δ^{13}C as indicator of soil water availability and drought stress in *Pinus radiata* stands in South Africa

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Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Forestry at the Faculty of AgriSciences , University of Stellenbosch

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March 2011

Declaration

By submitting this thesis electronically I, the undersigned, declare that the entirety of the work contained therein is my own, original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:... Date:..

Abstract

This study investigated the use of carbon isotopes as a potential measure for water availability and drought stress in *Pinus radiata* in the Western and Southern Cape, South Africa. An understanding of water availability and its variation in space is fundamental to the implementation of increasingly site-specific management regimes that have the potential to greatly improve productivity across sites in the region.

Fifteen plantation compartments situated on water shedding sites were identified where good weather data existed and a water balance model could be run. In addition, late wood samples were analysed from four co-dominant trees in the same stand to determine the $\delta^{13}C$ values of five tree rings, each representing a specific growth year before first thinning. Detailed water balances were constructed for each trial site and drought stress indicators (a) relative canopy conductance (after Granier *et al., 2000*) and (b) the ratio of actual to potential evapotranspiration (supply / demand ratio), were related to $\delta^{13}C$ values in latewood.

Maximum available soil water ranged from 52 to 313 mm across trial sites. The water balance model used adequately described soil water availability throughout each growing season and indicated that stand stress due to the lack of available soil water mainly occurred during the summer months of the study period (November to April). The supply / demand ratio for this period as well as the relative canopy conductance proved to be good measures of drought stress. The six-month supply demand ratio (calculated for the period November to April) ranged from 0.04 to nearly 1 (winter rainfall zone) and 0.35 to 1 (all-year rainfall zone) and were strongly related to $\delta^{13}C$ values ($p < 0.001$; $r^2 = 0.7822$).

It appears that using $\delta^{13}C$ values, it may be possible to classify sites into three water availability classes. This classification may assist in the implementation of intensive silvicultural operations on an increasingly site-specific basis. Where sites are enriched with water from lateral flow or upslope positions, $\delta^{13}C$ may be the only reliable technique to quantify soil water availability.

Opsomming

Hierdie studie ondersoek die gebruik van koolstof isotope as 'n moontlike maatstaf vir die beskikbaarheid van water en droogtestremming in *Pinus radiata* in die Wes-en Suid-Kaap, Suid-Afrika. 'n Begrip van die beskikbaarheid van water en die ruimtelike variasie daarvan is fundamenteel vir die implementering van groeiplek-spesifieke bestuur sisteem wat die potensiaal het om baie verbeterde produktiwiteit oor persele in die streek teweeg te bring.

Vyftien plantasievakke, geleë op waterskeidingsterreine is geïdentifiseer waar goeie weer data bestaan en 'n water balans model uitgevoer kon word. Daarmee saam is laathout monsters vanuit vier ko-dominante bome in dieselfde kompartement geanaliseer en die $\delta^{13}C$ waardes van laathout in vyf jaarringe bepaal wat elk 'n spesifieke jaar van groei voor die eerste dunning verteenwoordig. Gedetailleerde water balanse is vir elke proef perseel bereken en aanwysers van droogtestremming, nl.: (a) relatiewe kroon geleiding (na Granier *et al.*, 2000) en (b) die verhouding van die werklike teenoor potensiële evapotranspirasie (vraag / aanbod verhouding) is gekorreleer met $\delta^{13}C$ waardes in laat hout .

Die maksimum hoeveelheid water beskikbaar op die verskeie proefpersele wissel van 52 tot 313 mm. Die water balans model wat gebruik is beskryf die beskikbare grondwater met genoegsame akkuraatheid. vir die hele groeiseisoen. Die model dui ook aan dat die kompartemente droogtestremming as gevolg van die gebrek aan beskikbare grond water ervaar gedurende die somer maande van die studie tydperk (November tot April). Die vraag / aanbod verhouding vir hierdie tydperk, asook die relatiewe kroon geleiding is geskik om as maatstawwe van droogtestremming gebruik te word. Die vraag / aanbod verhouding (bereken vir die tydperk November tot April) het gewissel van 0,04 tot byna 1 (Winter reënval gebied) en 0,35 tot 1 (die heel jaar reënval sone) en is sterk verwant aan $\delta^{13}C$ waardes ($p < 0{,}001$; $r^2 = 0{,}7822$).

Dit blyk dat met die gebruik van $\delta^{13}C$ waardes, dit moontlik kan wees om kompartemente te klassifiseer in drie klasse van water beskikbaarheid. Hierdie klassifikasie kan help met die implementering van intensiewe boskultuur

bedrywighede op 'n meer vak-spesifieke basis. Waar vakkeverryk is met water vanuit laterale vloei of hoër liggende posisies, mag $\delta^{13}C$ dalk die enigste betroubare tegniek wees om die beskikbaarheid van water te kwantifiseer.

Acknowledgements

I would like to acknowledge:

My supervisors, Dr. Ben du Toit and Dr. Caren Jarmain for their support and guidance.

Professor Thomas Seifert who's input and discussions has been invaluable.

Colleagues, staff and students at the Department of Forest and WoodScience for their help with field work, sample preparation and support.

Mountain to Ocean Forestry for allowing the research to be carried out on their properties.

The FORSIM project for creating the opportunity to obtain and parameterise the HyMo water balance model for plantation stands in the Southern and Western Cape.

THRIP for financial support.

The ARC, SAWS and SAEON for supplying weather data.

My family, friends and loved ones for their patience, support and encouragement.

Our Heavenly Father, His blessings and grace knows no bounds.

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List of Abbreviations

ARC	Agricultural Research Council
BA	Basal Area
DBH	Diameter at Breast Height (1.3 m)
Dq	Quadratic Mean Diameter
ETa	Actual Evapotranspiration
ETa/Etp	Supply/demand ratio
Eto	Evapotranspiration
Etp	Potential Evapotranspiration
FC	Field Capacity
g_c	Canopy Conductance
g_{cmax}	Maximum Canopy Conductance
GDD	Growth Degree Days
GPP	Gross Primary Production
G_r	Growth Degree Days weighted against precipitation
IRMS	Isotopic Ratio Mass Spectrometry
MTO	Mountain to Ocean Forestry
PDB	Pee Dee Belemnite
REW	Relative Extractable Water
RH	Relative Humidity
Rs	Solar Radiation
SAEON	South African Environmental Observation Network
SAWS	South African Weather Services
SDI	Stand Density Index
SI	Site index at age 20
VPD	Vapour Pressure Deficit
WP	Wilting Point
Δ	Carbon Isotope discrimination
δ	Stable Isotope Composition

Chapter 1: Introduction

Water lies at the heart of many problems in Southern Africa (Jones and Van der Walt, 2004). One of the major problems is the distribution and the reliability of water resources. Plant-available water plays an important role in tree growth; it is a major consideration when decisions on plantation establishment and site-species matching are made (Smith *et al.*, 2005). There are also financial implications to water when looking at the legal aspects of growing plantations. Water licenses and water tax make up part of the cost of a plantation. Other issues include ethical and environmental considerations which does have an effect on how water is utilised within the forest industry and within the landscape as a whole. The potential for expanding forests into marginal areas (areas with low precipitation) does require knowledge of how water is utilised during stand growth (Pammenter, 2002). For the forest manager, it is critical to understand how tree stands will react to changes in the availability of water and other resources. This knowledge will assist in predicting the response to management practices like thinning and fertilisation in the presence of varying degrees of drought stress across different sites. Drought stressed trees are less likely to respond to increased availability of other growth resources. For example, drought stressed trees seldom show significant growth responses following fertilisation (Linder *et al.*, 1987) and fertilising drought stressed stands would constitute an unnecessary financial expense.

By knowing if a stand is under drought stress, unnecessary expenses that would have been incurred by fertilising unresponsive stands can be minimised. However, currently there are no quick, cost effective techniques available to plantation managers to determine the amount of drought stress a stand is experiencing and how it will affect management practices. Meteorological data like precipitation, temperature and humidity can assist to some degree in determining drought stress, but the data provided by weather stations are not always representative of all the compartments on an estate. In most cases (in South Africa) weather stations are concentrated around agricultural production areas while the coverage of the commercial forest area is inadequate. Stations that do cover plantation estates most often only measure rainfall, and data which would have been useful to compile water balances i.e. A-pan readings, temperature data and relative humidity data sets are seldom recorded.

Carbon isotope fractionation has been studied since the 1980's and it has been proven that a relationship exists between the extent of carbon isotope discrimination and plant water availability (Zhang *et al.*, 1996, Marshall and Zhang, 1994, Farquhar *et al.*, 1989). Using isotope fractionation could possibly allow us to be able to predict the amount of drought stress a stand is experiencing over specific periods during its lifetime. Using this information, decision makers will be able to make more informed decisions about silvicultural practices such as fertilisation, thinning, and site-species matching.

Research Questions

To be able to use carbon isotope fractionation as a management tool, some issues have to be researched in order to prove the usefulness and worth of this technique. The issues are:

- The correlation of $\delta^{13}C$ between individual trees.
- $\delta^{13}C$ and its correlation with water availability.
- $\delta^{13}C$ as a useful management tool.

Usable correlations in the above will enable decision makers to get a reliable estimate of the degree of plant-available water in a stand over a specified time period. The use of carbon isotope fractionation could thus replace complex, data-intensive calculations to determine water availability, and at the same time increase accuracy of this estimate especially on compartments remote from weather stations. Carbon isotope fractionation may thus become an instrument to improve decision making in forest management and planning.

Chapter 2: Literature Review

2.1 Introduction

The timber production in forests depends on trees obtaining growth resources from their environment and using these resources to fix atmospheric CO_2 into biomass (Binkley *et al.*, 2004). Intensive management of pine plantations has become the norm throughout the world as research have shown that production can be improved through the manipulation of resource availability. Such manipulations include soil preparation, the deployment of genetically improved planting stock, vegetation management and fertilisation (Albaugh *et al.*, 2004). The application of these methods stems from the knowledge that productivity is mostly dependant on resource availability with emphasis on nutrients and water availability. There is however one aspect of the aforementioned that is not entirely controllable. Water availability can to some extent be manipulated through thinning practices and the negative effect of soil water shortages can be mitigated to some degree by proper site species matching. Improving soil water availability in forests by implementing large scale irrigation is not an economically viable option in a water-scarce country. Soil water availability is therefore largely determined by annual precipitation and its distribution, atmospheric conditions and the site characteristics on which a stand has been established.

If nutrition level and water availability on a site is low, then the stand would have low productivity. This is largely true, but low productivity can be improved somewhat by implementing silvicultural practices aimed at improving productivity to the most responsive stands, as is argued below. There are numerous factors that contribute towards stand growth. Temperature, radiation, water and nutrients all have an integral role in stand growth (Byrne *et al.*, 1986; McMurtrie *et al.*, 1990b; Snowdon *et al.*, 1998). The concentration of O_2 and CO_2 in the soil and air also affects stand growth (Carey *et al.*, 1996; Sands *et al.*, 2000). Plants themselves also contribute to some of the factors that affect growth. Some of these factors include their water use efficiency, radiation-use efficiency and drought response mechanisms like stomatal regulation (Sheriff *et al.*, 1986; Ripullone *et al.* 2000). The last mentioned factors are governed by the interaction between the planted genotype and the site on which it is planted (Stape *et al.,* 2004; McKeand *et al.,* 2006; du Toit *et al.,* 2010). Silvicultural operations carried out in forest stands aim to manipulate some of these factors in order to enhance growth and increase productivity. Even though factors like nutrition, intercepted solar radiation and plant genetics can be manipulated, factors like the CO_2 and O_2 concentrations in the environment cannot be manipulated by forest managers. In the discussion that follows the focus will be on the most important factors driving growth, the interactions of which are (to some extent) controllable by the silviculturalist.

The interaction between nutrient availability, soil water supply and stand growth factors eg. genetic material, silvicultural management, can be demonstrated graphically by a simplified growth triangle as depicted in *Figure 2.1*. Stand productivity (the annual increase in timber volume) will be high when there is an adequate supply of water and nutrients. The efficiency with which the stand will utilise these resources is strongly dependent on the availability of the resources because it will affect photosynthetic efficiencies as well as carbon allocation patterns in stands (Binkley et al., 2004, Stape et al., 2008, du Toit, 2008). If any component of this triangle is limiting, e.g. the stocking of the stand is sub optimal or a poor species choice was made, stand productivity will not be optimal. Extensive tree breeding programs are found within every forestry company in the world. The best plants within these programs are used to produce enhanced genotypes which deliver optimal timber characteristics under given growing conditions. The water component of the growth triangle is indirectly incorporated into the breeding programs as the species used would be adapted to the growing conditions to which the genotypes would be subjected. The effect of available soil water on stand productivity has not been investigated very intensively in South African forestry; it appears rather that research on the effects of afforestation on stream flow reduction has been the highest priority over the last half century (Van Lil *et al.*, 1980; Scott and Smith, 1997; Hughes 2006; Dye and Versfeld, 2007).

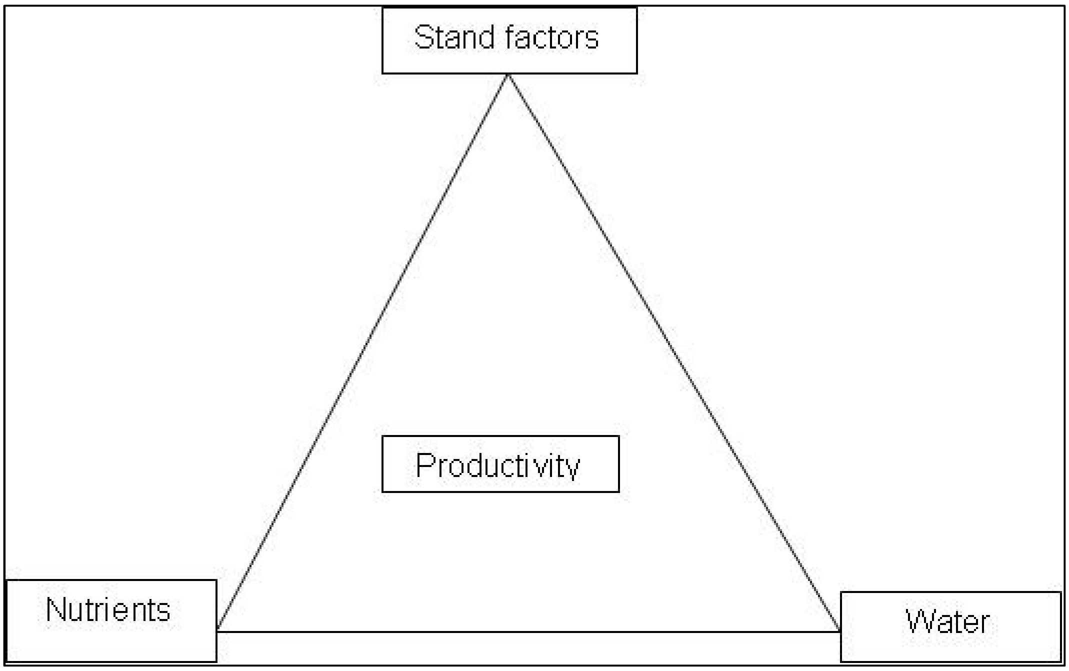

Figure 2.1: The Simplified Growth Triangle. Limited supply of water and nutrients to a stand will lead to sub-optimal or stunted growth of the stand.

Available soil water and nutrients, and in particular the interaction between them, strongly influences stand productivity (McMurtrie *et al.,* 1990b; Boosma and Hunter, 1990; Nambiar, 1990; Sands and Mulligan, 1990; Trichet *et al.,* 2008; Stape *et al.*, 2008). Although available soil water of a stand is largely outside the direct control of the forester, it is the interaction between soil water availability and other resources that can be manipulated by implementing a site-specific management style. In this study we will however focus on understanding the water component of the triangle in Figure 2.1.

A major determinant of growth under water-limited conditions is the efficiency with which a tree will use the available water (Korol *et al.*, 1999). This can clearly be seen from the production ecology equation which states the following (based on Monteith, 1977): As the efficiency of the resource use increase, the Gross Primary Production (GPP) of a stand will increase.

Gross Primary Production (GPP) = resource availability x proportion of resource supply captured x efficiency of resource use (Eq. 1).

To illustrate the effect of Eq. 1, Binkley *et al.* (2004) presented data on an irrigation trial on a clonal *Eucalyptus* stand in Brazil. Irrigation of this trial increased the annual water availability from 1.21 to 2.17 m^3 m^{-2}. The percentage water supply used by the stands decreased from 74 (rainfed) to 58 % (irrigated). These figures seem to be counter intuitive. How can trees use a smaller fraction of water if they are supplied with more? Also, incoming light was not altered by the irrigation, but the percentage light capture increased from 63 to 71 %. The increase in the amount of light capture and the decrease in water use percentage were both notably less than the percentage increase in GPP (increased from 6.1 to 11.3 kg m^{-2} per year). The fact that fewer resources were used per unit of GPP produced indicate that the efficiency with which these resources were used was increased. But what exactly is the driving force behind this increase in resource use efficiency?

Trees absorb water from the soil and it is transported through the xylem to the canopy from where it evaporates *via* the stomata on the leaves through a process called transpiration. Various climatic factors, including solar radiation, air temperature, relative humidity and wind are the most important factors that determine the magnitude of transpiration. The amount of moisture that can evaporate from the leaves is principally determined by the vapour pressure deficit (VPD) which exists between the leaf and the air, resulting in a close relationship between transpiration and VPD (Morris and Benyon, 2005). The effect of VPD on tree water use is however complicated by the sensitivity of the stomata to climatic conditions. As the VPD increase, the stomata will progressively close to minimize the amount of water loss through transpiration. In addition, transpiration may also be limited by the xylem pressure potential which is related to the soil water content (Landsberg and Waring, 1997; Granier *et al.*, 2000). To be able to absorb CO_2 from the atmosphere during photosynthesis, the stomata of a tree has to be open. Because less CO_2 is now taken up into the leaves, the proportion of the resource captured (Eq. 1) decreases which will lead to a decrease in GPP. A leaf which is adequately supplied with water can fix more CO_2 per unit of intercepted light than a leaf experiencing drought stress and of which the stomata are partially closed (Binkley *et al.*, 2004). Logically it will follow that by increasing the supply of one limiting resource, the efficiency by which other resources are used should be increased throughout the stand (Harrington *et al.,* 2001; Binkley *et al.,* 2010; Stape *et al.,* 2010).

In short, if the supply of water to a stand is increased, or the water-use efficiency of the stand improves, the efficiency with which the stand will use available nutrients will also increase. This poses a new challenge to the forest manager: When exactly is the availability and use of water in a stand substantial enough so that available nutrients will be used efficiently and carbon assimilation will be maximised? Researchers have already touched on this phenomenon superficially in empirical fertiliser trials. Researchers observed that fertilised stands yielded comparatively lower responses on dry sites or on sites fertilised during drought conditions (Schönau and Herbert, 1980; Herbert, 1990). Simply put: although the availability of water is beyond the direct control of the forester, the response to other silvicultural practices (e.g. fertilization) can still be improved or even optimised on the landscape or plantation level by selectively implementing these practices on more responsive compartments only.

To answer the question of resource use efficiency more comprehensively, an understanding of the amount of drought stress a stand is experiencing is needed. As mentioned, trees under drought stress will have partially closed stomata and when adding the shortage of the water resource and the decreased proportion of assimilates produced by photosynthesis into Eq. 1, the GPP of a stand will be greatly reduced. If drought stress in a stand can be quantified, the next step would be to predict whether management practices such as fertilisation would be effective, economically viable and advisable on the site. But just how can the degree of water-stress a stand is experiencing over seasons and years be quantified for use in management decisions (i.e. on a forest compartment or landscape scale)? This question can potentially be answered by a number of approaches, and some of the most promising ones are listed below:

1. Constructing a daily water balance using detailed climatic and soils information (Running and Coughlan, 1988; McMurtrie *et al*., 1990a; McMurtrie and Landsberg, 1992; Myers and Talsma, 1992; Dye *et al*., 2008). This will only be feasible where a very dense network of detailed weather data is present on the landscape and

detailed soil information is available, which is often not the case in Southern African forests.

2. Establishing a relationship between stand drought stress and remotely sensed indices of evapotranspiration on a limited number of experimental sites and extrapolating this across the landscape (Ahmad *et al.*, 2005; Bastiaanssen and R. Harshadeep, 2005; Bastiaanssen *et al.*, 2005; Van Dam *et al.*, 2005). This technique relies on an array of satellite images of a particular forest estate that are well spaced in time intervals which is not always available.

3. Establishing a relationship between stand water use and the ratio of carbon isotopes sequestered by the stand during photosynthesis and extrapolating this across the landscape (Farquhar and Richards, 1984; Ehleringer and Cooper, 1988; Warren *et al.*, 2001). One major advantage of this technique is that the isotope ratio is preserved in the tree rings and that it can be sampled retrospectively.

For this thesis, the focus is on the carbon isotope fraction option. Carbon isotope discrimination is closely linked to the intrinsic transpiration efficiency (Korol *et al.*, 1999). Intrinsic transpiration efficiency, as used here, is defined as the ratio of CO_2 assimilated and water transpired at a given VPD. It is determined by the difference between the atmospheric (c_a) and the intercellular (c_i) CO_2 concentrations. The mean c_i/c_a value can also be inferred from the analysis of carbon isotope discrimination (Δ) of samples (Farquhar *et al.*, 1982; Farquhar *et al.*, 1989; Geuhl *et al.*, 1995; Duquesnay *et al.* 1998;.Osório *et al.*, 1998; Feng, 1999).

At this point it is important to distinguish between carbon isotope discrimination (Δ) and the stable isotope composition (δ) of a plant. The former refers to the degree of discrimination which occurs while the latter refers to the physical amount measured within a plant. Isotopic composition values of plants are negative, whereas the discrimination taking place during diffusion of CO_2 into the plant through the stomata and Ribulose-1,5-biphosphate carboxylation has positive discriminations (Farquhar *et al.*, 1982).

2.2 Carbon isotopes

Atmospheric CO_2 consists of approximately 98.9% carbon-12 (^{12}C) isotope and 1.1% carbon-13 isotope (^{13}C) (O'Leary, 1988). Both of these are stable isotopes of carbon. When a plant absorbs CO_2 from the atmosphere during photosynthesis, it discriminates against ^{13}C due to small differences in its chemical and physical properties. The isotopic composition of carbon in tree rings has proved invaluable to scientists who have successfully reconstructed historic palaeo-climates from $\delta^{13}C$ values. This is due to the fact that the isotopic composition can "document" variations of atmospheric CO_2 and the physical response to increasing global atmospheric CO_2 concentrations (Lipp *et al.*, 1991, February and Stock, 1999). Since the beginning of industrialisation in 1850 AD there has been an increase of ^{13}C depleted CO_2 released into the atmosphere due to the combustion of fossil fuels and therefore palaeoclimatologists have to adjust $\delta^{13}C$ values to obtain a clear pattern of climate change.

2.2.1 Measuring carbon isotopes

The amount of ^{13}C in carbon dioxide is normally measured using a mass spectrometer and is expressed as the ratio R, defined by:

$$R = {}^{13}CO_2/{}^{12}CO_2 \qquad \text{(Eq. 2)}$$

To obtain CO_2 from plant material, the material is converted using combustion. Natural materials produce an R of approximately 0.0112 and only the last digit of this ratio varies. R values are generally converted to values of $\delta^{13}C$ for convenience:

$$\delta\ {}^{13}C = \left[\frac{R(sample)}{R(s\tan dard)} - 1\right] x\ 1000 \qquad \text{(Eq. 3)}$$

where the standard is the CO_2 obtained from Pee Dee Belemnite (PDB) (Craig, 1957). PDB as primary reference material has been exhausted since the 1950's and the calibration of measurements are now done using new reference materials

(Gröning, 2004). A more in depth discussion about various reference materials and standards used are presented by Gröning (2004). It is important to note that all materials used as reference materials, whether they are inorganic, organic or synthetic, have to meet standards set by the International Atomic Energy Association (IAEA) with regards to, among others, the homogeneity and stability of the material. The units of $\delta^{13}C$ are expressed in "per mill" or ‰. More negative values of $\delta^{13}C$ indicates an enrichment in ^{13}C of the material and a more positive $\delta^{13}C$ is indicative of a depletion in ^{13}C (O'Leary, 1988).

2.2.2 The extent of isotope discrimination

One of the most widely used models to explain changes in the isotopic sequences of tree rings were described by Francey and Farquhar (1982). This model states that there are two stages during which discrimination against ^{13}C takes place. During the absorption of atmospheric CO_2 through the stomata, the isotopic content of the CO_2 is altered by fractionation. The leaf CO_2 is then altered by Ribulose-1,5-biphosphate carboxylation.

Farquhar *et al.* (1982) determined that the extent of discrimination that takes place during the uptake of CO_2 from the atmosphere is determined by the ratio of intercellular to ambient CO_2 concentrations (c_i/c_a). This is the balance between the inward diffusion of CO_2 which is controlled by stomatal conductance (g_c) and CO_2 assimilation determined by photosynthesis (A) (Walcroft *et al.*, 1997). If stomatal diffusion is rapid (g_c is high), the predicted $\delta^{13}C$ value will be more negative (more enriched with respect to ^{13}C) than when stomatal diffusion is slow (g_c is low) (O'Leary, 1988). Therefore, environmental factors such as water availability, or physiological factors such as nutrition levels which affects both A and g_c will be reflected in the $\delta^{13}C$ of plant tissues.

The theory behind the relationship between the isotopic composition of C_3 plant leaves and c_i/c_a is described by Farquhar *et al.*, (1982). Tree development is dependent upon the climatic variables that occurred during the growing season and the growth rings (tree rings) represent the integration of these variables (Pawelczyk and Pazdur, 2000). The carbon isotope composition of tree rings is therefore dependant on the isotopic conditions that prevailed at the time the carbon was fixed into the plant's biomass and the relationship between carbon isotope discrimination (Δ) and c_i/c_a can be expressed as:

$$\Delta = a + (b - a) \, {}^{c_i}\!/_{c_a} \qquad \text{(Eq. 4)}$$

where Δ is calculated as:

$$\Delta = \frac{{}^{13}C_a - {}^{13}C_p}{1 + {}^{13}C_p} \qquad \text{(Eq. 5)}$$

where $\delta^{13}C_a$ is the estimated current atmospheric isotopic composition of -8‰, $\delta^{13}C_p$ is the isotopic composition of the plant material, a is the discrimination associated with diffusion (4.4‰) and b is a fitted parameter of which the value is determined by the discrimination of ribulose biphosphate carboxylase/oxygenase (27‰). By calculating Δ (Eq 5) and substituting its value into (Eq 4), together with a and b, Eq 4 can be rearranged to estimate the mean intrinsic transpiration efficiency as follows:

$$c_i/c_a = (\Delta - 4.4‰)/22.6‰ \qquad \text{(Eq. 6)}$$

2.3 $\delta^{13}C$ determination

2.3.1 Basic overview of mass spectrometry

The actual measurement of $\delta^{13}C$ values is done using a technique called Isotopic Ratio Mass Spectrometry (IRMS). Mass spectrometry is used to obtain a quantitative assessment of a sample (Brand, 2004). The quality of the data obtained from mass spectrometry is dependent on the ability of the equipment to detect all components that a sample consists of with a constant sensitivity no matter how complex the chemical makeup of the sample might be.

As the principles on which mass spectrometry is based and how the equipment works fall outside the scope of this project, only a brief explanation of this process will be given. There are various models of mass spectrometers available on the market. Duel inlet systems rely on a mechanism called the “changeover valve”. This valve allows the system to switch between two gasses, each of which then enters the mass spectrometer. The duel inlet system will typically allow a subsample to the sample gas to enter the mass spectrometer after which the reference gas is injected and the composition of the sample is determined.

It rarely happens that the sample to be analysed is in the form of a pure gas. For this reason samples are converted into a gaseous form using combustion prior to analysis. Products derived from the combustion include gasses like $CO_{2,}$ carbon monoxide, hydrogen, sulphur dioxide, methane, ethane, nitrous gasses etc. Many of these gasses are toxic and carcinogenic and they are filtered out of the gas mixture using copper columns. The remaining gasses are then separated using their chemical properties before being fed to the mass spectrometer through thin tubes.

Once inside the mass spectrometer, sample molecules enter an ion source and some of the molecules are ionized by being bombarded with electrons. The ions that resulted from the bombardment are then sent through a magnetic field or sector which acts like an optical prism in geometrical optics. As light enters a prism it is refracted and different wavelengths within pure white light travels through different parts of the prism. Ions passing through a magnetic field will act in the same way. Lighter ions will follow shorter paths through the magnetic field and the heavier ions have longer paths. The resultant ion beams are then focussed on a focal plane where multiple Faraday Cups are positioned which detect the various ions and the masses of different compounds within the sample is then measured after various corrections and scaling of δ values.

There are various methods used to determine the $\delta^{13}C$ content of timber. These are based on the analysis of single constituents of timber or the analysis of extractive free or whole wood samples.

2.3.2 Lignin analysis

Due to the fact that lignin is one of the components in timber which has the slowest rate of decay, the analysis of lignin to determine $\delta^{13}C$ is one which is preferred by palaeaoclimatologists to reconstruct past climates. This is a very complicated method which involves the cryogenic separation of gasses. Due the complexity of the analysis, this method was not feasible within this study.

2.3.3 Alpha cellulose analysis

During this analysis, timber samples are digested (pulped) and then purified to produce alpha cellulose (hereafter α-cellulose). α-cellulose as used here is defined as the proportion of holocellulose that is insoluble in a 17% solution of NaOH. The reason for this purification is that holocelluose can be enriched in ^{13}C by as much as 0.39 ‰ relative to the purified α-cellulose.

There are already various ways by which the α-cellulose can be extracted from the timber. Most of these methods deliver a result that agree within the precision range of the isotope ratio mass spectrometry (IRMS) method that is used (±0.2‰) (Boettger *et al.* 2007). A widely used technique for extracting α-cellulose from whole wood samples has been described fully by Green (1963). This technique has been modified to facilitate the rapid batch processing of small samples. Between 25 and 50 samples can be treated at once (Loader *et al.* 1997)

Macfarlane *et al.* (1999) describes a process where an acid-catalyzed solvolytic method has been adapted for this purpose. Wood meal was treated with acidified di-glycol methyl ether (diglyme) and then analysed using a mass spectrometer. This method delivers results within 24 hours and can be used to process more than 100 samples per day. Assuming that there was 5% residual lignin content in the crude cellulose residue and that the difference between the $\delta^{13}C$ values of lignin and cellulose was a constant 3‰, it was concluded that the crude cellulose extracted using the modified diglyme-HCL method underestimated $\delta^{13}C$ of cellulose with an almost constant value of 0.2 – 0.3‰.

2.3.4 Whole wood analysis

The analysis of whole-wood samples is often found in literature. This method is more complicated than simply using α-cellulose since variation of ^{13}C content exists in the different components of timber. The $\delta^{13}C$ of lignin is generally 2 – 4‰ less than cellulose and is thought to be the greatest source of difference between $\delta^{13}C$ of cellulose and holocellulose (Wilson and Grinstead, 1977, Benner *et al.*, 1987). Because of this, Walcroft *et al.* (1997) suggested that the $\delta^{13}C$ of whole wood samples can be estimated by the simple mass equation:

$$\delta^{13}C_{wood} = (0.40 \text{ cellulose})(-23.8‰)+(0.31 \text{ hemicellulose})(-24.3‰)+(0.27 \text{ lignin})(-27.9‰)+(0.02 \text{ others})(-25.2‰) \qquad \text{(Eq. 7)}$$

The values given here were taken from Uprichard (1991). The question that arises from this is just how accurate will the determination of $\delta^{13}C$ from whole wood samples be? Warren *et al.* (2001) determined a positive correlation ($r^2 = 0.92$) between $\delta^{13}C$ of cellulose and $\delta^{13}C$ of whole wood. This correlation is depicted in Figure 2.2.

West *et al.* (2001) did similar trials and found that the regressions of leaf tissue and whole wood sample $\delta^{13}C$ against holocellulose $\delta^{13}C$ produced r^2 values of 0.74 and 0.84 respectively. Thus, it is possible to determine the $\delta^{13}C$ of whole wood samples and simply using the regression equation to convert the values to $\delta^{13}C$ for cellulose but this method could induce some additional variation when the data is going to be used for future extrapolations.

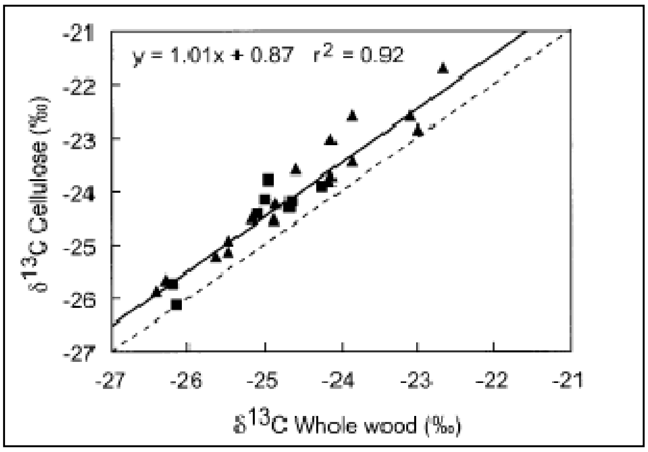

Figure2.2: The relationship between the stable C isotope composition ($\delta^{13}C$; ‰) of whole wood and $\delta^{13}C$ of cellulose extracted with Diglyme-HCl (Macfarlane *et al.* 1999). Data points are single measurements of wood samples pooled from five trees of *Pinus radiata* (■) and *P. pinaster* (▲) (From Warren *et al.*, 2001).

2.3.5 Whole wood sampling *vs*. α-cellulose extraction

Various studies have shown that there is a close relationship between the $\delta^{13}C$ values of cellulose and whole-wood samples. This has been proven for beech (*Fagus sylvatica*) (Elhani *et al.*, 2005), mangrove (*Rhizophora mucronata*) sapwood (Verheyden *et al.*, 2005), *Eucalyptus globulus*, *Pinus radiata* and *Pinus pinaster* (McFarlane *et al.*, 1999) and a few other species. Borella *et al.* (1998) described good correlations (r^2>0.8) between the cellulose and bulk wood $\delta^{13}C$ values for two hardwood species. However, it was also recommended that the extractives should be removed from coniferous species as these species tend to have variable and high resin content.

The use of extracted cellulose could be preferred theoretically because:

1) Cellulose is immobile and does not migrate from the tree ring in which it was formed.
2) During the synthesis of cellulose from photosynthates, there are fewer reactions (fractionations) that occur along the biosynthetic pathway than in the case of lignin and other wood constituents.

When analysing whole-wood samples, $\delta^{13}C$ values are more affected by lignin, resins and other mobile carbon reserves like carbohydrates which can migrate between adjacent tree rings. Also the amount of these other constituents may vary widely between sapwood and heartwood (Tans *et al.*, 1987, Leavitt and Danzer, 1993, Sheu and Chiu, 1995). Wilson and Grinstead (1977) suggested that because the ratio of lignin and cellulose concentrations of whole-wood is variable, the use of whole-wood to determine $\delta^{13}C$ would represent the mixing ratio of lignin and cellulose and not the water use efficiency of the plant being studied. D'Alessandro *et al.* (2004) found that the $\delta^{13}C$ of whole wood *Pinus radiata* samples deviate significantly ($P<0.01$) from the $\delta^{13}C$ of cellulose. This has been attributed to the possible effect of secondary compounds, such as oleoresins and lignin. The amount of these compounds can vary from year to year as well as migrate between and within tree rings. In such cases, cellulose $\delta^{13}C$ would be a more stable and reliable index to use compared to whole-wood samples.

2.4 $\delta^{13}C$ in wood and water availability

Water availability on a site will have an influence on $\delta^{13}C$ of plant tissues. This has been illustrated by various trials which have been conducted in the past. Walcroft *et al.* (1997), working on *Pinus radiata*, reported a difference of 3‰ between Kawerau forest (long term rainfall of 1791mm) and Balmoral Forest (long term rainfall of 658mm) for the period 1991 – 1993 (Kawerau) and 1993 – 1995 (Balmoral). The seasonal difference between $\delta^{13}C$ values for each plantation is depicted in Figure 2.3. The $\delta^{13}C$ values of each forest are clearly less negative during the late summer months when water availability is lower, suggesting a lower transpiration rate. Lipp *et al.* (1991) have also found a strong relationship between mean annual precipitation and the $\delta^{13}C$ values of Fir trees (*Abies alba*) from the black forest ($r = -0.68$).

The relationship between $\delta^{13}C$ and indicators of drought stress, such as pre-dawn water potential (Ψ), has not been very well examined in conifers (Warren *et al.*, 2001). It is thought that by linking $\delta^{13}C$ with the water status of the plant a much better relationship can be produced than when only using rainfall values. Measuring

Ψ and $\delta^{13}C$ in coniferous stands allowed Warren *et al.* (2001) to produce a correlation between the two variables, and the correlation coefficient (r) values for the linear regression between Ψ (x-axis) and $\delta^{13}C$ (y-axis) ranged from $r^2 = 0.38$ to $r^2 = 0.58$. Although this seems like a weak correlation, the overall trend does show that $\delta^{13}C$ is less negative during periods of drought stress (when Ψ has large negative values), and that wetter periods are associated with less negative values of Ψ and enriched (more negative) $\delta^{13}C$ values.

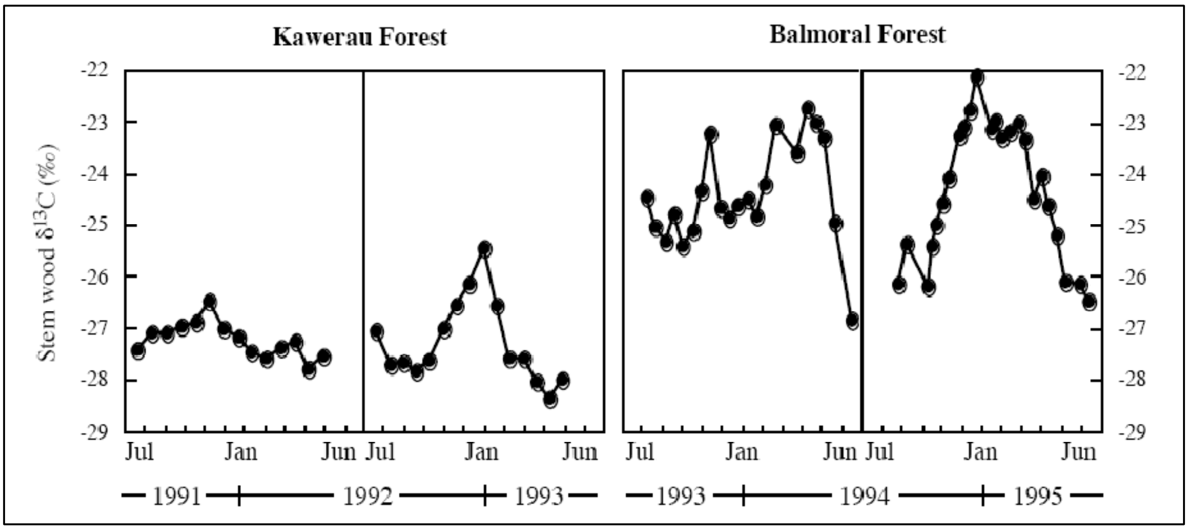

Figure 2.3: Seasonal changes in $\delta^{13}C$ of whole wood across two annual growth rings from the two forest sites. Since stem diameter growth was not measured for the Kawerau Forest site and for the 1993-94 season at the Balmoral Forest site, it was assumed that growth was linear over the growing season (August to June) so the dates shown are only approximate. Two weekly dendrometer band readings during the 1994 - 1995 season at Balmoral Forest allowed accurate dating of the seasonal $\delta^{13}C$ pattern. (From Walcroft *et al.*, 1997).

In a trial by Klein *et al.* (2005) *Pinus halepensis* grown in a semi-arid area were irrigated and the $\delta^{13}C$ of tree growth rings were compared to that of trees which were not under irrigation. They reported that the $\delta^{13}C$ of trees being irrigated were on average 1.5‰ lower than those of the control plots. Clear seasonal cycles were also visible with the lowest $\delta^{13}C$ values occurring during the cool humid winter and the highest values recorded during the dry hot summer.

From these studies it can be seen that $\delta^{13}C$ is definitely influenced by the availability of soil water. During periods where water availability is low, the $\delta^{13}C$ values are less negative which will imply that the discrimination against ^{13}C is greater. This is due to the fact that g_{sc} is low because transpiration is minimised through the closing or partial closing of stomatal guard cells. Research results are summarised in Table 2.1.

2.5 $\delta^{13}C$ in wood and plant nutrition

Very few trials have been conducted to describe the effect of fertilisation on $\delta^{13}C$ of stem wood. Warren *et al.* (2001) reported the addition of fertiliser significantly affected the $\delta^{13}C$ of annual rings ($P<0.04$) of *Pinus radiata* in New Zealand. Fertilised plots showed on average 0.6‰ less $\delta^{13}C$ than unfertilised plots. The addition of fertiliser also affected the seasonal pattern of $\delta^{13}C$ in such a way that the maximum value was reached later in the year. These effects however diminished over time. Thinning carried out during this trial also significantly affected $\delta^{13}C$ values, with an average difference of 0.75‰ between plots thinned to 250 and 750 spha respectively. It was found that the mean differences in summer (1.5‰) and autumn (1‰) were greater than in spring (0.6‰) and winter (0.1‰). Figure 2.4 illustrates the responses that were obtained from this trial.

2.6 Other factors influencing $\delta^{13}C$ signatures in wood.

Various other factors have been reported in literature which could also have an effect on the $\delta^{13}C$ values found in timber. Altitude (Warren *et al.,* 2001), the type of nitrogen available to the stand (Martínez-Carrasco *et al.,* 1998) and the age of the stand (Duquesnay *et al.,* 1998; McCarroll and Pawellek, 2001) could all contribute towards differences in $\delta^{13}C$ values.

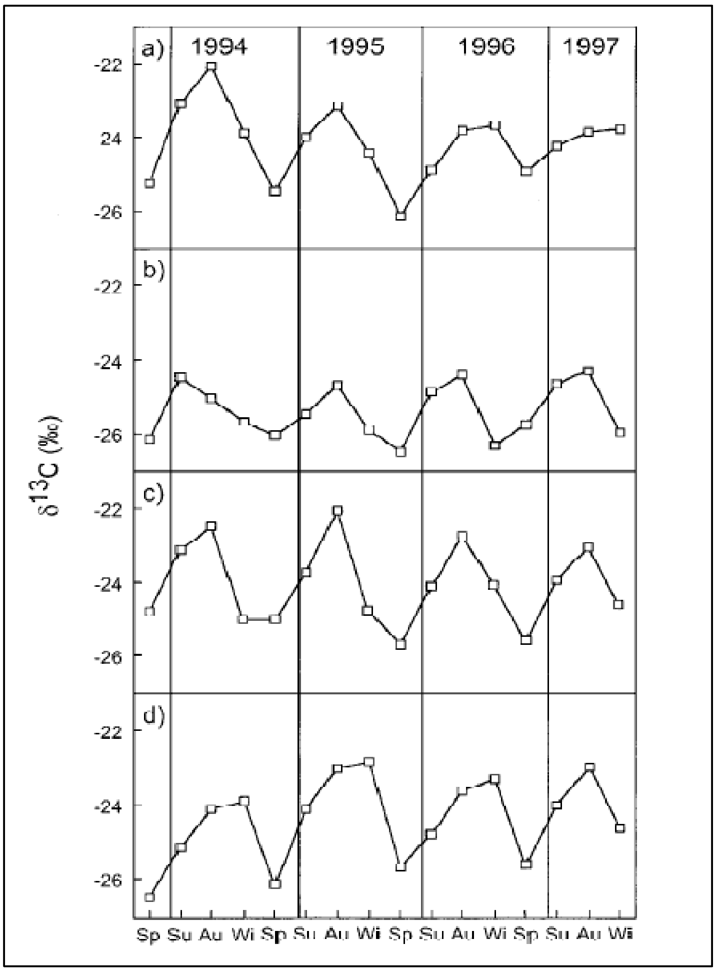

Figure 2.4: The seasonal course of stem wood δ^{13}C (‰) in **a** *P. radiata* 750 stems ha–1 plus fertiliser, **b** *P. pinaster* 750 stems ha–1 plus fertiliser, **c** *P. pinaster* 250 stems ha–1 plus fertiliser, and **d** *P. pinaster* 250 stems ha–1 minus fertiliser. Results are means; *n*=5; 1 SE was <0.7‰ in all cases. *Sp* Spring, *Su* summer, *Au* autumn, *Wi* winter (From Warren *et al.*, 2001).

Table 2.1: Summarised research results found in literature.

Author	**Species**	**Response**
Klein *et al.*, 2005	*Pinus halapensis*	δ^{13}C of irrigated trees 1.5‰ less than control. δ^{13}C exhibited annual cycles. δ^{13}C decreased with increasing rainfall.
Warren *et al.*, 2001	*Pinus radiata*	δ^{13}C positively related to altitude. δ^{13}C increase by 2.53‰ per kilometre rise in altitude (r^2=0.49). δ^{13}C of fertilised trees were 0.6‰ lower than unfertilised trees. Fertiliser application affected annual δ^{13}C patterns. In thinned plots δ^{13}C was on average 0.75‰ greater than unthinned. δ^{13}C negatively related to plant drought stress (r^2 = 0.38, 0.50, 0.58).
Walcroft *et al.*, 1997	*Pinus radiata*	δ^{13}C for forests in higher rainfall area is 3‰ less than low rainfall areas.
McNaulty and Swank, 1995	*Pinus strobus*	Carbon Isotope discrimination positively correlated with soil water potential (r^2=0.74). Carbon isotope discrimination significantly related to annual basal area growth (r^2=0.78, P< 0.0002).
Stewart *et al.*, 1995	Various	Relationship between foliar δ^{13}C and long-term rainfall average (r^2=0.78). Relationship between foliar δ^{13}C and number of rain days (r^2=0.70). Relationship between foliar δ^{13}C and 5-year rainfall average (r^2=0.70). Relationship between foliar δ^{13}C and moisture balance (r^2=0.74).
McCarroll and Pawellek 1998	*Pinus sylvestris*	Correlation coefficient for trees on the same sites range from 0.62 to 0.80. Correlation coefficient declines as the distance between sites increase.

2.7 Summary

Water and nutrients are two important factors when it comes to growing healthy, productive trees. Both of these factors influence the physiological processes within a tree, especially stomatal conductance and photosynthesis. By optimizing water availability and nutrition, the production of assimilates through photosynthesis will be increased along with tree growth. In order to fertilise stands optimally, it is not only necessary to know the current nutrient status of the stand, but it is also important to keep the prevailing soil moisture status of the stand in mind. The use of $\delta^{13}C$ could not only be a useful tool to indicate prevailing stand drought stress, but if it is more thoroughly researched, it could become a useful management tool which could indicate when fertilisation of a stand could be economically justifiable, given the soil water status of the stand. It could also be incorporated into process based models to indicate the minimum amount of soil water availability during a growing season which will simplify water balance modules usually incorporated into such models.

2.7 Hypotheses and study questions

In order to answer the problems posed in Chapter 1 the following hypotheses have been formulated and will act as basis for the study.

Hypothesis 1:
H_0: The $\delta^{13}C$ values of tree ring latewood from *P. radiata* stands of similar age but under different climatic conditions are not significantly different from each other.
H_A: $\delta^{13}C$ values of tree ring latewood from *P. radiata* stands of similar age but under different climatic conditions are significantly different from each other.
To be able to test this hypothesis, the following questions will need to be addressed:

- What range of values does $\delta^{13}C$ analysis yield in South African *P. radiata* stands?
- How do the $\delta^{13}C$ values correlate with each other across years (i.e. do dry years show the same trend across sites)?

Hypothesis 2:

H_0: $\delta^{13}C$ of *P. radiata* latewood rings from stands of similar age are not significantly less negative with a decrease in the available soil water levels across different sites and years.

H_A: $\delta^{13}C$ of *P. radiata* latewood rings from stands of similar age are significantly less negative with a decrease in the available soil water levels across different sites and years.

To be able to test this hypothesis, the following questions will need to be addressed:

- What range of values do $\delta^{13}C$ analysis yield?
- Which indices of drought stress are useful in describing tree drought stress? Do the $\delta^{13}C$ values differ significantly across a gradient of water availability?
- How do the $\delta^{13}C$ values correlate with the drought stress indices?
- Which time period is best suited to describe drought stress during late wood formation?
- How does the summer water availability affect the $\delta^{13}C$ values of the late wood?

Hypothesis 3:

H_0: The $\delta^{13}C$ values in late wood rings cannot be implemented as a useful management tool (i.e. as indicator of drought stress) in pine plantation forests.

H_A: The $\delta^{13}C$ values in latewood rings can, under certain conditions, be implemented as a useful predictor of drought stress in pine plantation forests.

After standardization on a given set of stand conditions, questions relating to this topic would be:

- Is the use of $\delta^{13}C$ analysis practical in the industry?
- Can single tree rings be used or will it be desirable to work with averages of several rings?
- What is the predictive ability of $\delta^{13}C$ as indicator of stand drought stress?
- Is the cost of using $\delta^{13}C$ analysis justified when it is used as a management tool?

Chapter 3: Methods and Materials

3.1 Site selection and description

3.1.1 Pilot Study

The main objective of the pilot study was to determine whether the differences between $\delta^{13}C$ values on different sites were significant enough to allow the continuation of the main trial. Two sites were selected for the pilot study. These sites formed part of an allometric study and the sample disks for $\delta^{13}C$ analysis were taken from the trees chosen for allometric sampling. This resulted in different numbers of samples taken on the pilot trial sites and the main trial sites. Compartment M4 is located in the Jonkershoek plantation. It receives a mean annual rainfall of 1060mm. Compartment B39 is located in the Kluitjieskraal plantation. This site has a mean annual rainfall of 840mm. Both sites are located on chromatic soils and are 14 years old.

3.1.2 Main trial

Using compartment information supplied by Mountain to Ocean Forestry (MTO), compartments were selected which fell between the ages of 12 and 16 years. The removal of trees from a stand during thinning practises has a significant effect on the $\delta^{13}C$ signature found in the timber (Warren *et al.,* 2001) and for inclusion in the shortlist, these compartments further needed to be scheduled for a first thinning during the year of sampling or would have had a first thinning the previous year. After selection of the sites from the compartment information, field inspections were carried out to determine the suitability of each stand for inclusion into the main trial. The slope of the stand, stand uniformity and soil type played an important role in the selection as this would determine whether available soil water would be influenced by the movement of water from another are into the study area.

Trial sites were selected in such a way that it would include sites from the Boland area as well as the all year rainfall area of South Africa, to ensure that a wide range of water availabilities will be covered. This approach would have the added benefit that it will facilitate the extrapolation of the results to most areas where *P. radiata* is grown in South Africa.

In order to simulate water availability more accurately, the chosen compartments had to be located close to weather stations with reliable weather data. For this reason, all sites had to be within two kilometres of such a weather station. The aim was to end up with sampling sites that had the necessary meteorological and soil information that the daily water balance could be modelled for each site. The daily water balance would then be used to calculate indices of stand drought stress for specific years or growing periods, which could be regressed against $\delta^{13}C$ in latewood tree rings. Stand characteristics of the chosen trial sites as well as summarised meteorological information are given in Table 3.2 and trial sites are depicted graphically in Figure 3.1. Note that sites using the same weather station were chosen such that soil profiles (and hence water storage characteristics) were markedly different.

After the sites were selected, an area with a uniform distribution of trees was located and then inspected on foot. To ensure that the available soil water would not be influenced by sub-soil drainage or that soil water could be replenished by the lateral movement of water within the soil, the trial plots were placed in topographical positions that are water-shedding rather than water-accumulating within the chosen stands. Also, where stands were in close proximity to mountain foothills, stands with a deep soil profile were avoided as it would be easier to determine the possibility of lateral soil water movement into the shallower soils by a visual inspection of the soil profile.

A plot of 25 x 25 trees were marked out and the diameters at breast height (DBH) for each tree was recorded using a diameter tape. The length and width of each plot was also determined in order to calculate basal area (BA) for each sample plot. The starting point of each of these length measurements were between trees in the specific row of each plot side.

Table 3.1: Trial site locations and characteristics of compartments used in the main trial.

Compartment	Plantation	Latitude	Longitude	Altitude	Age	Dq	BA	SI	PTPH	CTPH	Age of first thinning	Mean annual rainfall	Soil profile water	Mean Air Temp
L35b	Haweqwas	-33.71000	19.05200	408	12	17	20	23	1111	989	9	772	178	12.5
M4	Jonkershoek	-33.97100	18.93500	246	14	20	22	25	816	727	13	1073	118	10.4
G36	Grabouw	-34.07400	19.07400	394	18	30	29	25	1372	413	10	954	115	11.9
E18	Grabouw	-34.12900	19.01500	350	14	19	23	23	816	727	13	1188	75	9.1
N15a	Grabouw	-34.18100	19.12500	368	11	13	12	23	1087	967	13	773	178	11.3
A35c	La Motte	-33.90400	19.08700	241	17	25	25	25	816	543	14	880	177	12.2
D3d	Bluelilliesbush	-33.97400	23.86900	228	15	25	36	29.3	1372	473	8	1060	99	12.7
D10	Bluelilliesbush	-33.95900	23.89400	343	10	19	27	29.1	1372	648	8	1060	52	12.7
B5a	Bluelilliesbush	-33.99200	23.93000	231	11	26	34	29.1	1372	645	7	1060	59	12.7
D60	Bluelilliesbush	-33.95700	23.88000	332	9	14	16	28.0	1372	1219	9	1060	105	12.7
E2a	Kruisfontein	-34.02400	23.11100	270	16	22	28	26.3	1372	530	14	791	195	13.3
F14c	Kruisfontein	-34.03400	23.12900	247	13	22	33	24.2	1111	540	12	791	313	13.3
G19d	Kruisfontein	-34.05000	23.10900	156	13	22	27	27.2	1372	567	10	791	312	13.3
G4c	Kruisfontein	-34.04500	23.12200	205	9.8	20	26	32.7	1111	650	8	791	56	13.3

Dq: Quadratic mean diameter (cm)
SI: Site Index at age 20
BA: Basal Area (m^2)
PTPH: Planted trees per hectare
CTPH: Counted trees per hectare
Soil profile water: The maximum available soil water in mm if the profile is at field capacity.

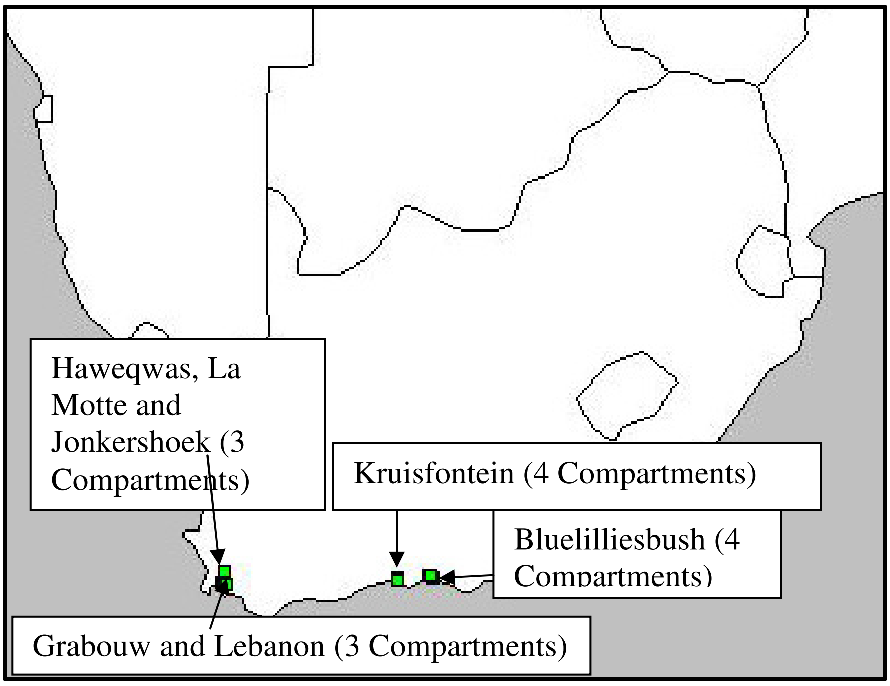

Figure 3.1: Plantations and compartments where main trial sites are located.

3.2 Soil sampling

After the samples plots were laid out, a soil pit measuring about 1.5 by 0.5 m were dug in the centre of the plot. The depth of the plots averaged 90 cm.

Top soil samples (0 – 10 cm) were taken at four random points on the plot. All organic matter and forest floor litter were carefully removed to expose the mineral soil and a cylindrical sampler with a volume of 200 cm^2 was hammered into the soil and then carefully removed by digging it out. All material inside the sampler was collected and the four samples were bulked to represent the topsoil layer.

Inside the soil pit, the different soil horizons were visually identified and the depth of each horizon recorded. Samples were taken of each horizon from the four sides of the pit from 10 cm below the soil surface up to the bottom of the pit. The cylindrical samplers were hammered into the soil face using a flat piece of wood on the back to minimise damage to the samplers as well as to make sure that the sampler enters the soil evenly. Care was taken not to compress the soil inside the sampler when it was close to being inserted all the way. In order to determine how deep the soil profile would be if it was opened up completely, a dutch auger was used to drill down further than the bottom of the pit. Materials retrieved with the auger was also collected and bulked per depth.

Soil samples were sent to Bemlab commercial laboratories for textural analysis as well as coarse fragment percentage determination.

In order to determine the field capacity (FC) and wilting point (WP) of each soil profile the computer program "SOILPAR", developed by Marcello Donatelli and Marco Acutis (Acutis and Donatelli, 2003) was used. It is available online from the Research Institute for Industrial Crops (http://www.isci.it). The process by which the FC and WP was determined is known as pedotransfer functions. In small areas with homogenous soils it is easy to determine FC and WP from just a few sampling sites, however, once the area gets larger determination of these parameters become difficult and costly. In cases like these it is important to find a fast and inexpensive way to obtain these parameters (Schaap *et al.*, 2001). Pedotransfer procedures are grouped into two classes. These are "Point Pedotransfers" and "Fuction Pedotransfers" (Cornelis *et al.*, 2001; Acutis and Donatelli, 2003). Point Pedotransfers estimate soil water content at certain matrix potentials while Function Pedotransfers predict the parameters of a closed form analytical equation (Givi *et al.*, 2004).

Using the textural analysis obtained from the laboratory as well as the bulk density for each of the soil classifications (Schulze, 1995), the data was input into SOILPAR and the point pedotransfer function developed by Hutson and Cass (1987) was chosen for use. This function was developed for the model LEACHM (Leaching Estimation and Chemistry Model) (Hutson and Wagenet, 1992). Care was taken to assign particles to the correct individual size classes since the names of size fractions in the model were not completely congruent to that of the South African texture classification system used in local laboratories. Appendix 1 contains the soil descriptions as well as textural analysis, wilting point and field capacity for each of the samples taken.

Soil classification was done according to the South African soil classification system. (Soil Classification Working Group, 1991)

3.3 Meteorological data

The meteorological data used in the study was obtained from various sources. Most of the Boland sites were located close to stations run by the Agriculture Research Council (ARC). This enabled the acquisition of data from their stations. Data for the Jonkershoek site was obtained from the South African Environmental Observation Network (SAEON). In the Outeniqua and Tsitsikamma regions, weather data was obtained at the plantation offices of the forestry company. Meteorological data was also obtained from the South African Weather Services (SAWS) in order to complete the datasets obtained from the ARC and the plantation offices. In order to simulate soil water availability the following weather parameters were needed as input on a daily basis: Maximum and minimum temperatures, relative humidity, wind speed, precipitation and solar radiation. The weather stations from which the data was obtained are listed in Table 3.

3.3.1 Missing data

When working with weather data it is inevitable that some data sets will not be complete. This can be due to weather station failure or some unforeseen problem that caused the station or data capture process to malfunction. It is however important that incomplete data sets are patched, using data from nearby stations, in order to obtain more reliable results.

3.3.1.1 Temperatures and wind speed

Where any of these two parameters were missing from the data set, long term averages were calculated for the specific days using existing data if a surrogate station was not available in the immediate vicinity for patching data. An average of at least five years was used for temperatures and wind speed.

3.3.1.2 Solar radiation

It often happens that weather stations measure solar radiation, uses this value to calculate various other parameters like evapotranspiration, but the radiation value is

Table 3.2: Weather stations used to obtain meteorological data.

Station name	**Source**	**Latitude**	**Longitude**	**Altitude**	**Data Period**
		(Degrees decimal)		(m)	(calendar years)
Ashanti	ARC	-33.72356	19.04239	249	2002 – 2009
Nederburg	ARC	-33.71349	19.01295	144	2002,2003,2007, 2009
Drostersnes	ARC	-34.07473	19.07687	394	1998 – 2009
Old Nursery	ARC	-34.13813	19.04301	279	2005 – 2009
Slangkop	ARC	-34.13245	19.06414	383	2005 – 2009
De Rust	ARC	-34.17120	19.10813	326	2002 – 2009
Haasvlakte	SAWS	-34.22100	19.12900	488	2000 – 2009
La Motte	ARC	-33.88132	19.07148	207	2002 – 2009
Kruisfontein	MTO	-34.04200	23.11300	300	2005 – 2009
Knysna	SAWS	-34.05900	23.09100	54	2002 – 2009
Bluelilliesbush	MTO	-33.99600	23.95400	300	2005 – 2009
Tsitsikamma	SAWS	-34.026	23.90800	5	2002 – 2009
Stormsrivier Dorp	SAWS	-33.975	23.88800	230	2002 – 2009

then discarded and not logged. This is most probably done because of the costs involved to download a lot of data using wireless methods. Other reasons for the lack of solar radiation could include dirt on the sensor, an accumulation of water, shading of the sensor or inaccurate calibration (Abraha and Savage, 2008). Solar radiation is a very important parameter and the absence of it restricts the application of crop simulation models (Hook and McClendon, 1992). For this reason researchers have been developing various methods to estimate solar radiation from other more readily available meteorological observations (e.g. Bristow and Campbell, 1984; Hunt *et al.*, 1998; Thornton and Running, 1999; Mahmood and Hubbard, 2002).

For the purposes of this study, a temperature based model developed by Hargreaves and Samani (1982) was chosen to estimate solar radiation from measured daily minimum and maximum temperatures, for stations where it was not recorded. See Appendix 2 for more detail on the estimation of parameters.

3.3.1.3 Relative Humidity

On some weather stations values for relative humidity were also absent. In order to calculate relative humidity, it is first necessary to determine what the dew point temperature would be for each day as dew point temperature is a precise measure of atmospheric moisture (Hubbard *et al.*, 2003). Using the model described in Hubbard *et al.*, (2003), the daily dew point temperatures were calculated and then used to calculate the actual vapour pressure. Following the guidelines by Allen *et al.* (1998), the relative humidity was calculated for each day. See Appendix 2 for more detail on the estimation of parameters.

3.3.1.4 Reference Evapotranspiration

This parameter is not always logged on all weather stations. Evapotranspiration (ET) is the combination of the two processes evaporation and transpiration. Through these processes, liquid water gets converted into water vapour which is transferred into the atmosphere. The ETo parameter was not a requirement for the water balance model used in this study but for the sake of having complete data sets, it was determined using the FAO 56 Penman-Monteith equation. See Appendix 2 for more detail on the estimation of parameters.

After estimating the parameters, data logged by a weather station run by the Department of Forest and Wood Science (University of Stellenbosch) was used to evaluate the performance of the estimated data visually. It was found that on some of the weather station data obtained from certain ARC stations, the solar radiation measured did not provide a good match with the estimated solar radiation. On closer inspection it seemed that the calibration of the weather station sensors might be faulty as the values logged for the parameter are far below the minimum monthly values of solar radiation as given by Shulze *et al.*, (1997) for the study area. Data from the weather station at the Department of Forest and Wood Science was however complete and the equipment calibrated. There was a much better correlation between the measured and estimated data and it was decided to use only solar radiation which was estimated by using Hargreaves and Samani (1982). The estimated solar radiation values were adjusted downwards by 15% for the months, March to November as described in Appendix 2.

Using all the techniques described in Appendix 2, full weather data sets were thus prepared for use within the soil water balance model HyMo (Rötzer *et al.*,2004).

3.4 Sample processing and analysis

On each of the pilot trial study sites, six trees were felled and disks with a thickness of ± 25 mm were cut at diameter at breast height (DBH). The number of trees sampled on main trial sites were four trees per site as suggested by McCarroll and Loader (2004). The disks were dried in an oven overnight at 105 ^{0}C. The oven dried disks were cut in half using a small bandsaw. The halves were inspected and the one with the least reaction wood was selected for further analysis.

3.4.1 Early – and latewood separation

Literature studies have highlighted that carbohydrates which formed during the previous growing season is utilised during the formation of earlywood cellulose (Robertson *et al.*, 2004, Lipp *et al.*, 1991, Pawelczyk and Pazdur, 2000). For this reason, the latewood from samples was chosen for analysis. The selected half-disk was again cut in half and the resulting quarters were halved again. This continued until the disk was cut into eight segments. These segments were lightly sanded on their sides to improve the visibility for annual growth rings. To minimise the variability of $\delta^{13}C$ within each tree ring, four segments, representing four cores or radii, were randomly chosen for further processing (McCarroll and Pawellek, 1998, Leavitt and Long, 1984).

Starting from the youngest latewood growth ring on each segment, the late- and earlywood was split off using a hammer and sharp chisel. The split off pieces were placed in Petri dishes and labelled with tree number and year. After all the selected rings were split off the segments, all visible earlywood which was still present were removed by slicing it off with a scalpel. The sides of the pieces which were sanded down earlier during the process were also sliced off to avoid cross contamination which might have occurred during the sanding process. Using the chisel, all the pieces of wood were reduced in size to be small enough for milling.

Sample pieces were then milled with a Wiley Mill (Arthur H Thomas Co., Pa., U.S.A.) and all pieces which passed through a 40 mesh sieve were collected for further extraction. Milled samples were stored in clean glass bottles with screw cap lids, placed in desiccators.

3.4.2 Extraction of non-cellulosic material

The non-cellulosic materials in milled samples were extracted using an acid-catalyzed solvolytic method which has been developed and described by Macfarlane *et al.* (1999). Milled samples were oven dried (105 ^{0}C) overnight. Samples (0.5 g) were weighed (± 0.002 mg) and placed in clean 28 ml McCartney bottles. Exactly 2.65 ml of diglyme (Diethylene glycol dimethyl ether) and 0.665 ml 10M HCl were added and the bottles were sealed with screw caps lids lined with teflon-butyl liners placed in a shaking waterbath (SHWBD36, Scientific Manufacturing, Table View) at 90 ^{0}C for three hours. Increasing the amount of diglyme-HCl and using a longer reaction time was implemented as Wallis *et al.* (1997) found that all non-cellulosic materials were removed from *Pinus radiata* when using the values described earlier. After three hours the caps were removed and the residue was collected by gravity filtration. Filter paper (150 mm diameter, grade: 391) were weighed, folded into cones and placed in glass funnels in 250 ml Erlenmeyer flasks. 20 ml of methanol was used to rinse the bottles and wash the residue. The residue was further washed three times with boiling water and oven-dried overnight. The mass of the dried samples were determined after it cooled down in a dessicator. Taking care not to contaminate the sample with cellulose from the filter paper, residues were collected and placed in 2 ml Eppendorf tubes. The residues were dark brown in colour and retained its shape during the extraction process.

3.5 δ^{13}C analysis

3.5.1 Pilot trial

Samples were sent to the Stable Light Isotope Unit at the University of Cape Town where the δ^{13}C content of each sample was determined. Samples were weighed into tin cups (± 1 mg) using a Sartorius micro balance after which the cups were squashed to enclose the sample.

The samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Milan, Italy). The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Bremen, Germany), *via* a Conflo III gas control unit (Thermo Finnigan, Bremen, Germany).

Two in-house standards, MG (Merck Gel), a proteinaceous gel produced by Merck and Nastd (dried nasturtium leaves collected from Woodbine Lane) were used. All the in-house standards have been calibrated against IAEA standards by the Stable

Light Isotope Unit or by other labs. The carbon values obtained were expressed in terms of its value relative to Pee-Dee Belemnite.

3.5.2 Main trial

Samples taken for the main trial were sent to IsoEnvironmental cc. at the Botany Department, Rhodes University for analysis due to equipment unavailability at the University of Cape Town. The analysis was carried out on an Europa Scientific Elemental analyser and 20-20 IRMS. Each batch of samples analysed contained 29 internal standard samples of “refmix2” which is made up of beet sugar and ammonium sulphate. Also included were five standards of Casein, a certified protein standard which had been calibrated against IAEA-CH-6 and IAEA-N-1. The overall C precision obtained from this analysis was 0.14. The results were comparable with results obtained from the Stable Light Isotope unit.

3.6 Soil water availability

3.6.1 Model description

In order to model soil water availability, it was decided to use the hydrological model called HyMo, which had been developed by Rötzer *et al.* (2004). The decision to use this model was based on the fairly simple input requirements needed to run the model. Various other models were investigated for potential use including “SAPWAT 3” (van Heerden *et al.*, 2009) and ACRU (Schulze, 1995; Smithers and Schulze, 2004). SAPWAT was found to be too basic and couldn’t easily be adapted to fit the needs for this study while ACRU needed more data than what was available for the study.

Basic inputs needed for HyMo are temperature, precipitation, radiation or sunshine duration, wind speed and humidity. Furthermore some site information has to be supplied which include latitude, longitude, altitude, as well as some soil depth, soil texture and stand characteristics.

The model estimates actual evapotranspiration, interception, runoff and calculates the daily change in soil water availability as:

$$\Delta\Psi = rr - et_a - \text{int} - ro - cr + irr \qquad \text{(Eq. 8)}$$

With

$\Delta\Psi$: change in soil water content in $mm.d^{-1}$
rr : Precipitation in $mm.d^{-1}$
et_a : actual evapotranspiration in $mm.d^{-1}$
ro : total runoff in $mm.d^{-1}$
int : rainfall interception by canopy in $mm.d^{-1}$
cr : capillary rise in $mm.d^{-1}$
irr : irrigation in $mm.d^{-1}$

Details on how each of the parameters are calculated by the model can be obtained in the original literature. The initial model was developed for European stands of Beech (*Fagus sylvatica* L.) and Spruce (*Picea abies* L. Karst) and was adapted for the use in pine (*Pinus* spp.) stands by the author of the model, Dr. Thomas Rötzer. The model has been validated by various authors in the past.

Rötzer (1996) found a difference between measured and simulated soil water content of 7.3% for winter wheat, 8.2% for grass and 8.3% for maize for periods of 4 to six years. Würlander (1997) compared the measured and modelled runoff for two different catchment areas in Germany. Using HyMo, the modelled runoff for the Regen River deviated -7.7% from measured values while values for the Naab River deviated +5.9%.

Rötzer (2001) investigated a warm, dry region Northwest of Bavaria having high potential evapotranspiration values and low actual evapotranspiration values. He found deviations from actual soil water content measurements of between 6% and 9%. Kremb *et al.* (2000) compared the runoff totals calculated by HyMo with measurements using a lysimiter and found a mean difference between the measured and simulated values of 4.5%. HyMo was also tested in the forest stand “Höglwald” northwest of Munich by Kreutzer and Weiss (1998). During this trial, the mean absolute error of the simulated and measured soil water contents were found to be 12 mm for beech and spruce stands and on the basis of maximum available soil water, the deviation of simulated values were 2.8% for spruce and 4.0% for beech.

From these results it can be assumed that HyMo is a reliable soil water balance model with relatively simple data requirements.

3.6.2 Relative extractable water

In order to determine whether a stand experiences stress due to the unavailability of water, the relative extractable water (REW) was calculated according to Granier *et al.* (2000).

$$REW = \frac{W - W_m}{W_{FC} - W_m} \qquad \text{(Eq. 9)}$$

Where:
W = Soil water content in the root zone (modelled daily soil water content)
W_m = Lower limit of water availability
W_{FC} = Soil water content at field capacity

The use of REW is beneficial to the study since

- predawn water potential measurements were not available in this study;
- soil water content in the root zone can have large ranges while REW only ranges between 0 and 1;
- predawn water potential and REW are strongly related (Bréda *et al.*, 1995).

Drought stress is assumed to occur when REW drops below 0.4, i.e. below 40% of the maximum extractable water (Granier *et al.*, 1999). This gave a clear indication of when stands started to experience stress due to inadequate water availability. Poyatos *et al.* (2005) described a strong limitation of transpiration in *Pinus sylvestris* when REW dropped below 0.4, and for that reason the value was maintained here.

From the REW values, periods where stands experience drought stress were identified. The stress period during a year is usually from November to April. From this the growing year was defined as August to July.

3.6.3 Relative canopy conductance

Granier and Bréda (1996) developed a model to describe canopy conductance using environmental drivers (climate and soil water availability). Using daily values of REW they calibrate a logarithmic curve which describes the relationship between canopy conductance (g_c) as a fraction of the maximum canopy conductance (g_{cmax}) and REW :

$$g_c/g_{cmax} = 1.05 + 0.59 * \log_{10}(\mathrm{REW}) \qquad \text{(Eq. 10)}$$

This relationship holds for pine species as well, as described in Granier *et al.* (2000).

Using the output generated by HyMo and Eq. 10, the relative canopy conductance was estimated on a daily basis.

3.6 4 Water supply/demand ratio

The supply / demand ratio (ETa/ETp) is the ratio of actual (ETa) to potential (ETp) evapotranspiration. This indicates the amount of moisture that a stand could supply to the atmosphere as a fraction of what the atmosphere could accept. ETa reflects the simultaneous availability of biological usable energy and water in the environment and as a result it reflects the magnitude and length of conditions that are favourable for plant growth (Major, 1963; Rosenzweig, 1968; Stephenson, 1998). Small ETa/ETp values indicate that the stand cannot supply the atmosphere's evaporative demand. This is mainly due to low soil water content or large vapour pressure deficits.

As part of its output, HyMo calculates the daily actual and potential evapotranspiration. The supply / demand ratio was calculated accordingly and also incorporated as a second measure of stand stress.

3.7 Growing degree days (GDD)

In cases where water and nutrients are non-limiting factors, temperature is the main factor that influences growth (Whitehead *et al.*, 1994). Growth begins above a lower (or base) temperature and above this temperature the growth increases with increasing temperature until an optimum temperature is reached. If the temperature continues to increase above the optimum, the growth rate will decline. This concept of thermal time was described by Monteith (1981) and can be expressed when $T_j > T_b$ as:

$$GDD = \sum_{j=1}^{n} \left(T_j - T_b \right) \quad \text{(Eq. 11)}$$

Where T_j is the mean temperature on day j, T_b is the base temperature and n is the number of days in the growing season.

Growing degree days assumes that water and nutrients are not limiting factors to growth. Dickson *et al.* (2000) proposed a weighting procedure where G is modified by using the daily precipitation as a fraction of the total precipitation for the period being studied. This allowed some measure of accountability towards drought stress. The GDD weighted against rainfall is calculated as:

$$G_r = \left(\sum_{j=1}^{n} \left(T_j - T_b \right) \right) x \left(\sum_{i=1}^{n} \frac{r}{m} \right) \quad \text{(Eq. 12)}$$

Where r is the daily rainfall at day i and m is the mean rainfall during period n. G_r was calculated on a daily basis to investigate any possible relationships between $\delta^{13}C$ and the accumulated GDD weighted by daily rainfall.

3.8 Calculation periods

In order to ensure that the best models were selected during the analysis of the data, six periods were used for the calculation of the average g_c/g_{cmax}, ETa/ETp and G_r. The first period started in the middle of the six month stress period as indicated by REW and took the months January and February into account. From there, a month was added on each side and the results were analysed again. This continued until a period of 12 months were reached. Figure 3.2 is a graphic representation of the periods which were investigated.

Table 3.3 summarises the abbreviations used for the different study periods and parameters

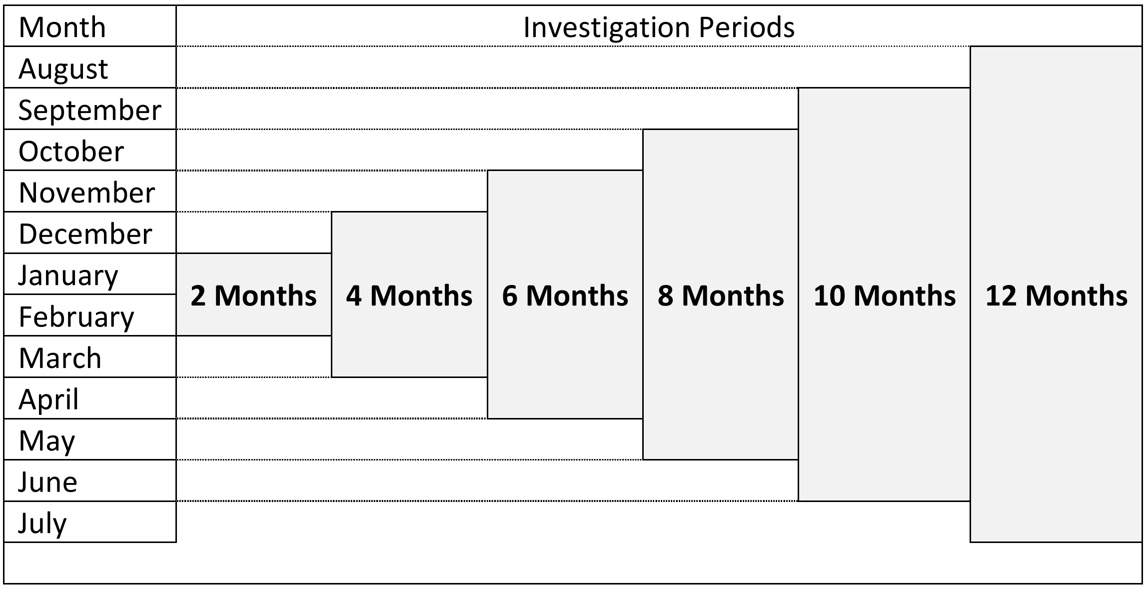

Figure 3.2: Graphic representation of the different investigated stress periods starting from mid-summer (Jan-Feb) and gradually increasing until encompassing the whole year.

Table 3.3: Summary of abbreviations concerning study periods.

g_c/g_{cmax}	Relative Canopy Conductance
ETa/ETp	Supply / demand ratio
5-yr means	The mean value of five years for either $\delta^{13}C$, g_c/g_{cmax} or ETa/ETp
6M - RCC	Average value for six months relative canopy conductance
6M - ETa/ETp	Average value for six months supply / demand ratio

3.9 Statistical analyses

All statistical calculations were done using the Statistica software package (Statsoft Inc., USA, version 9). Differences between the pilot trial sites were investigated using a t-test. Due to the uneven number of trees sampled on each pilot trial site, the Least Square Means procedure was used Also, taking samples over a continuous number of years meant that a Repeated Measures ANOVA (RMANOVA) could be done to investigate any interactions between the specific site where the samples were taken and the years for which the $\delta^{13}C$ values were determined.

To analyse the main trial results, two types of linear regressions were done. The first was a set of normal linear regressions with only $\delta^{13}C$ as independent variable. The second set of regressions included the stand density index as a covariate. The predictive confidence interval was determined on a 95 % level.

Chapter 4: Results

4.1 Pilot trial

The mean values for $\delta^{13}C$ across sites are presented in Figure 4.1. From the RMANOVA the site means differed significantly, $F_{(1,10)} = 8.0392$ ($p = 0.01769$).

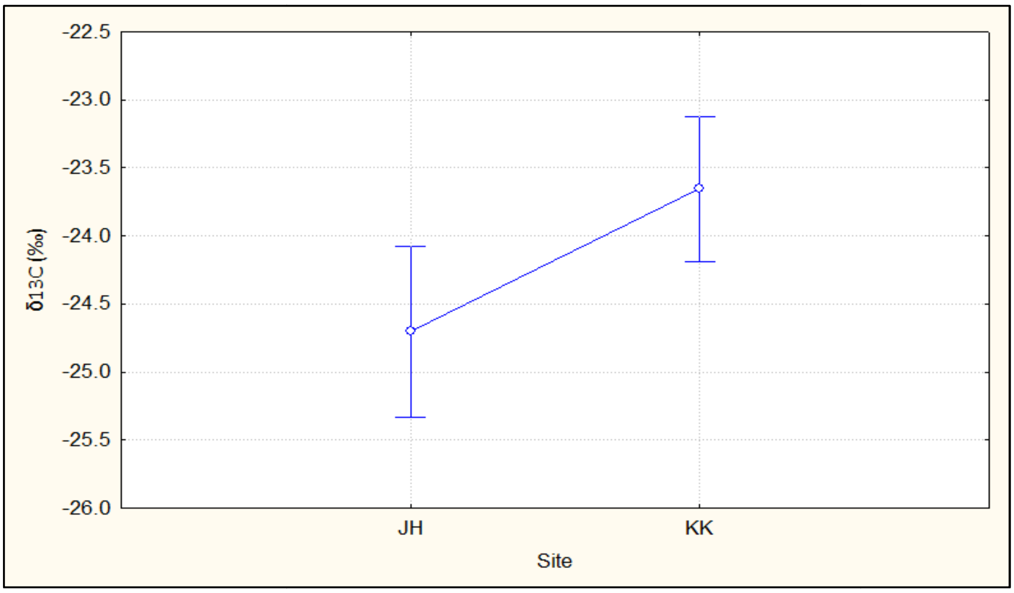

Figure 4.1: Differences between timber $\delta^{13}C$ values for pilot trial sites using Least Square Means for Jonkershoek (JH) and Kluitjieskraal (KK).

Table 4.1 describes the results of the RMANOVA. It is clear that there is a significant difference ($p = 0.17$) between the Jonkershoek and Kluitjieskraal sites when looking at the $\delta^{13}C$ values. The strong correlation between mean annual rainfall and $\delta^{13}C$ at Jonkershoek is shown in Figure 4.2. Due to a rainfall gradient that exists, there was also a significant difference between the years used in the pilot trial, $F_{(4,40)} = 3.93$ ($p= 0.008769$). The year 2006 was a particularly dry year and $\delta^{13}C$ values in Jonkershoek were almost the same as in Kluitjieskraal. To investigate this trend the $\delta^{13}C$ values were correlated with the mean annual precipitation. For Jonkershoek a correlation coefficient of $r = -0.64$ ($p = 0.001$) was obtained and for Kluitjieskraal $r = 0.014$ ($p = 0.935$) (Figure 4.3).

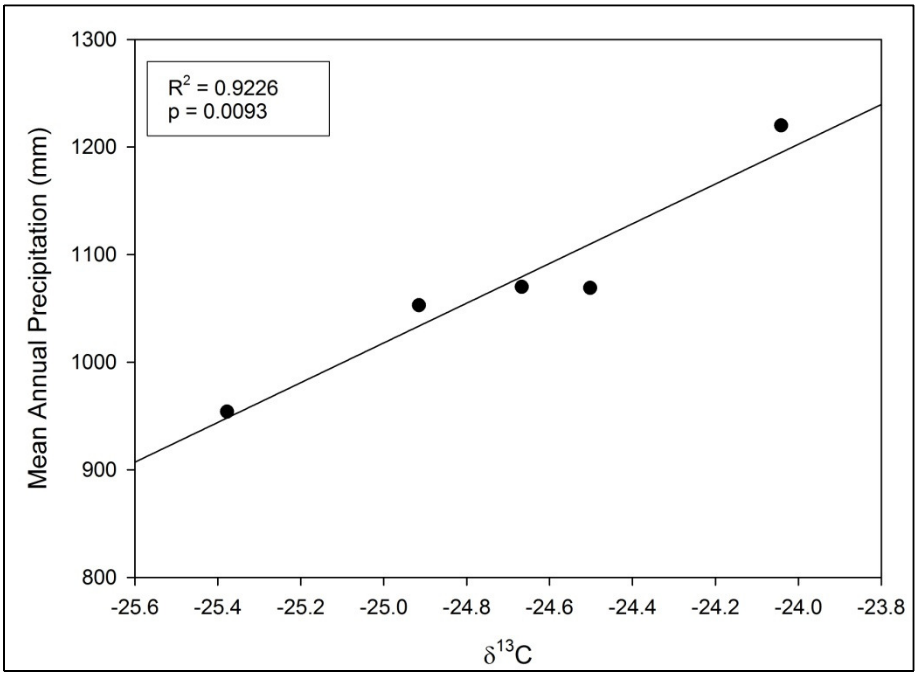

Figure 4.2: The relationship between mean annual precipitation and the mean δ^{13}C value for five year rings on the Jonkershoek site.

Table 4.1: Repeated measures ANOVA for the pilot trial

	SS	Degrees. of Freedom	MS	F	*p*
Intercept	34098.85	1	34098.85	17165.01	0.000000
Site	15.97	1	15.97	8.04	0.017689
Error	19.87	10	1.99		
YEAR	4.12	4	1.03	3.93	0.008769
YEAR*Site	3.82	4	0.96	3.65	0.012611
Error	10.47	40	0.26		

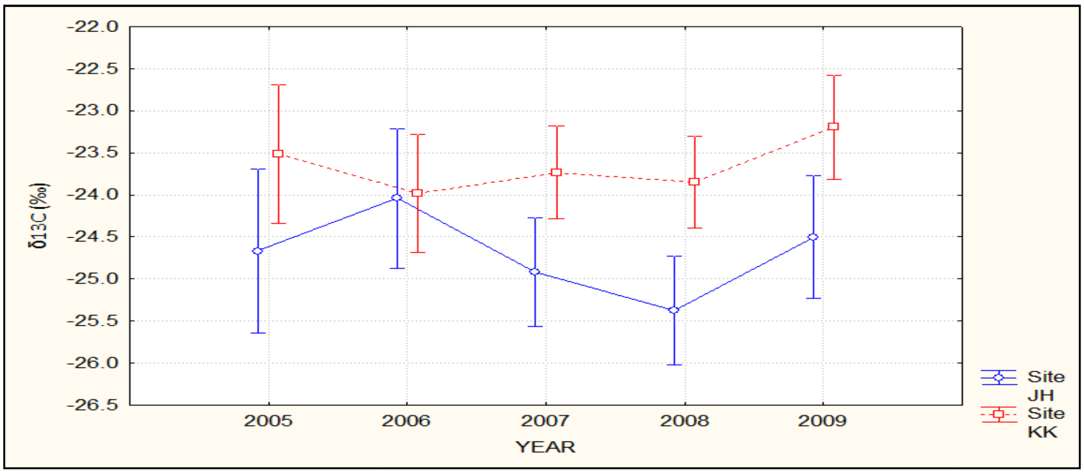

Figure 4.3: Year x Site interaction over time for the Jonkershoek (JH) and Kluitjieskraal (KK) sites. Each value represents the average of five growth rings for the specific year.

4.2 Main trial

4.2.1 Soil water balance and relative extractable water

From the output generated by HyMo the soil water content was plotted over time to better understand the change in soil water content over time. Several sites were investigated in each region and a unique seasonal pattern could be observed for each region. This pattern remained similar among different sites within a region despite the fairly wide ranges in soil profile water (Table 3.1). Three distinct trends in water availability became clear over the study area and accordingly the results and discussion will be presented according to each region. The three regions are the Boland region (Haweqwas, La Motte, Jonkershoek and Grabouw plantations), Knysna region (Kruisfontein plantation) and Stormsrivier region (Bluelilliesbush plantation). Figures 4.4 to 4.6 depict the typical soil water content patterns for sites in the Boland, Knysna and Stormsrivier areas.

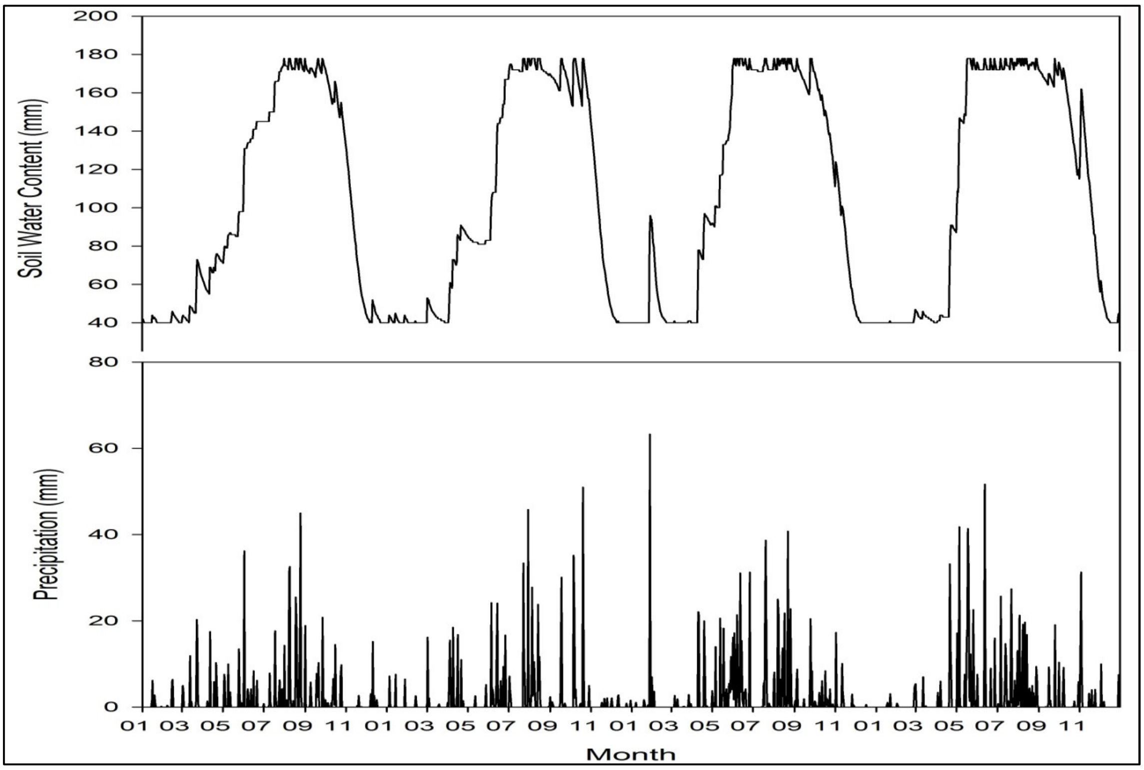

Figure 4.4: Soil water content (top section, as modelled with HyMo) compared to daily precipitation (bottom section) for Compartment L35b (Boland) for the period 2003 – 2006.

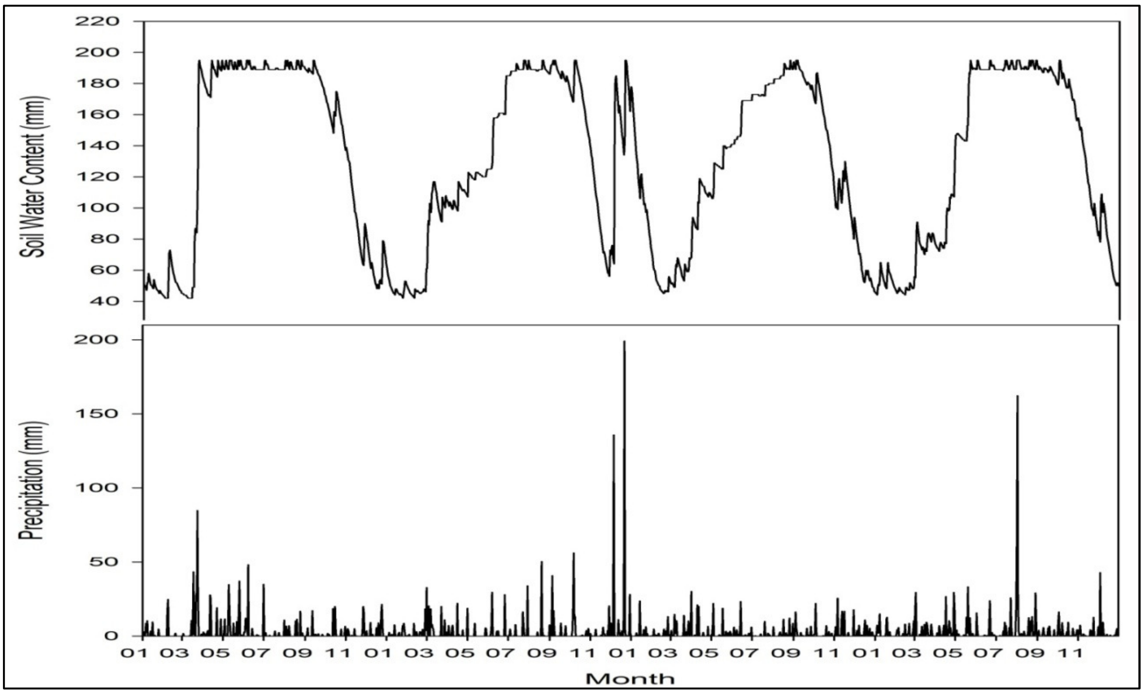

Figure 4.5: Soil water content (top section, as modelled with HyMo) compared to daily precipitation (bottom section) for Compartment E2a (Knysna) for the period 2003 – 2006.

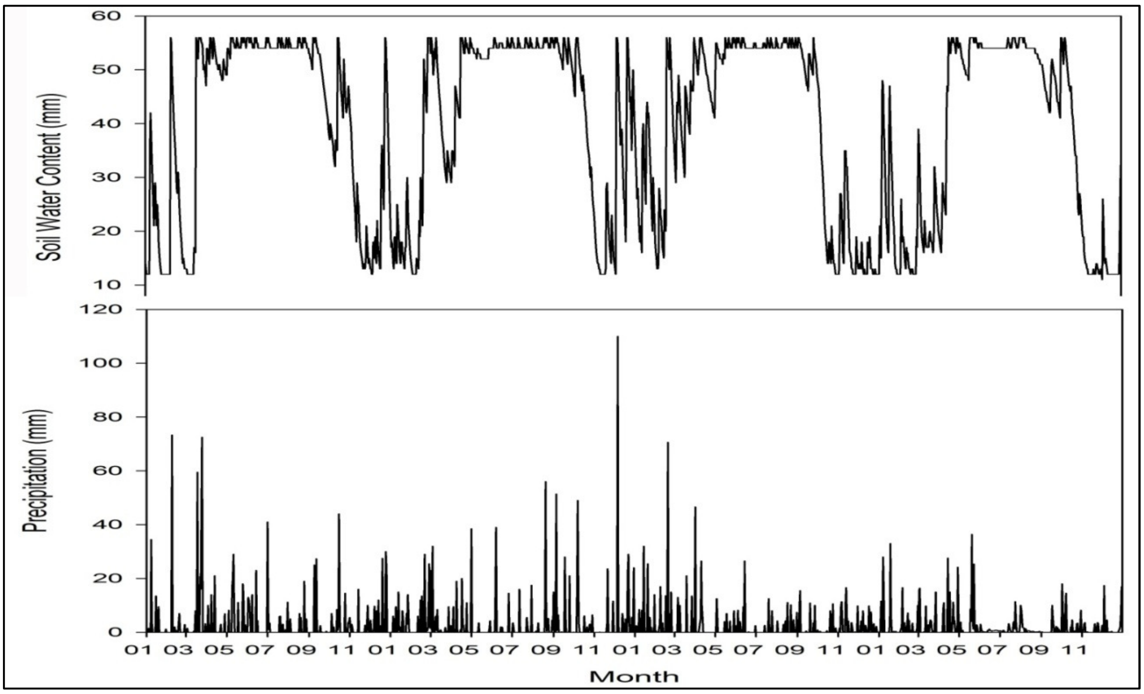

Figure 4.6: Soil water content (top section, as modelled with HyMo) compared to daily precipitation (bottom section) for Compartment B5a (Stormsrivier) for the period 2003 – 2006.

The difference between the sites is clearly shown with the Boland region having a pronounced seasonal effect on the soil water content with long periods where the soil water content is at the lower limit of water availability. Sites in the Knysna area still show some seasonal effects, however it is not as clear as in the Boland.

The Stormsrivier sites are located in a high rainfall area of the all year rainfall zone of South Africa and there are fewer periods where the soil water content is at the lower limit of water availability.

Trees start to experience drought stress when the relative extractible water (REW) is less than 0.4 and this threshold has been tested for *Pinus* species (Granier et al., 2000). Figures 4.7 to 4.9 show the typical pattern of REW available in compartments throughout the study area. The dashed line in each figure represents the stress threshold where the relative canopy conductance (g_c/g_{cmax}) will begin to decline.

Again, clear differences can be seen between the sites. Sites in the Boland region have more pronounced periods where the REW is below the threshold of 0.4 than sites in the Knysna area. The Stormsrivier sites typically experience a lot less moisture stress as precipitation during the year constantly recharges the soil water content.

Using REW as a measure of moisture stress it was possible to determine that the moisture stress period experienced by trees in the study areas are between late spring and early autumn (November to April). This period was used as the set stress period for the study.

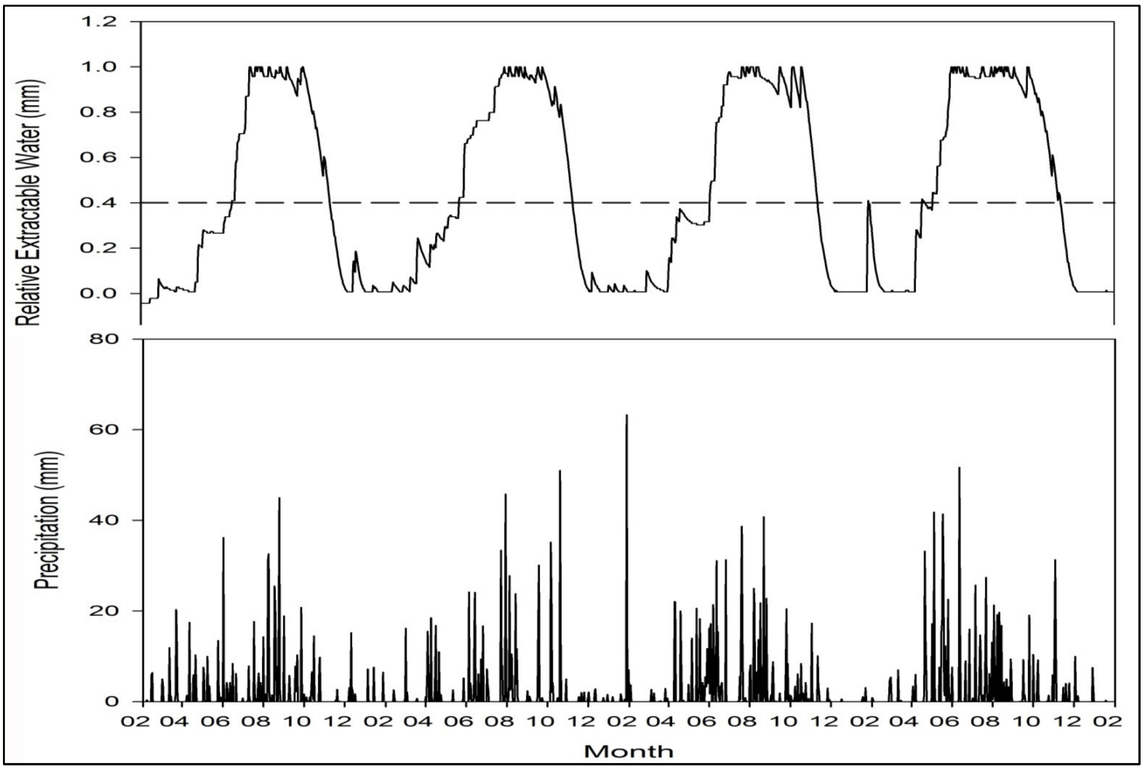

Figure 4.7: Relative extractable water (top section) compared to daily precipitation (bottom section) for Compartment L35b (Boland) for the period 2003 – 2006. The dashed line indicates the stress threshold.

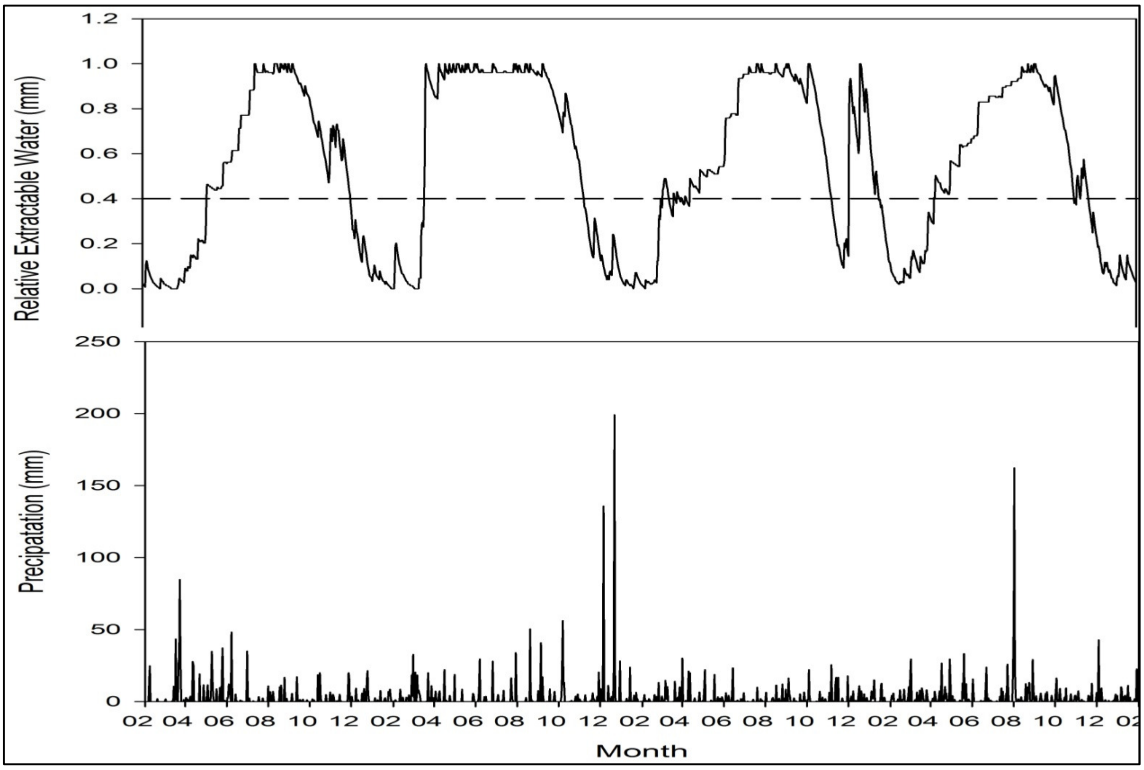

Figure 4.8: Relative extractable water (top section) compared to daily precipitation (bottom section) for Compartment E2a (Knysna) for the period 2003 – 2006. The dashed line indicates the stress threshold.

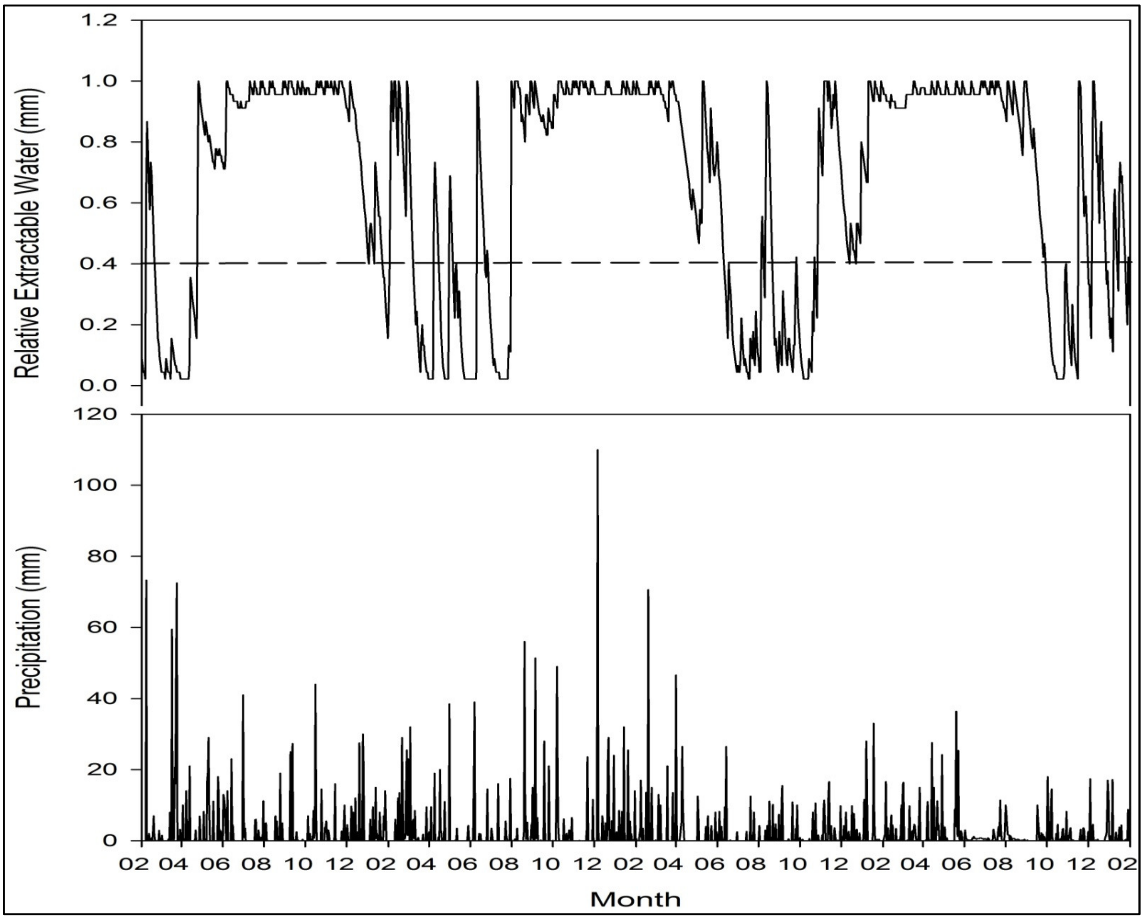

Figure 4.9: Relative extractable water (top section) compared to daily precipitation (bottom section) for Compartment B5a (Stormsrivier) for the period 2003 – 2006. The dashed line indicates the stress threshold.

4.2.2 Drought influences on study sites

For the two years (2008 – 2010) prior to sampling stands in the Knysna and Stormsrivier area, the region experienced a severe drought, a uncommon event when viewed against climate data for the last century for this area. The influence of the drought can clearly be seen in the soil water content of the sites (Figure 4.10) and the $\delta^{13}C$ values of those particular drought years (Table 4.2). Care was taken not to include such years in the study as these are atypical years which will not represent an overall view of growing conditions for these sites.

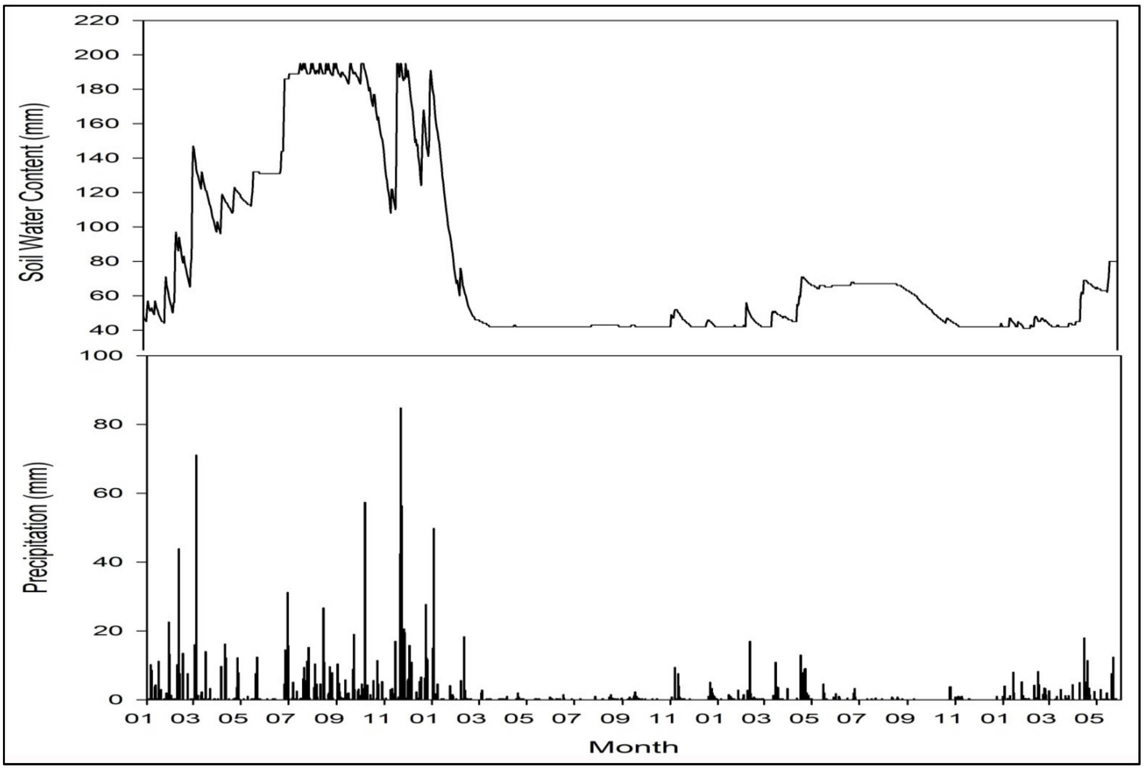

Figure 4.10: Soil water content (top section) and daily precipitation (bottom section) for Compartment E2a during a two year drought in the Knysna and Stormsrivier areas (2008 - 2010).

Table 4.2: $\delta^{13}C$, mean annual precipitation values and yearly average REW depicting the drought between 2008 and 2010 for the Knysna area.

Growth year	$\delta^{13}C$	MAP	REW
2004-2005	-27.4287	1104.7	0.590
2005-2006	-27.2141	841.9	0.549
2006-2007	-27.5217	1066.8	0.569
2007-2008	-26.7366	753.7	0.426
2008-2009	-26.7207	185.3	0.054
2009-2010	-26.3725	138.3	0.053

4.2.3 Relative canopy conductance and supply/demand ratio

Using the output generated by HyMo the relative canopy conductance (g_c/g_{cmax}) was calculated using the logarithmic function describe by Granier and Bréda (1996). As part of the HyMo output the actual to potential evapotranspiration (*ETa/ETp)*, or supply / demand ratio, is also calculated. To investigate how these two different methods of assessing tree stress are correlated with each other, the calculated values are described in Figure 4.11.

Lower values of *ETa/ETp* (or g_c/g_{cmax}) would indicate that the stand is under stress and not performing optimally. These values coincided with the REW calculated for the six month stress period which was below 0.4. The average g_c/g_{cmax} values for the Boland and the Southern Cape (Knysna and Stormsrivier) areas were 0.265 and 0.533 respectively. Average *ETa/ETp* values were 0.227 and 0.552 the Boland and the Southern Cape areas respectively.

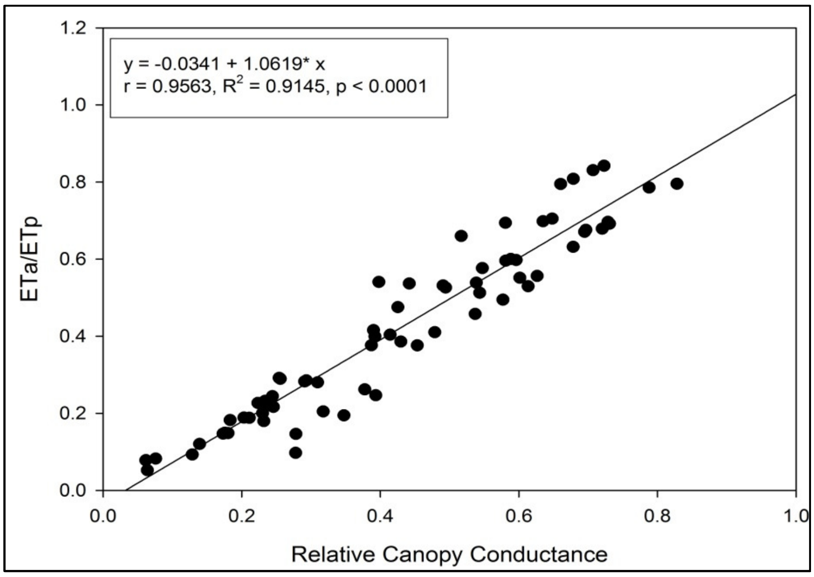

Figure 4.11: The relationship between Relative Canopy Conductance calculated using Granier and Bréda (1996) and the Supply/Demand ratio as derived from HyMo outputs.

This indicates that stands in the Southern Cape region experiences less moisture stress than sites in the Boland and that either g_c/g_{cmax} or *ETa/ETp* would be good response variables to use to indicate water or drought stress.

4.3 δ^{13}C and stress indicators

The calculated relative canopy conductance and supply / demand ratios were plotted against δ^{13}C to investigate the relationship between the variables. On an annual basis, no clear relationships between the data sets were apparent. (Figures 4.12 And 4.13).

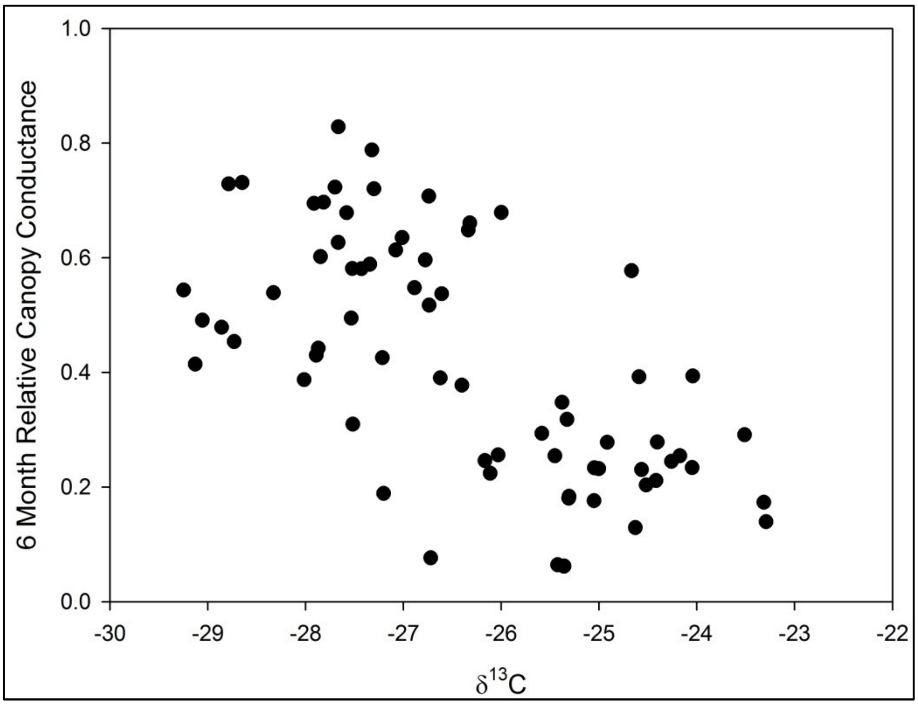

Figure 4.12: Annual values for 6M – RCC calculated for each growing season (tree ring) and the corresponding δ^{13}C value for that specific season.

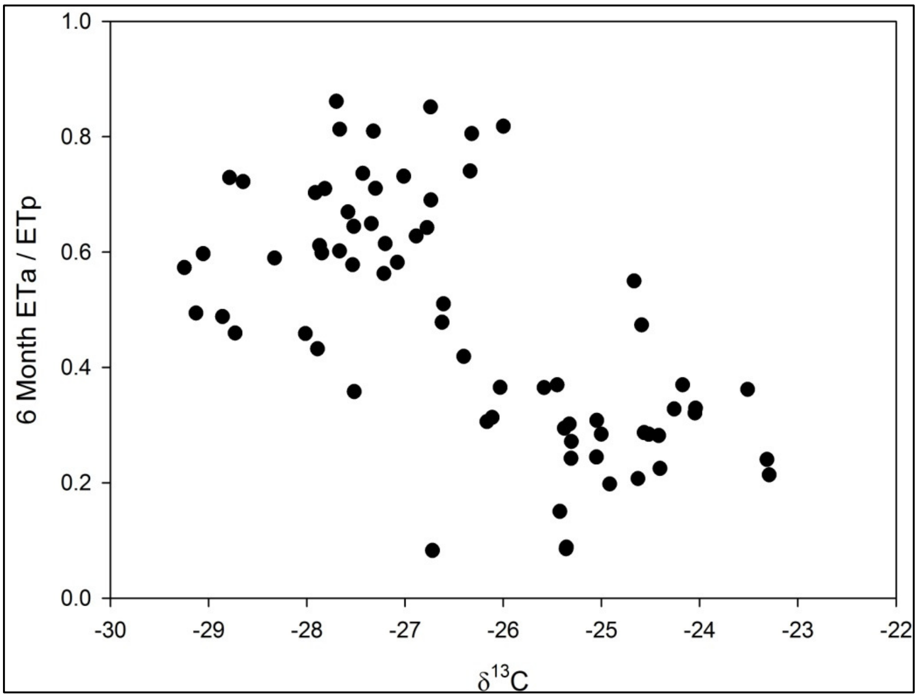

Figure 4.13: Annual values for 6M – ETa / ETp calculated for each growing season (tree ring) and the corresponding δ^{13}C value for that specific season.

In order to try and minimise some of the variation within the data, it was thought that the competition between trees on the stand could be a useful covariate. The stand density index (SDI) was calculated for each year to act as a measure of competition between the trees. Warren et al. (2001) has shown that the stand density influences the $\delta^{13}C$ values and by employing SDI as a covariate in the regression analysis, it was hoped that it would remove some of the covariance. This did not yield significant improvements on the results for either g_c/g_{cmax} (p = 0.701) or *ETa/ETp* (p = 0.300) (data not shown).

In practice however, a plantation manager is not interested in a single year's value as this would be a poor indicator of the average water availability of a particular stand. In order to get an idea about the typical situation in the stand, the average of five yearly values for $\delta^{13}C$, g_c/g_{cmax} and *ETa/ETp* (hereafter 5-yr means) were used for further analysis. Table 4.3 is a summary of the stress factors calculated for each site for six (6M) and twelve (12M) months. The graphic investigation of the data yielded Figures 4.14 and 4.15. In both cases, site G19d in Knysna was an outlier. For a few years this site yielded $\delta^{13}C$ values in the region of -29‰ while the other three sites had $\delta^{13}C$ values ranging from -25‰ to -28‰. It is thought that this site might receive seepage water in the subsoil and it was decided to exclude this site from the study.

With site G19d excluded, linear regressions were done with 5-year means of $\delta^{13}C$ as independent variable and 5-yr means of g_c/g_{cmax} and *ETa/ETp*, in turn, as response variables. These regressions were done for various stress periods throughout the year starting with the two months in the middle of the stress period and adding a month on either side of these until a period of 12 months was reached. This was done to ensure that the best model for stress, as indicated by $\delta^{13}C$ could be selected.

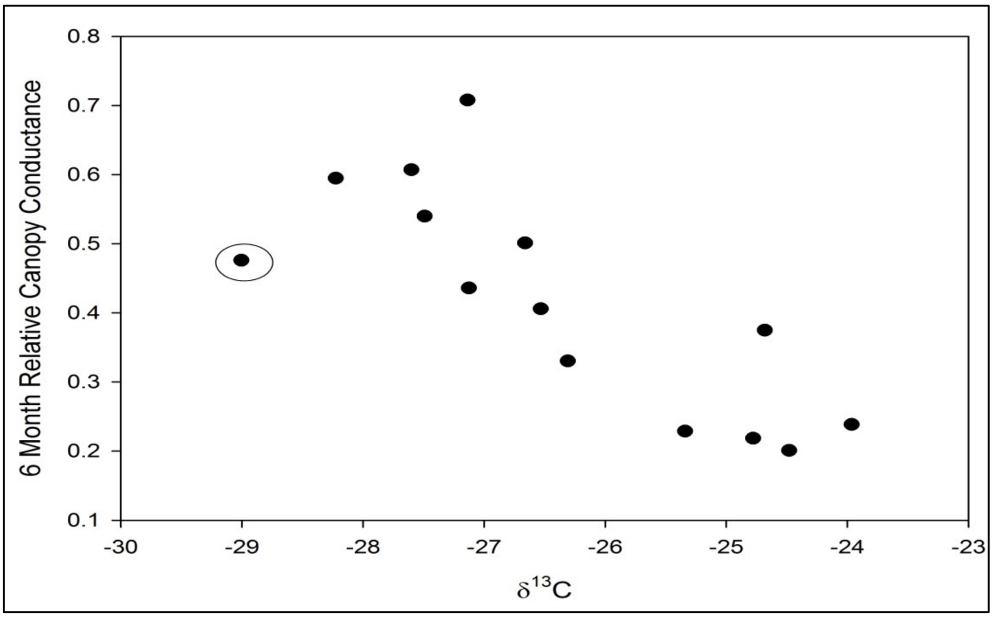

Figure 4.14: The relationship between 5-yr mean values of the 6M - RCC and $\delta^{13}C$. Each dot represents the mean of the last 5 years leading up to the mid-rotation thinning. The outlier data point, Site G19d, is marked with a circle.

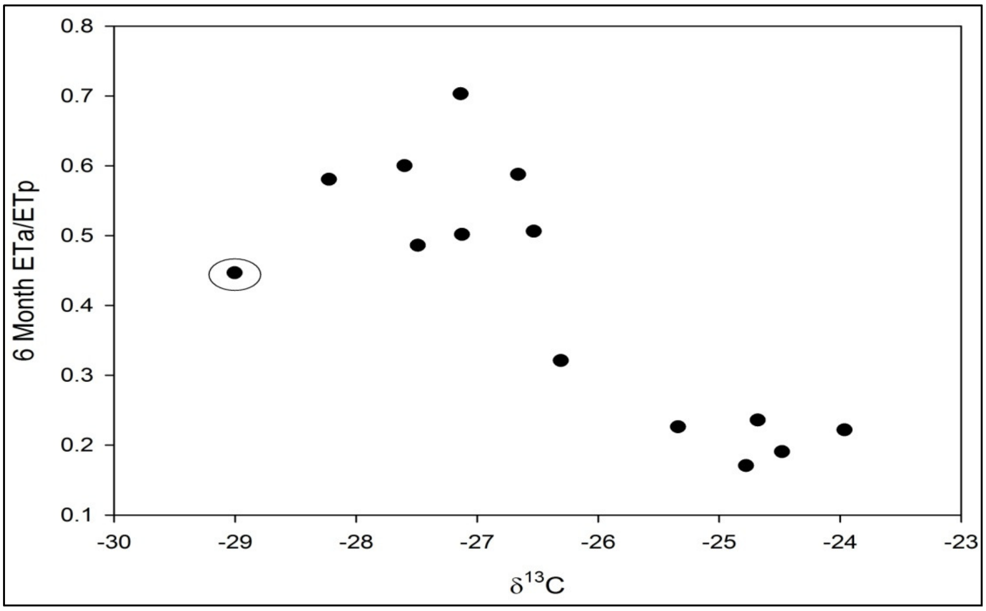

Figure 4.15: The relationship between 5-yr mean values of 6M - ETa/ETp and $\delta^{13}C$. Each dot represents the mean of the last 5 years leading up to the mid-rotation thinning. The outlier data point, Site G19d, is marked with a circle.

For relative canopy conductance the best two models were obtained when using the average values for four and six months during the stress period. Periods above eight months did not deliver a good fit on the data. Using the supply / demand ratio, all the models, except for the two months in the middle of the stress period, produced a very good fit of the data. Standardising on the models to be used, the six months stress period was chosen. Although the four month stress period models have a similar fit, the six month period would cover the whole stress period during a growing year.

The ANOVA results for the two models chosen to describe stand stress using $\delta^{13}C$ are shown in Tables 4.4 and 4.5. Figures 4.16 and 4.17 show the fitted regression lines with the 95% predictive confidence intervals for both response variables. Both models fit the data very well, yielding $r^2 = 0.7347$ ($p = 0.002$) for 6M-RCC and $r^2 = 0.7822$ ($p < 0.001$) for 6M-*ETa/ETp.*

Table 4.3: Summarised stress indicators for study sites for two measurement periods.

		6 Months		**12 Months**	
Site	**Area**	**g_c/g_{cmax}**	**ETa/Etp**	**g_c/g_{cmax}**	**ETa/Etp**
L35b	Boland	0.23	0.23	0.59	0.31
N15a	Boland	0.20	0.19	0.58	0.28
G36	Boland	0.22	0.17	0.59	0.27
M4	Boland	0.37	0.24	0.68	0.32
A35c	Boland	0.24	0.22	0.60	0.31
E18	Boland	0.33	0.32	0.65	0.39
E2a	Knysna	0.44	0.50	0.58	0.54
F14c	Knysna	0.41	0.51	0.43	0.56
G4c	Knysna	0.50	0.59	0.60	0.62
G19d	Knysna	0.48	0.45	0.72	0.52
D3d	Stormsrivier	0.71	0.70	0.85	0.73
D10	Stormsrivier	0.61	0.60	0.79	0.64
D60	Stormsrivier	0.59	0.58	0.79	0.63
B5a	Stormsrivier	0.54	0.49	0.74	0.54

The 95% predictive confidence interval values for 6M-RCC were -0.06268 (upper limit) and -0.1458 (lower limit) while for 6M-*ETa/ETp* these limits were -0.07787 (upper limit) and -0.16182 (lower limit). Data for the other tested models are not presented here.

Table 4.4: ANOVA for using mean δ^{13}C per site to describe 6M-RCC.

	Degrees of Freedom	Conductance SS	Conductance MS	Conductance F	Conductance p
Intercept	1	0.177847	0.177847	21.86570	0.000676
δ^{13}C	1	0.247822	0.247822	30.46883	0.000181
Error	11	0.089470	0.008134		
Total	12	0.337292			

Table 4.5: ANOVA for using mean δ^{13}C per site to describe the mean 6M-*ETa/ETp.*

	Degrees of Freedom	ETa/ETp SS	ETa/ETp MS	ETa/ETp F	ETa/ETp p
Intercept	1	0.246845	0.246845	29.76459	0.000199
δ^{13}C	1	0.327529	0.327529	39.49353	0.000060
Error	11	0.091226	0.008293		
Total	12	0.418755			

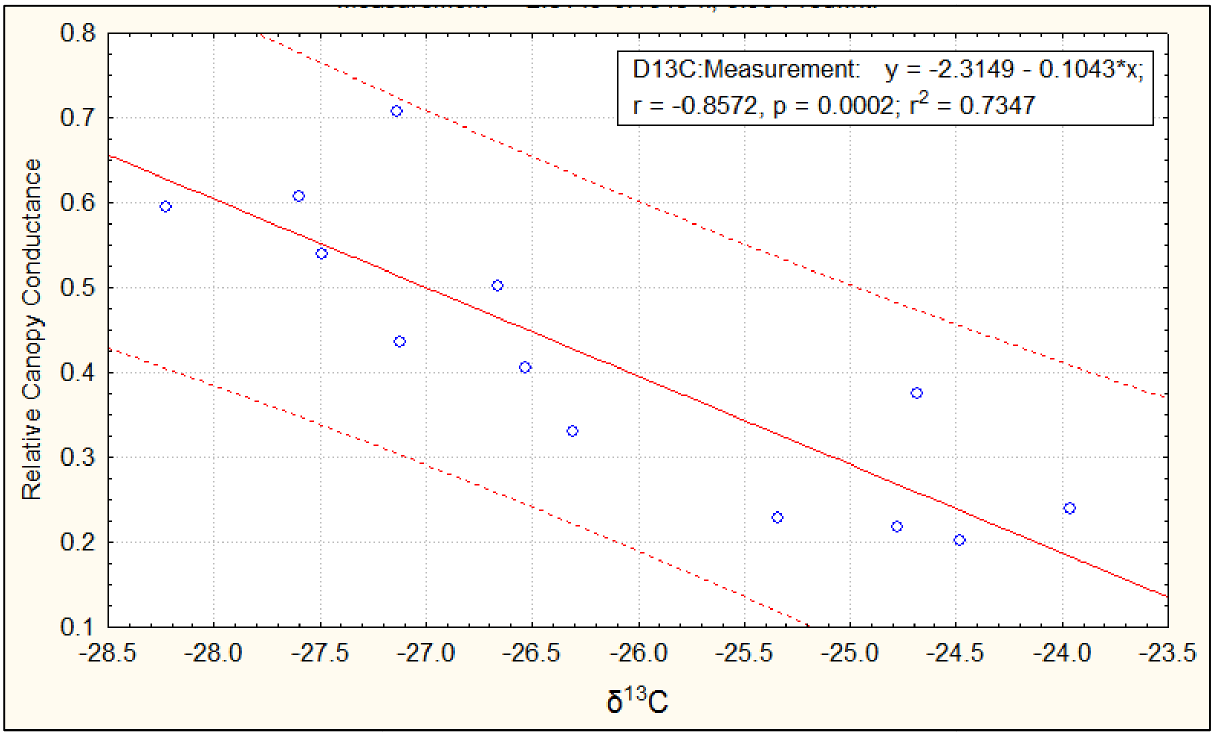

Figure 4.16: Fitted regression for 5-yr mean values of 6M-RCC and δ^{13}C.

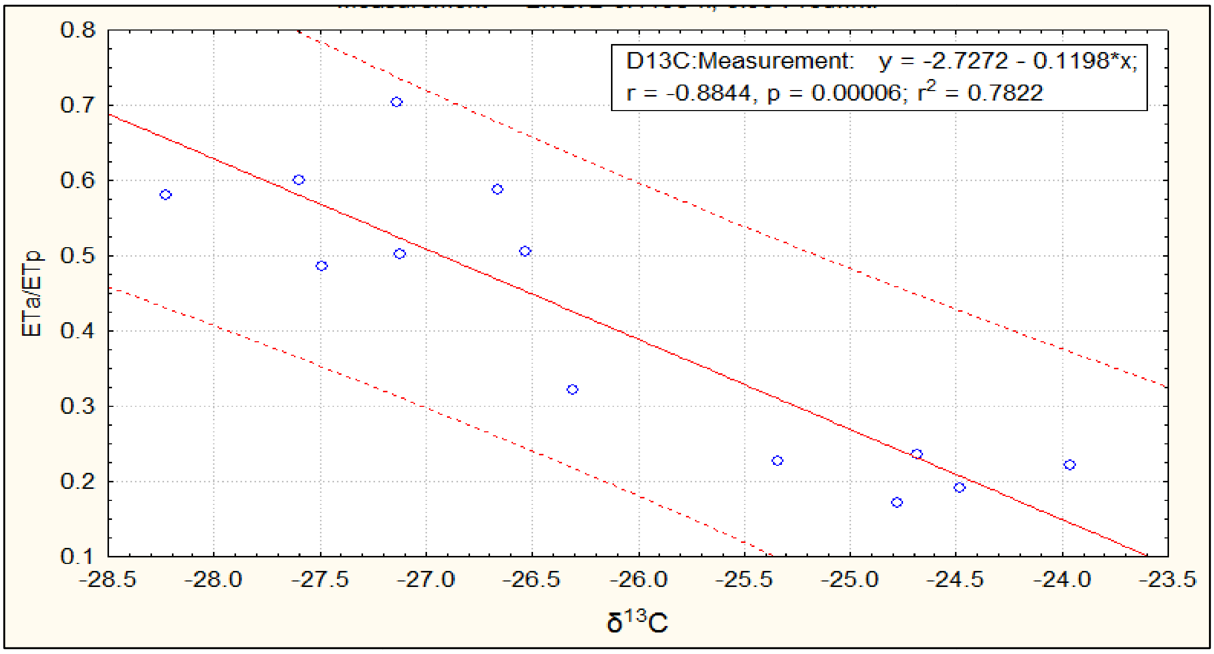

Figure 4.17: Fitted regression for 5-yr mean values of 6M-ETa/ETp and $\delta^{13}C$.

4.4 Accumulated growth degree days

As a further investigation into possible stress indicators, the relationship between 5-yr means of $\delta^{13}C$ and accumulated growth degree days was investigated. Using the same methodology of stress period selection, a number of regressions were made to determine the relationship. The models for g_c/g_{cmax} and *ETa/ETp* only produced an r^2 value of 0.5618 ($p = 0.0031$) and 95% predictive confidence intervals of -62.967 (upper limit) and -241.26 (lower limit). Two other periods (two and four months) were comparable to this while the rest yielded poorer fits. The ANOVA table for the six month model is shown in Table 4.6. Data for the other tested models are not shown.

Table 4.6: ANOVA for using the 5-yr mean $\delta^{13}C$ to describe the accumulated growth degree days for six months.

	Degrees of Freedom	G_r SS	G_r MS	G_r F	G_r p
Intercept	1	357709.5	357709.5	9.56236	0.010244
$\delta^{13}C$	1	527619.5	527619.5	14.10442	0.003179
Error	11	411489.0	37408.1		
Total	12	939108.5			

Chapter 5: Discussion and Recommendations

5.1 Pilot trial

Hypothesis 1 (Section 2.7) states that the $\delta^{13}C$ values of tree ring latewood from *P. radiata* stands under different climatic conditions are not significantly different from each other. The differences between the $\delta^{13}C$ values on the pilot trial sites indicated that sites with differing MAP but within the same rainfall region have significantly different $\delta^{13}C$ values. This means that the alternative formulation of Hypothesis 1 should be accepted. Because of the differences in $\delta^{13}C$ values it was possible to continue with the main trial and also to extend the study area to areas with a different rainfall regime. The fact that MAP showed a significant correlation with $\delta^{13}C$ values in Jonkershoek (Figure 4.2), and seeing that the year with lowest precipitation also yielded the least negative $\delta^{13}C$ value, was encouraging. However, this relationship was obtained with data from one site and the introduction of more sites would likely introduce more variance. West et al. (2001) found a range of $\delta^{13}C$ values of between -21‰ and -27‰ for samples taken from indigenous species in the Diepwalle and Hilltop forests in South Africa. Macfarlane et al. (1999) reported a range of values between -21‰ and -28‰ for *Eucalyptus globulus* and *Pinus radiata* while McCarroll and Loader (2004) report values ranging from -20‰ to -30‰. $\delta^{13}C$ values found in the pilot study ranged between -23 ‰ and -25.5‰ and is thus representative of the drier end of the spectrum of values reported in the literature for comparable forestry examples. This was to be expected as both sites are located in the winter rainfall area with a pronounced summer drought period.

The weak correlation between $\delta^{13}C$ and mean annual precipitation at Kluitjieskraal can partly be attributed to the location of the site. The compartment from which the samples were taken is situated between the weather station and a mountain range. A precipitation gradient is present with more precipitation taking place towards the mountain (judging from rain gauges near the foot and the top the mountain, respectively). At the Jonkershoek site, the sample compartment is situated closer to the weather station with no topographical changes or other evidence of a precipitation gradient being present. This was an indication that sample compartments for the main trial had to be selected as close to a weather station as possible and that care should be taken to ensure that any precipitation gradient effects were not present.

5.2 Main trial

5.2.1 Soil water availability

Due to sub-optimal weather station network and a lack of soil water availability modelling, foresters generally base their silvicultural decisions on indices as crude as mean annual precipitation or mean monthly precipitation. The soil water content over time, as modelled by HyMo gives us a much more comprehensive understanding of the available soil water in forest stands over seasons and years in the Southern and Western Cape forestry areas (Figures 4.4 to 4.6). The Boland region which receives winter rain is subject to a strong seasonal pattern of soil water availability. During the winter, the soil profile stays fully charged for long periods of time because precipitation events are frequent and evapotranspiration is very low (the average evapotranspiration rate for June/July in the modelled sites were less than 0.1 mm/d, Appendix 3). With the change from winter to spring, the available soil water content declines rapidly due to increases in ETa to a point where the soil water approaches wilting point during the hot, dry summer (Figure 4.4). The rapid depletion of available soil water stems from the fact that profile available water in modelled Boland sites ranges from 75 to 178 mm (Table 3.1) and the corresponding daily evapotranspiration rates for October-November average around 3 mm day^{-1} . This rapid depletion of soil water is also reflected in the g_c/g_{cmax} and *ETa/ETp* values for the Boland region.

The sites in the Southern Cape show a more gradual change in soil water content over time (Figures 4.5 and 4.6). The periods where soil water content is almost at wilting point are shorter and these are frequently interrupted by precipitation events. The result is that g_c/g_{cmax} and *ETa/ETp* for these areas are higher, indicating less moisture stress. Appendix 3 depicts the monthly values of *ETa*, *ETp* and *ETa/ETp*, averaged over five years for three sites typical to each region. During June and July, the VPD in the region is generally so low that evapotranspiration drops to very low levels. As the year progresses, the *ETp* rises sharply from October to January in the

Boland. The *ETa* however decreases rapidly from November to January as the soil profile dries out and stand stress sets in. In the Knysna and Stormsrivier areas, the increase in *ETp* from October to January is not as severe as in the Boland. The intervals between rainfall events are also shorter which provides stands with larger amounts of available water. This leads to smaller decrease in *ETa* than in the Boland. The amount of solar radiation that the regions receive also differs. The Knysna and Stormsrivier areas regularly receives mist which reduces solar radiation while the Boland area has more could free days.

In this study, the locations of sample sites were chosen so that they were positioned on water-shedding locations. The effect of this selection process was that the sample sites represent the drier end of the spectrum, on the plantation estates. The average 12 month *ETa/ETp* values for the study sites ranged from 0.30 in the Boland to 0.56 in the Knysna and 0.63 in the Stormsrivier area. When comparing these results with relative extractable water typically held in the soil profiles (Figures 4.7 to 4.9), it becomes clear that the shorter interval between rainfall events in the Knysna and Stormsrivier area, especially during the summer months, makes more water available in the soil profile with a reduction in stand stress, leading to increased stand productivity. Various authors have reported on ETa / ETp ratios (reported over a full 12 month period) for different types of vegetation. Wang and Klinka (1996) have reported ETa / ETp values between 0.71 and 1 in white spruce (*Picea glauca*) in British Colombia, Canada. Lutz *et al.,* (2010) reported values of 0.56 to 0.85 for various tree species within the Yosimite National Park, USA. The 6M ETa / ETp values are listed in Table 5.1 as well as the corresponding 12 month ETa / ETp (12M ETa / ETp) values for each region.

Table 5.1: 6M – and 12 month supply / demand ratio calculated for the study region.

Region	**6M ETa / ETp**	**12M ETa / ETp**
Boland	0.22	0.31
Knysna	0.51	0.56
Stormsrivier	0.59	0.63

Garbulsky *et al.* (2010) conducted a study on the control and patterns of radiation use efficiency (RUE) and primary productivity across a wide range of ecosystems (tundra to rainforest). Their results showed that, except for rainfall, the actual evapotranspiration positively influence the spatial variation of gross primary production in forests. In colder ecosystems, savanna and Mediterranean forests, RUE increased with an increase in *ETa/ETp.* For these environments actual evapotranspiration accounted for a high proportion of the variability in gross primary production (R^2 = 0.70, p < 0.0001). Beadle (1997) adapted work from various authors (Myers *et al.,* 1996; Honeysett *et al.,* 1996; White, 1996) and showed that greater leaf area indices were measured in stands with a higher *ETa/ETp* value. In short, assuming that nutrition is not a limiting factor, not only will areas with higher *ETa/ETp* values allow for the development of a greater leaf area index, but the RUE and GPP will be higher.

Keeping in mind that the most stressed compartments were used for this study, and following results from various authors (Zhou and Zhang, 1996; Garbulsky *et al.,* 2010; Lutz *et al.,* 2010), it can be postulated that the productivity potential of many of the Boland sites planted on water shedding locations are extremely low. This raises the question of management intensity on such stressed sites. The higher stress levels in the Boland underlines the importance of proper stand management in this area. To maintain a high level of productivity and get the maximum economic return, intensively managed *P. radiata* stands must be restricted to sites which exhibit less drought stress. Sites with significant drought stress could be managed less intensively with regard to thinning, pruning and fertilization (Turner and Lambert, 1987; Cole *et al.*, 1990, McMurtrie *et al.,* 1990; Zwolinski *et al.*,1998) or in extreme cases, even be earmarked for different species/genotypes.

The positive interaction between water availability and fertilization has been described by various authors (Raison et al., 1990; Benson et al., 1992; Ewers et al., 2000; Albaugh et al., 2004). The cost of fertilization is an investment made by the forest company. The return on an investment would be the deciding factor on the implementation of fertilization in forest stands. If a stand experiences large amounts of stress due to the unavailability of soil water, it is highly likely that the growth response to fertilization on such sites would be very low and that such an investment would lead to a financial loss. By knowing how much water is available on a site, more informed decisions can be made in terms of whether fertilization is a viable option.

However, as stated in Chapter 2, forests occur on many landscape positions where water may enter the soil profile by means of lateral flow, making it impossible to use simple rainfall-based soil water balance models (such as HyMo) to track water availability and stress. This is where $\delta^{13}C$ may become a useful surrogate for stand drought stress.

5.2.2 $\delta^{13}C$ and drought stress indicators.

On an individual year basis, the weak correlation between $\delta^{13}C$ and either g_c/g_{cmax} or *ETa/ETp* indicates that there still is a lot of unexplained variation. This could be due to sampling error, measurement error or a possible "carry over" effect that could be present between growing seasons. Warren et al. (2001) working on *Pinus radiata* in New Zealand reported that a significant component of variation in $\delta^{13}C$ values was due to altitude with discrimination decreasing 2.53‰ km-^{1} altitude in their study. It is thought that this relationship is can be explained by the autocorrelation between altitude and drought stress observed in the study, rather than to altitude per se. They also reported that fertilization had an effect on $\delta^{13}C$ values. The nutrition status of the stands in the study areas were not determined as it was thought that this signal would not have been as strong as soil water related $\delta^{13}C$ signals. Combined with this, Martínez-Carrasco et al. (1998) reported a difference in $\delta^{13}C$ for *Casuarina equisetifolia* grown under well-watered and stressed conditions and fed with different sources of nitrogen (NH_4^+ and NO_3^-). This would indicate that not only does plant nutrition affect the $\delta^{13}C$ values, but that the type of nutrition could lead to differences in carbon discrimination. No records of historical fertilization operations were available and this could have influenced the $\delta^{13}C$ values that were obtained in this study.

Tree age has also been suggested to have an effect on $\delta^{13}C$ values. McCarroll and Pawellek (2001) reported age-related trends in *Pinus sylvestris* ranging in age from less than 36 years to more than 130 years, while Duquesnay *et al*. (1998) reported similar results in high forest beech trees which were sampled in three age classes of 10, 14 and 24 years.

Donald (1988) investigated using ^{14}C as a method for the early ranking and grading of *Eucalyptus grandis* clones in South Africa. This method did not yield significant results, yet it was the first step in the direction of carbon isotopes as bio-indicators in South African Forestry. Bond and Stock (1990) investigated a similar matter in the former Eastern Transvaal using $\delta^{13}C$ as an indicator to assess the possibility of using the carbon discrimination as indicator when screening clones in genotype / environment interaction trials for the most water efficient genotypes. Their results showed a significant difference between clones ($p<0.05$). However, Corcuera *et al.* (2010) and Rowell et al. (2009) suggests that tree ring $\delta^{13}C$ values is a result of uniform, stomatal driven response to drought stress and that environmental factors, rather than genotype affects carbon discrimination.

The lack of adequate solar radiation data in this study and the use of modelled values could also have introduced a degree of variation which cannot be quantified in this study. The rooting depth and soil profile depths were determined as accurately as possible but the possibility still exists that there could have been an influence of sub-soil water on the $\delta^{13}C$ values obtained for the trial sites. This would have caused an enriched ^{13}C fraction and thus more negative $\delta^{13}C$ values indicating higher water availability than what could have been modelled using HyMo.

All these sources of variation or a combination thereof could be the cause for the weak correlation between $\delta^{13}C$ and g_c/g_{cmax} or ETa/ETp on a year-to-year basis. To be able to work with data at such a resolution, further studies will have to be carried out to determine the effects of these factors in a South African context.

However, from a managerial point of view it would be worth more to know what the longer term trends in plantation stands are. Mean annual precipitation is often used as one of the criteria to make site-species matching decisions. In seasonally dry climates, especially those with limited soil water holding capacity, mean annual rainfall will be a poor indicator of stand water stress. By taking the average $\delta^{13}C$ value over a number of years, forest management can get a more representative idea as to the severity of soil moisture stress that a stand experiences at mid rotation age. This also minimises the amount of variation that could have been caused by any of the factors mentioned above. Following the protocol set out here, co-dominant trees which are removed during the first thinning operation can be sampled as they are removed from the stand, eliminating the need to destroy standing trees. A further improvement on the sampling technique would be the taking of timber cores at breast height instead of whole disks.

By integrating the $\delta^{13}C$ values obtained with each thinning into a geographic information system, representative values for stand stress across a whole plantation can be mapped and used for future site/species matching and silvicultural decisions. Management decision on whether to fertilise a particular stand would then be made easier as the likelihood of the response to fertilization becomes more certain. $\delta^{13}C$ indicating lower stress conditions would mean that more soil water is available for use by the stand and thus the likelihood and magnitude of response to fertilization would be greater (Bergh et al., 1999; Carlson, 2000, Campion, 2005; du Toit, 2006; Chikumbu, in process). Stands experiencing drought stress (as indicated by low g_c/g_{cmax} or *ETa/ETp* values), will shift their carbon allocation patterns and more carbon will be allocated to root growth (Erricsson et al, 1996, Axelsson and Axelsson, 1986). Under such conditions fertilization will not have the desired effect of improving volume growth and may result in a financial loss to the forest company.

With the data presented from the regression analysis and the discussion above, it is clear that we can accept the alternative formulation of Hypothesis 2, as stated in Section 2.7. $\delta^{13}C$ values do become less negative with a decrease in the calculated available soil water levels during the dry season across different sites and years. However, the amount of variation accounted for by the relationship is not strong enough to predict annual drought stress from $\delta^{13}C$ on an individual latewood ring basis.

5.3 Accumulated Growth Degree Days Weighted against Rainfall

The results obtained for the regression between G_r and $\delta^{13}C$ did not yield very strong correlations ($p = 0.003$, $r^2 = 0.561$). Even though this method can easily be used as a quick way of assessing the situation in field with regards to $\delta^{13}C$, it is not a very good indicator of stress. It is thought that this relationship can be due to the fact that as it was used here as the temperature term for calculating GDD using a base temperature of 10^0C and this is not necessarily the optimum temperature for growth. To refine the temperature term it is suggested that a temperature modifier, similar to the modifier used in the 3-PG model (Landsberg and Waring, 1997), be used to adjust the base temperature for the GDD calculation in order to use the optimum growth temperature for every specific day.

Another shortfall of this method is that the water component of the equation uses daily rainfall as a surrogate for water availability. Not all precipitation is available for use by the stand. Some is lost through run-off and canopy interception, or it percolates too deep into the mineral soil that it is out of reach of the roots (Loustau et al., 1992; Kelliher et al., 1992). The fraction that is lost is not accounted for when calculating G_r. The moisture term used when calculating G_r can be refined by using a soil water balance model like HyMo, the daily REW can be calculated and used instead of the daily precipitation. This leaves the question of whether this method would then be worthwhile implementing as a management tool since using the detailed soil water balance on its own would already be more meaningful than G_r alone.

This method might not deliver the precision that is needed in order to assist in management decision, but it could still prove useful as a rapid screening method where decisions have to be made quickly. After the initial screening, $\delta^{13}C$ values have to be obtained in order to get a better handle on stand conditions.

5.4 Recommendations

For management purposes it would be suitable to use an 80% predictive confidence interval instead of the 95% used in Figure 4.17. Adding more sites to the dataset from which the current proposed model has been developed will also increase the predictive power of the model. Work done by Donald (1987) and Chikumbu (in progress) on sites similar and in close proximity to the E18 study site have produced significant, long term responses to fertilization. The 6M ETa / ETp value for compartment E18 is 0.32. This compartment is the wettest site of the Boland trial sites. Using the 6M Eta / ETp value and historic responses to fertilization the 6M ETa / ETp value of 0.3 has been chosen as a threshold between “Dry” and “Intermediate” stands. In the Knysna area the average ETa / ETp value for all study sites is 0.51. From the literature it appears that forest stands below an ETa / ETp ratio of 0.5 are low productivity sites. The 6M ETa / ETp value of 0.51 (0.56 if 12M ETa / ETp is used) has for this reason been chosen as a threshold value between “Intermediate” and “Wet” sites. Each of these three classes, dry, intermediate and wet, will exhibit a response likelihood to silvicultural operations such as fertilization. Using Figure 4.17, these three classes has been grouped with $\delta^{13}C$ values measured in the study. The result of this grouping (Table 5.2) is proposed as a possible management tool which will allow forest managers to apply more site specific silviculture. The limited number of study sites does not allow for the creation of more than three classes but with further research the number of proposed classes can be increased.

The null hypothesis for Hypothesis 3 can now also be disproved and the alternative formulation can be accepted. $\delta^{13}C$ can be used as a useful management tool, however, provided that bulked samples across several years are used.

Table 5.1: Fertilization response likelihood classes based on $\delta^{13}C$ values.

$\delta^{13}C$ Value (‰)	**Site Class**	**Response magnitude to fertilization ***	**Relative Canopy conductance**	**6 Month ETa / ETp**	**12 Month ETa / ETp**
-23 to -25	Dry	None to low	0 - 0.3	0 - 0.3	0 – 0.35
-25 to -27	Intermediate	Intermediate	0.3 - 0.5	0.3 - 0.5	0.35 – 0.55
-27 to -29	Wet	High to very high	0.5 - 0.8	0.5 - 0.75	0.55 – 0.80

* Only an estimate of potential responses on nutrient poor sites based on various authors (Donald 1987; Donald *et al.,* 1987; Payne *et al.,* 1988; Chikumbu, in progress)

For future work it would be advised that more precise measurements of solar radiation be used in order to further improve on the suggested models. Forestry companies should investigate the possibility of upgrading existing weather stations to include solar radiation measurement as well as a proper database management system to make access and use of meteorological data for research less cumbersome. Chikumbu (in progress) has also reported on significant differences in timber volume increment within the Boland region following fertiliser application to mid-rotation stands. Further studies on these sites will allow for a refinement of the three proposed moisture classes (as indicated in Table 5.1) and could also include the addition of more classes.

The sample preparation for $\delta^{13}C$ determination can be enhanced by investigating the different methods used in literature to obtain the cellulose used for isotopic ratio mass spectrometry. The possibility exists that whole wood samples can be processed without the need for extracting non-cellulosic material which will cut down on sampling costs. Also the use of late – and early wood as a single sample can reduce preparation time of samples, but this needs further investigation. The use of glass fibre filters instead of cellulosic filter paper could also improve the results obtained from the extraction process.

5.5 Conclusion

Understanding how much water is available to plantation stands and during which period of the growing season this amount of water is available is of critical importance to both scientists and plantation managers alike. This allows for more in depth scientific studies to be carried out and serves to strengthen any decisions made on the management intensity of a stand. This study has shown that on water shedding sites in the Boland, the amount of water available to stands reaches a maximum between May and October. During the rest of the year, these sites experience moderate to extreme drought stress. The seasonal pattern of drought stress is present in the Southern Cape forestry regions as well; however, the intensity of the drought stress is less than in the Boland.

Carbon isotope fractionation serves as a good measure of drought stress in plantation stands. $\delta^{13}C$ values differ not only between rainfall regions, but also within the same region. Lower soil water availability results in less negative $\delta^{13}C$ values and is strongly related to the different measures of stand stress investigated in this study. The use of $\delta^{13}C$ as a predictor of the soil moisture stress that plantation stands experience is a powerful tool that will enhance site management. By knowing the amount of stress in a particular stand, better site/species matching decisions can be made and first choice species can be planted on the optimum sites, thus improving the possibility of a more productive crop. Decisions on thinning and pruning intensities and whether or not to fertilise stands can now also be made with greater confidence and a higher likelihood of return on investment.

Appendix 1
Soil Classification and Information

Site	L35b
Plantation	Haweqwas
Soil form	Tukulu 1110
Estimated Soil Depth	160 cm
Soil profile Wilting Point (vol%)	2
Soil profile Field Capacity (vol%)	8.55

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
L/35b/1	0 - 10cm	4	6	90	55.08	12.64	22.28	Sa	19.39
L/35b/2	10 - 20cm	12	11	77	48.3	13.08	15.62	SaLm	16.24
L/35b/3	20 - 60cm	22	10	68	41.92	9.6	16.48	SaKlLm	30.68
L/35b/4	60 - 90cm	22	10	68	40.8	11	16.2	SaKlLm	16.78
L/35b/5	90 - 100cm	22	13	65	40.8	10	14.2	SaKlLm	1.35
L/35b/6	100 - 120cm	20	16	64	41.2	9.8	13	SaLm	13.57
L/35b/7	120 - 140cm	22	16	62	40.76	8.8	12.44	SaKlLm	1.58
L/35b/8	140 - 150cm	20	16	64	43.8	9.2	11	SaLm	0.86
L/35b/9	150 - 160cm	15	14	71	43.8	10.2	17	SaLm	29.66

Site	E18
Plantation	Grabouw
Soil form	Cartref 1200
Estimated Soil Depth	200 cm
Soil Profile Wilting point (Vol%)	3.15
Soil Profile Field Capacity (Vol %)	8.6

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
E 18/1	0 - 10cm	2.4	0.4	97.2	55.18	23.8	18.22	Sa	0.00
E 18/2	10 - 20cm	2.2	2.2	95.6	36.2	30	29.4	Sa	0.00
E 18/3	20 - 30cm	2.2	0.2	67.6	38.16	3	26.44	Sa	0.00
E 18/4	30 - 70cm	2	2	96	22.6	23.6	49.8	Sa	17.18
E 18/5	70 - 80cm	2.2	2.2	125.6	50.96	16.64	58	Sa	39.53
E 18/6	80 - 100cm	2	1	97	30.2	16.6	50.2	Sa	50.77
E 18/7	100 - 110cm	2	1	97	32.14	21.66	43.2	Sa	47.06
E 18/8	110 - 120cm	4	8	88	52.16	28.8	7.04	LmSa	16.74
E 18/9	120 - 130cm	8	8	84	55.4	24	4.6	LmSa	8.89
E 18/10	130 - 140cm	8	8	84	49.54	31.4	3.06	LmSa	5.05
E 18/11	140 - 170cm	10	8	82	51.34	26.46	4.2	LmSa	2.64
E 18/12	170 - 180cm	10	8	82	32.12	23.08	26.8	LmSa	3.32
E 18/13	180 - 190cm	12	6	82	50.6	27.4	4	SaLm	3.93
E 18/14	190 - 200cm	8	8	84	57.2	22	4.8	LmSa	3.03

Site	G36
Plantation	Grabouw
Soil form	Bainsvlei 1100
Estimated Soil Depth	45cm
Soil Profile Wilting point (Vol%)	8.4
Soil profile Field Capacity (vol%)	24.8

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
G 36/1	0 - 10cm	8	26	66	61.94	1.46	2.6	LmSa	5.394605
G 36/2	10- 20cm	18	26	56	47.56	4.44	4	SaLm	2.638191
G 36/3	20 - 40cm	24	28	48	42.6	0.8	4.6	SaKlLm	40.6071

Site	N15a
Plantation	Grabouw
Soil form	Oakleaf 2110
Estimated Soil Depth	60 cm
Soil Profile Wilting point (Vol%)	4.3
Soil profile Field Capacity (vol%)	19.0

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
N15a/1	0 - 10 cm	3.6	11.6	84.8	71.58	4.6	8.62	LmSa	49.45
N15a/2	10 - 20cm	13.2	18	68.8	63.6	1	4.2	SaLm	74.91
N15a/3	20 - 30cm	17.2	16	66.8	52.16	2	12.64	SaLm	74.14
N15a/4	30 - 60cm	19.6	11.8	68.6	52.6	6.6	9.4	SaLm	82.22

Site	A35c
Plantation	La Motte
Soil form	Tukulu 2110
Estimated Soil Depth	200 cm
Soil Profile Wilting point (Vol%)	2.0
Soil profile Field Capacity (vol%)	8.5

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
A35c/1	0 - 10cm	2.8	2	95.2	66.8	23.4	5	Sa	0.0
A35c/2	10 - 20cm	2.8	2	95.2	63.56	26.04	5.6	Sa	0.0
A35c/3	20 - 30cm	3	2.2	94.8	61.86	29.66	3.28	Sa	0.0
A35c/4	30 - 110cm	3	0.2	96.8	72.4	20	4.4	Sa	0.0
A35c/5	110 - 120cm	1.2	2	96.8	70.8	20	6	Sa	0.0
A35c/6	120 - 160cm	1.2	3	95.8	68.52	20.8	6.48	Sa	1.0
A35c/7	160 - 200cm	1.2	2	96.8	68.7	20.68	7.42	Sa	2.2

Site	E2a
Plantation	Kruisfontein
Soil form	Pinegrove 1000
Estimated Soil Depth	200 cm
Soil Profile Wilting point (Vol%)	2.1
Soil profile Field Capacity (vol%)	9.4

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
E2a/1	0 - 10cm	2.8	1.4	95.8	62.8	32.6	0.4	Sa	0.0
E2a/2	10 - 20cm	2.8	3.4	93.8	53.0	40.4	0.4	Sa	0.0
E2a/3	20 - 40cm	1.0	2.0	97.0	68.8	28.0	0.2	Sa	0.0
E2a/4	40 - 60cm	3.2	1.2	95.6	63.7	31.4	0.5	Sa	0.0
E2a/5	60 - 80cm	1.0	4.0	96.0	64.9	30.8	0.3	Sa	0.0
E2a/6	80 - 100cm	3.0	6.0	91.0	62.5	28.3	0.2	Sa	0.0
E2a/7	100 - 140cm	3.0	2.0	95.0	63.2	31.6	0.2	Sa	0.0
E2a/8	140 - 180cm	3.2	2.2	94.6	59.2	35.0	0.4	Sa	0.0
E2a/9	180 - 200cm	7.3	4.0	86.0	55.8	30.0	0.2	Sa	0.0

Site	F14c
Plantation	Kruisfontein
Soil form	Kroonstad 2000
Estimated Soil Depth	180 cm
Soil Profile Wilting point (Vol%)	3.4
Soil profile Field Capacity (vol%)	16.7

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
F14c/1	0 - 10cm	1	14	85	76.28	8.28	0.44	LmSa	0.1
F14c/2	10 - 50cm	5	9	86	76.32	9.48	0.2	LmSa	0.0
F14c/3	50 - 70cm	7	10.4	82.6	74	8.4	0.2	LmSa	0.1
F14c/4	70 - 80cm	10	7.4	82.6	72.8	9.6	0.2	LmSa	0.0
F14c/5	80 - 100cm	9	10	81	73.2	7.6	0.2	LmSa	0.2
F14c/6	100 - 140cm	19.2	7.8	73	65.4	7.4	0.2	SaLm	0.2
F14c/7	140 - 180cm	21	8	71	58.72	11.4	0.88	SaKlLm	0.4

Site	G4c
Plantation	Kruisfontein
Soil form	Pinedene 1200
Estimated Soil Depth	160 cm
Soil Profile Wilting point (Vol%)	4.5
Soil profile Field Capacity (vol%)	18.8

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
G4c/1	0 - 10cm	2.20	8.00	89.80	59.96	29.40	0.44	Sa	0.07
G4c/2	10 - 40cm	5.20	10.00	84.80	59.32	25.08	0.40	LmSa	0.05
G4c/3	40 - 60cm	19.20	8.00	72.80	50.16	22.24	0.40	SaLm	0.15
G4c/4	60 - 80cm	27.20	4.00	68.80	43.80	24.60	0.40	SaKlLm	0.40
G4c/5	80 - 120cm	21.60	3.60	74.80	35.92	38.68	0.20	SaKlLm	0.00
G4c/6	120 - 160cm	13.20	4.00	82.80	41.00	41.60	0.20	SaLm	0.17

Site	G19d
Plantation	Kruisfontein
Soil form	Sepane 2210
Estimated Soil Depth	190 cm
Soil Profile Wilting point (Vol%)	1
Soil profile Field Capacity (vol%)	5.05

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
G19d/1	0 - 10cm	5.00	11.00	84.00	70.96	10.40	2.64	LmSa	0.00
G19d/2	10 - 50cm	7.00	12.00	81.00	65.40	12.00	3.60	LmSa	0.00
G19d/3	50 - 70cm	10.00	12.00	78.00	64.60	11.20	2.20	SaLm	0.00
G19d/4	70 - 90cm	19.00	8.00	73.00	60.80	10.00	2.20	SaLm	0.17
G19d/5	90 - 110cm	21.00	6.00	73.00	63.36	8.00	1.64	SaKlLm	0.37
G19d/6	110 - 130cm	27.00	7.00	66.00	63.40	2.40	0.20	SaKlLm	0.56
G19d/7	130 - 150cm	25.00	12.00	63.00	62.20	0.60	0.20	SaKlLm	0.00
G19d/8	150 - 170cm	17.00	10.00	73.00	63.34	9.00	0.66	SaLm	0.00
G19d/9	170 - 190cm	3.20	24.80	72.00	67.20	4.20	0.60	LmSa	0.00

Site	B5a
Plantation	Bluelilliesbush
Soil form	Longlands 1000
Estimated Soil Depth	60 cm
Soil Profile Wilting point (Vol%)	2.0
Soil profile Field Capacity (vol%)	9.0

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
B5a/1	0 - 10cm	3.00	1.80	95.20	69.74	23.00	2.46	Sa	0.0
B5a/2	10 - 30cm	2.80	0.40	96.80	91.74	3.00	2.06	Sa	0.0
B5a/3	30 - 40cm	2.80	1.40	95.80	58.52	33.68	3.60	Sa	0.0
B5a/4	40 - 60cm	2.80	1.60	95.60	59.60	34.00	2.00	Sa	0.0

Site	D3d
Plantation	Bluelilliesbush
Soil form	Longlands 1000
Estimated Soil Depth	80 cm
Soil Profile Wilting point (Vol%)	2.7
Soil profile Field Capacity (vol%)	13.6

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
D3d/1	0 - 10cm	1.8	6.4	91.8	86.4	4.8	0.6	Sa	0
D3d/2	10 - 30cm	0.8	16.4	82.8	73.72	7.6	1.48	LmSa	0
D3d/3	30 - 45cm	3.2	18	78.8	70.6	7	1.2	LmSa	0
D3d/4	60 - 70cm	13.2	12	74.8	37.18	14.02	23.6	SaLm	0.9456

Site	D10
Plantation	Bluelilliesbush
Soil form	Cartref 1200
Estimated Soil Depth	50 cm
Soil Profile Wilting point (Vol%)	2.0
Soil profile Field Capacity (vol%)	10.0

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
D10/1	0 - 10cm	3.40	2.40	94.20	76.12	13.28	4.80	Sa	0.00
D10/2	10 - 20cm	3.00	8.00	89.00	65.40	20.20	3.40	Sa	18.45
D10/3	20 - 50cm	3.00	9.00	88.00	60.92	21.60	5.48	Sa	6.54

Site	D60
Plantation	Bluelilliesbush
Soil form	Cartref 1200
Estimated Soil Depth	60 cm
Soil Profile Wilting point (Vol%)	2.0
Soil profile Field Capacity (vol%)	9.5

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
D60/1	0 - 10cm	3.40	2.40	94.20	62.74	26.00	5.46	Sa	0.00
D60/2	10 - 30cm	3.40	2.40	94.20	62.20	27.00	5.00	Sa	0.00
D60/3	30 - 40cm	1.00	8.00	91.00	59.96	26.00	5.04	Sa	0.00
D60/4	40 - 60cm	1.00	10.00	89.00	57.36	26.64	5.00	Sa	0.22

Site	M4
Plantation	Jonkershoek
Soil form	Cartref 1100
Estimated Soil Depth	80 cm
Soil Profile Wilting point (Vol%)	2.0
Soil profile Field Capacity (vol%)	9.0

Sample	Depth (cm)	Clay (%)	Silt (%)	Total Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Classification	% Coarse fragments
M4/1	10	3.2	1.4	95.4	39.2	48	8.2	Sa	0
M4/2	20	3	0.2	96.8	37.4	50.4	9	Sa	6.684142
M4/3	60	3	2.2	94.8	37.8	52.4	4.6	Sa	97.06266
M4/4	80	3	2.2	94.8	37.8	52.4	4.6	Sa	68.76525

Appendix 2
Estimating Missing Meteorological Parameters

A full set of meteorological data is worth its weight in gold. It does however happen that some meteorological parameters are either not measured or not logged by the weather station. In such cases the missing values have to be estimated from the existing data using models. Models can describe the real world situation that we are interested in, however, the answers that are derived from such models are not 100% accurate. Due to experimental or modelling error, a model will always over-or underestimate any parameter that we are interested in.

In order to obtain full data sets for use with a soil water availability model, missing data had to be estimated using published prediction models. The parameters that were most often absent from weather station data were solar radiation, relative humidity and evapotranspiration.

Unless otherwise stated, all calculations were done according to the guidelines set forth in Allen *et al.* (1998).

Solar Radiation Estimates

This specific model we used was chosen due to its simplicity and used to estimate the solar radiation where it was missing. The Hargreaves and Samani (1982) model to estimate solar radiation (R_S) is described as follows:

$$R_s = KT * R_a * TD^{0.5} \qquad \text{(Eq 1.1)}$$

Where TD = the daily temperature difference (maximum minus minimum) (^{0}C); R_a = extraterrestrial solar radiation (MJ/day) and KT = an empirical coefficient.

The empirical coefficient used depends on where the sites are located. For inland sites, 0.17 is used as the value for KT, and 0.2 is used for coastal regions.

Throughout this study, a value of 0.2 was assigned to KT. The extraterrestrial solar radiation is the amount of radiation that reaches the top of earth's atmosphere. This will be determined by the position of the earth around the sun. For each day and at different latitudes it can be determined from the solar constant, the solar declination and time of year as follows:

$$R_a = \frac{1440}{\pi} \left(G_{SC} \ . \ d_r \right) \left[\psi_s \sin(\phi) \sin(\delta) + \cos(\phi) \cos(\delta) \sin(\psi_s) \right] \qquad \text{(Eq 1.2)}$$

Where:

G_{SC} = solar constant = 0.0820 $MJ.m^{-2}.min^{-1}$
d_r = inverse relative distance from earth to sun

$$= 1 + 0.033 \cos \left[\frac{2 \ \pi \ (JD)}{365} \right]$$

and

JD = Day of the year
Ψ_s = Sunset hour angle (rad)
= arcos[-tan(φ)tan(δ)]
δ = solar declination (rad) = $0.409 \sin \left(2\pi \frac{JD}{365} - 1.39 \right)$
φ = latitude of Location (rad)

Using calculations obtained from the National Oceanic and Atmospheric Administration's, Surface radiation branch, the sunset hour angle, and solar declination, as well as sunset and sunrise times were calculated (http://www.srrb.noaa.gov/).

In order to assess how good the modelled data describes the real world scenario, the estimated sunlight radiation was placed on a graph along with the measured values. This was done for data obtained from a weather station that was not used in the study. Figure A2.1 shows that the estimated values slightly overestimates the radiation values, however it does follow the same trend over time and that the answers are within reason of what we'd expect.

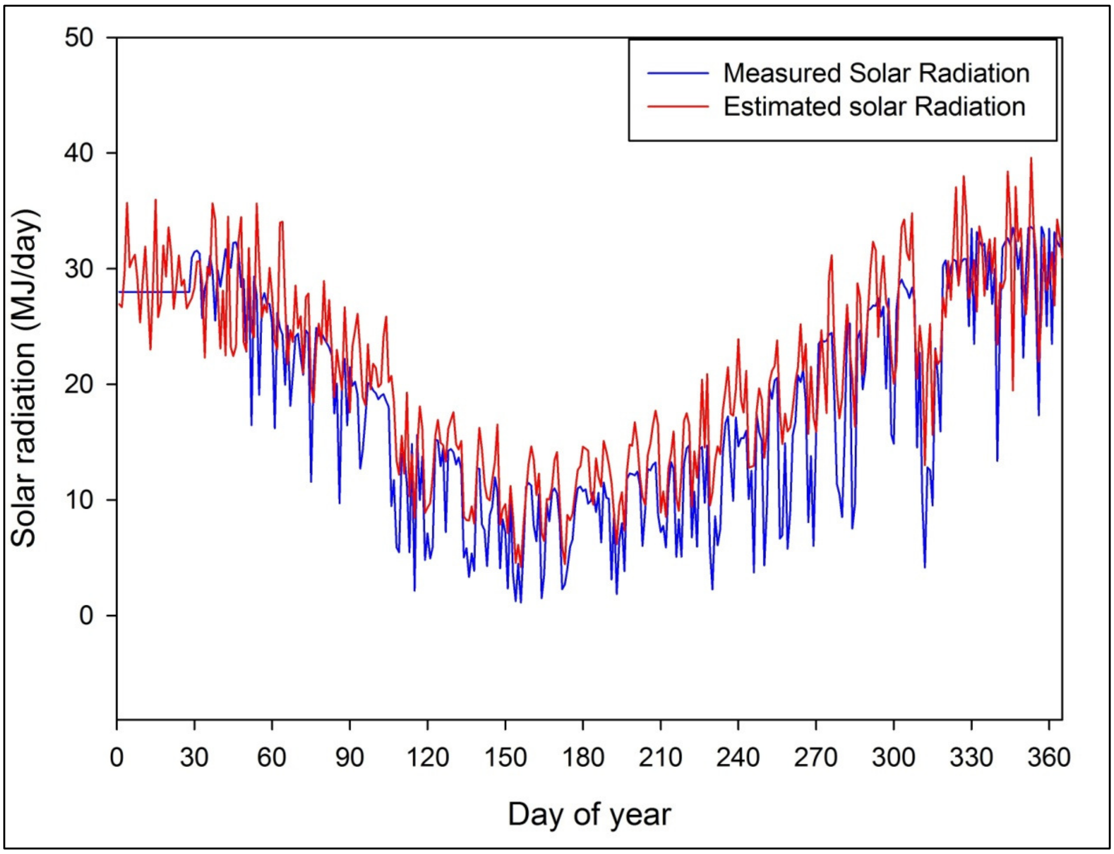

Figure A2.1.Measured and unadjusted Estimated solar radiation values for Bosbou Nursery.

Plotting monthly average Rs values of estimated against measured, it became clear the Hargreaves and Samani model's estimates of Rs values for the months December to February (Summer), are fairly accurate with very little difference between the monthly measured and estimated values. From March to November (day of year 61 to 335) the model does over predict the Rs values. This can be attributed to the fact that the model does not explicitly take cloud cover into account. In the model it is assumed that could cover would affect the air temperature and as such it is indirectly included in the model. This statement is however not entirely true as even on a fairly sunny day it can be cold due to a passing cold front. It was found that by adjusting the daily Rs values down by an empirically determined value of 15%, the measured and estimated monthly average values are virtually the same. Figures A2.2 and A2.3.

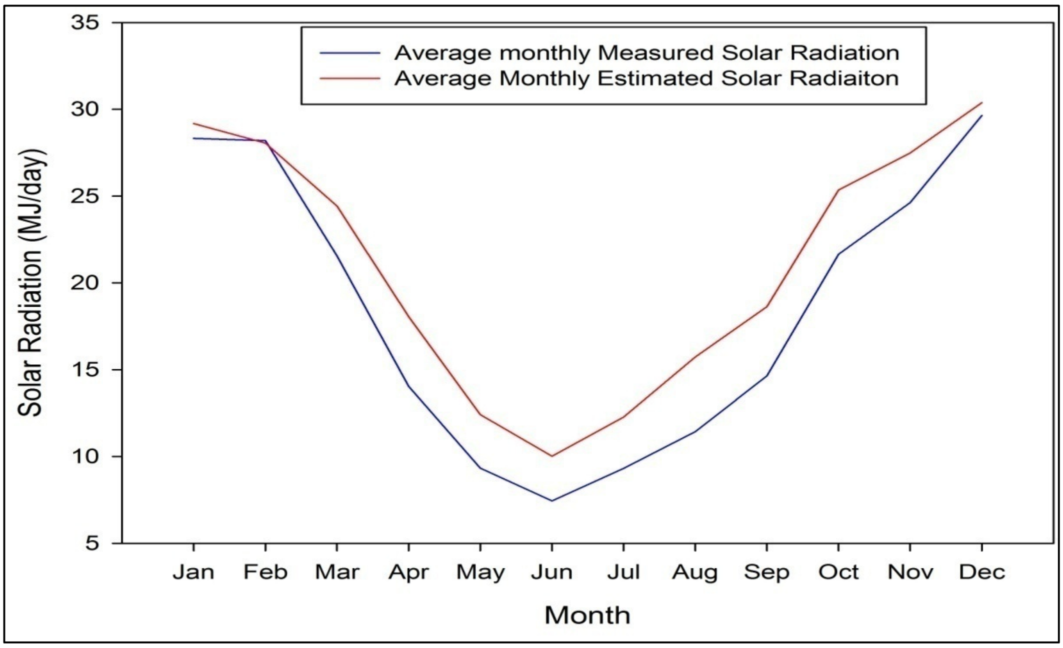

Figure A2.2: Average monthly values for measured and estimated solar radiation.

The adjusted values were still over estimating Rs in some cases and under estimating in others. A sensitivity analysis was done to determine the effect of

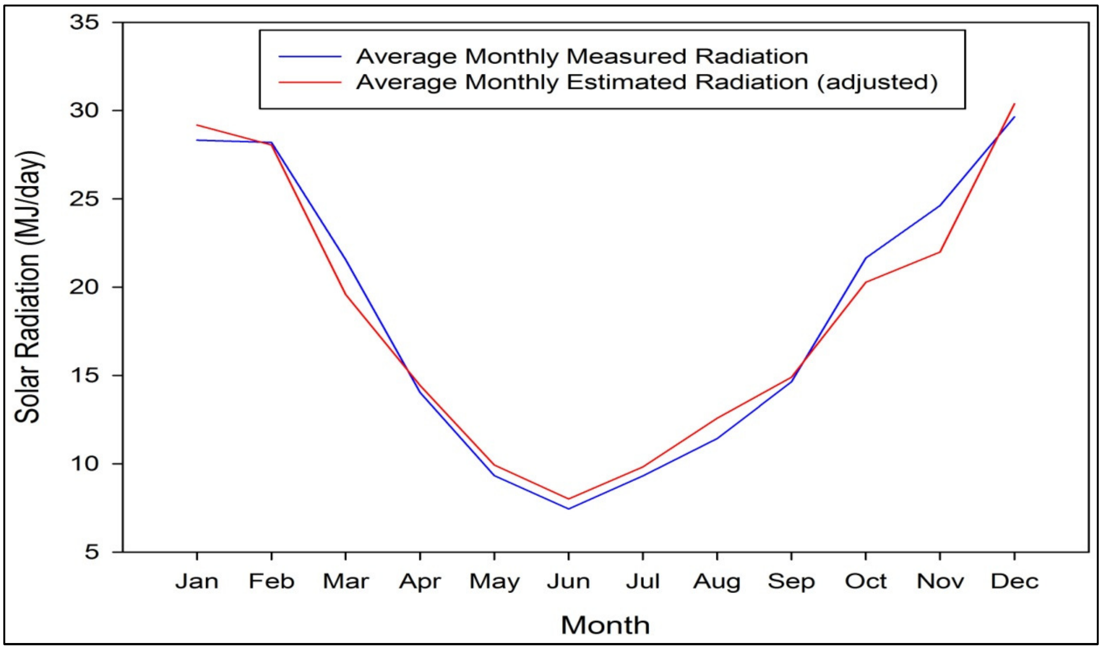

Figure A2.3: Average monthly values for measured and estimated solar radiation after 15% adjustment of estimated values.

Rs on evapotranspiration (ETo). The difference between measured and estimated (unadjusted) Rs was 14.55% which was reduced to 3.29% after adjusting the Rs values for March to November, down by 15%.

Before this adjustment was done, the difference between ETo calculated from measured data and ETo calculated using estimated Rs was 10.41%. This difference was lowered to 2.77% after the 15% adjustment in Rs. This suggested that the use of this adjustment had to be done for all data sets.

To further reduce the over- and underestimates, three additional sets of data were used to estimate Rs which were all adjusted down by 15%. The daily differences between the measured and adjusted values were calculated for each data set and the average of the daily differences were determined. This represents the average residuals for daily differences. The residuals were plotted on a graph and a trend line was added. The polynomial line described as:

$$y = 5E\text{-}09x^4 - 4E\text{-}06x^3 + 0.0011x^2 - 0.1181x + 4.6555 \qquad \text{(Eq 1.3)}$$

best described the residual plot and this function was used to further adjust daily estimated Rs values in order to better describe the measured values. Figure A2.4 shows the measured Rs values for the La Motte site for 2008 and the adjusted estimates of Rs.

The daily Rs value in this study was then calculated by adjusting the Hargreaves and Samani estimate down by 15% for the months March to November and then applying Eq. 1.3 to the estimated values.

All estimated values for Rs were found to be similar to those given by Shulze *et al.,* (1997)

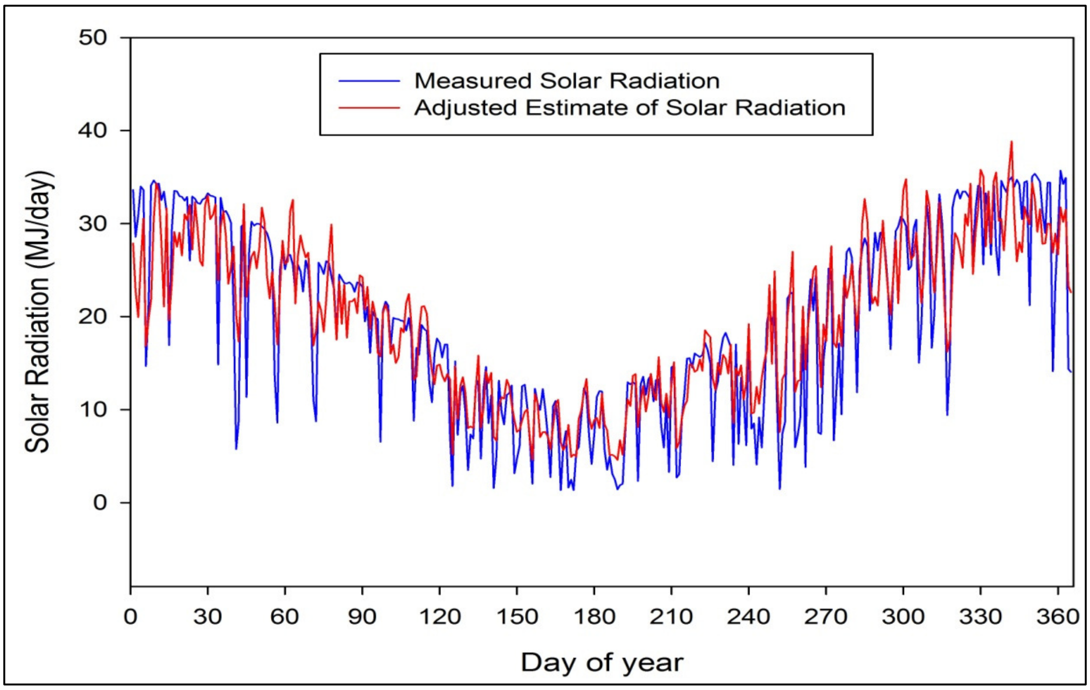

Figure A2.4: The final adjusted solar radiation estimate against the actual measured values.

Relative Humidity

This parameter will determine how much moisture can be taken up by the atmosphere. If the atmosphere is saturated with water vapour, the evapotranspiration demand will be lower due to the fact that the air is not able to store any more water (Allen *et al.,* 1998). This highlights the importance of relative humidity as an input parameter into any crop simulation model.

It is also one of the more difficult parameters to model as the movement of air, minimum and maximum temperatures, recent precipitation as well as sudden weather changes will affect its value.

When trying to calculated relative humidity (RH), the actual vapour pressure (the vapour pressure exerted by the water in the air), and the saturation vapour pressure (the pressure at which the air can no longer hold any moisture) should be calculated. To find the temperature to which the air must be cooled before becoming saturated, the dew point temperature (T_d) was estimated using the model described by Hubbard et al. (2003):

$$T_d = -0.0360\ (T_m) + 0.9679\ (T_n) + 0.0072\ (T_x - T_n) + 1.0119 \quad \text{(Eq 1.4)}$$

Where T_x, T_n, T_m are the daily maximum, minimum and average air temperatures.

Using the estimated dew point temperatures, the relative humidity was calculated foreach site. Plotting the estimated RH against the measured values resulted in Figure A2.5. The estimated value does sometimes overestimate and other times underestimate the actual RH, but the trend over the year and between the days are extremely similar. For that reason it was decided that the estimated values for RH will be used where measured values are absent from data sets obtained from other sources.

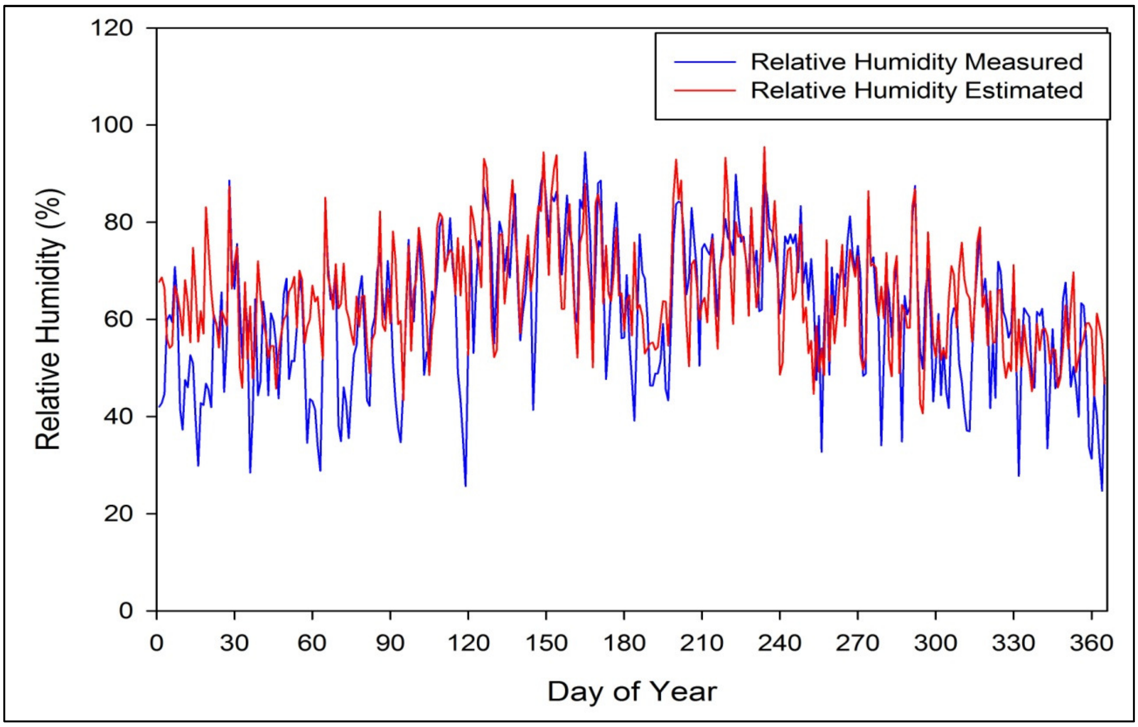

Figure A2.5: Estimated and Measured relative humidity for Ashanti 2005.

Evapotranspiration

This process consists of evaporation, where liquid water is converted to vapour form and removed into the atmosphere, and transpiration, where liquid water in plant tissue is converted to vapour and also removed into the atmosphere (Allen *et al.,* 1998). These processes occur simultaneously and it is difficult to differentiate between the two. This value gives us an idea of the amount of water that is lost into the atmosphere on a daily basis and is used to "dry out" a soil profile in a crop simulation model. Some weather stations calculate this value and logs it while other stations don't. For this reason, it was decided to calculate the reference evapotranspiration for each day as part of the input for a soil water balance model.

This value is calculated using the Penman-Monteith equation and it is fully described in the FAO 56 report as a standard for reference evapotranspiration calculation.

Appendix 3

Daily *ETp*, *ETa* and *ETa/ETp* values (calculated as an average per month) for three typical sites, averaged over five years.

Boland: L35b			
Month	ETp (mm/d)	ETa (mm/d)	ETa/ETp
January	6.60	0.24	0.04
February	5.50	0.72	0.13
March	2.87	0.56	0.19
April	1.10	0.45	0.41
May	0.24	0.21	0.89
June	Near 0	Near 0	Near 1
July	Near 0	Near 0	Near 1
August	0.08	0.08	0.99
September	0.84	0.80	0.95
October	2.39	2.25	0.94
November	4.14	3.24	0.78
December	6.02	1.07	0.18

Knysna: G4c			
Month	ETp (mm/d)	ETa (mm/d)	ETa/ETp
January	5.13	2.60	0.51
February	4.25	1.60	0.38
March	2.63	1.49	0.57
April	1.16	0.90	0.78
May	0.34	0.30	0.87
June	0.01	0.01	0.80
July	0.01	0.01	0.80
August	0.11	0.11	0.98
September	0.96	0.93	0.97
October	2.51	2.39	0.95
November	3.82	3.41	0.89
December	5.06	4.06	0.80

Stormsrivier: B5a			
Month	ETp (mm/d)	ETa (mm/d)	ETa/ETp
January	4.57	2.32	0.51
February	3.85	1.42	0.37
March	2.18	1.43	0.66
April	1.00	0.73	0.74
May	0.29	0.24	0.84
June	0.01	0.01	1.00
July	0.01	0.01	1.00
August	0.09	0.09	0.99
September	0.78	0.75	0.97
October	2.22	1.66	0.75
November	3.58	1.26	0.35
December	4.80	2.05	0.43

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