY 51 and 52 with the latter being the day that stressed plants began to show signs of drought stress through leaf temperature.

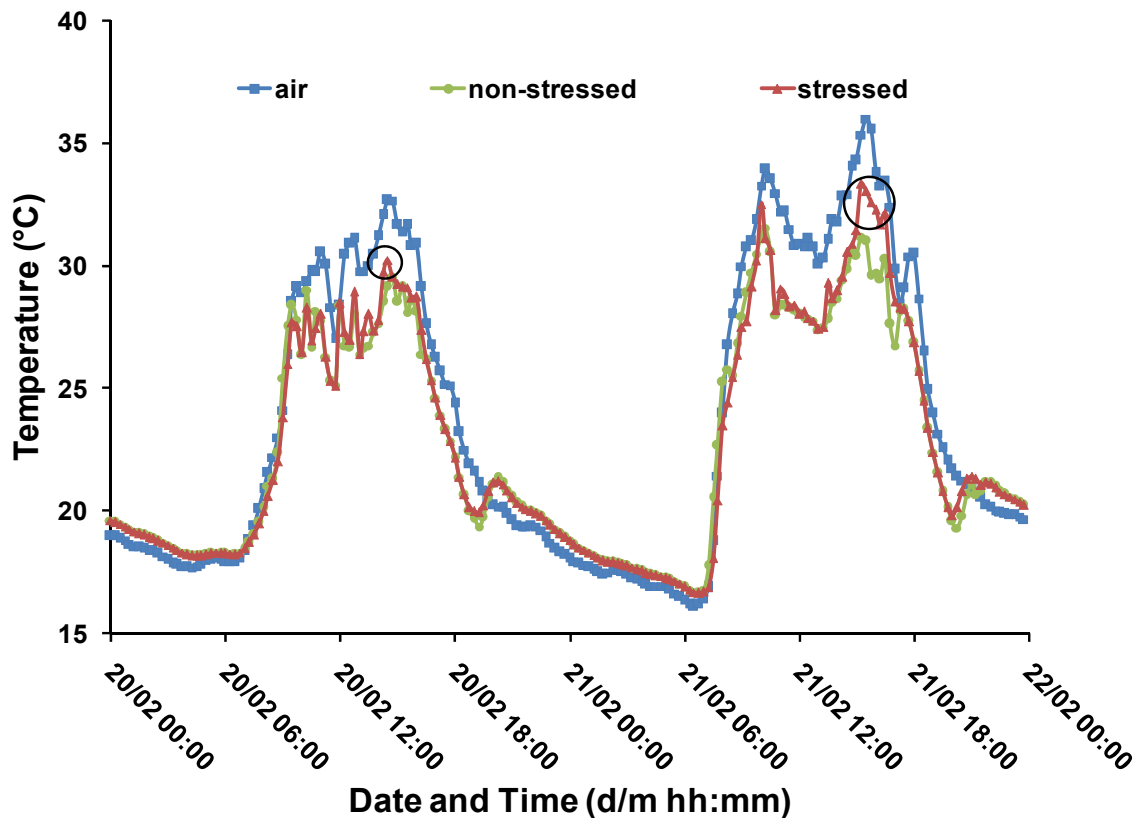


Figure 4.23: Leaf temperatures and air temperature on DOY 51 and 52 showing high leaf temperatures (approaching air temperature) at certain times of day in the two treatments subjected to water stress

Looking at DOY 52 shows that at certain times of day stressed plants will have higher leaf temperature than non-water stressed plants. This graph also indicates that drought stress most likely manifests itself in the form of higher leaf temperatures between 3:00

pm and 4:45 pm. Vermeulen *et al* (2007) stated that any such unexpected increase in leaf temperatures should be attended to immediately.

There is still a difficulty in determining at what leaf temperature or what leaf to air temperature difference can a grower conclude that a plant is under stress. The leaf to air temperature difference of both stressed and non-water stressed plants is shown in Figure 4.24.

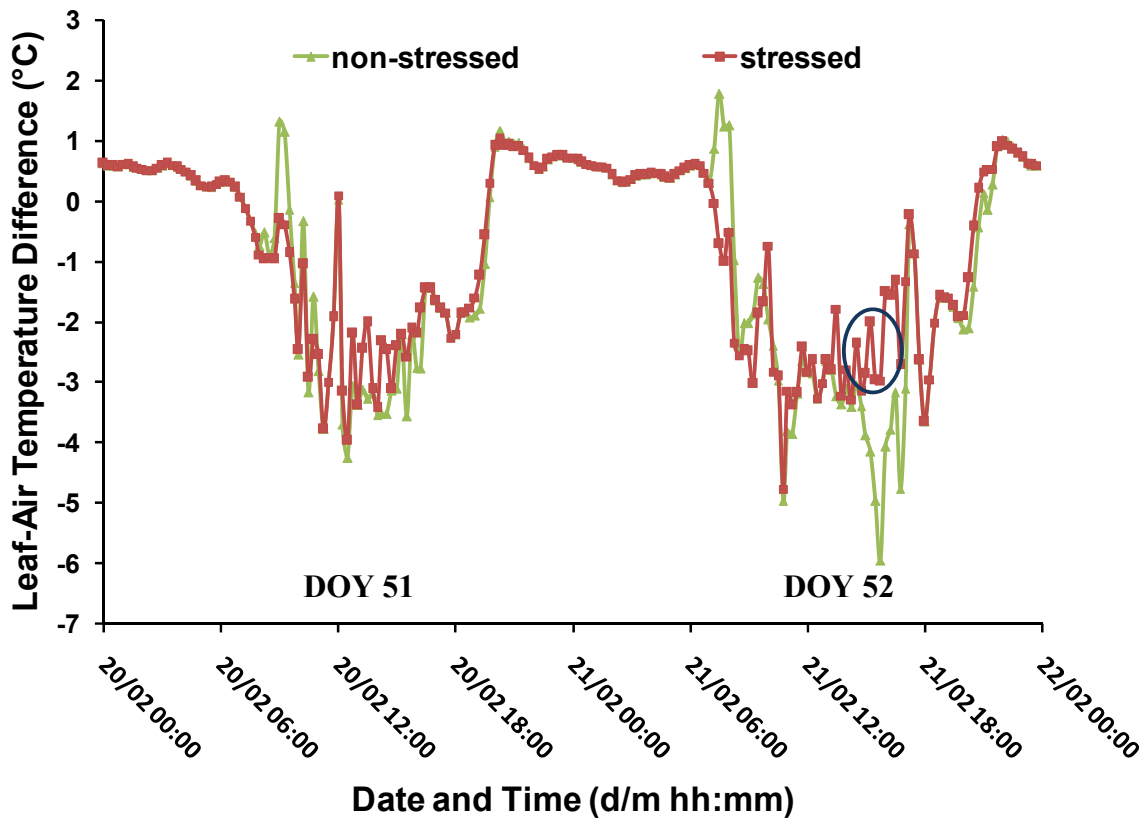


Figure 4.24: Leaf to Air temperature difference in stressed and non-water stressed plants on DOY 51 and 52

The graph shows that while there is a definite difference between the leaf to air temperature difference of stressed and non-water stressed plants there is no set threshold above which one can safely say that the plant is undergoing stress. Anomalies may occur as shown on DOY 51 between 11:15 am and 12:30pm (circled) when leaf temperatures

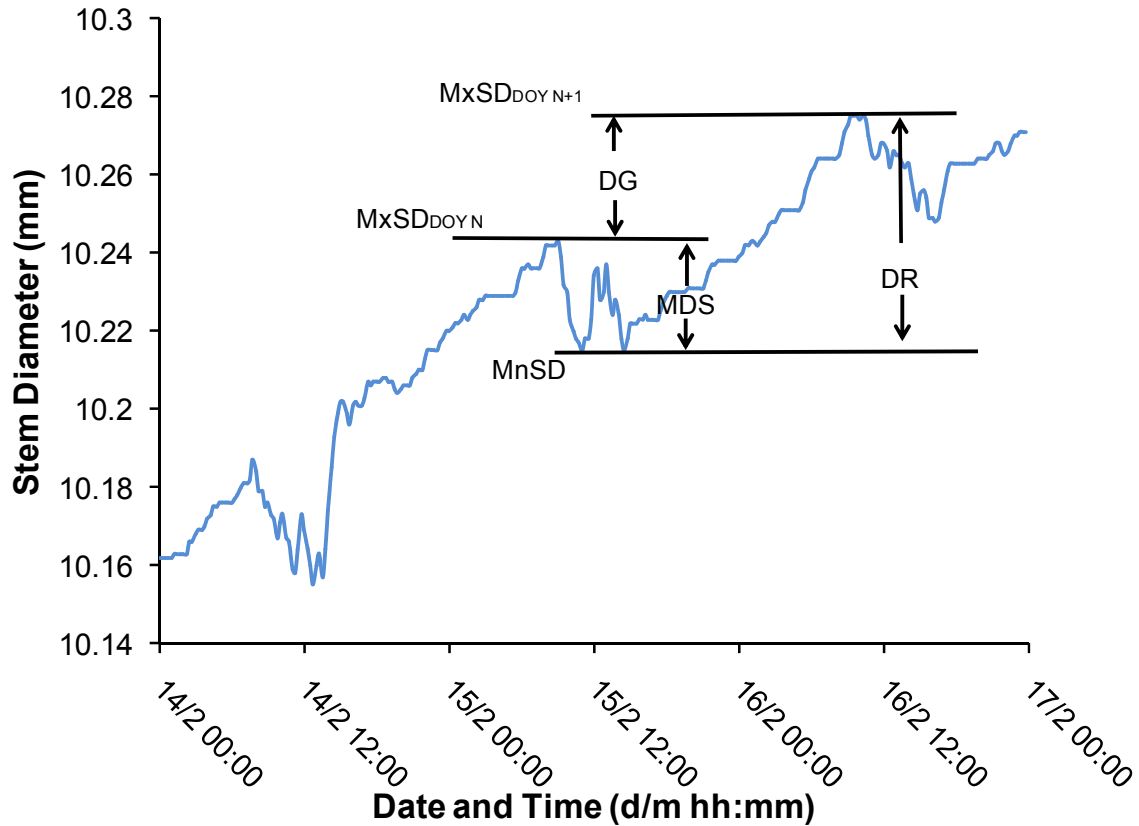
seem to suddenly increase above air temperatures resulting in a positive leaf to air temperature difference. However, this may be attributed to sudden cloudiness or even rain outside the greenhouse resulting in a quick reduction in air temperatures. However, it can be said that if the leaf-air temperature difference between 3:00pm and 4:45pm approaches -2°C or greater then misting of the plants may be necessary to reduce the chances of possible physiological damage of the leaves.

4.5.2 Stem Diameter

Stem diameter proved difficult to measure with the micrometer screw gauge since often daily fluctuations are very small and human error plays a large role in adding to the uncertainty of these measurements. However, the use of stem dendrometers in the later stages of the project proved extremely useful and should be considered a necessity when measuring plant physiological responses to stress. The results of the stem diameter measurements are discussed in detail in the next sections.

4.5.2.1 Stem Diameter Derived Indices

Before analysing stem diameter it was crucial to identify and extract the indices required for their analysis. The indices are shown in Figure 4.25 which plots a graph of stem diameter fluctuations over the course of three typical days (DOY 45 - 47). The trend of the graph was as per expectations and was consistent with what was documented by Fernandez and Cuevas (2010) in olive trees.



Where MxSD = Maximum stem diameter for a given day (day N)

MnSD = Minimum stem diameter for a given day (day N)

MDS = Maximum daily shrinkage = $MxSD - MnSD$

DG = Daily growth = $MxSD_{DOY\ N+1} - MxSD_{DOY\ N}$

DR = Daily recovery = $MxSD_{DOY\ N+1} - MnSD_{DOY\ N}$

Figure 4.25: Stem diameter derived indices of a non water stressed tomato plant over three typical days (DOY 45 – 47)

The most important of these indices is the maximum daily shrinkage (MDS) which it has been said can be used to determine irrigation quantity and timing in greenhouse crops. From Figure 4.25 it was then possible to compare plant water requirements calculated using the FAO-Penman-Monteith equation with MDS or one of the other stem diameter derived indices. The graph also shows that maximum stem diameter occurred at approximately 11:00 to 11:30 am while the minimum stem diameter occurred at around 1:00 to 2:00 pm and that measurements taken between these times would be sufficient in

determining MDS which is the most important stem diameter derived index in terms of irrigation scheduling. Figure 4.26 shows that on a daily basis there is a similar pattern between ET_o and MDS fluctuations.

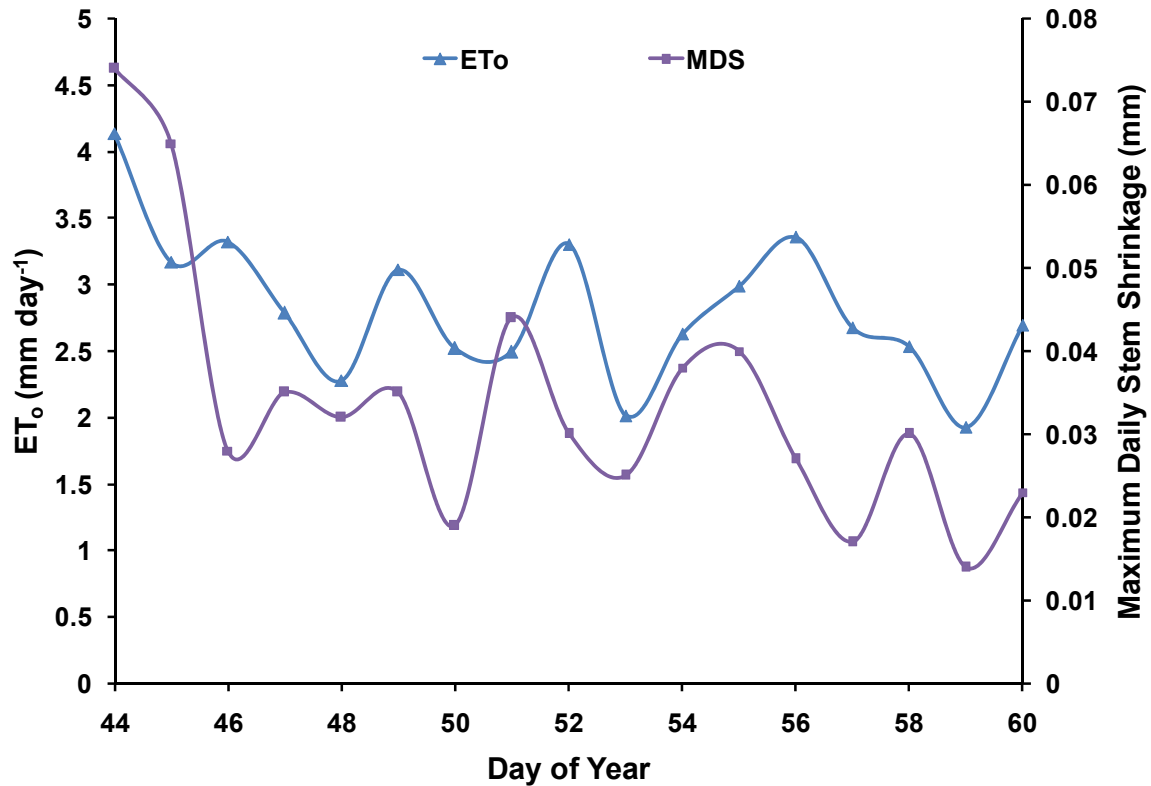


Figure 4.26: The variation of daily MDS and ET_o over the course of the experiment

The graph in Figure 4.26 indicates that either the amount of water applied to the plant affects MDS or that MDS can possibly determine the amount of water that the plant requires. It is also possible that MDS is correlated to one or more of the parameters used in the FAO-Penman-Monteith Equation.

A correlation between MDS and ET_o was done and the results are shown in Figure 4.27. The correlation is a medium strength one ($R^2 = 0.401$) but is strong enough for use in irrigation scheduling on a daily basis. This compared well with results from Intriglio and

Castel (2006) who observed R^2 values of between 0.73 and 0.24 (for the fruit growth and post harvest stages respectively) in their study of fully irrigated plum trees.

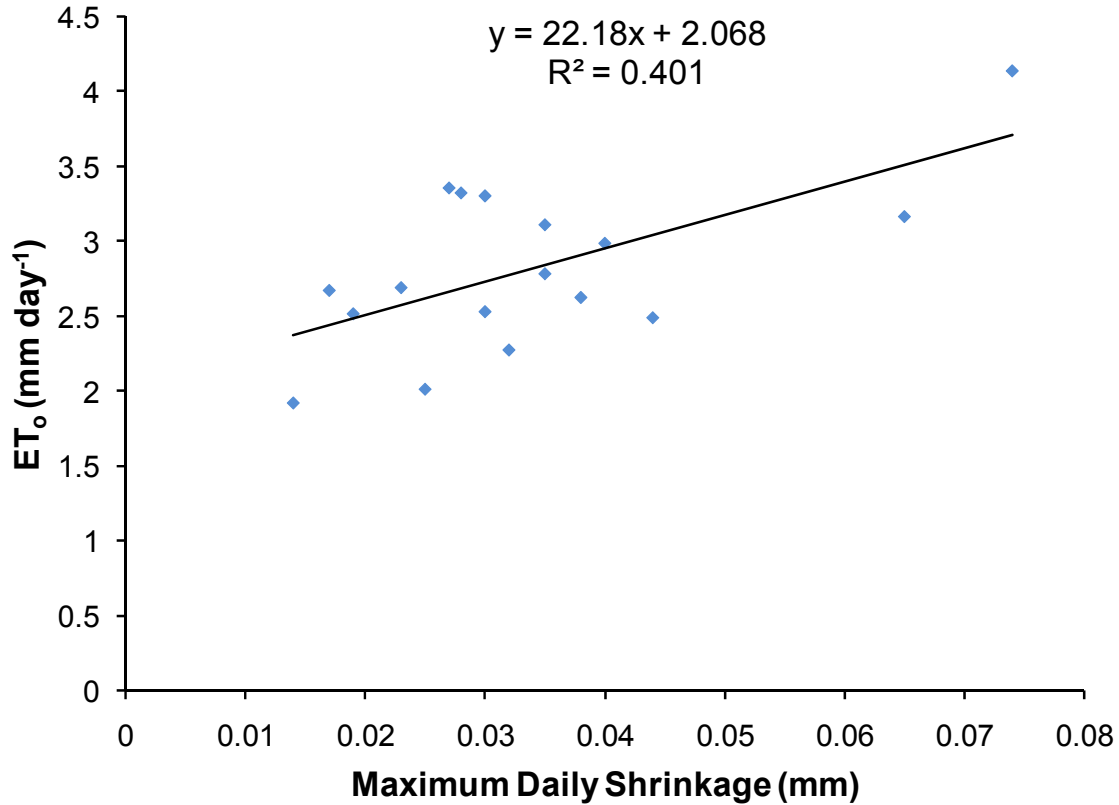


Figure 4.27: The correlation between maximum daily stem shrinkage (MDS) of a non water stressed tomato plant and ET_0 for 16 days (DOY 44 to DOY 60)

However, at intervals shorter than a day however, it is not possible to use stem diameter fluctuations in irrigation scheduling. For example, at intervals of 15 minutes or even an hour none of the stem diameter derived indices could be used to calculate irrigation quantity.

For irrigation scheduling based on MDS both the maximum and minimum stem diameter occurs before 3:00 pm and thus it is possible to take measurements before the end of the day and calculate irrigation amounts. Irrigation can then be done in the late afternoon or early evening each day.

4.5.2.2 The Use of Stem Diameter in Stress Detection

In terms of stress detection there is no true difference between the MDS of a stressed and non-water stressed tomato plant and it is hence difficult to use this indicator for the detection of stress. The difficulty comes in determining a reference MDS below which the plant can be said to be under stress conditions and requiring water urgently. Figure 4.28 shows that the MDS of healthy and water stressed tomato plants is out of sink (DOY 47 – DOY 52) and only goes back into sink when normal watering is returned (DOY 53 onwards).

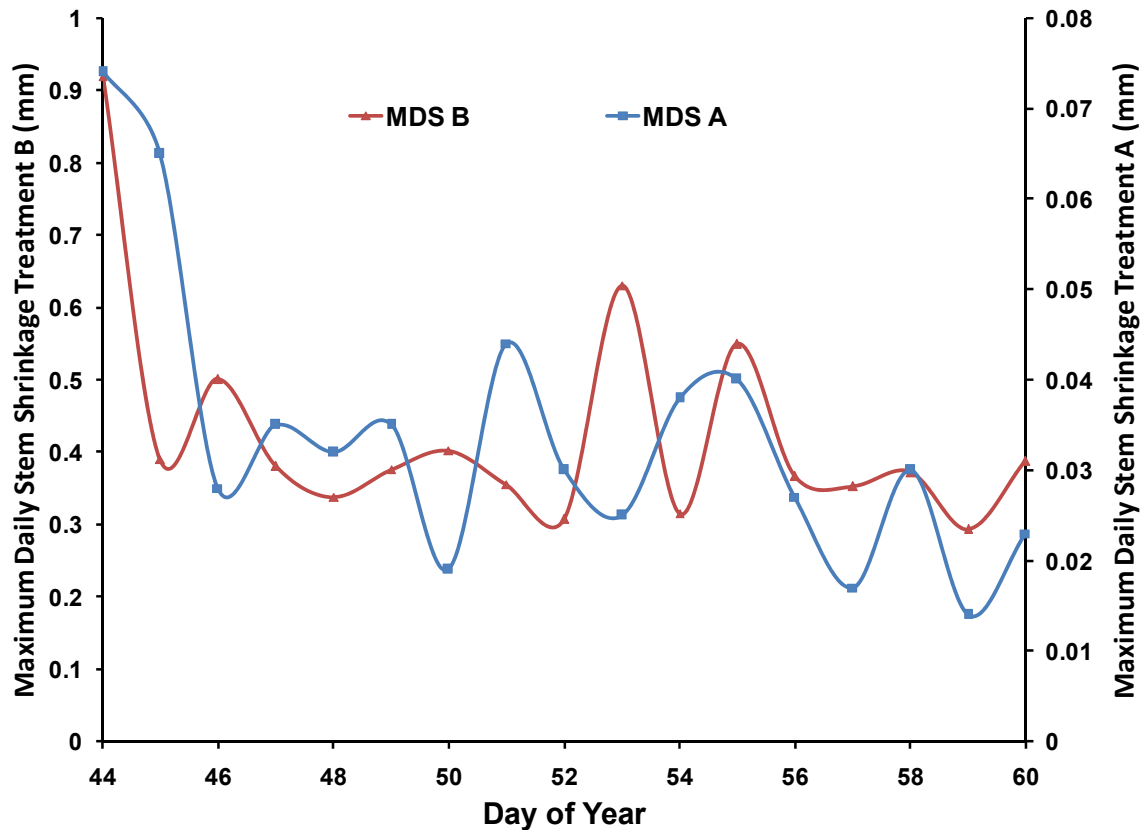


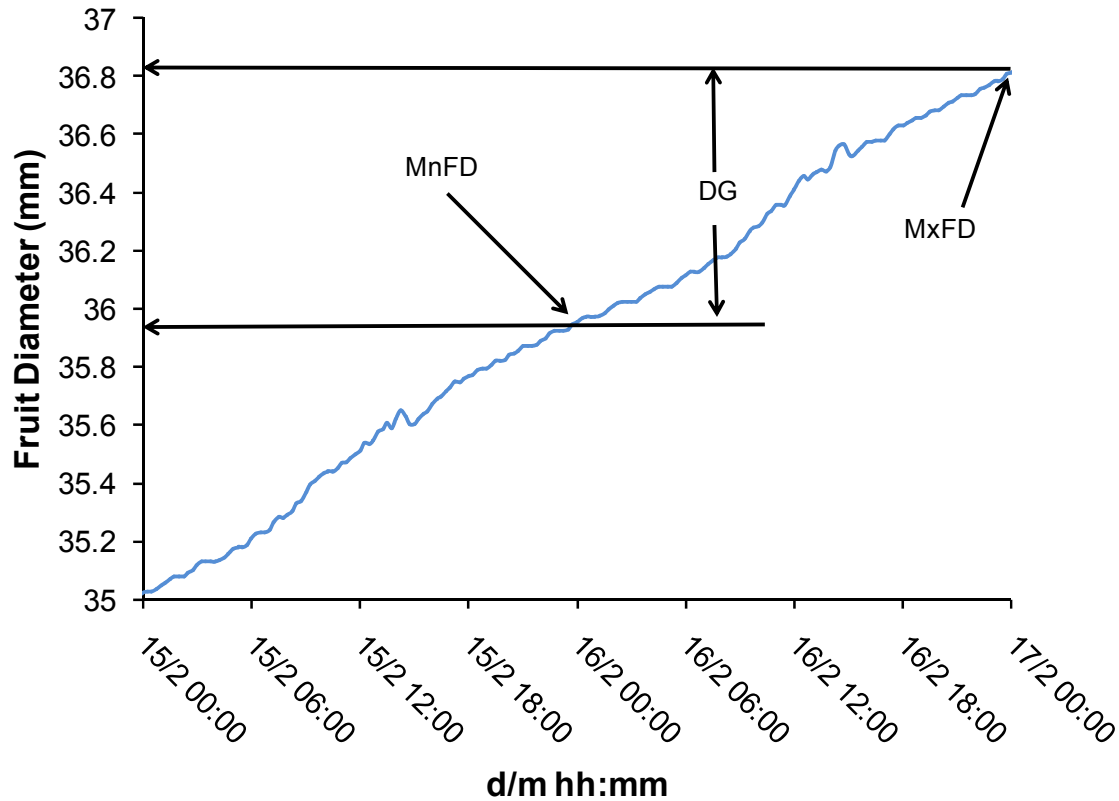
Figure 4.28: Comparison of the MDS of a stressed (MDS B) and non-stressed (MDS A) tomato plant for DOY 44 – 60 (stressing started DOY 47)

The results shown in Figure 4.28 are not conclusive and need to be further tested so that reference values can be determined as was done by Ortuno *et al* (2005). There is also a

need to ensure that stem diameter fluctuations are a result of water content or stress rather than growth. Daily growth rates can possibly be used in stress detection using stem diameter however, once again a reference daily growth rate below which it can be said that stress is occurring needs to be determined. This is difficult because it was found from the experiment that stems of the same crop planted at the same time still have different growth rates and also grow to different maximum diameters (views shared by Fernandez and Cuevas, 2010). It is possible for the growth rate of two different well watered healthy plants to have different growth rates or to reach maximum diameter at different times. Still Fernandes and Cuevas (2010) stated that in some cases and for some plants, stem growth rate may be a better indicator of water stress. More research in the early detection of stress using stem diameter derived indices needs to be done. However, for irrigation scheduling MDS would be suitable for calculating irrigation quantity on a daily basis.

4.5.3 Fruit Diameter

Fruit diameter fluctuations were found to be different to those of stem diameter as shown in Figure 4.29. Figure 4.29 shows that fruit diameter growth is more steady and constant as compared to stem growth, and while there is a reduction in diameter every day between 2pm and 5pm this reduction is small and fairly insignificant. The numbers of fruit diameter derived indices are fewer than those of stem diameter and are in fact limited to the minimum fruit diameter (i.e. the fruit diameter at the beginning of the day), the maximum fruit diameter (i.e. the fruit diameter at the end of the day) and the daily growth (maximum minus minimum). Further not much can be derived from these minimum and maximum values as can be done with daily stem shrinkage.



MnFD = Minimum fruit diameter for a given day

MxFD = Maximum fruit diameter for a given day

DG = Daily Growth = MxFD - MnFD

Figure 4.29: The diurnal course of fruit diameter of a medium sized, healthy, non water stressed, growing fruit over two typical days (DOY 46 – 47)

However, since it was not possible to plot MDS of a tomato fruit the daily growth was thus plotted and correlated with plant water requirements as shown in Figure 4.30. It shows that there is a weak correlation ($R^2 = 0.221$) between daily fruit growth and ET_o . This indicates that fruit diameter cannot be reliably used as a determinant of irrigation quantity in greenhouse grown tomato plants.

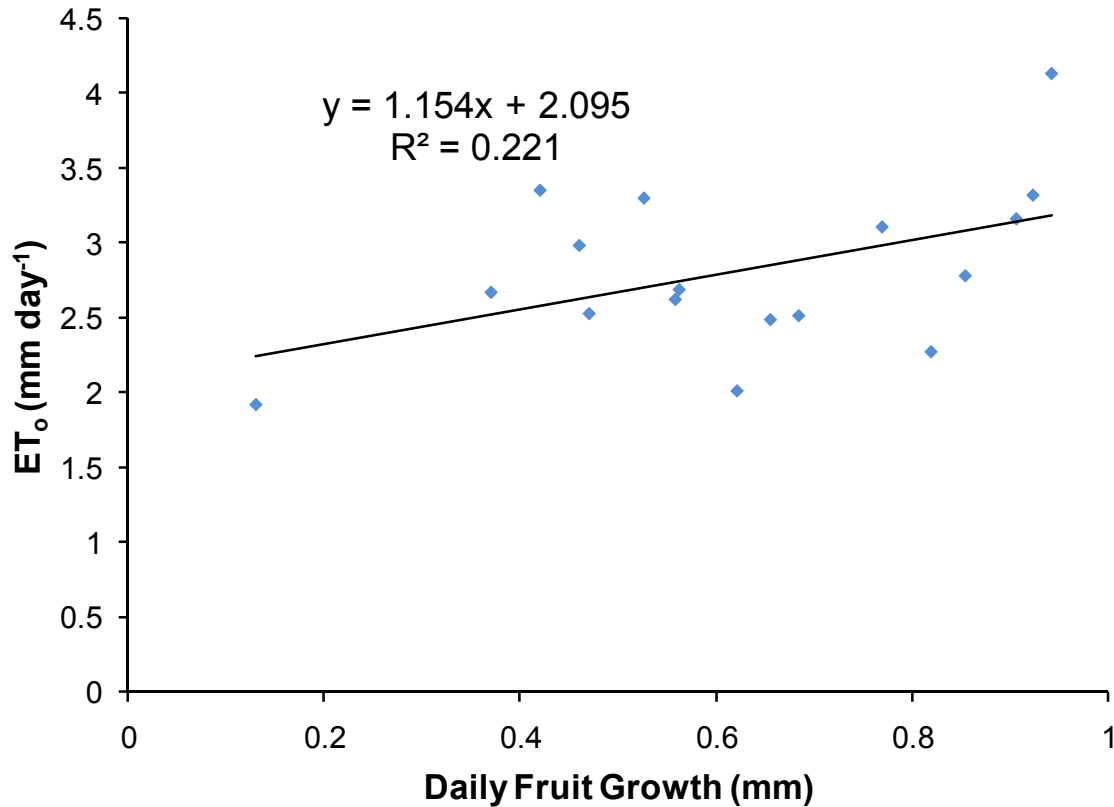


Figure 4.30: The correlation between the daily fruit growth (DG) of a non water stressed tomato plant and ET_o for 16 days (DOY 44 to DOY 60)

As a determinant of water stress however, it may be possible to use daily growth rates to ascertain whether the plant is getting enough water to grow at its maximum rate possible. This is demonstrated in Figure 4.31 which shows the daily growth of a tomato fruit subjected to water stress. As can be seen there is a reduction in growth rate from DOY 52 which may be a result of the lack of water to the plant. However, this drastic reduction in fruit growth only occurred five days after water had been cut off from the treatment and this may not be good enough for detection of stress as after five days the plant may already have suffered enough to permanently affect growth and yield.

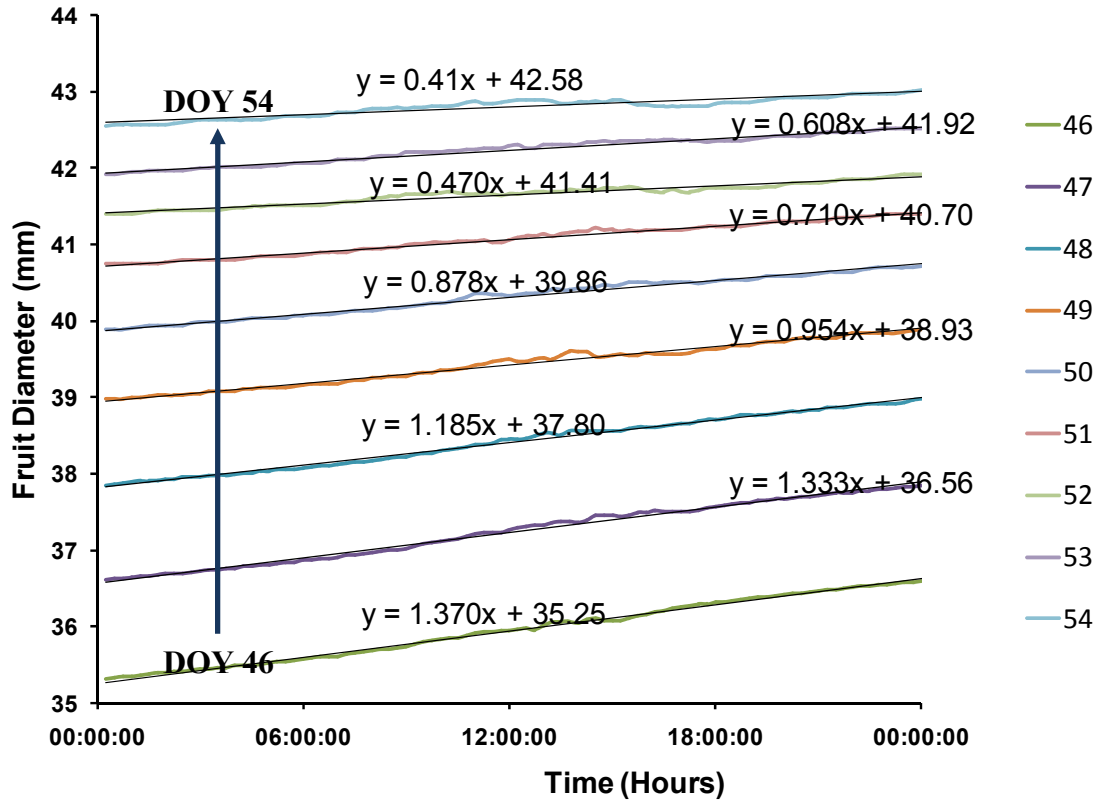


Figure 4.31: Daily equations of fruit growth rates for a water stressed tomato plant for nine days (DOY 46 – DOY 54)

In general fruit diameter is not suitable for calculating irrigation quantity but may be used for the detection of drought stress. It is important to use small growing fruit for this purpose though as large fruit may seem to give signs of drought stress through reduced daily growth when in fact they are under no stress at all but are just reaching their maximum diameter. Also it is important to note that at the fruit growth stage fruits are often the last to be affected by stress as the plant partitions nutrients and water more to the fruits than to other plant parts (Jones, 1990b).

4.5.4 Stomatal Resistance

The use of stomatal resistance in the irrigation scheduling of a tomato crop was difficult to determine. This is due to the difficulty in automating the measurements as well as the difficulty in using a porometer in an environment whose temperature and humidity

changes greatly within the space of a few hours. It was not possible to take stomatal resistance measurements at regular enough intervals to determine its progression in stressed and non-water stressed plants. However, measurements of stomatal resistance were made in all treatments at one hour intervals on one day (DOY 52). In non-water stressed plants it was found that stomatal resistance during daylight hours is dependant on the amount of solar radiation incident on the plant leaf as shown in Figure 4.32 This shows that the bottom of the leaf has a medium correlation ($R^2 = 0.693$) with the radiation incident upon it while the top of the leaf has a weak correlation ($R^2 = 0.304$). It can also be seen from Figure 4.32 that at any given PAR value resistance of the top of the leaf is higher than that of the bottom of the leaf. This is because even though tomato leaves are amphistomatous (i.e. have stomata on both top and bottom of the leaf) the majority of stomata are concentrated on the bottom and this is where the majority of transpired water is lost from.

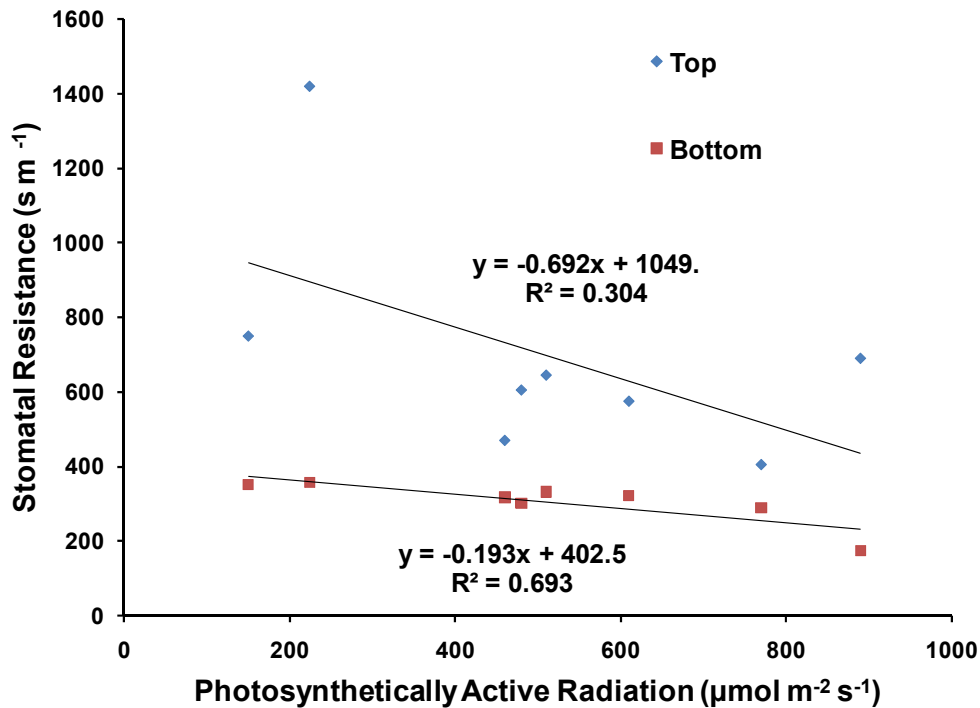


Figure 4.32: The variation of stomatal resistance with PAR of leaves in a healthy non-water stressed tomato plant on DOY 52

For water stressed plants it was surprisingly found that stomatal resistance increased with increasing PAR as shown in Figure 4.33. With non-water stressed plants the bottom of the leaf had a medium correlation ($R^2 = 0.571$) with incoming radiation while the top had a weak correlation ($R^2 = 0.287$) with incoming radiation. The reversal in the relationship with incoming radiation in water stressed plants may be a survival mechanism of the plant to conserve water at high irradiance since it is well known that stomata close to avoid tissue dehydration (Vermeulen *et al*, 2007). From comparing Figure 4.32 and 4.33 it can be seen that the stomatal resistance of drought stressed plants is much greater than that of non-water stressed plants and this fact can possibly be used in the early detection of drought stress if suitable threshold values can be determined.

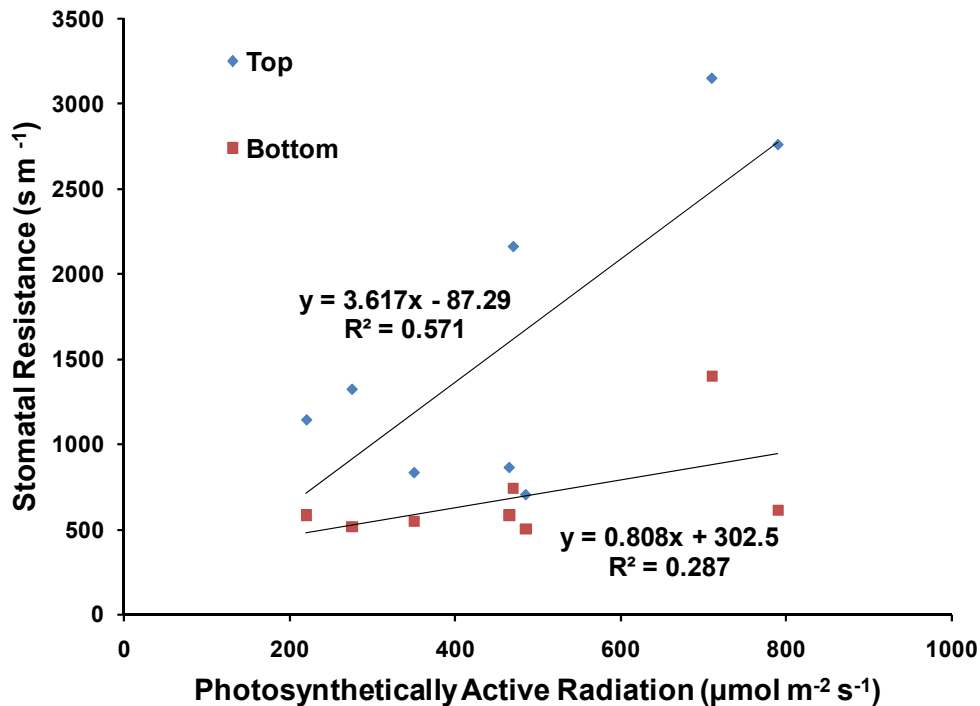


Figure 4.33: The variation of stomatal resistance with PAR of leaves in a water stressed tomato plant on DOY 52

In general it was found that stomatal resistance cannot be used in determining irrigation quantity. However, stomatal resistance shows good response to drought stress and can possibly be used for the timing of irrigation. However, the high dependence of stomatal

resistance on factors such as incoming solar radiation and vapour pressure deficit (Anderson and Peterson, 2007) makes it difficult to give recommendations to growers on stress detection using this indicator. One recommendation is that if stomatal resistance increases with increasing irradiance then the plant is suffering from drought stress. It is important to note that the regressions shown in Figures 4.32 and 4.33 are linear (as was done by Anderson and Peterson, 2007) because all readings of stomatal resistance were taken between 9am and 5pm. If measurements had been taken starting at sunrise and ended at sunset the trend lines would have been exponential curves falling very steeply adjacent to the y-axis then leveling out to a straight line adjacent to the x-axis. This is because stomatal resistance increases exponentially around those times.

4.5.5 Leaf Water Potential

From the experiment it was found that midday leaf water potential varied from day to day between -1200 kPa to -1500 kPa. It was hypothesized that this variation was due to a combination of factors including plant water content, incoming solar radiation, air temperature and vapour pressure deficit. The variations of LWP_{midday} between the water stressed and non-water stressed treatments throughout the experiment are shown in Figure 4.34 which shows the average values between the water stressed treatments (B and D) and the non-water stressed treatments (A and C). The graph shows that midday leaf water potential shows good response to water stress as shown by the dips in LWP_{midday} in treatments B and D during the two stressing periods of the experiment. LWP also showed an immediate response to drought stress and a drop in LWP_{midday} was detected as early as two days after stressing began. Similar results were seen by Intriglio and Castel (2006) who noted lower values of predawn leaf water potential in their deficit irrigation treatments. This means that LWP_{midday} can be a good indicator for use in the early detection of plant water stress. Intriglio and Castel (2006) however, noted that midday leaf water potential and stem water potential did not perform as well as predawn leaf water potential.

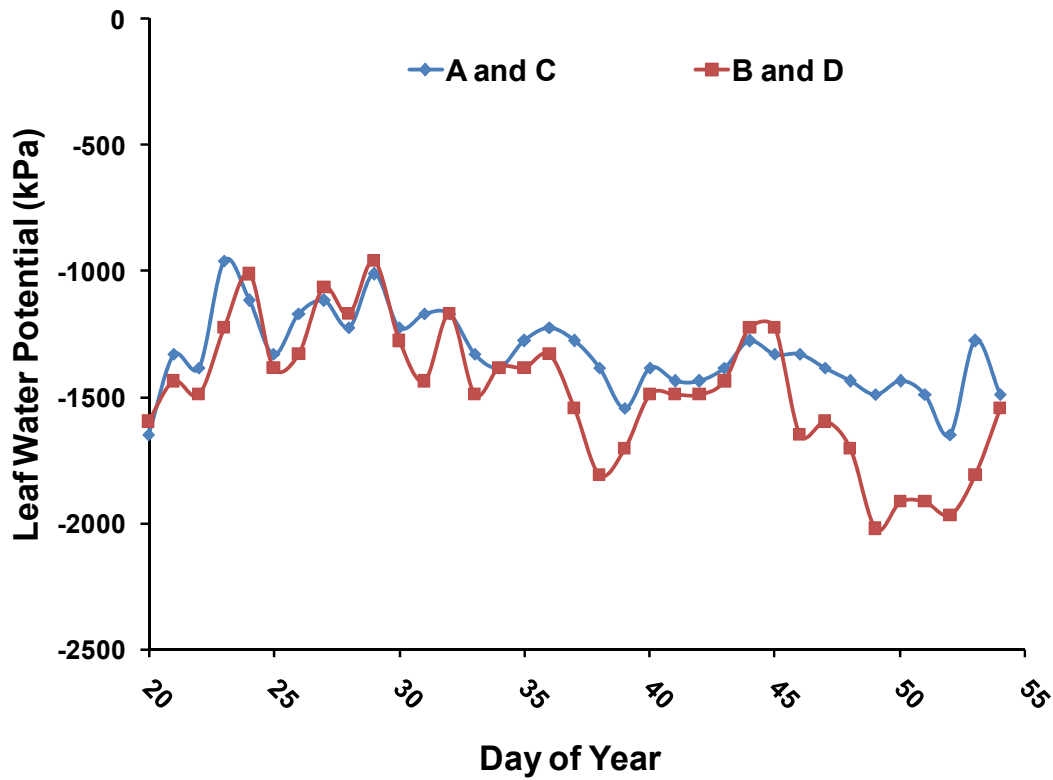


Figure 4.34: The variation of midday leaf water potential from DOY 20 to DOY 58

In terms of actual figures it can be said that if LWP_{midday} of a greenhouse grown tomato plant falls below -1600 kPa then the plant can be said to be suffering from a water deficit within its internal organs which usually translates to a root zone water deficit. One drawback was that the thermocouple psychrometer used could only accurately show changes of 100 kPa (0.5 μV) or more (i.e. it has a low resolution). The use of an instrument with a higher resolution would aid in detection of water stress using midday leaf water potential. In terms of irrigation scheduling, leaf water potential showed no correlation with ET_o ($R^2 = 0.070$) as shown in Figure 4.35.

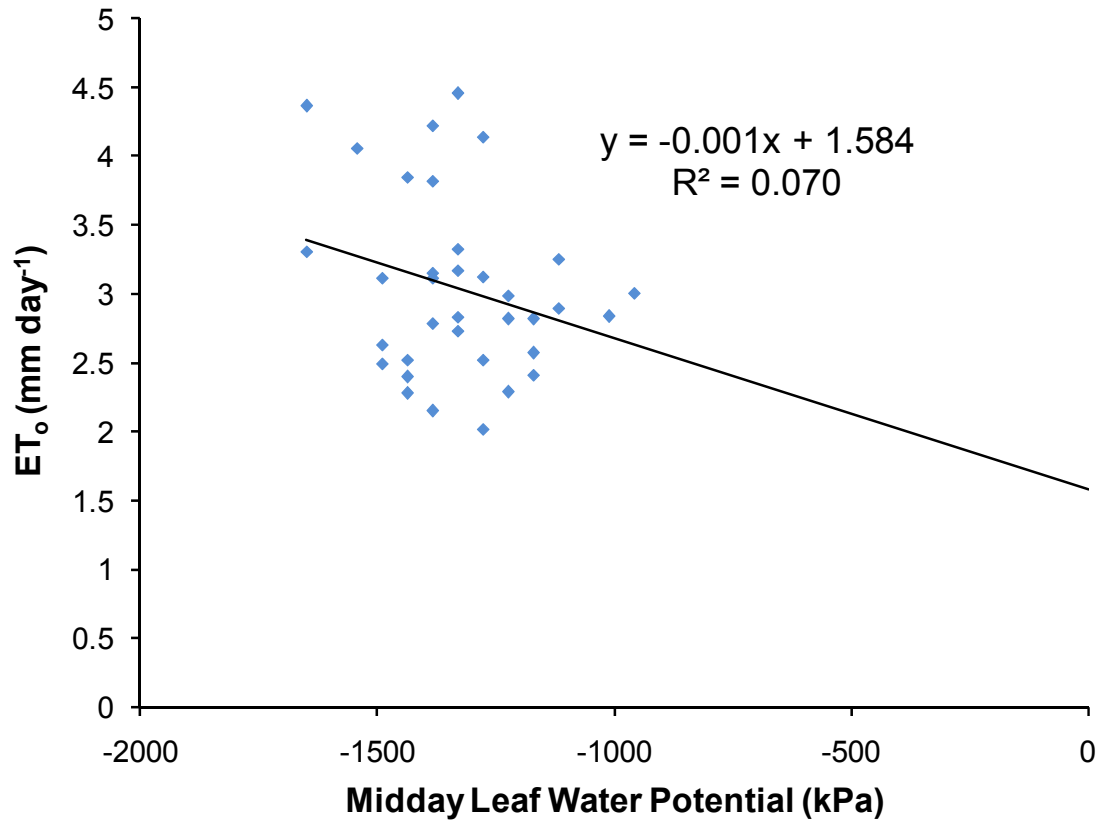


Figure 4.35: The correlation between midday leaf water potential and ET_o of a non water stressed tomato plant from DOY 20 to DOY 58

For this reason LWP_{midday} cannot be used alone to determine the quantity of irrigation water required. However, in terms of the timing of stress detection and irrigation timing it can be said that there is a possibility in using leaf water potential measurements to determine if plants require water or not. It can be seen from Figure 4.34 that for greenhouse grown tomatoes midday leaf water potential is usually between -1200 kPa and -1500 kPa. If midday leaf water potential falls below -1600 kPa then the plant is under water stress and requires water immediately.

In general, LWP_{midday} bore little correlation to daily water requirements but only served as an indicator of plant water content (or stress) itself. Thus while LWP may not be useful in predicting water requirements it would be useful in determining the timing of

irrigation since farmers can use LWP_{midday} to detect when plant is under or nearing water stress or when water status is low and hence irrigate.

4.5.6 Sap Flow

4.5.6.1 The Use of Sap Flow to Calculate Daily Irrigation Quantity

From monitoring sap flow at 15 minute intervals it was possible to calculate total daily sap flow for each day of the experiment. The results of this are plotted in Figure 4.36 and compared to daily ET_c calculated using the FAO-Penman-Monteith Equation and a crop coefficient of 0.8.

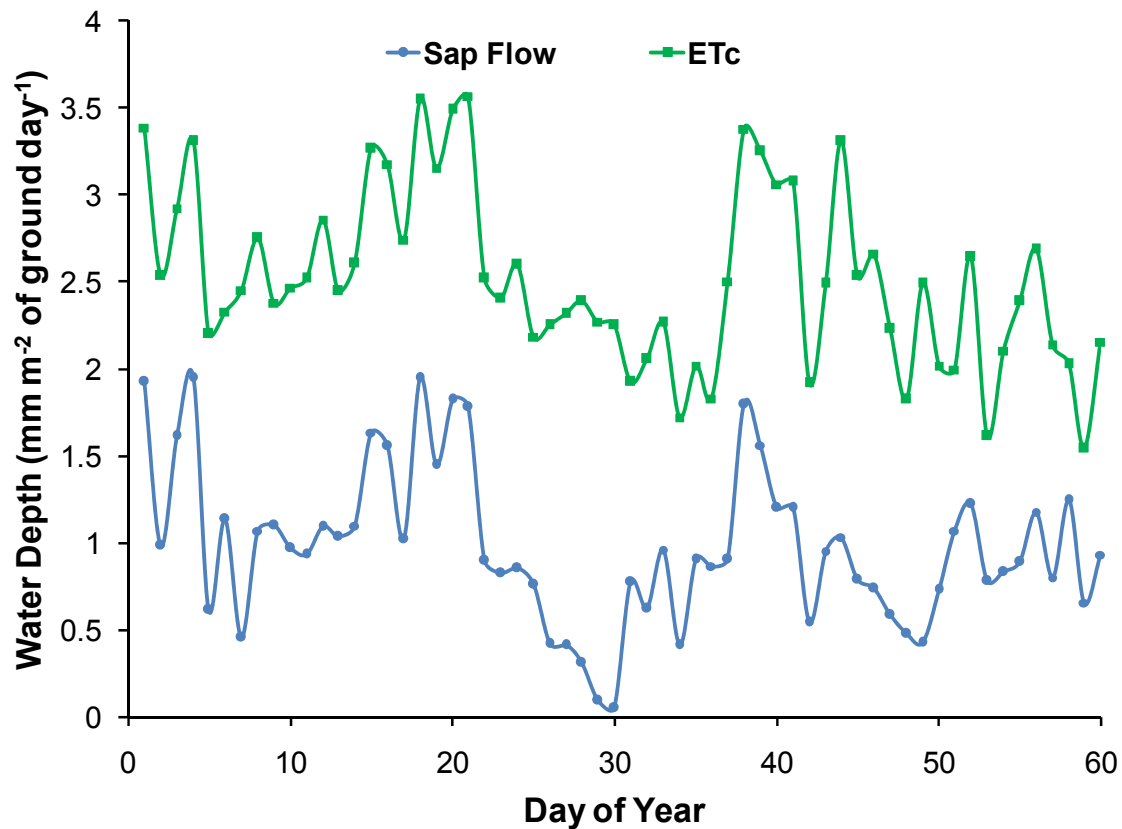


Figure 4.36: The total daily sap flow and daily ET_c for each of the 60 days of the project.

Results and Discussion

From the graph it can be seen that there is a similarity in the pattern of daily total sap flow and daily ET_c . The troughs and peaks coincide with each other and it is possible that the same factors which affect ET_c calculated using the FAO-Penman-Monteith Equation similarly affect sap flow on a daily basis. Sap flow ranges from approximately 13.1% to 61.5% of ET_c with the average being 38.1% and this may point to the possible use of lower crop coefficients (i.e. approximately 0.4) when using the FAO-Penman-Monteith equation for greenhouse grown tomatoes thus leading to great water savings.

The correlation between sap flow and ET_o is shown in Figure 4.37 which shows that there is a medium leaning to high correlation ($R^2 = 0.64$) between sap flow on a daily basis and ET_o . This fairly good correlation may be due to the fact that their driving variables are similar. However, sap flow seems to be affected by some other outside variables. The use of sap flow to calculate irrigation quantity on a daily basis is very possible and can be done according to the regression equation in Figure 4.37. There is also a possibility of using sap flow to optimize water use since sap flow is a direct indicator of plant water use.

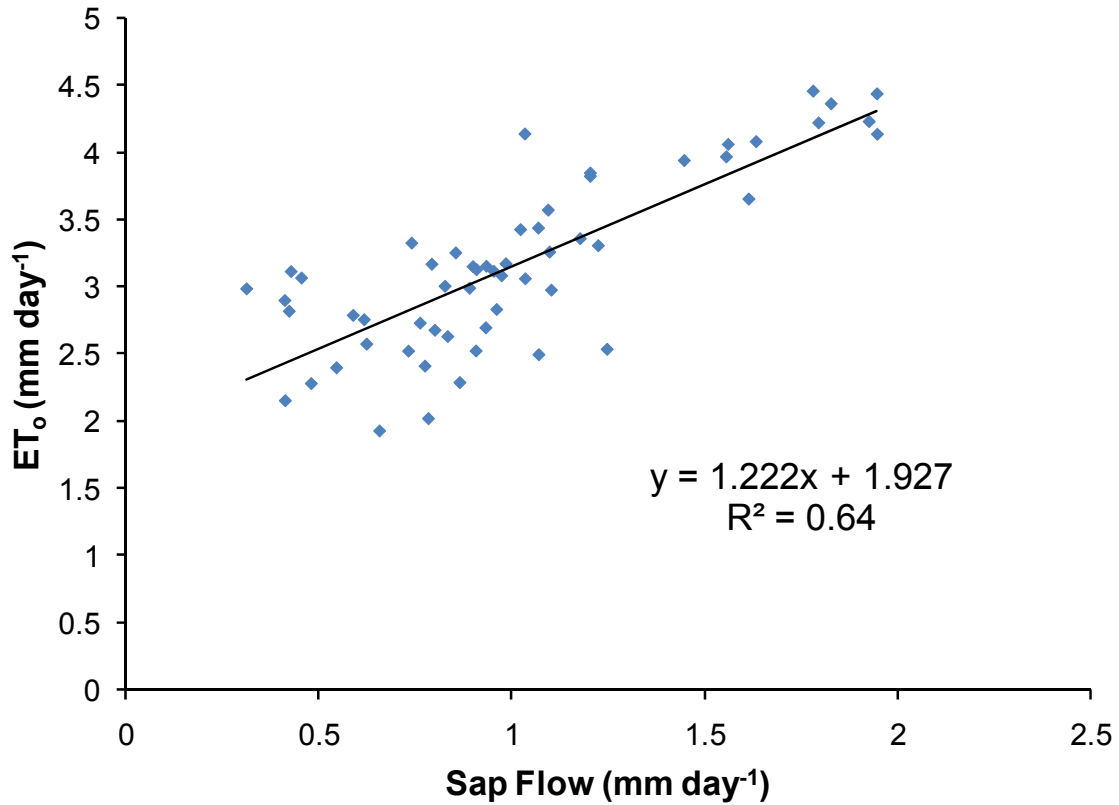


Figure 4.37: The correlation between daily total sap flow and ET_o calculated using the FAO-Penman-Monteith Equation for the 60 days in which the experiment was done.

At intervals of 15 minutes sap flow (in g hour^{-1}) also seems to follow a similar pattern as ET_o (or ET_c) however, sap flow is more variable and has many troughs and peaks while ET_o is steadier throughout the day. Also while sap flow may be zero at most times during the night ET_o is never zero. The comparison between the diurnal variation of sap flow and ET_c is shown in Figure 4.38

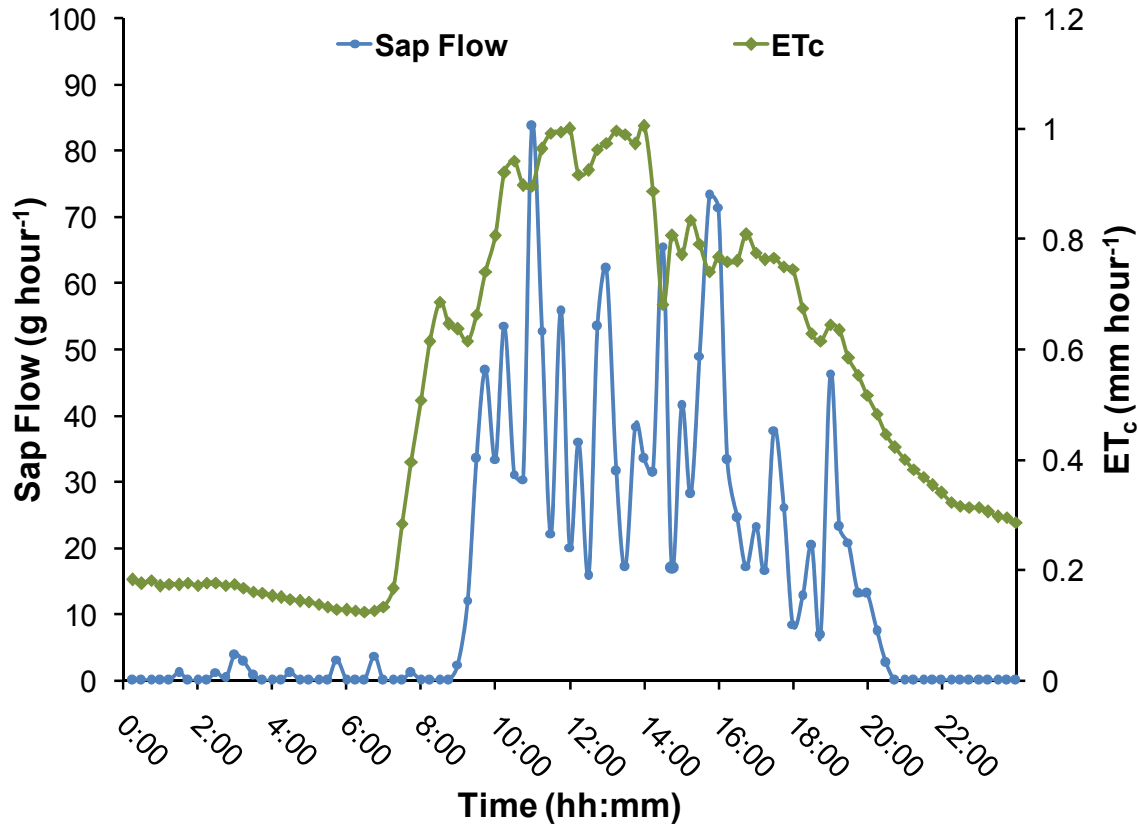


Figure 4.38: The diurnal course of sap flow and ET_c on a typical clear sunny day (DOY 38)

The correlation between sap flow and ET_0 at hourly intervals is also medium to high ($R^2 = 0.569$) as shown in Figure 4.39. The variability of sap flow in the short term negatively affects the relationship; however, the relationship is good enough for calculation of plant water requirements at intervals of 15 minutes to an hour. The advantage of being able to calculate and apply water at short intervals is that there is less water lost to runoff and evaporation (i.e. while the total for the day is the same applying little amounts at regular intervals does not saturate the substrate and lead to unnecessary loss through dripping). The equation shown in Figure 4.39 can be used to calculate water requirements based on sap flow at 15 minute intervals while that shown in Figure 4.37 can be used to calculate irrigation amounts based on sap flow on a daily basis.

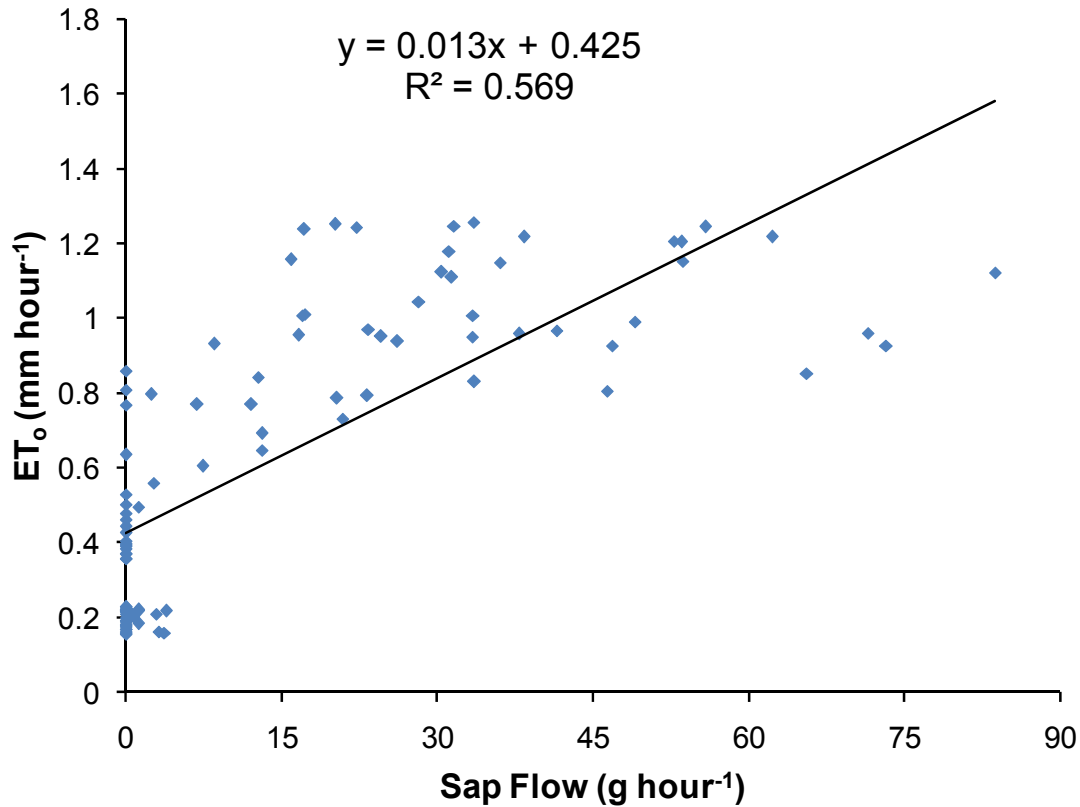


Figure 4.39: The relationship between sap flow and ET_o on a typical clear sunny day (DOY 38)

In general sap flow is a good indicator of actual plant water use and hence plant water requirements and as such can be effectively used in irrigation scheduling of greenhouse grown tomato plants. Also the use of sap flow to schedule irrigation at short intervals is very promising and can lead to great water savings since sap flow responds quickly to changes in meteorological conditions. It is also possible that the same variables driving sap flow are the same ones that determine plant water use and this is discussed next.

4.5.6.1.1 The Main Variables Driving Sap Flow

It was found that sap flow was dependant on two main variables namely solar radiation and leaf temperature. By comparing the daily course of sap flow and that of solar radiation it was found that they followed similar patterns as shown in Figure 4.40.

However, sap flow is more variable in the short term and has many peaks and troughs as compared to solar radiation which rises and falls steadily from dawn to dusk. Also it seems that sap flow has a delayed reaction to solar radiation and an increase in solar radiation is only met with an increase in sap flow over 1 hour later. This could be because of plant capacitance which is a result of the buffering effect of the water stored in the plant causing a delay between stomatal opening or closing and the reaction of sap flow.

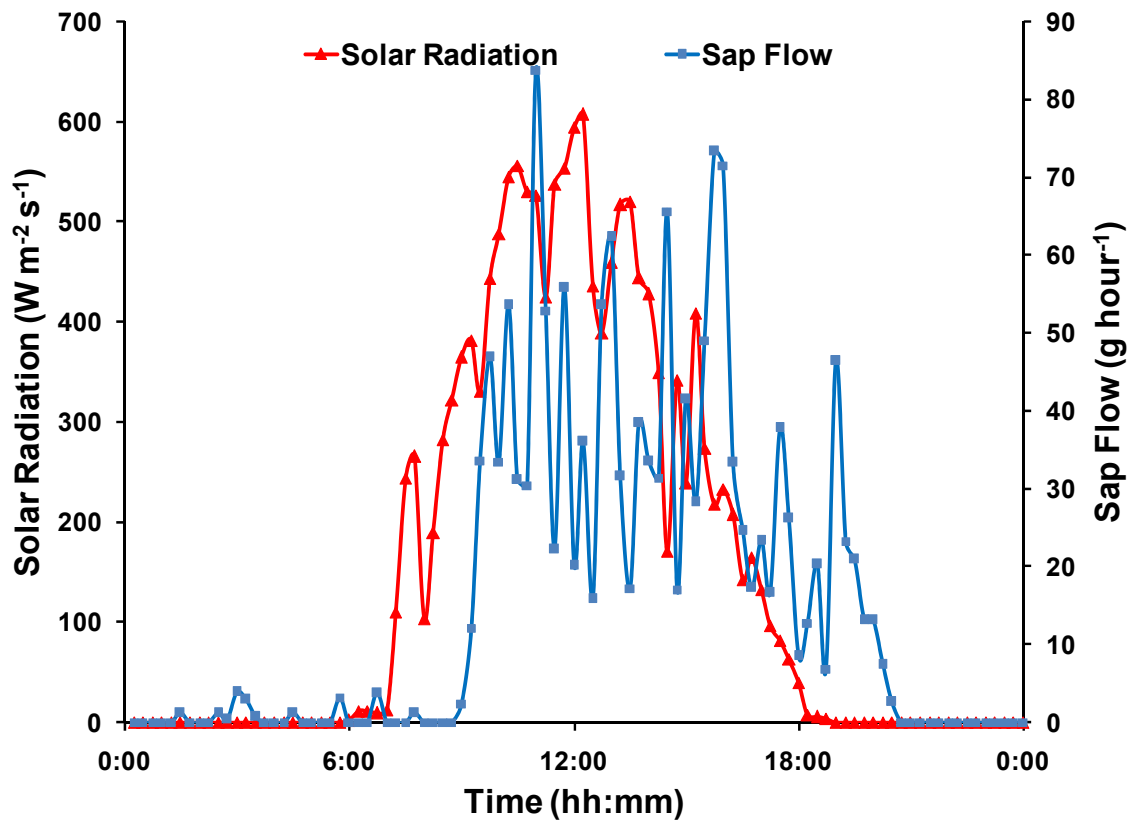


Figure 4.40: The diurnal course of sap flow (blue line) and solar radiation (red line) on a typical, cloudless sunny day (DOY 38)

Leaf temperature and sap flow also seem to follow similar patterns as shown in Figure 4.41. Just like solar radiation leaf temperature rises and falls steadily from dawn to dusk and does not follow the many peaks and troughs of sap flow. However, from the two graphs shown in Figure 4.40 and 4.41 it can be seen that there is possibly good

correlations between solar radiation and sap flow as well as between leaf temperature and sap flow.

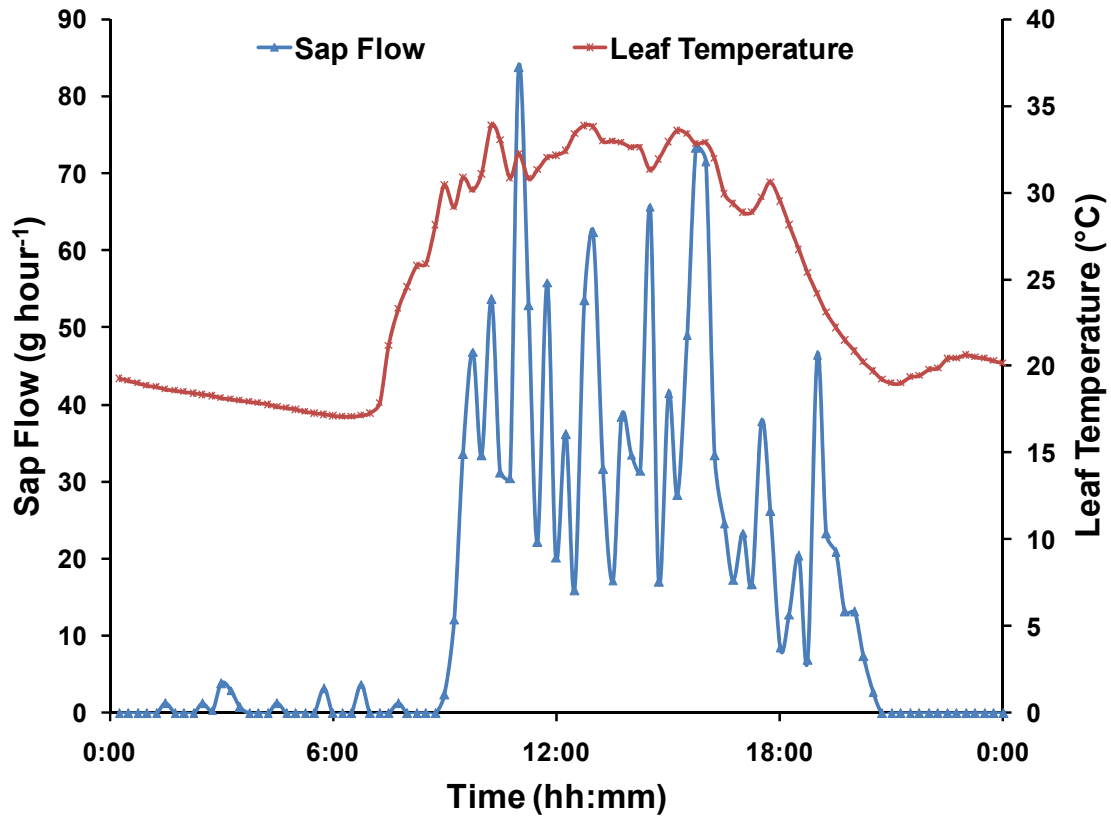


Figure 4.41: The diurnal course of sap flow (blue line) and leaf temperature (red line) on a typical, cloudless sunny day (DOY 38)

The closest pattern was seen however, between sap flow and vapour pressure deficit which is plotted in Figure 4.42 and shows how VPD rises steadily through the day and falls towards evening.

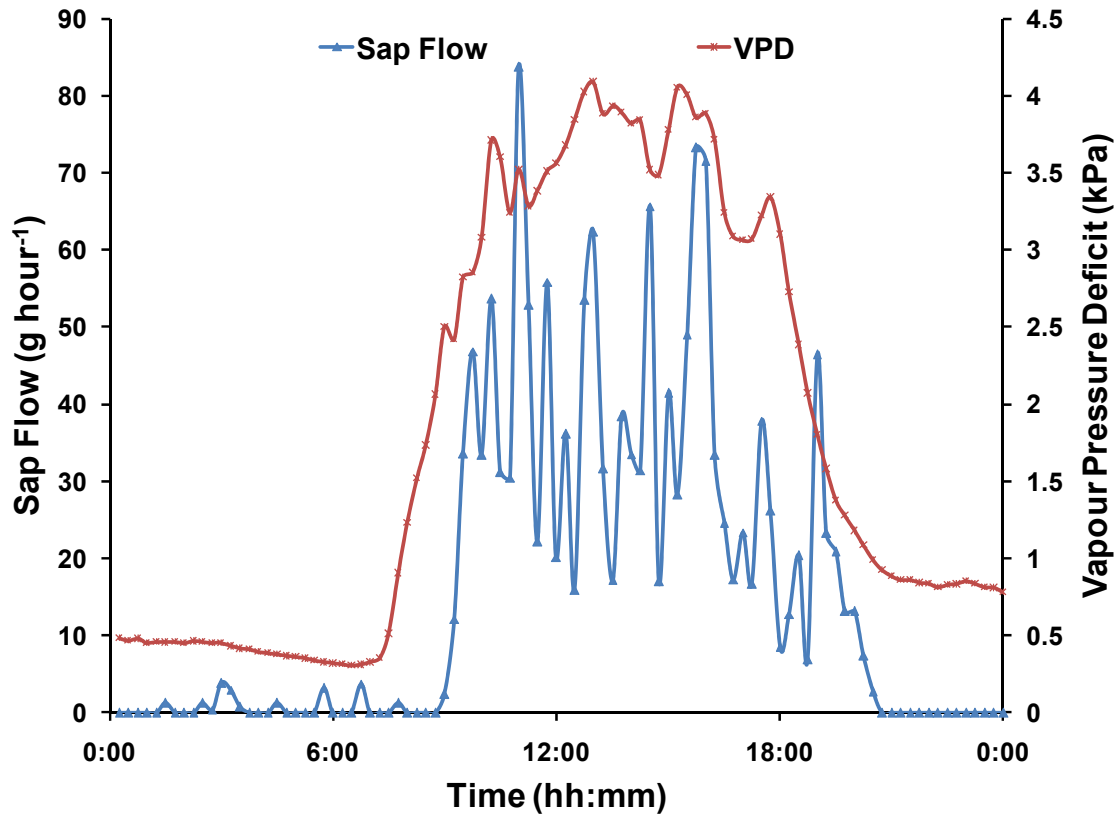


Figure 4.42: The diurnal course of sap flow (blue line) and vapour pressure deficit (red line) on a typical, cloudless sunny day (DOY 38)

It is possible that of the three variables shown in Figure 4.40, 4.41 and 4.42 vapour pressure deficit could have the greatest influence on sap flow and this was tested using correlation. The relationship between sap flow and solar radiation was found to be a medium strength one ($R^2 = 0.414$) as shown in Figure 4.43. While a higher relationship was expected this value was not surprising given the high variability of sap flow throughout the day as well as the high variability of both sap flow and solar radiation within the 15 minute averaging intervals used by the data logger.

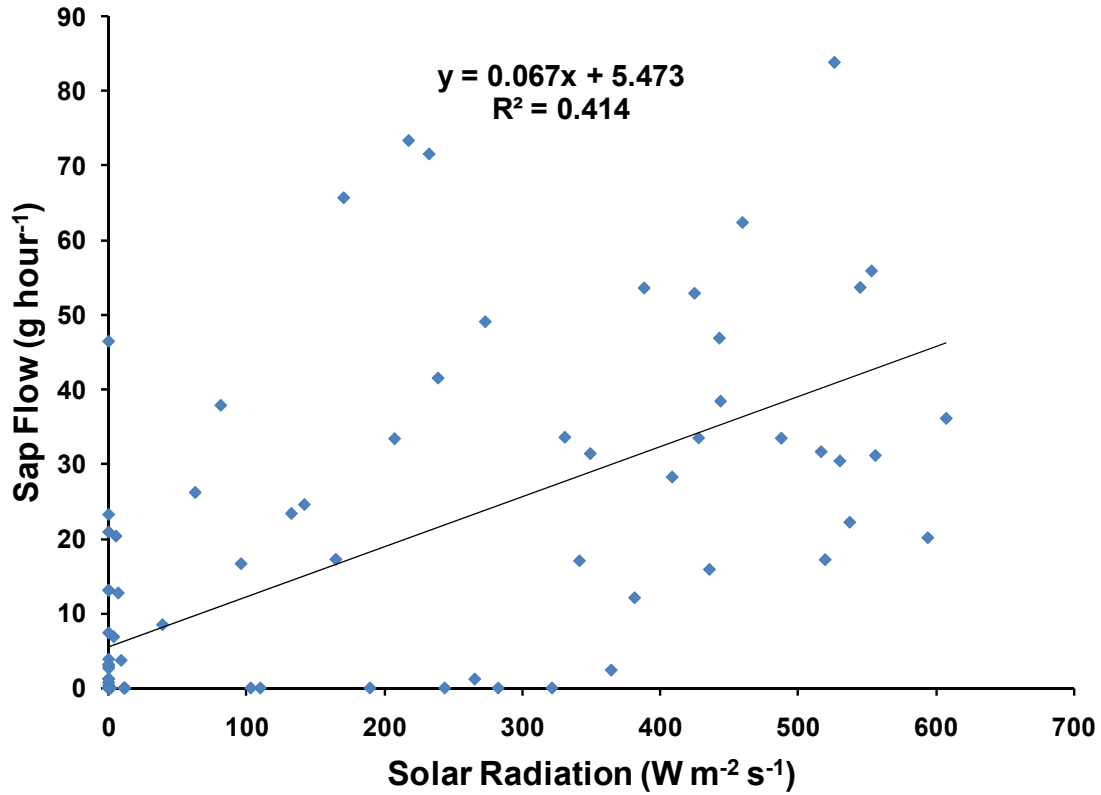


Figure 4.43: The correlation between sap flow and solar radiation on a typical sunny day (DOY 38) showing a medium dependence of sap flow on solar radiation

Leaf temperature consistent with what was read in literature was found to have a high medium correlation ($R^2 = 0.627$) with sap flow throughout the day as shown in Figure 4.44. This meant that of the meteorological parameters leaf temperature played a fairly high role in determining sap flow.

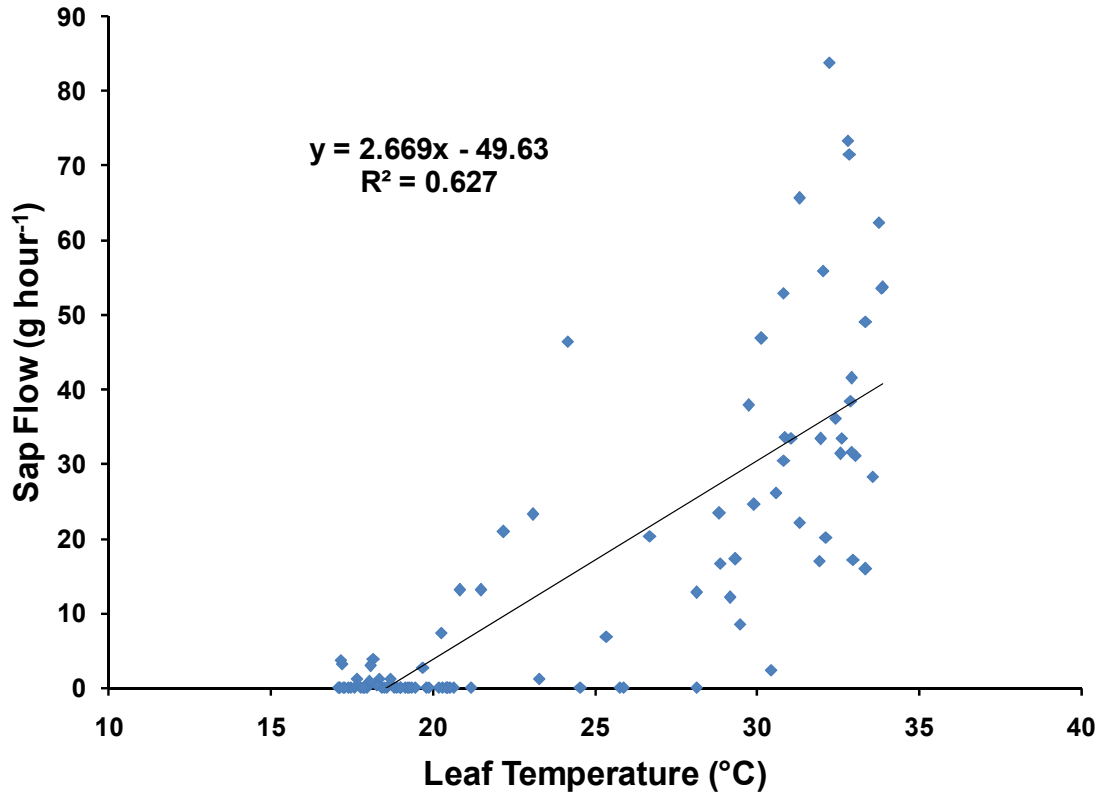


Figure 4.44: The relationship between leaf temperature and sap flow on a typical sunny day (DOY 38) showing a high medium correlation between the two variables.

However, it was found that vapour pressure deficit had the highest correlation ($R^2 = 0.663$) with sap flow as shown in Figure 4.45. This meant that of all the three factors leaf to air vapour pressure deficit had the greatest influence on sap flow rate and hence water use. Dziki (2010) and Ortuno *et al* (2005) concurred with this.

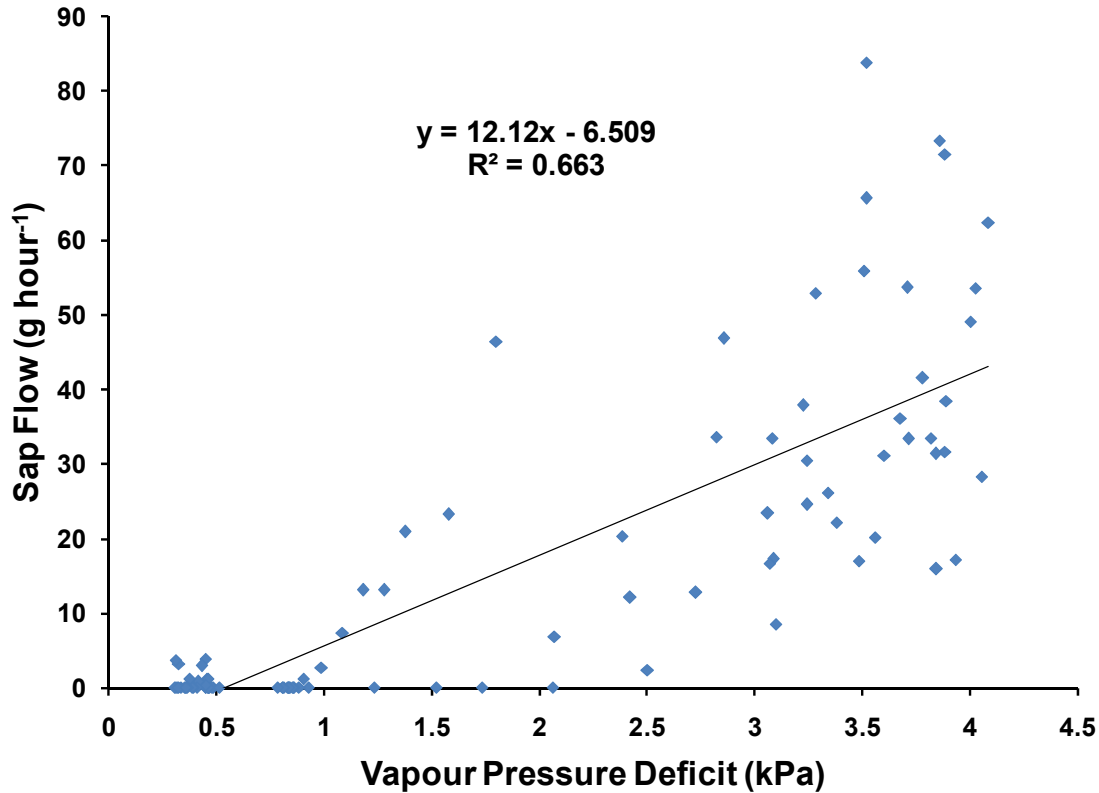


Figure 4.45: The relationship between vapour pressure deficit and sap flow on a typical sunny day (DOY 38) showing a medium to high correlation between the two variables.

However, these correlations while satisfactory point to the fact that other outside influences may play a role in determining sap flow. These factors may be physiological and have something to do with factors such as the high variability of stomatal resistance for example.

4.5.6.2 The Response of Sap Flow to Water Stress

The response of sap flow to water stress was observed and is shown in Figure 4.46. On the day that stressing began sap flow in the non-stressed and stressed treatments was approximately similar. However, after approximately three days differences began to be seen. Sap flow in the water stressed treatment was less than that in the non water stressed

treatment and the trend continued for the next few days with the difference between sap flow of the two treatments getting larger and larger everyday. It took approximately four days for sap flow to reduce significantly enough for a difference to be detected. It should be noted that in this experiment the days in question were relatively cool days and thus the evaporating power of the atmosphere was low. On hotter days it is expected that the response of sap flow to water stress will be quicker and possibly immediate as seen by Ortuno *et al* (2005) in their study on young lemon trees. In a similar study on tomatoes Vermeulen *et al* (2007) noticed a reduction in sap flow on the second day of stressing.

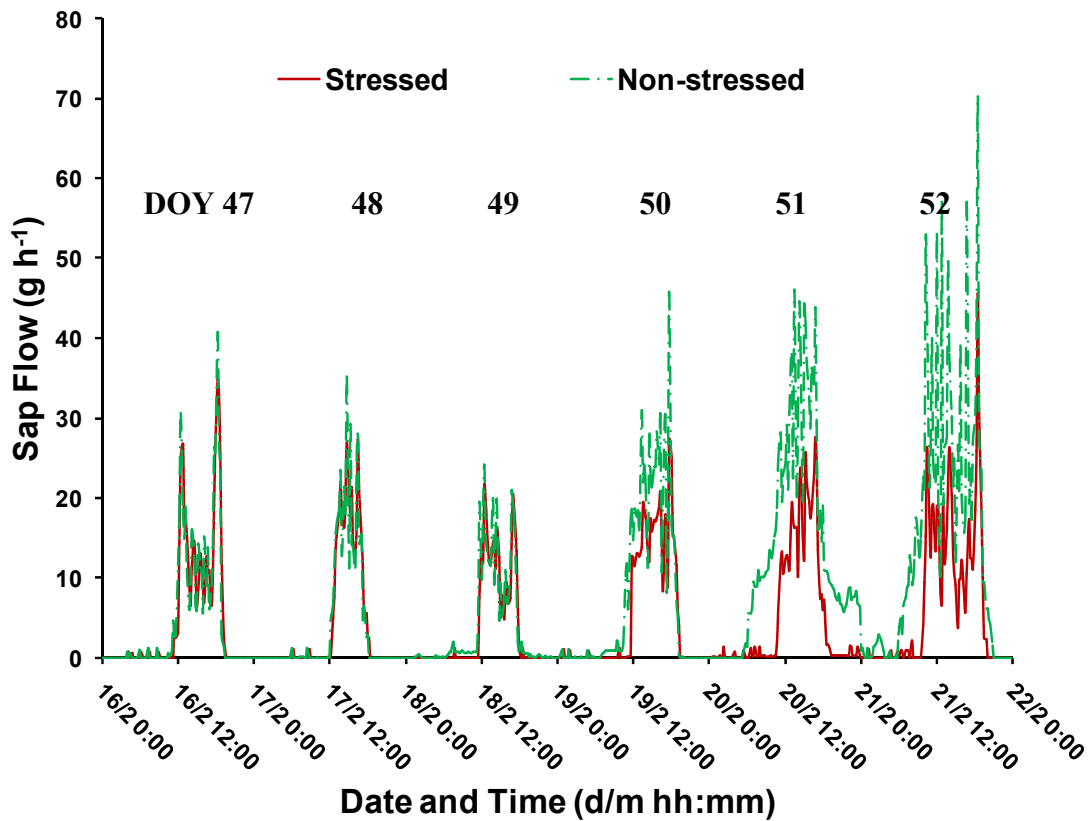


Figure 4.46: Sap flow in treatment A and D showing the number of days for sap flow to reduce from the day that stressing began (DOY 47).

DOY 47 and 52 were further analysed as shown in Figure 4.47. This was done to show that in healthy non-water stressed plants (such as those on DOY 47) sap flow will be similar (but not exactly equal). It was also done to dispel any suggestions of problems with the sap flow gauges as from Figure 4.46 it seems that the two are exactly equal on

DOY 47 which would be possible but highly unlikely. Statistically however, they can be said to be significantly equal ($P = 0.05$). The slight differences in sap flow in the two treatments on DOY 47 come from differences in leaf area as well as exposure to the elements due to positioning in the greenhouse. However on DOY 52 it is clearly shown that there is a significant difference ($P = 0.05$) between sap flow in stressed and non-stressed plants (see Table A-3 and A-4 in Appendix A for the significance tests).

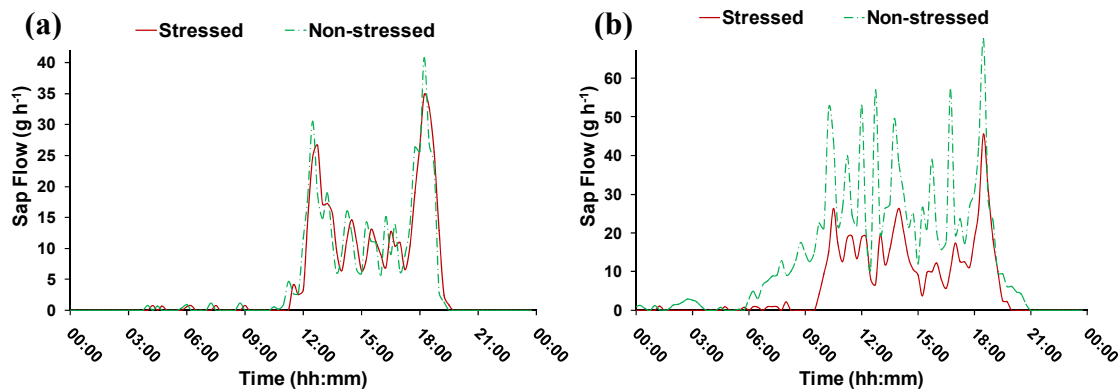


Figure 4.47: Sap flow in treatment A and D on a) DOY 47 and b) DOY 52 showing that sap flow was similar in the two treatments on DOY 47 but very different on DOY 52

Still despite the good response of sap flow to drought stress it was still not possible to determine some threshold value below which a grower can say that his plants are under drought stress. This is because sap flow is dependant on other environmental variables namely solar radiation, stomatal resistance, vapour pressure deficit, air temperature and others. Thus sap flow can only be interpreted in the context of these other environmental variables.

4.6 Summary

In summary, a number of plant indicators were examined for their use in determining irrigation quantity and timing as well as for their use in the early detection of drought stress in greenhouse grown tomato plants. Table 4.4 summarises the parameters/indicators that were investigated and their usefulness in determining irrigation quantity of

Results and Discussion

a greenhouse grown tomato crop, while Table 4.5 summarises their usefulness in drought stress detection and irrigation timing of a greenhouse grown tomato crop.

Table 4.4: The usefulness of the investigated plant indicators in determining water requirements (quantity) for a greenhouse tomato crop

Rank	Parameter	Units	Correlation to ET _o (R ²)	Equation For Calculating ET _o (units mm day ⁻¹) where <i>x</i> is the parameter
1	Mean Daily Leaf temperature	°C	0.648	ET _o = 0.476x - 7.894
2	Sap Flow Daily	mm day ⁻¹	0.640	ET _o = 1.222x + 1.927
3	Sap Flow Hourly	g hour ⁻¹	0.569	ET _o = 0.013x + 0.425
4	Daily Maximum Leaf Temperature	°C	0.454	ET _o = 0.193x - 3.001
5	MDS	mm	0.401	ET _o = 22.18x + 2.068
6	Fruit Growth	mm	0.221	ET _o = 1.154x + 2.095
7	LWP _{midday}	kPa	0.070	ET _o = -0.001x + 1.584
8	Stomatal Resistance	s m ⁻¹	-	-

In terms of irrigation quantity a number of indicators proved suitable with sap flow, leaf temperature and stem diameter leading the way. Sap flow is useful for irrigation scheduling on a daily basis as well at intervals of as little as 15 minutes. Stem diameter and leaf temperature on the other hand are only useful for scheduling irrigation at a minimum interval of a day or more. Fruit diameter, stomatal resistance and leaf water

potential were found to be not very useful in determining irrigation quantity. However they can work in conjunction with other indicators to do this. For example LWP_{midday} can be used in conjunction with measurements of LWP_{predawn} to determine the quantity of irrigation on a given day. This aspect needs to be tested though.

Table 4.5: The usefulness of the investigated plant indicators in the detection of drought stress and irrigation timing

Rank	Parameter	Units	Response Time	Threshold value (i.e. plant is stressed if this value is surpassed)
1	LWP_{midday}	kPa	2 days	$\leq -1600\text{kPa}$
2	Sap Flow	g hour^{-1}	3 days	None
3	Leaf – Air Temperature Difference	$^{\circ}\text{C}$	4 days	$\geq -2^{\circ}\text{C}$ between 3:00pm and 4:45pm
4	MDS	mm	5 days	None
5	Fruit Growth	mm	5 days	$\leq 0.470 \text{ mm day}^{-1}$ for fruit not close to maturity or maximum diameter
6	Stomatal Resistance	s m^{-1}	-	Increases with increasing irradiance in stressed plants

In terms of stress detection and the timing of irrigation most of the parameters have possibilities for indicating to the grower when plant water status is low and plants require irrigation. Leaf temperatures proved very useful in detecting stress and so did sap flow. However, threshold values could not be determined for sap flow but with leaf temperature it is advised to mist or irrigate crops if leaf to air temperature difference between 3:00 pm and 4:45 pm approaches -2°C . Fruit and stem growth rates can also be monitored, with any drastic reduction in their daily growth rates possibly being a result of

drought stress. LWP_{midday} was found to be one of the best indicators of drought stress and responds almost immediately to a deficit in plant water content. A threshold value of $\leq -1600\text{kPa}$ can be used and if LWP_{midday} falls below this then plants are in immediate need of water. Stomatal resistance on its own is difficult to use and should be interpreted in the context of the amount of radiation falling on the sampled leaf. However, if at any time stomatal resistance increases with increasing radiation then the plant is under drought stress and requires water urgently.

Thus, the experiment proved fruitful in answering the questions posed in Chapter One. More research however needs to be done in using these indicators and the results obtained operationally to determine if there are any significant differences in the amount of water used for irrigation and also if these differences result in differences in yield and fruit quality.

Chapter 5: Conclusions and Recommendations

For modern greenhouse tomato growers wishing to practice direct crop monitoring the use of five main indicators is suggested. These are sap flow, leaf temperature, stem diameter, fruit diameter and leaf water potential. Stomatal resistance, while useful for scientific purposes, was found to have no real operational value. However, more research on the use of stomatal resistance and the automation of its measurements could possibly change this. For operational automation of irrigation scheduling, sap flow is recommended as one of the most reliable methods. Care must be taken, however, to properly install the gauges and shield them from direct radiation which may cause errors. The sap flow gauges should also be periodically moved to prevent damage to the plant stem. The number of sap flow gauges to be used in a greenhouse to give a fair representation of the entire crop is also an issue which should be researched. Leaf temperature and stem diameter (specifically maximum daily shrinkage, MDS) can also be used in the automation of irrigation scheduling of greenhouse crops. When using thermocouples to measure leaf temperatures, care should be taken during installation and use as they require constant checking to see that they are in contact with the leaf and are taking correct readings. When using an infra-red thermometer to measure leaf temperatures care must be taken to only measure leaf temperature, and not the temperature of the surroundings. The use of dendrometers to measure stem diameter fluctuations, while useful are subject to disturbance when used in a commercial environment. Care must be taken to place dendrometers where they will not be disturbed by workers or other plants so as to reduce errors. Fruit diameter cannot be used in irrigation scheduling but should form an integral part of a system designed to monitor the health of greenhouse grown tomato plants.

The linear equations presented in many of the correlations done in Chapter Four can be used to determine irrigation amounts based on their respective plant parameters. However, they should still be tested and compared *in situ* against systems already being used for irrigation scheduling and the water use and yields used to determine whether

Conclusions and Recommendations

they are useful operationally or not. Also the opportunity for water saving exists through use of sap flow in irrigation scheduling. Rather than correlating sap flow to ET_c , calculated using the FAO-Penman-Monteith equation, it may be possible to use sap flow measurements alone as the quantity of water required for irrigation. This needs to be tested however to be sure that this does not result in drought stress. In terms of stress detection each of the parameters tested has value in their own respective way. A combination of all the indicators would be recommended. However sap flow, leaf temperature and LWP_{midday} are ideal and sensitive indicators of drought stress and at least one of these should be used for detection of plant water status and drought stress detection. Fruit diameter can also be useful but only in combination with one or more of the three suggested indicators.

For future projects the main recommendation would be to test the indicators against each other in operational automated systems to see how they perform in a commercial setting. This can be done to test the water use when irrigating based on each of these indicators as well as testing the difference between yield and growth when irrigating different treatments according to different indicators. Another important research area would be that of discovering and testing ways of automating stomatal resistance measurements and leaf water potential measurements. Other indicators may also need to be investigated such as chlorophyll fluorescence and predawn leaf water potential (LWP_{predawn}). The experiment was also only done during the fruit bearing and harvest stages and there is a need to do the experiment from the early development stage throughout the growth period to see if the results differ.

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Appendix A: Significance Tests

The following significance tests were based on the hypothesis H_0 and H_1 .

H_0 : There is no significant difference between the means of the samples in question.

H_1 : There is a significant difference between the means of the samples in question.

A p value of 0.05 was used in all cases.

Table A-1: The significance test relating to Figure 4.19a

t-Test: Two-Sample Assuming Equal Variances		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	24.74494792	23.73432292
Variance	38.29967868	24.69956612
Observations	96	96
Pooled Variance	31.4996224	
Hypothesized Mean Difference	0	
df	190	
t Stat	1.247550062	
P(T<=t) one-tail	0.106865659	
t Critical one-tail	1.972528138	
P(T<=t) two-tail	0.213731318	
t Critical two-tail	2.259304757	

Table A-2: The significance test relating to Figure 4.19b

t-Test: Two-Sample Assuming Equal Variances		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	22.42161458	22.25182292
Variance	10.274265	9.832232168
Observations	96	96
Pooled Variance	10.05324858	
Hypothesized Mean Difference	0	
df	190	
t Stat	0.371008431	
P(T<=t) one-tail	0.355522296	
t Critical one-tail	1.972528138	
P(T<=t) two-tail	0.711044592	
t Critical two-tail	2.259304757	

Table A-3: The significance test relating to Figure 4.47a

t-Test: Two-Sample Assuming Equal Variances		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	4.61166128	4.716890915
Variance	65.25853213	68.92202599
Observations	96	96
Pooled Variance	67.09027906	
Hypothesized Mean Difference	0	
df	190	
t Stat	-0.089007967	
P(T<=t) one-tail	0.464584664	
t Critical one-tail	1.972528138	
P(T<=t) two-tail	0.929169329	
t Critical two-tail	2.259304757	

Table A-4: The significance test relating to Figure 4.47b

t-Test: Two-Sample Assuming Equal Variances		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	6.398935342	14.11600427
Variance	82.35289716	261.3324323
Observations	96	96
Pooled Variance	171.8426648	
Hypothesized Mean Difference	0	
df	190	
t Stat	-4.078566037	
P(T<=t) one-tail	3.3305E-05	
t Critical one-tail	1.972528138	
P(T<=t) two-tail	6.661E-05	
t Critical two-tail	2.259304757	