THE USE OF ISOTOPE ($\delta_{13}C$) TECHNIQUES TO DEFINE THE RIPARIAN ZONE IN COMMERCIALLY AFFORESTED CATCHMENTS

Final report submitted to the Water Research Commission

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Executive Summary

The riparian habitat is defined as *the physical structure and associated vegetation of the areas associated with a watercourse which are commonly characterised by alluvial soils, and which are inundated or flooded to an extent and with a frequency sufficient to support vegetation of species with a composition and physical structure distinct from those of adjacent land areas* (National Water Act 1998). These areas are typically adjacent to and affected by surface and subsurface hydrological features, such as rivers, streams, lakes or drainage ways. Because of the unique hydrological and biotic features of riparian areas, they are of fundamental importance in the functioning of watercourses and they support very high levels of biodiversity.

Riparian habitats often represent the deepest, most fertile soils in a catchment, and the relative abundance of water leads to particularly vigorous growth of comparative upland plant communities or species. This makes the habitat an attractive prospect for commercial exploitation by the forestry and agricultural industries. However increased riparian tree biomass leads to decreased riparian functioning, and the forestry industry in South Africa adopted a proactive approach to optimise water use, primarily by protecting the riparian zone. This was formalised in the 1969 Soil Conservation Act, and the Amendment of Forestry Act (1968) further restricted afforestation in areas in which water resources need protection.

The National Water Act (1998) presented the framework by which the South African government assumed responsibility for the national water resource. With reference to forestry the Act targets “stream flow reduction activities” (SFRA) for control. SFRAs are defined as any activity *that is likely to reduce the availability of water in a watercourse to the Reserve, to meet international obligations, or to other water users significantly*. The classification of forestry as a SFRA is on the basis of the ability of forest trees to intercept rainwater and to transpire water that has already entered the soil. In particular the transpiration of trees is classified under Sections 21of the Act as consumptive use of water. The latter is the subject of this research. Whether the water tapped by the trees is phreatic (retained in the soil above the saturated zone) or vadose (from at or below the saturated zone), it remains relevant in the policy framework as the Act considers a holistic concept of the “water reserve”.

The National Water Act (1998) also presents the National Water Resource Strategy that specifies measures to protect, use, develop, conserve, manage and control water resources. Together with the National Forests Act (1998), the legislation requires water to be administered at a catchment level and managed by a *catchment management agency* with registered users that pay an equitable rate for water. The National Environmental Management Act (Act 107 of 1998) delegates the implementation of the policies to the Departments of Water Affairs and Forestry (DWAF) and Environmental Affairs and Tourism (DEAT). SFRA will have to be licensed in accordance with declared ways of calculating the volumetric effects of the activity.

The policy framework therefore requires commercial forest owners to pay for the water that their forests use, and to protect the riparian habitat. In both instances the precise delimitation of the riparian area is required. Although the riparian habitat is defined in the National Water Act in terms of the plant communities that it supports,

the practical delimitation requires further consideration of the mechanisms that lead to the unique environmental conditions. The wetlands handbook (Department of Water Affairs 2001) links the definition of the riparian habitat to three structural components: the soil, the water and the biological communities. In practice the soil and biological communities are the dominant factors in delimiting the riparian zone and there are currently no techniques that provide a rapid and practical means of assessing the hydrology of a riparian zone.

This research uses trees as integrators of the edaphic conditions in the vicinity of their roots. The objective is to explore the physical and chemical characteristics of wood that relate to water uptake of the trees and to use these to demonstrate reduced water stress associated with riparian water access. Models that relate SFRA to tree productivity are applied at the catchment scale, and are not able to differentiate water-use efficiency of trees across the riparian habitat to the adjacent watershed within any catchment. The spatial differentiation of characteristics that proxy water-use can therefore be combined with the established criteria for defining the riparian habitat to give a more precise measure of the water-use or savings associated with the protection of the riparian habitat.

Two transects of *Pinus elliottii* trees (n=13 and n=6) from Usutu Forest, Swaziland, and another of *Pinus patula* trees (n=10) from the Riverside Forest, Eastern Cape, were felled for this study. Each transect comprised trees from the immediate stream bank within the riparian habitat to the top of the adjacent slope. Temporal resolution was obtained using dendrochronlogical analysis to link annual growth rings between trees. For each growth ring of each tree the $\delta^{13}C$ value, average tracheid size and tracheid density as well as the growth ring widths were measured as proxies of water transpiration.

The following conclusions were reached:

1 Dendrochronology

- The annual growth rings of *Pinus elliottii* trees from Usutu Forest, Swaziland, correlate with one another to form a valid tree-ring chronology. Similarly the annual growth rings of *Pinus patula* from the Riverside Forest, Eastern Cape form another valid tree-ring chronology that is distinct from that from the Usutu Forest. These regional chronologies are among the first in Africa, and they ensure valid inter-tree comparisons of rings.

2. Riparian Water use

- The hydrological functioning of the trees used in this research cannot be used as a proxy for the hydrological functioning of the soil in which they grow. Accordingly the techniques cannot be used to determine the lateral extent or variability of the riparian habitat.

3. Future research: tree anatomy

- The anatomical structures for water transpiration are coherently related to one another within any tree. Both *Pinus patula* and *Pinus elliottii* have a coherent response in which large growth rings have smaller tracheid cells and higher tracheid density. Carbon isotopes are more positive.

- The anatomical relationships that exist within any individual tree cannot be extrapolated to other trees. This means that the method cannot be used to ascertain spatial variations in water availability. The most likely reason for the different responses between trees is presumed to be genetic variability.

4. Future research: water use adaptive strategies
    - The tracheid complex in *Pinus patula* is capable of substantially higher water conductance than *Pinus elliottii*, but the system may be regulated by stomatal aperture reduction during periods of water stress.
    - The high productivity associated with high water transpiration during times of water abundance, combined with the ability to minimize water loss through stomatal control during periods of water stress in *Pinus patula* is interpreted as an adaptation to relatively low, intermittent rainfall conditions.
    - Although it seems that Pinus elliottii is not particularly well adapted to very wet conditions, it relies on very little stomatal control of transpiration, and this is interpreted as a dry-intolerant adaptive strategy.

5. Future research: optimal rainfall conditions
    - The data produced in this study suggest that carbon assimilation (growth) of *Pinus patula* is optimal when rainfall exceeds 800mm.
    - Carbon assimilation (growth) of *Pinus elliottii* is optimal when rainfall exceeds 950mm and is less than 1500mm.

This research suggests that forestry management strategies should focus on tree genetics to manage both the fiber quality and water-use efficiency of the forests.

This project represents a pilot study into the use of isotope and related techniques to determine water-use strategies of trees. Capacity building occurred in the infrastructure that was developed and commissioned at both the CSIR and the University of Cape Town to handle bulk analyses of tree isotope samples, dendrochronology and tree anatomy analysis. In addition the network of capability between these institutions was greatly enhanced.

Acknowledgements

We are indebted to Rob Guy and the management of SAPPI forest for assisting us in site selection, and allowing us to fell trees in the Usutu Forest, Swaziland. Rob Pallett was very helpful in providing site data on the Usutu Forest. We are also indebted to Dave Butt and the management of MONDI Forests for giving us access to the Riverside forest and allowing us to fell trees. Simon Lorenz (University of Natal) provided the soil and rainfall data for the Weatherly catchment. We are also very grateful to Mrs. Swart of the South African Weather Bureau who continues to be a most efficient supplier of rainfall records. Fieldwork was undertaken with the assistance of Cynthia Froyd and Kath Ficken (University of Wales, Swansea). Laboratory work was undertaken by Gill Collett, Annalie Havenga, Rose Newton and Zewdu Eshetu.

The steering committee comprising: Dr. S. Mkhize (chair), Prof. J Midgley, Prof. W.D. Stock, Prof. N.W. Pammenter, Prof. E. Witkowski, Prof. K.H. Rogers, Mr. J. Bosch, Dr. P.Dye, Dr. C. Everson, Prof. P.J.T. Roberts, Mr. M Warren and Dr. B.W. van Wilgen, were both critical and supportive in the execution of this research.

Archiving of Data and Samples

Analytical results and field notes derived from this research will be retained in the appropriate project files at the Quaternary Dating Research Unit (QUADRU), Environmentek, CSIR. This includes all analyses done in this laboratory, as well as summary spreadsheets of the data generated in the Department of Botany at the University of Cape Town. All electronic data will be stored on CDs with the documents in the project file. Any residual sample remaining after the isotope analyses will be archived at QUADRU in the existing sample archive. The samples used for the dendrochronology and anatomical analyses will be archived at the University of Cape Town.

Glossary of water related terms used in this text

Phreatic water – also called groundwater is the subsurface water in the saturated zone below the watertable (Department of Water Affairs 2001, Whitten and Brooks 1972)

Vadose water – water that is found between the ground surface and the watertable (Whitten and Brooks 1972).

Watertable – the upper surface of groundwater or that level below which soil is saturated with water (Department of Water Affairs 2001).

Edaphic - pertaining to soil

Chapter 1

Introduction

1.1 The riparian habitat and forestry policy framework

The riparian habitat is defined as *the physical structure and associated vegetation of the areas associated with a watercourse which are commonly characterised by alluvial soils, and which are inundated or flooded to an extent and with a frequency sufficient to support vegetation of species with a composition and physical structure distinct from those of adjacent land areas* (National Water Act[1] Ch1 1 (xxi)). These areas are typically adjacent to and affected by surface and subsurface hydrological features, such as rivers, streams, lakes or drainage ways. Riparian areas are distinct from wetlands in that water saturation is neither frequent nor prolonged, and the associated hydromorphic soil features are not found in the top 50cm (Department of Water Affairs 2001). Nevertheless the riparian habitat either supports distinct plant communities, or the relative abundance of water leads to particularly vigorous growth of comparative upland communities or species. Because of the unique hydrological and biotic features of riparian areas, they are of fundamental importance in the functioning of watercourses and they support very high levels of biodiversity. As such they need to be conserved.

Riparian areas often represent the deepest, most fertile soils in a catchment, and the high productivity is an attractive prospect for the forestry and agricultural industries. Unfortunately there is evidence that links riparian tree biomass and decreased riparian functioning, such as the increased stream flow that results from tree clearing in the riparian area (Rycroft 1955, Nänni 1972, Dye and Poulter 1995, Prinsloo and Scott 1999). In response the forestry industry in South Africa has adopted a conservative approach. From as early as 1932 the industry has put in place measures to conserve water, primarily by protecting the riparian zone (Görgens and Lee 1992). This was formalised when the Department of Forestry introduced a policy that prevented commercial planting within 20m of surface water sources (entrenched in the 1969 Soil Conservation Act), and restricted further afforestation in areas in which water resources needed protection (Amendment of Forestry Act 1968). The Revision of the Forestry Act (1984) allowed the 20m riparian protection zone to be reduced if deemed acceptable by the Minister.

More recently, the National Water Act (1998) presented the framework by which the South African government assumed responsibility for the national water resource. The emphasis is on the sustainable utilisation of the water *reserve*, which is the very broadest definition of available water, including the present reality and the anticipated future demand. Most industries can accurately measure the amount of water they use, but there are certain industrial practices that are not as easy to quantify but

[1] Note: Although policy documents are published by the “Office of the President of South Africa” in the *Government Gazette*, the reference format adopted in this text presents the name of the relevant Act. The reference list presents the details under the authorship of the “Office of the President of South Africa”.

clearly they have an impact on the on downstream users. The Act recognises less tangible users of water and targets “stream flow reduction activities” (SFRA) for control. SFRAs are defined as any activity *that is likely to reduce the availability of water in a watercourse to the Reserve, to meet international obligations, or to other water users significantly*. The classification of forestry as a SFRA is on the basis of the ability of forest trees to intercept rainwater and to transpire water that has already entered the soil. The water that is intercepted and retained in the canopy is often overlooked although the effect of increased evaporation and consequent enhanced atmospheric advection presents a substantial water loss mechanism (Calder and Dye 2001). Nevertheless the unique biota of the riparian zone is a function of the flooding and flow regime of the watercourse, and the rainwater interception by forests will have downstream impact. The impact on the immediate riparian zone is through the transpiration of either vadose or phreatic water. Even if the phreatic water is hydrologically linked to aquifer recharge rather than stream flows, the focus on the “reserve” nevertheless validates the classification. Irrespective of the precise mechanism that is involved, the impact of forests on the hydrological cycle is classified under Sections 21of the Act as consumptive use of water.

The National Water Act (1998) also presents the National Water Resource Strategy (Sections 5 and 6), which specifies measures to protect, use, develop, conserve, manage and control water resources. Together with the National Forests Act (1998), the legislation requires water to be administered at a catchment level and managed by a *catchment management agency* with registered users that pay an equitable rate for water. The National Environmental Management Act (1998) delegates the implementation of the policies to the Departments of Water Affairs and Forestry (DWAF) and Environmental Affairs and Tourism (DEAT). SFRA will have to be licensed in accordance with declared ways of calculating the volumetric effects of the activity. The models of Le Maitre *et al.* (1997) and Scott and Smith (1997) are specified as the means of calculating forest water-use, although the prospect of paying for water will likely lead to detailed scrutiny of the model and possibly some refinement.

1.2 Research focus

The current policy framework will require commercial forest owners to pay for the water that their forests use, and to protect the riparian habitat. In both instances the precise delimitation of the riparian area is required. Although the riparian habitat is defined in the National Water Act in terms of the plant communities that it supports, the practical delimitation requires further consideration of the mechanisms that lead to the unique environmental conditions. The *Wetlands Handbook* (Department of Water Affairs and Forestry 2001) links the definition of the riparian habitat to that of wetlands which are seasonally or occasionally flooded. It comprises three structural components: the soil, the water and the biological communities. In practice the soil and biological communities are the dominant factors in delimiting the riparian zone. Certain plant communities can only exist in a riparian zone, and indicator species have been listed, while chemical changes that take place in soils as a result of seasonal inundation are also well defined, and are particularly useful in identifying riparian zones even after it has ceased to function as such. Thus where plant communities may change in response to impacts on the riparian zone, the soils may

preserve the indicator characteristics. For this reason the plant-community-based and soil-structure-based criteria need not all be simultaneously met. Although the hydrological functioning of the riparian zone is the cornerstone of both the soil structure and the biological communities that it supports, there are no techniques that provide a rapid and practical means of assessing the hydrology of a riparian zone.

Having established the extent of the riparian zone by the definition given above there are reasonably sophisticated models that relate SFRA to tree productivity at the catchment scale (Bosch *et al.* 1995, Scott and Smith 1997, Scott *et al.* 1998). However these models lack precision as they focus on above ground productivity and lose the important contribution that root development has on water-use efficiency by trees (Dye 2000). In particular the scientific evidence for spatially differentiated water-use by trees across the riparian habitat to the adjacent watershed has not been linked to the definition of the riparian zone. From the perspective of a forestry management tool aimed at responsible water-use and accounting, the catchment scale approach does not provide the desired resolution.

This research focuses on trees as integrators of the edaphic conditions in the vicinity of their roots. The objective is to explore the physical and chemical characteristics that relate to water uptake of trees and to use these to demonstrate reduced water stress associated with riparian water access. The spatial differentiation of these characteristics can then be combined with the established criteria for defining the riparian area to give a more precise measure of the water-use or savings associated with the current framework for the protection of the riparian habitat. In order to integrate the results into the understanding of the riparian habitat it will ultimately be necessary to integrate specialised pedological data with botanical data that make up the existing framework. However this report is limited to a test of the tree physiology to determine if there is merit in the approach.

Chapter 2

Tree Physiology Background

2.1 Tree anatomy and water conductance

The physiological organs that make up the “water-use system” of trees, comprise the stem, roots, and leaves (Fritts 1976). The roots constitute the underground part of the plant and are important for absorbing water and minerals from the soil, storage of food reserves, and the synthesis of certain growth hormones. Roots and stems share many structural and developmental characteristics. They are physically continuous and commonly treated as two parts of the same axis, with similar terms applied to their tissue systems (Esau 1965). The stem provides mechanical support, conducts water upward from the roots, transports the products of photosynthesis from the leaves, and acts as a storage medium for water, carbohydrates and minerals. Leaves are the major photosynthetic apparatus of woody plants. The central strand of the procambium develops into the leaf midrib and therefore, the leaf is continuous with the vascular tissue in the stem. Together, the leaves and stem form the shoot of the plant and store both carbohydrates and mineral nutrients. Most of the water lost from the plant escapes through the leaves during transpiration.

At a cellular level the way in which water and photosynthates are conducted through the tree is well known. *Xylem cells* are the principal water conducting tissues. In general, water is conducted in two pathways. The *apoplast* is the route along the interconnecting walls and spaces between the cellulose fibres; water moves quickly through these dead cells without crossing any membranes. These cells may be either *tracheids* or *vessel elements.* Tracheids are elongated hollow cells with highly lignified walls. Tracheid cells have tapered ends that overlap. Pits in the end part allow water to move from one tracheid into the next. Water may also move into adjacent cells through pits in the side-walls. Vessel elements tend to be shorter and wider than tracheids with perforated end plates. Unlike tracheids, which are arranged in overlapping vertical files, vessel elements are stacked end-to-end. The transport of water in the plant is limited to the younger xylem vessels, which are referred to as the *sapwood*. Resistance to water flow is relatively high in gymnosperms, owing to the smaller diameter of vessel elements to tracheids (Salisbury and Ross 1992). The older vessels of the xylem that no longer conduct water are referred to as the *heartwood* (Greulach 1973). During the transformation to heartwood, xylem parenchyma cells may form protuberances that penetrate through the pits of adjoining cells. These invaginations are called *tyloses*. They are common in the xylem vessels of angiosperms, and block water transport in the vessels, sometimes causing dehydration. Tyloses may also be found in the sapwood as a response to wounding, invasion by fungus pathogens or virus infection (Beckman *et al.* 1953). The *symplast* is the slower route of water through individual cells and along fine strands of cytoplasm that pass through the cell walls and connect the living parts of the cell.

The leaves contain *stomata* that permit gas exchange between the internal air spaces and the ambient environment. Guard cells surround each stoma, and in most cases, the guard cells are themselves surrounded by specialised, differentiated epidermal cells called *subsidiary* or *accessory cells*. This stomatal complex controls gas exchange and therefore, plays an essential role in the physiology of plants. The guard cells control the size of stomata by reversible changes in their turgidity. Guard cells take up water and swell to open the pore when CO_2 is required for photosynthesis, and lose water to reduce the aperture of the pore when CO_2 is not required or when water stress overrides the photosynthetic needs of the plant.

Growth occurs through the formation of new cells on either side of the vascular cambium that constitutes the inner bark. These cells are quite distinct and are known as *xylem* and *phloem* cells. The secondary phloem is formed on the outside of the vascular cambium, and secondary xylem inside. The *phloem* is the principal tissue for the translocation of photosynthates, and unlike the passive transport in the xylem cells, transport in the phloem cells is active and requires the expenditure of energy by the plant.

2.2 Tree transpiration model

It has been shown (Hubbard *et al.* 2001) that the resistance that is exerted by the water pathway from soil to needles in *Pinus ponderosa* is directly linked to the rate of transpiration (water loss) as well as the rate of carbon assimilation (growth). Transpiration and carbon assimilation are processes that are controlled by the passage of gases out of, and into the stomata respectively. It follows that growth rate is determined by water availability in the needles, which is a balance between the stomatal control of water loss, and the anatomical control of water supply.

Models that relate the transpiration of water by trees to their anatomical structure and stomatal control (eg Tyree and Zimmermann 2002, Tyree 2002) are based on short time scale responses. The model that forms the basis of this research is that presented by Tyree (2002). It assumes that the water conveyance mechanism of the tree, which includes the tracheid and stomatal structures, has a resistance that is responsible for the water potential difference between the soil and the leaf. This water potential difference between soil and leaf may limit transpiration, carbon binding and growth. The equation that relates these parameters is given by:

$$\Psi_{SOIL} - \Psi_{LEAF} = R_{PLANT} E$$

Where Ψ_{SOIL} and Ψ_{LEAF} are the water potential in the soil and in the leaf, R_{PLANT} is the hydraulic resistance of the tree and E is the evaporative flux density (transpiration). The physiological adaptation that a tree has to cope with variations in water availability all combine to maintain the leaf water potential (Ψ_{LEAF}). If this value is too low or too high photosynthesis is adversely affected and the tree may die. For the purpose of this research the value can be assumed to remain constant. Accordingly any variation in the soil moisture potential (Ψ_{SOIL}), either as a result of rainfall or the elevated availability of moisture in the riparian habitat, should manifest in variations in the hydraulic resistance of the tree (R_{PLANT}) or the evaporative flux (E), or both.

At any point in time the anatomical structures of the stem that convey water have a limited ability to respond to changing water potential in the soil, and the evaporative flux is maintained by stomatal control. The stomatal response time to changes in light intensity and water availability is in the order of minutes. In most instances the water potential of the soil will vary in response to rainfall, the local hydrological conditions, and the transpiration of vegetation in the time scale of days. The response of a tree to changing environmental circumstances will be to form anatomical structures that are best suited to the ambient conditions at the time. This study uses tracheid cell size and density as a measure of the hydraulic resistance of a tree (R_{PLANT}), and stable carbon isotopes ($\delta^{13}C$) as a measure of stomatal conductance (E).

2.3 Tracheid size and Density

There are two ways in which a tree may increase water transport efficiency. One is by producing more cross sectional xylem, the other is by changing anatomical features that affect conductivity such as vessel diameter, length and frequency. Of these features, vessel diameter and vessel frequency are probably the most relevant parameters in angiosperm wood. Hydraulic conductivity is proportional to the radius raised to the fourth power (Zimmerman 1978, February 1993 and Tyree and Zimmerman 2002), and even a slight increase in vessel radius is equivalent to an enormous increase in ability to transport sap. For example, three vessels with relative radii of 1, 2 and 4 and having cross–sectional areas proportional to 1, 4 and 16 will have relative conductivities of 1, 16 and 256 (Zimmerman 1983). While the anatomical structure of angiosperm wood has specialised into vessels as conductive elements and fibres for support these features, they are combined in gymnosperm wood where the tracheids conduct water as well as provide mechanical support. Within this study, we suggest that tracheid, like vessels, will increase in diameter and decrease in frequency with an increase in plant available water. The primary objective of this section of the study is to ascertain whether or not tracheid diameter decreases from the river into the upland area as a function of reduced plant water availability.

2.4 Carbon Isotopes in trees

An area of research that may elucidate the relationship between water-use and tree productivity is stable isotope analyses. It is broadly accepted that stable carbon isotopes in tree rings reflect a balance between the rate of photosynthesis (carboxylation) and stomatal conductance (Farquhar and Richards 1984). In schematic terms trees reduce their stomatal aperture during periods of water stress, which forces them to use more of the CO_2 that is trapped within the leaves for carbohydrate synthesis than they would if the stomata were open. Instead of selectively using $^{12}CO_2$ the tree must make more use of $^{13}CO_2$. At the same time the reduced stomatal conductance slows the replacement of CO_2 within the leaves and the enzymatic reaction that is central to photosynthesis is also slower. Thus in times of high water stress it is anticipated that trees will form tissue at a slower rate, and that the tissue will have a higher ratio of $^{13}C/^{12}C$ than in times of reduced water stress. Using delta notation to express the ratio of $^{13}C/^{12}C$ the control of leaf isotopic composition is given by the equation:

$$d\ {}^{13}C_{leaf} = d\,{}^{13}C_{atm} - a - (b-a)\,c_i / c_a \qquad \text{(Farquhar } \textit{et al.} \text{ 1982)}$$

Where *a* and *b* are fractionation constants and $\delta^{13}C_{atm}$ represents the isotopic ratio of the air which is also more-or-less constant. Therefore the term c_i/c_a, the ratio of intercellular to atmospheric CO_2 concentration that is controlled by stomatal conductance, is directly related to the stable carbon isotope ratio.

A key concept in understanding the physiological response of trees to their environment is the notion of Water-use Efficiency (WUE). There are many different definitions of WUE (Dye 2000), and the link with stomatal conductance needs to be clarified. In isotope ecology the traditional definition is the ratio of photosynthesis and water transpiration (A/E). The latter term is given by the equation:

$$E = g(LAVD) \qquad \text{(Farquhar and Richards 1984)}$$

where g is the conductance to water vapour and *LAVD* is the *leaf to air vapour deficit*, and the equation that describes A is:

$$A = (c_a - c_i)g\,/1.6$$

Since the term g is common to both A and E the variation of the ratio A/E is dependent on *LAVD* (which is constant within a species) and c_i. Hence the common determinant in WUE and $\delta^{13}C$ within a species is the concentration of CO_2 within the leaves and so $\delta^{13}C$ can be used as a proxy indicator of WUE. It has been shown (Farquhar *et al.* 1989) that more negative $\delta^{13}C$ values are associated with decreased WUE.

Chapter 3

Sampling and Measurement Protocol

3.1 Sample specifications

In order to test the assumption that tracheid size and density as well as carbon isotope values would vary within and between trees in response to water stress, it was proposed that the annual rings of eighteen trees from three transects in two different catchments should be analysed. Although the ultimate application of this research would be a generic approach that could be applied in any circumstance, there were several criteria that were to be met:

- Each transect should span the riparian zone and should be uniform in solar aspect.
- The trees should be older than 50 years to facilitate cross dating of the rings, and thus ensure valid inter-ring comparisons.
- The rainfall should be less than 900mm, and good rainfall records should be available.
- The forest should comprise pines in their first rotation without a history of fertilisation and with a recorded management history.
- The soil data should be available for the site, and deep soils should be avoided.
- Pre-dawn xylem pressure potentials should be determined.

Satisfying all of these criteria was not possible even with the assistance of the forestry industry. The first compromise was to sample more trees per transect, but to work with younger trees as 50-year-old stands were rare indeed. The second compromise related to the fact that forests in the riparian area are no longer tolerated in South Africa, and so forests in neighbouring states were sought. This eventually led to a major portion of the samples coming from an area with more than 900mm of annual average rainfall. Even with these compromises the site selection proved problematic, and eventually two different pine species were sampled.

3.2 Usutu Forest, Swaziland

The first two transects comprised *Pinus elliottii* Engelmann trees that were felled from the Usutu Forest in Swaziland with the kind permission of SAPPI (figure 1). These were first rotation trees that had not been subject to any fertilisation. The soils in the vicinity are “derived from Gabbro of the Usushwana complex”, and are described as “deep (600-800mm) red apedal soils with high organic carbon in the topsoil, and shallow humic A horizons. Clay content is high, with 40-50% in the topsoil and 45-55% in the subsoil” (Rob Pallett pers. comm.). However the soil-thickness along the length of each transect varied considerably.

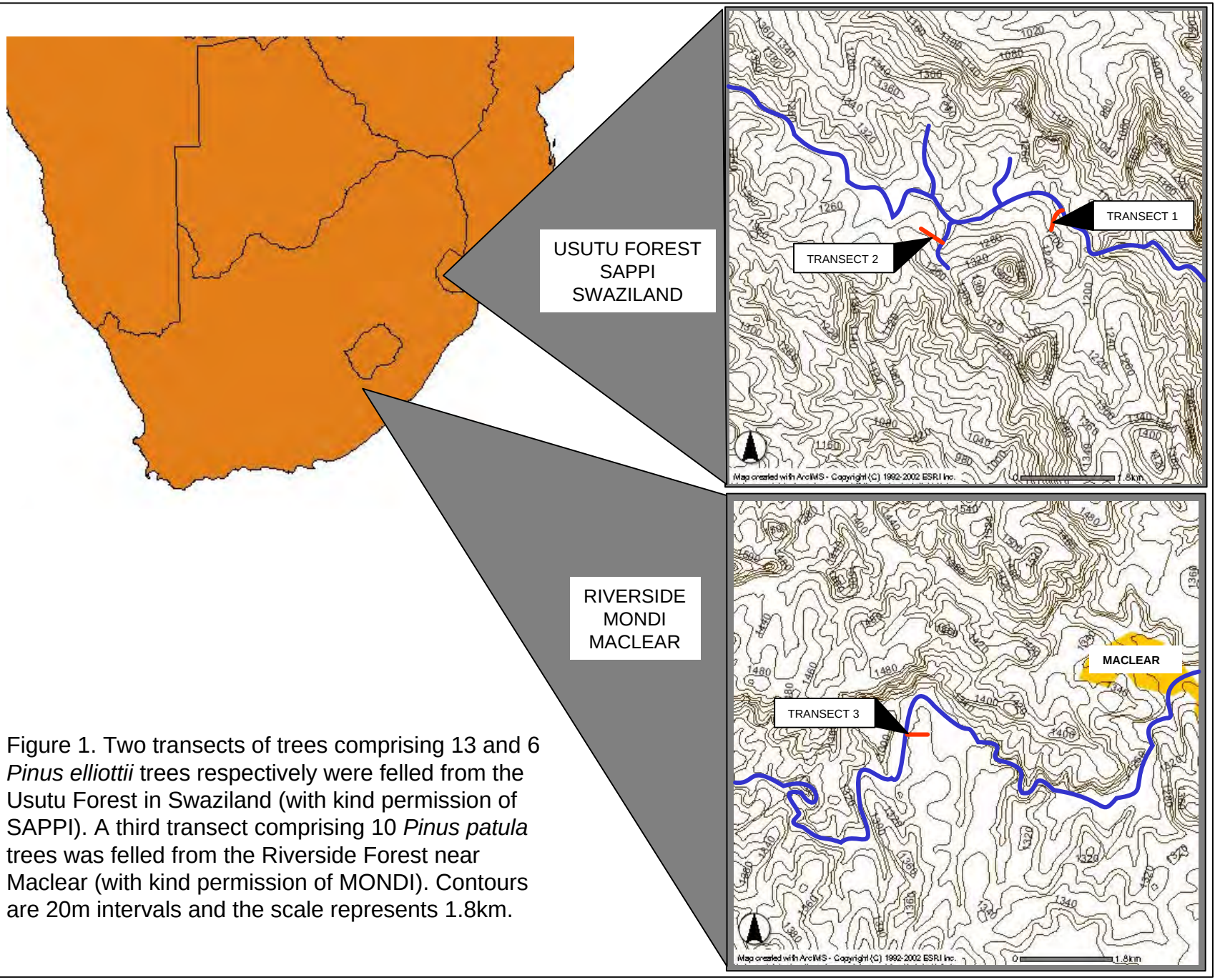

Figure 1. Two transects of trees comprising 13 and 6 *Pinus elliottii* trees respectively were felled from the Usutu Forest in Swaziland (with kind permission of SAPPI). A third transect comprising 10 *Pinus patula* trees was felled from the Riverside Forest near Maclear (with kind permission of MONDI). Contours are 20m intervals and the scale represents 1.8km.

The first tree of transect 1 was felled on the bank of the Mhlambanyati River (figure 2) and was rooted directly into the riverbank. This is a perennial river in an incised valley with a wide bank comprised of alluvial sediment. The spatial arrangement of the other 12 trees in this transect is illustrated in figure 3 with a schematic representation of the changing elevation. Trees 1-9 were in deep soils associated with the riverbed and represented an unambiguous sample of trees from the riparian area. Trees 10-13 were felled from a steep incline with very shallow soils and prominent outcrops of bedrock. The management record indicated that these trees should be 18 years old, but subsequent analysis of the growth rings showed that trees 1-9 were 20 years old, while 10-13 were 18 years old.

Figure 2. *Pinus elliottii* trees are planted to the banks of the Mhlambanyati River in the Usutu Forest of Swaziland. The felled trees in the foreground were part of transect 1 from the river to the adjacent watershed.

A second transect of 6 trees was felled from a slope higher in the same catchment (figure 1). The lowermost two trees were respectively found in and rooted into the bank of an ephemeral stream that formed a tributary to the Mhlambanyati River. Although there was water flowing in the stream at the time of sampling the flow is marginal and would likely cease in dry years. The soils are also very thin with occasional bedrock outcrops, and the alluvial sediments in the stream were almost non-existent. Along the course of the stream that was surveyed in the field the water flowed either over bedrock or shallow alluvial sediments.

Rob Pallett of SAPPI provided the monthly rainfall for the period 1980 to 1999 for the Usutu Forest. Tables 1 and 2 provide the size proxy measures (chest height circumference) and the annual rainfall record (July-June to correspond with the

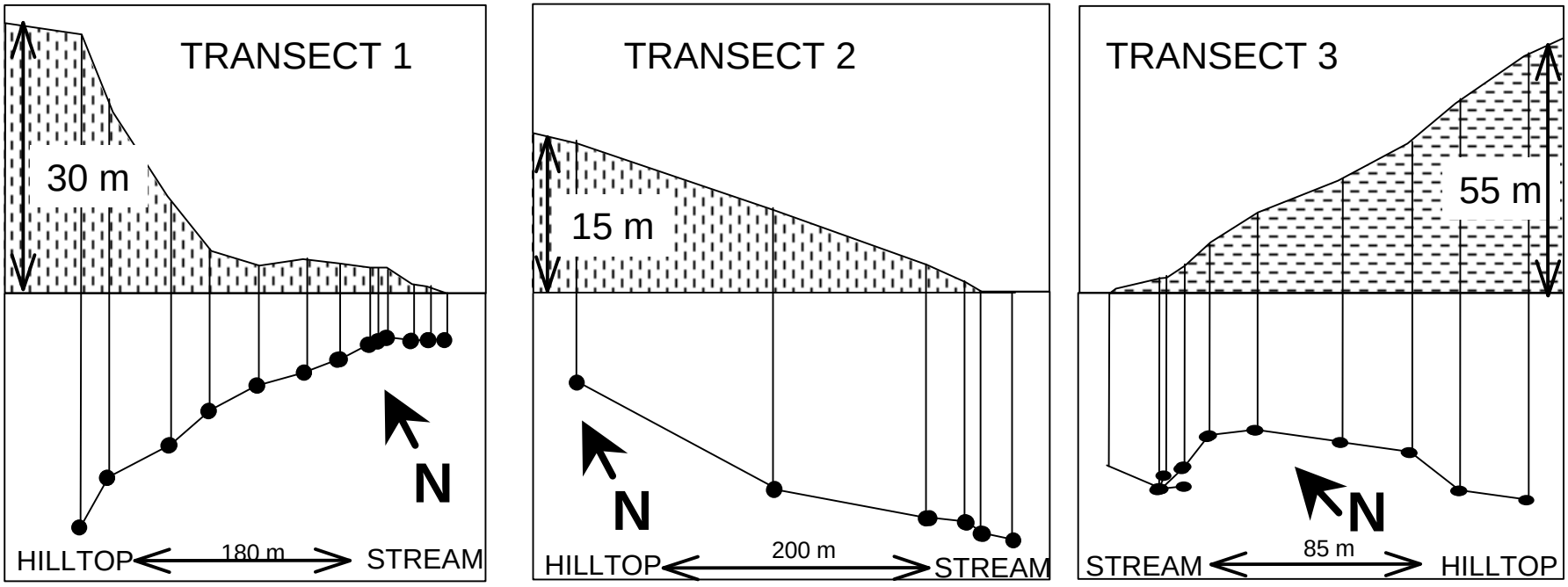

Figure 3. Schematic representations of the surface topography and spatial relationships between trees of transects 1 and 2 from the Usutu Forest, Swaziland, and transect 3 from the Riverside Forest near Maclear. The upper illustration represents the elevation relative to the stream bed, and the lower illustration represents the scaled orientation of the trees relative to one another.

summer growth rings) respectively. Pre-dawn xylem pressure potentials were not measured at this site.

Table 1. Chest height circumference measurements of *Pinus elliottii* trees felled in two stream-to-watershed transects in the Usutu Forest, Swaziland. There is no unidirectional trend in the size of the trees, although the uppermost trees in transect 1 were the smallest of the sample, and were similar in size to those in transect 2.

Transect 1 tree number	**Diameter (cm)**
1	41
2	33
3	33
4	33
5	37
6	41
7	45
8	37
9	46
10	32
11	29
12	28
13	26
Transect 2 tree number	**Diameter (cm)**
1	35
2	31
3	29
4	27
5	23
6	27

Table 2. Annual rainfall data (July – June) for the Usutu Forest.

Year	Rainfall (mm)
1981	1234.8
1982	933.9
1983	1794.3
1984	1635.6
1985	1290.1
1986	960.5
1987	1365.9
1988	1565.5
1989	1134.4
1990	126.9
1991	807.0
1992	1102.3
1993	923.6
1994	1010.0
1995	1088.2
1996	1239.2
1997	1090.1
1998	1047.1
1999	No data
Average	**1193.8**

3.3 Riverside Forest, Eastern Cape

A third transect of 10 *Pinus patula* Scheide ex Schlectendahl and Chamisso trees was felled from the Riverside plantation near Maclear (figure 1). The forestry industry in the Eastern Cape is relatively young, and few plantations are older than about 8 years. The site that was sampled was not originally planted as part of a coherent forestry strategy, but was rather an investment made by an entrepreneurial farmer that was later ceded to MONDI. The age of the trees was not known but it was subsequently established that they were 29 years old. They were a first rotation that had not been subject to any thinning, pruning or fertilisation. Details of the spatial distribution of the trees as well as a schematic representation of the elevation are presented in figure 3. Although there was a row of mature poplar trees cultivated along the riverbank, the lowermost pine trees were located on a slope break slightly above the perennial river and clearly within the riparian area. Higher density of sampling took place across the slope break, which seemed to delimit the riparian habitat and was associated with a transition from deep alluvial sediments to thinner soils that had weather in situ. Because the plantation was not subject to any management, the soils were not studied in detail. The soil reports for the adjacent Weatherly catchment indicate a great deal of variability in the soils, and any extrapolation of these analyses would be unfounded (Simon Lorenz *pers. comm.*).

The soil nevertheless had a high clay content that was apparent from the shrinkage cracks in road-cutting exposures.

The 10 trees from the Riverside transect were all felled before dawn to enable pre-dawn xylem pressure potential to be measured. Although this fieldwork took place on 19 November 2002, no substantial rain had been reported for the summer, and the local foresters and farmers reported “drought” conditions. Immediately a tree was felled the xylem pressure potential was measured, and the felling of the next tree did not take place until the measurement was completed. In this way the sample was taken within seconds of felling the tree, and the measurements were taken within minutes. The chest height circumference and the height of the trees were also measured. As was noted in the Usutu transects there is no consistent trend in tree size. The physical attributes are summarized in table 3. The nearest rainfall record comes from the Maclear police station which is located approximately 5km away. This was kindly made available by the South African Weather Bureau. The annual rainfall record (July to June) is presented in table 4. A lack of data for the 1999 calendar year prevents an accurate reflection of the rainfall during the 1998 and 1999 growing seasons. Rainfall records for 3 years are also available from the Weatherly catchment, but the short time series makes these of little value.

Table 3. Physical attributes of *Pinus patula* trees felled in transect 3, Maclear.

Tree number	**Diameter (cm)**	**Height (m)**	**Average pre-dawn Xylem Pressure potential (MPa, n=2)**
1	39	25	-13.75
2	41	26	-9.5
3	33	27	-10.75
4	38	27	-10.0
5	43	29	-13.0
6	38	29	-11.0
7	41	28	-11.5
8	33	27	-13.0
9	52	29	-8.25
10	41	28	-12.25

Table 4. Annual rainfall data (July – June) for the Riverside Forest, Maclear.

Year	Rainfall
1973	870.8
1974	665.9
1975	1047.3
1976	722.8
1977	733
1978	728
1979	527
1980	756.5
1981	627.5
1982	519.3
1983	891.4
1984	699.1
1985	958.7
1986	806.0
1987	1157.9
1988	1045.1
1989	846.8
1990	653.7
1991	863.3
1992	626.8
1993	1102.5
1994	789.6
1995	1009.2
1996	1095.8
1997	1009.5
1998	1074.4
1999	1352.8
2000	902.9
2001	882.6
Average	**860.9**

3.4 Measurement protocols

At both the Usutu and Riverside sampling sites, disks of approximately 5 cm thickness were cut from as low as possible from each trunk in order to incorporate as many growth rings as possible. Each disk was clearly labeled and air-dried. A slice through the center of the disk that included all the growth rings in two directions was later cut, although the remainder of the disk was retained for reference purposes. The sample slice was then split horizontally to yield two mirror image samples of the growth rings. One of the samples was used for the anatomical and dendrochronology studies and subsequent curation, and the second was used in the destructive isotope analyses. Initially the rings were numbered but with the confirmation of the dendrochronology it is now possible to assign calendar years to the rings. The naming convention assigns a ring to the year in which it started to grow (LaMarche

1979). Hence the most recent latewood ring that was found in trees felled in November 2002 would relate to the end of the previous growth season. Although this would physically have taken place between January and June of the same year, the ring is named after the year in which it started to grow, which would be 2001. The same nomenclature was applied to the rainfall and anatomical analyses.

In the laboratory, the samples were mounted permanently on backing blocks using epoxy wood adhesive and then sanded with progressively finer grades of abrasive paper until the annual growth rings were clearly visible (Stokes and Smiley 1963). In this study a belt-sander was used starting with ANSI 60 grit paper (250-297 µm) and using progressively finer paper, finally finishing with ANSI 400 grit paper (20.6-23.6 µm) (see Orvis and Grissino-Mayer 2002). Measurements of tracheid diameter and frequency were made directly from the wood using a Nikon Optiphot M Darkfield/Brightfield reflected light microscope at a magnification of 20X and the image analysing programme FIPS from the CSIR Pretoria. The ring-widths were measured to an accuracy of 0.001mm using a low-power binocular microscope and a Velmex measuring stage. These measures were in accordance with the procedure recommended by the International Association of Wood Anatomists (IAWA Committee 1989). Measures of tracheid diameter were made on the early wood only at the widest part of the tracheid as viewed in cross section and did not include the cell wall. Average values for tracheid diameter were based on 30 individual measures in each annual growth ring. Tracheid frequency was obtained by counting all the tracheids in an area 310 x 620 microns. The reported values for tracheid density relate to this area and are not normalised. The final result was calculated from the mean of three counts.

Potential measurement problems were identified using the program COFECHA. This program uses segmented time-series correlation techniques to verify cross dating amongst individual time-series and identifies potential outliers, absent rings and erroneous measurements (Cook and Holmes 1999, Grissino-Mayer 2001).

Standardisation was achieved using the program ARSTAN (Cook 1985, Cook and Holmes 1999). The site indices were created by fitting a negative exponential curve, a negative linear regression or a horizontal line through the mean of the data, depending upon which technique was most appropriate. Secondary detrending was achieved using a cubic smoothing spline. Although exponential smoothing has been used for many years to standardise North American conifers, other techniques may be more appropriate for dense forest stands (Fritts 1976).

Stable carbon isotope measurements were made on the latewood component of each growth ring. Although the preferred sample material for carbon isotope analysis is a-cellulose, it has been shown that wood has a stronger climatic signal (Loader *et al.* in press). In addition the pre-treatment protocol of Epstein *et al.* (1976) is too protracted to allow large sample numbers to be processed in a reasonable time. Instead the mobile resin fractions were extracted from ground latewood samples using an ethanol/toluene Soxhlet distillation (Loader *et al.* 1997).

The isotopic values are measured as deviations from standard reference materials using “delta” (δ) notation on a per mille (‰) scale. The equation used to derive the $\delta^{13}C$ value is

$$d = \left(\frac{R_{SAMPLE} - R_{STANDARD}}{R_{STANDARD}} \right) \times 1000$$

where $R = \frac{^{13}C}{^{12}C}$. Carbon isotopes in plants were measured relative to the PeeDee Belemnite (PDB) standard. The pre-treated samples were carefully weighed and combusted on-line in an elemental analyser (Thermoquest EA 1110) coupled a stable light isotope mass spectrometer (SIRA). Each sample was measured at least twice, and if the precision on replicates was unacceptable, further analyses were conducted. A beet sugar standard, and project specific sawdust standard were used to correct for sample size effects of the elemental analyser. The average precision on replicate results was <0.2‰.

Chapter 4

Results

4.1 Dendrochronology

The inter-comparison of tree rings is a mature science that has a well-developed methodology. In particular the ability to unequivocally assign any growth ring to a calendar year, which is essential to this research, can only be accomplished if false or missing rings are identified. This is precisely what the dendrochronology achieves. The chronology that emerges is specific to the region, and so both transects 1 and 2 from Usutu will form part of a single chronology, while transect 3 from Riverside will form another.

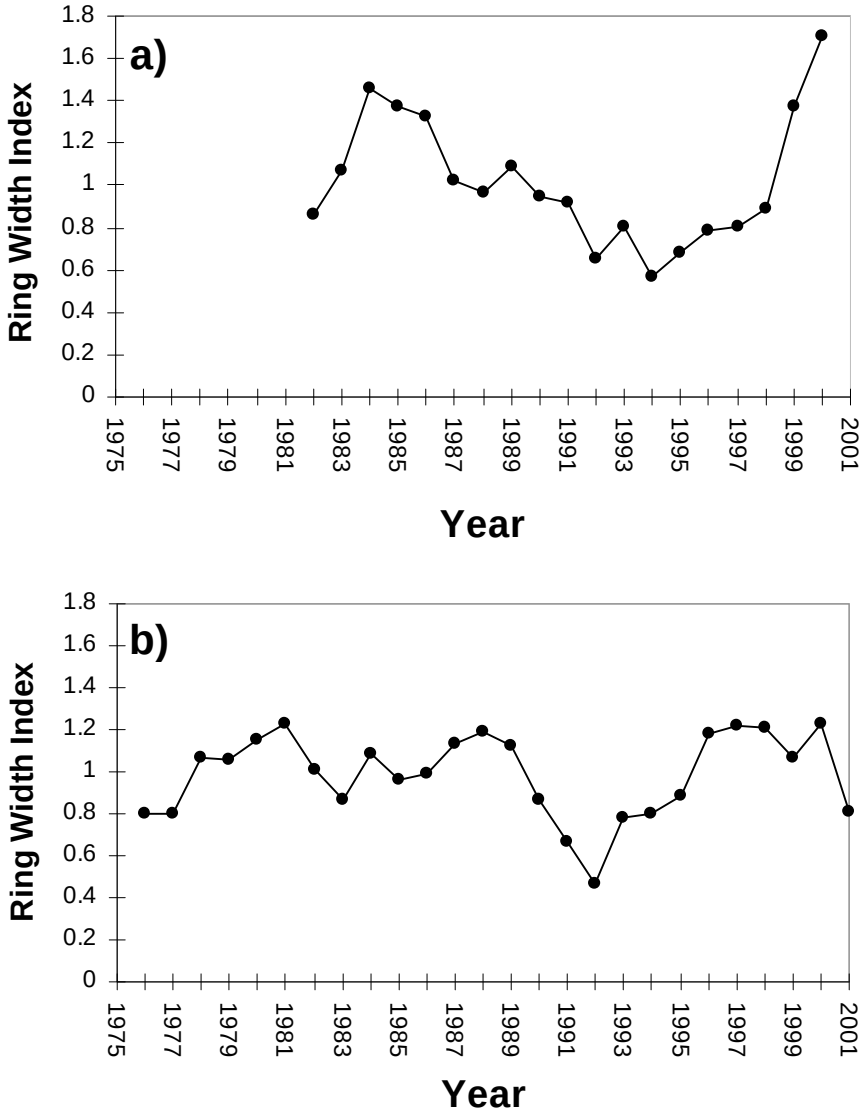

Figure 4. Standard ring-width chronologies at a) Usutu and b) Riverside calculated using ARSTAN (Cook and Holmes, 1999).

For each site, the *standard ring-width chronology* was calculated from tree-ring measurements that were detrended by a simple “curve fitting” process to remove the non-climatic signal. Means for each year were calculated from the individual time series as the bi-weight robust mean (Figures 4a and 4b).

The disks at each site were cross-dated successfully giving absolute confidence in the integrity of the calendar dates assigned in this study. This study represents one of the few successful attempts at cross dating in southern Africa. The only other published studies are for *Widdringtonia cedarbergensis* growing in the Cedarberg Mountains (LaMarche *et al.*, 1979; Dunwiddie and LaMarche, 1980) and *Pterocarpus angolensis* growing in western Zimbabwe (Stahle *et al.*, 1999).

4.2 Physiological changes through time

4.2.1 Stable isotope analyses

The underlying premise throughout this research is that the riparian habitat will have elevated soil moisture potential (Ψ_{SOIL}) relative to the non-riparian habitat, and that this distinguishing characteristic will be manifest in the hydraulic resistance (R_{PLANT}) of the trees that grow there. The isotopic ratio of carbon in trees varies in response to the relative concentration of CO_2 within, and outside of the leaf, and this is controlled by the stomatal aperture in the leaves. This is one of the main mechanisms that allow the tree to regulate its hydraulic resistance.

Before any differentiation between the $\delta^{13}C$ of trees in the riparian and non-riparian habitat can be made, it is necessary to empirically test the correspondence between stable carbon isotopes and variations in soil moisture potentials. Since rainfall varies through time it can be assumed that soil moisture potential does too, and this can be compared with the temporal variation of $\delta^{13}C$ to determine the sensitivity of this proxy measurement. The stable carbon isotope analyses of the transect 1, transect 2 and transect 3 tree rings are tabulated in tables 5a-5c in Appendix 1, and presented graphically in figures 5 to 7 respectively. These illustrations represent the average $\delta^{13}C$ values of all the rings that grew in any year in the transect sample as well as the associated standard deviation arising from inter-tree variation. No attempt is made at this point to differentiate individual trees from one another. In order to better elucidate the inter-annual response of the trees the first derivative (change in isotopic composition from one year to the next) is also graphed. This is a better reflection of the “response” rather than the “state” of the trees (Fitts 1976) and it provides a more sensitive basis to correlate with rainfall. The rationale is that the inter-individual variation in carbon isotopes may be constrained by genetic limitations on stomatal control, but that the inter-annual differences between stomatal conductance are still in response to a common forcing mechanism. The existing rainfall records are also graphed for comparison.

In the year with the lowest rainfall (1991) it appears that all of the transect 1 trees (figure 5) show a positive shift in the $\delta^{13}C$ values which is in line with the expected stomatal response to increased water stress. However the trees in transect 2 (figure 6), from the same catchment and presumably receiving very similar rainfall, show a

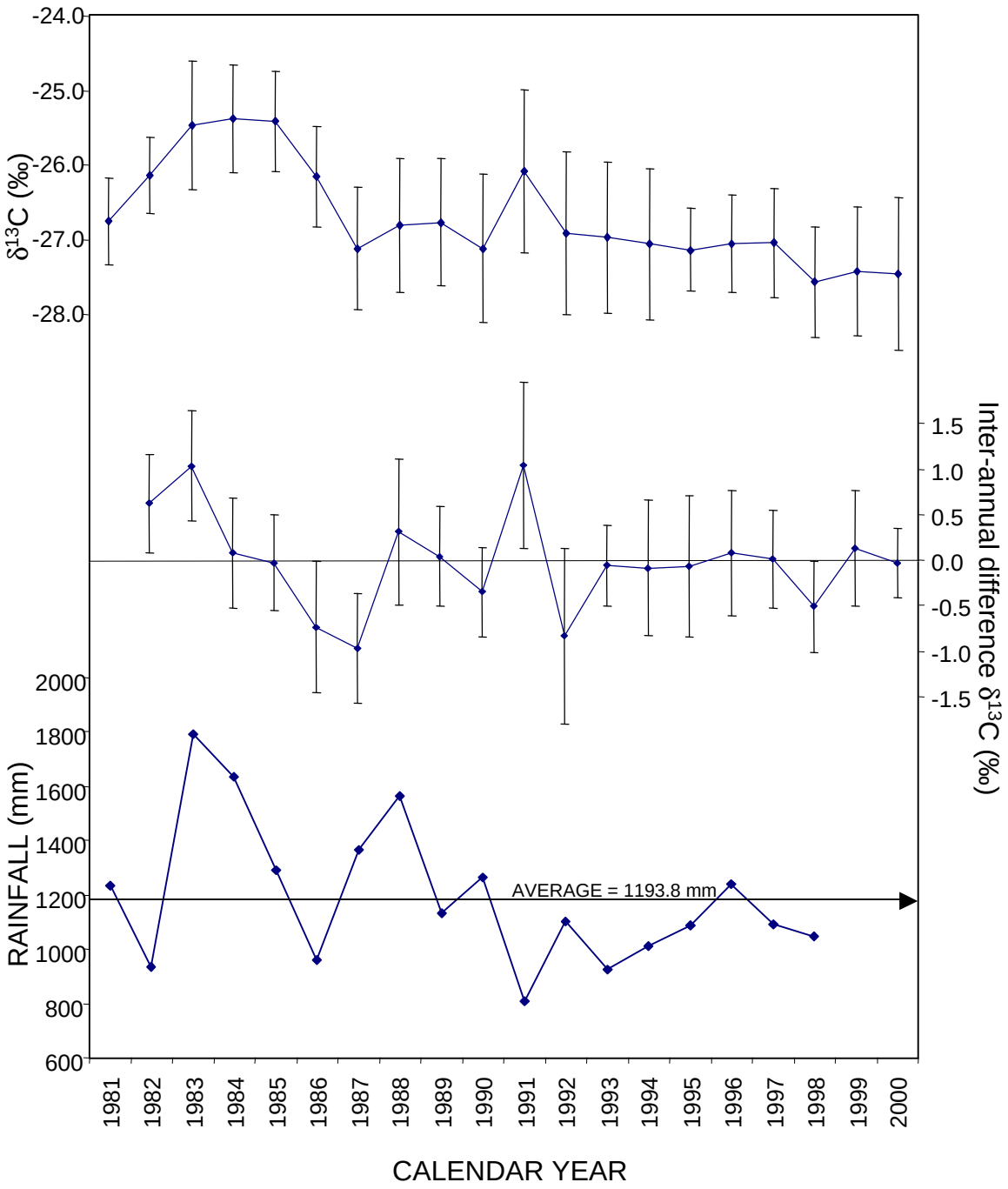

Figure 5. $\delta^{13}C$ of the latewood fraction of *Pinus elliotii* rings from transect 1, Usutu Forest, Swaziland (upper illustration). The mean and standard deviation values reflect the variability of the values in the corresponding growth ring in all the trees in the transect. In order to test if there is common forcing of the carbon isotope signal the year-to-year difference in isotope values is plotted (middle illustration). These are compared with the local rainfall record (lower illustration) to determine if the trees have a stomatal response to rainfall.

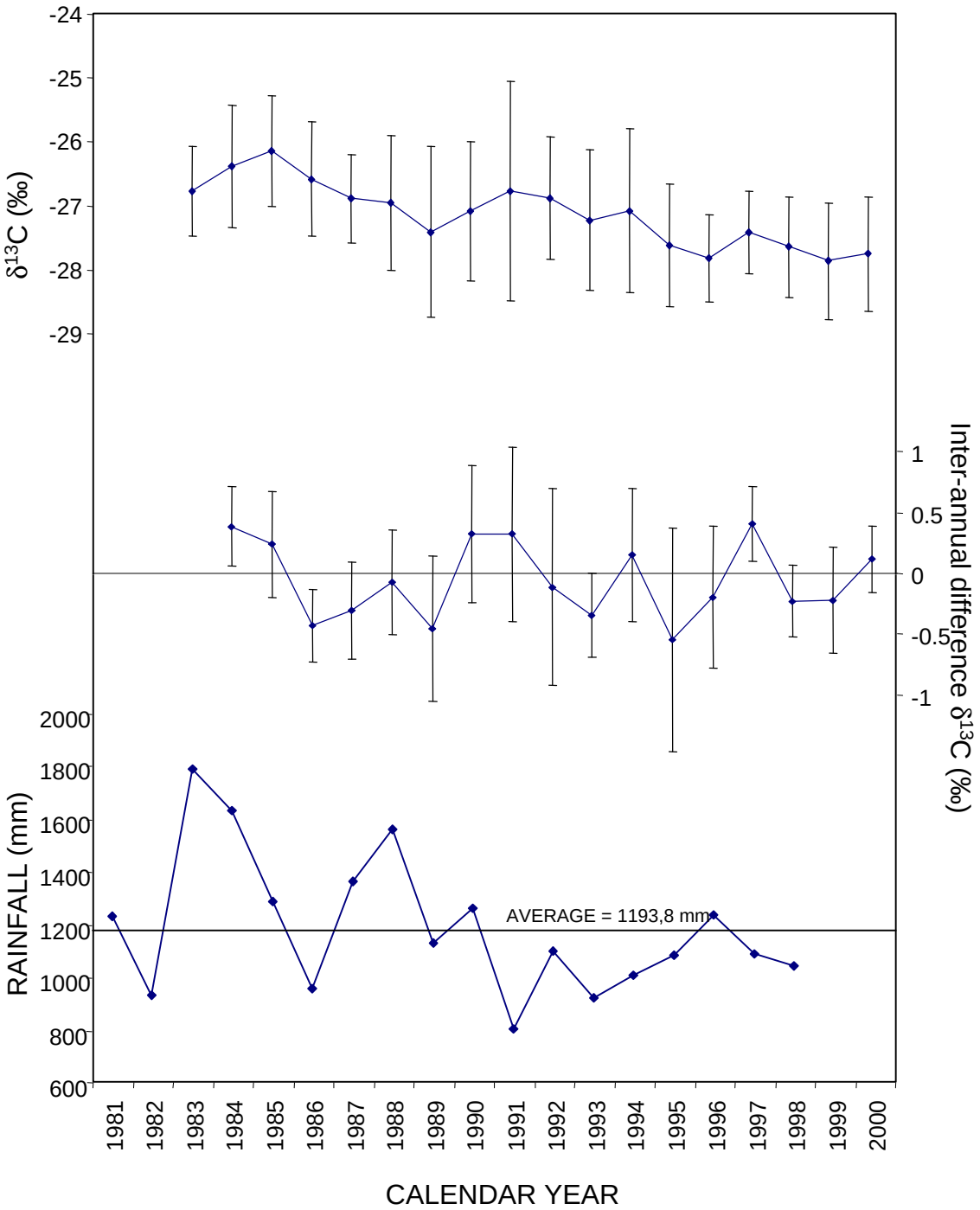

Figure 6. $\delta^{13}C$ of the latewood fraction of *Pinus elliotii* rings from transect 2, Usutu Forest, Swaziland (upper illustration). The mean and standard deviation values reflect the variability of the values in the corresponding growth ring in all the trees in the transect. In order to test if there is common forcing of the carbon isotope signal the year-to-year difference in isotope values is plotted (middle illustration). These are compared with the local rainfall record (lower illustration) to determine if the trees have a stomatal response to rainfall.

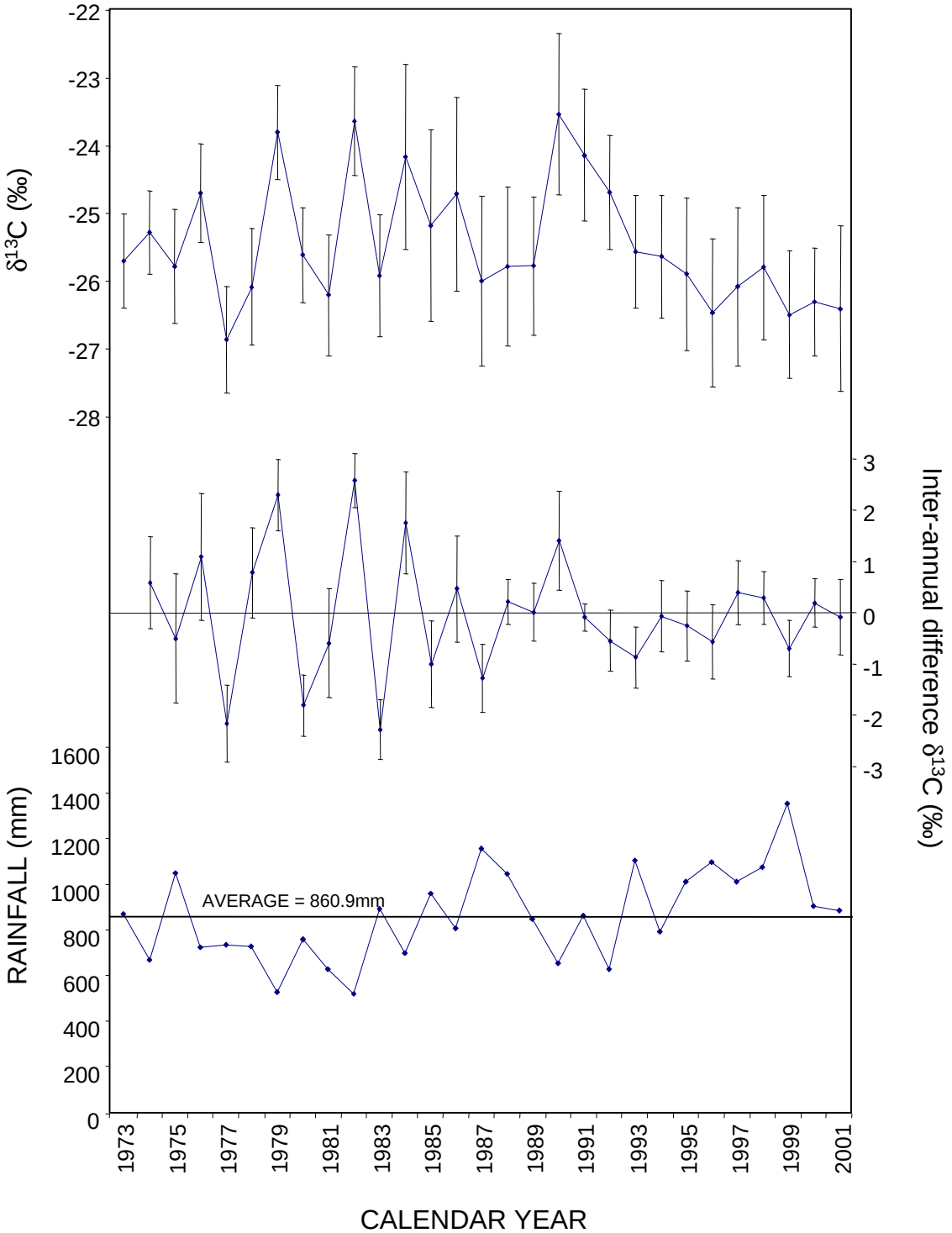

Figure 7. $\delta^{13}C$ of the latewood fraction of *Pinus patula* rings from transect 3, Riverside Forest, Maclear (upper illustration). The mean and standard deviation values reflect the variability of the values in the corresponding growth ring in all the trees in the transect. In order to test if there is common forcing of the carbon isotope signal the year-to-year difference in isotope values is plotted (middle illustration). These are compared with the local rainfall record (lower illustration) to determine if the trees have a stomatal response to rainfall.

less pronounced shift. Indeed the transect 2 analysis for the 1990 growth year shows a similar “dry” response relative to the 1989 growth year, even though the rainfall actually increased. With the exception of the juvenile years, which can be excluded from consideration, there are very few distinguishing features in the temporal analysis of the isotope values. Inter-annual variation is mostly less than 1‰ and positive shifts that should be associated with dry conditions also appear to be associated with excessively wet conditions. This suggests a rainfall optimum for *Pinus elliottii* in the Usutu environment, and this will be given further consideration in the chapter on further research.

The $\delta^{13}C$ trends in the *Pinus patula* trees from transect 3, the Riverside Forest (figure 7), presents a strong contrast to the Usutu results. The absolute $\delta^{13}C$ values for the trees vary within a ± 2‰ range for the first 10 years of growth, and thereafter the variability becomes less pronounced. The inter-annual variation shows a very coherent correspondence to changes in rainfall as predicted with higher rainfall associated with lower or negative shifts in the $\delta^{13}C$ values up to about 20 years of age.

The stomatal response of *Pinus patula* to rainfall in the initial growth period provides a promising indicator of a water stress response, but the declining correspondence through time needs to be considered. The hypothesis that is presented here is that the maturation of the root system of the trees will see a gradual shift from the exploitation of vadose water towards the tapping of the phreatic water at or below the watertable. This will be associated with a more reliable, but not necessarily more abundant water source. If the watertable is at great depth, the trees may still respond to rainwater or vadose water because of the reduced hydraulic lift required to access it. If this mechanism can be verified, it would indicate that the impact of mature trees such as those at the Riverside Forest on the water reserve, and probably also the water in the riparian habitat, is not limited to the trees that are planted in the riparian area.

4.2.2 Anatomical analyses

Just as stomatal aperture is one of the main mechanisms that allow trees to vary their hydraulic resistance (R_{PLANT}) in order to maintain leaf water potential, the other mechanism is to change the size and density of water conducting vessels in the stem. The tracheid cell size and density measurements for all transects are tabulated in tables 6a-c (tracheid diameters) and 7a-c (tracheid densities) in Appendix 1, and presented graphically in figures 8 to 10 (tracheid size) and figures 11 to 13 (tracheid density).

In all specimens there is a saturating increase in the size of tracheid cells associated with tree growth, particularly in the first five years. At the same time there is a corresponding decrease in the tracheid density. In terms of the absolute values and the year-to-year variation in both the tracheid size and density, there is no trend that suggests a link to rainfall in any of the trees. Accordingly there is no reason to anticipate a spatial trend that may help to delimit the riparian area.

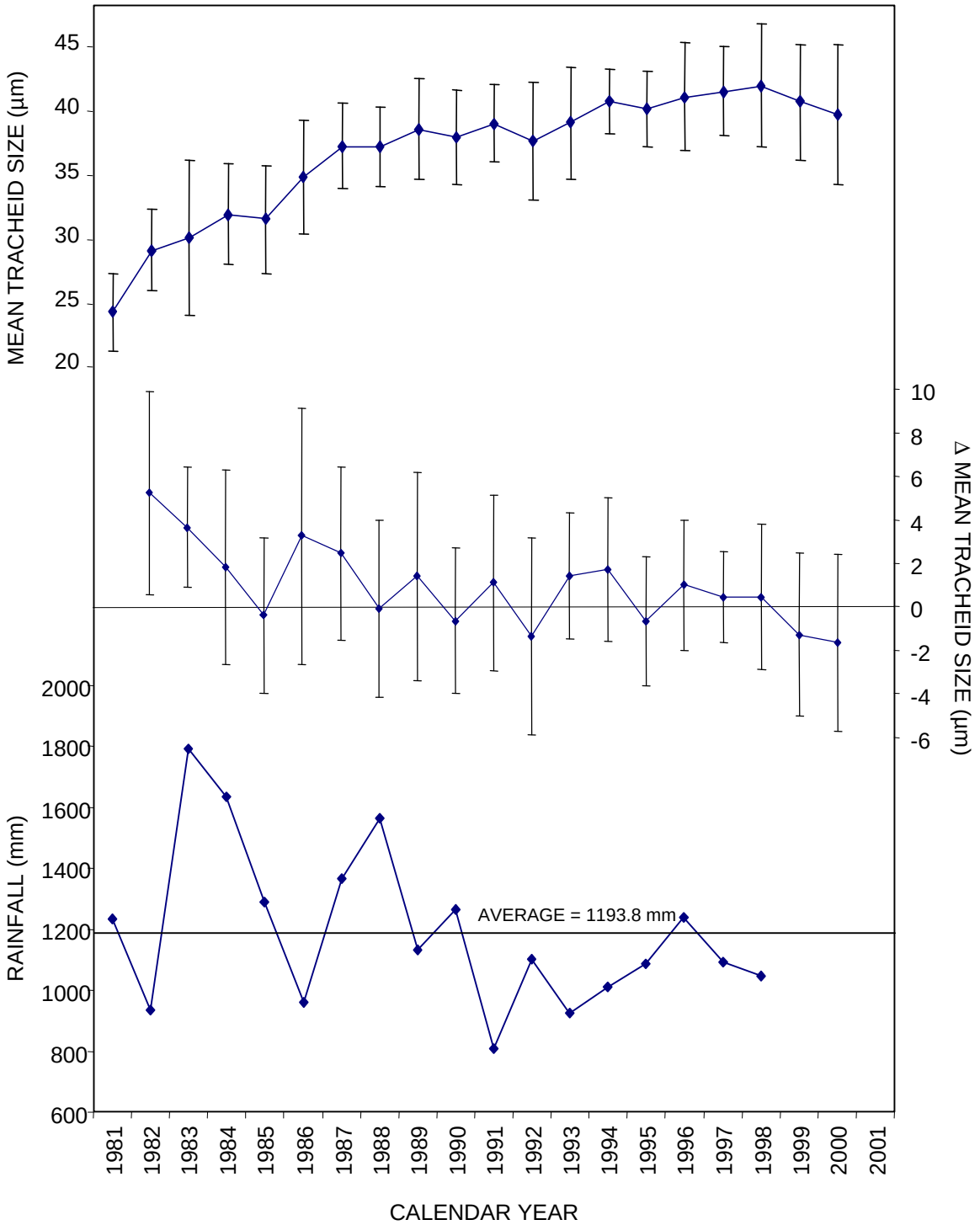

Figure 8. Tracheid cell diameter (µm) of the earlywood fraction of *Pinus elliottii* rings from transect 1, Usutu Forest, Swaziland (upper illustration). For each ring of each tree 30 cells were measured. The mean and standard deviation values reflect the variability of the values in the corresponding growth ring in all the trees in the transect. In order to test if there is common forcing of the carbon isotope signal the year-to-year difference in tracheid diameter values is plotted (middle illustration). These are compared with the local rainfall record (lower illustration) to determine if the trees have a physiological response to rainfall.

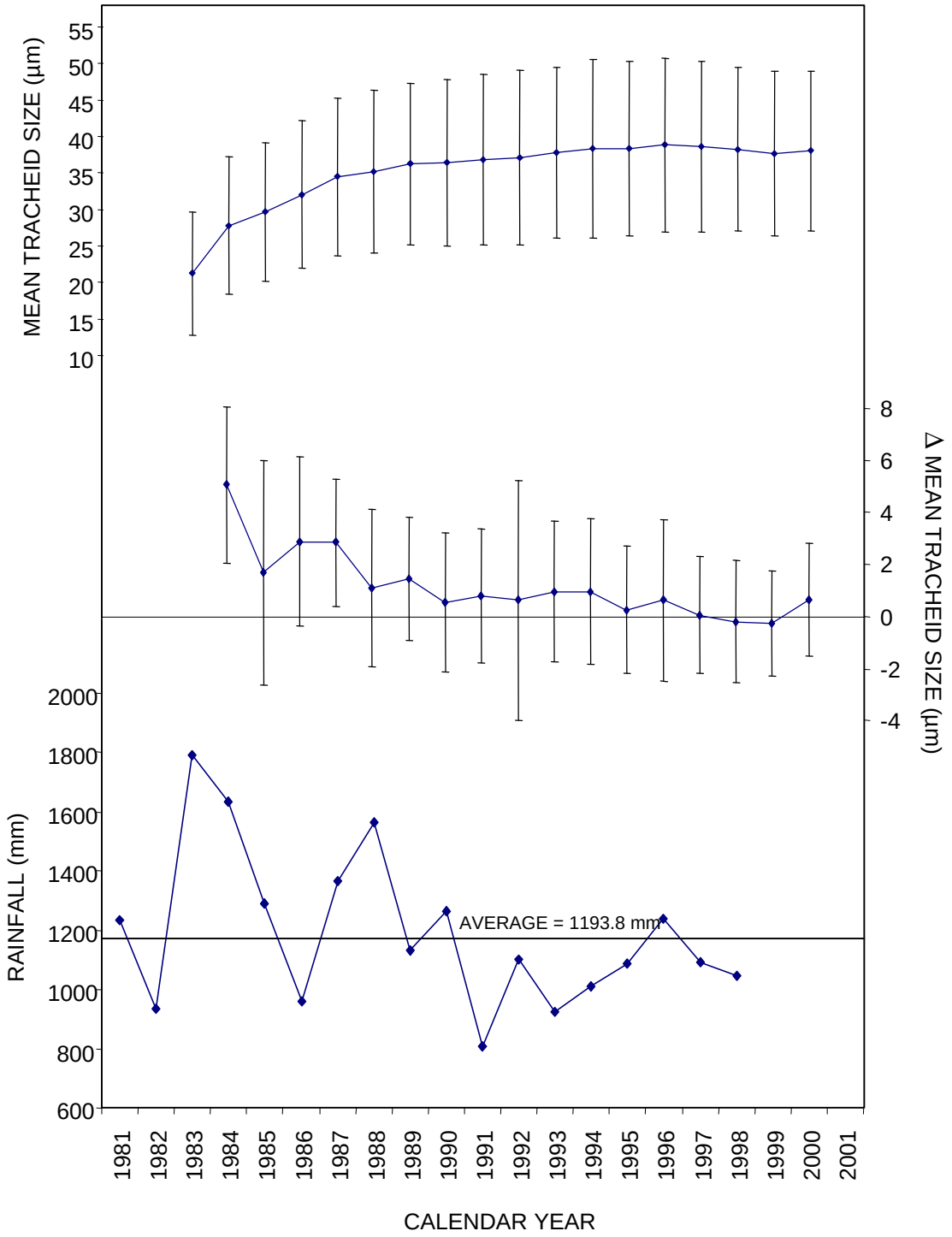

Figure 9. Tracheid cell diameter (µm) of the earlywood fraction of *Pinus elliottii* rings from transect 2, Usutu Forest, Swaziland (upper illustration). For each ring of each tree 30 cells were measured. The mean and standard deviation values reflect the variability of the values in the corresponding growth ring in all the trees in the transect. In order to test if there is common forcing of the carbon isotope signal the year-to-year difference in tracheid diameter values is plotted (middle illustration). These are compared with the local rainfall record (lower illustration) to determine if the trees have a physiological response to rainfall.

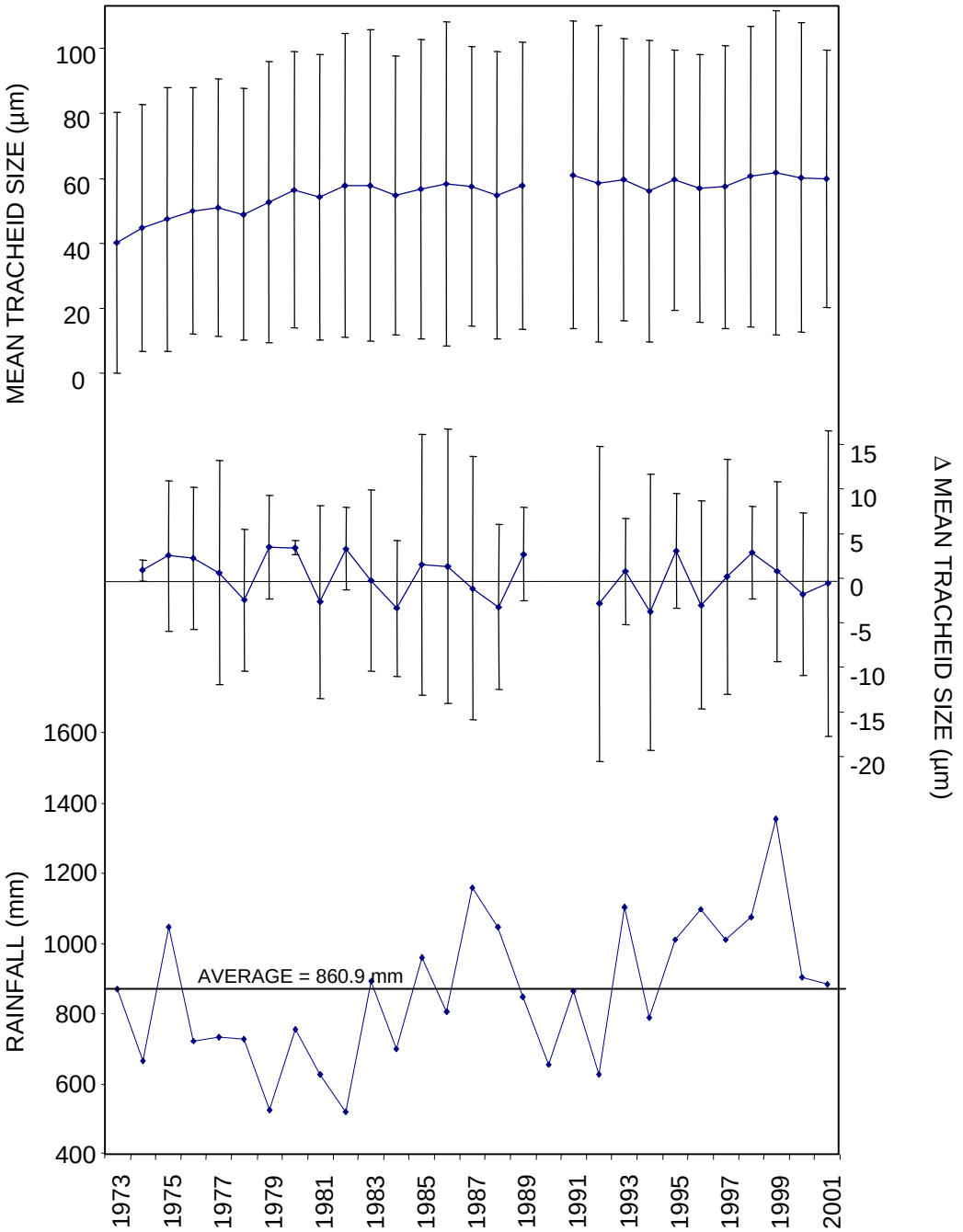

Figure 10. Tracheid cell diameter (µm) of the earlywood fraction of *Pinus patula* rings from transect 3, Riverside Forest, Maclear (upper illustration). For each ring of each tree 30 cells were measured. The mean and standard deviation values reflect the variability of the values in the corresponding growth ring in all the trees in the transect. In order to test if there is common forcing of the carbon isotope signal the year-to-year difference in tracheid diameter values is plotted (middle illustration). These are compared with the local rainfall record (lower illustration) to determine if the trees have a physiological response to rainfall. The data gap corresponding to the year 1990 relates to a marginal ring that was not initially identified

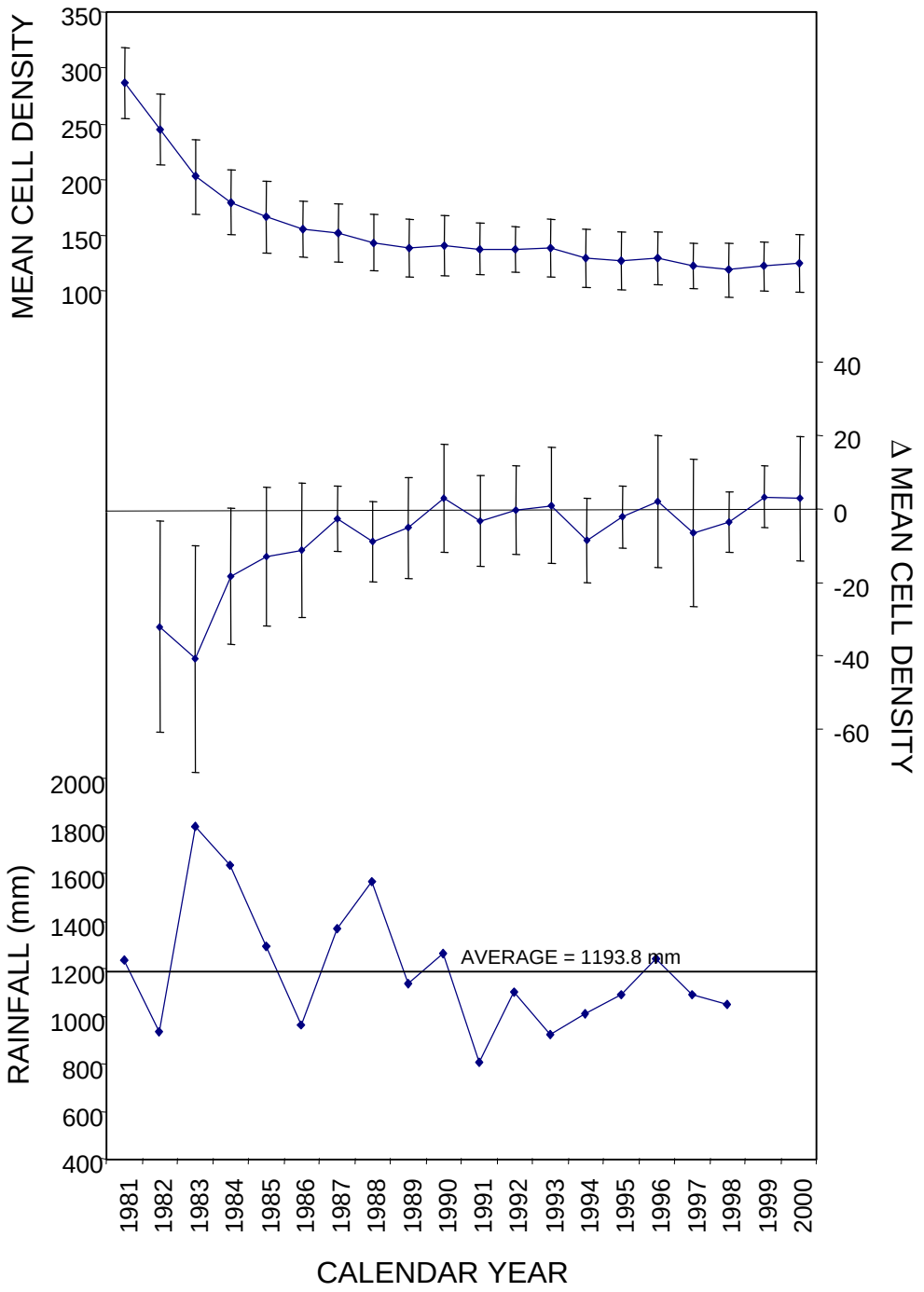

Figure 11. Tracheid cell density (average cell count in a 310x620 µm grid repeated in three areas of the earlywood fraction) of *Pinus elliottii* rings from transect 1, Usutu Forest, Swaziland (upper illustration). The mean and standard deviation values reflect the variability in corresponding growth ring in all the trees in the transect. In order to test if there is common forcing of the tracheid cell density the year-to-year difference in tracheid density is plotted (middle illustration). These are compared to the local rainfall record (lower illustration) to determine if the trees have a physiological response to rainfall.

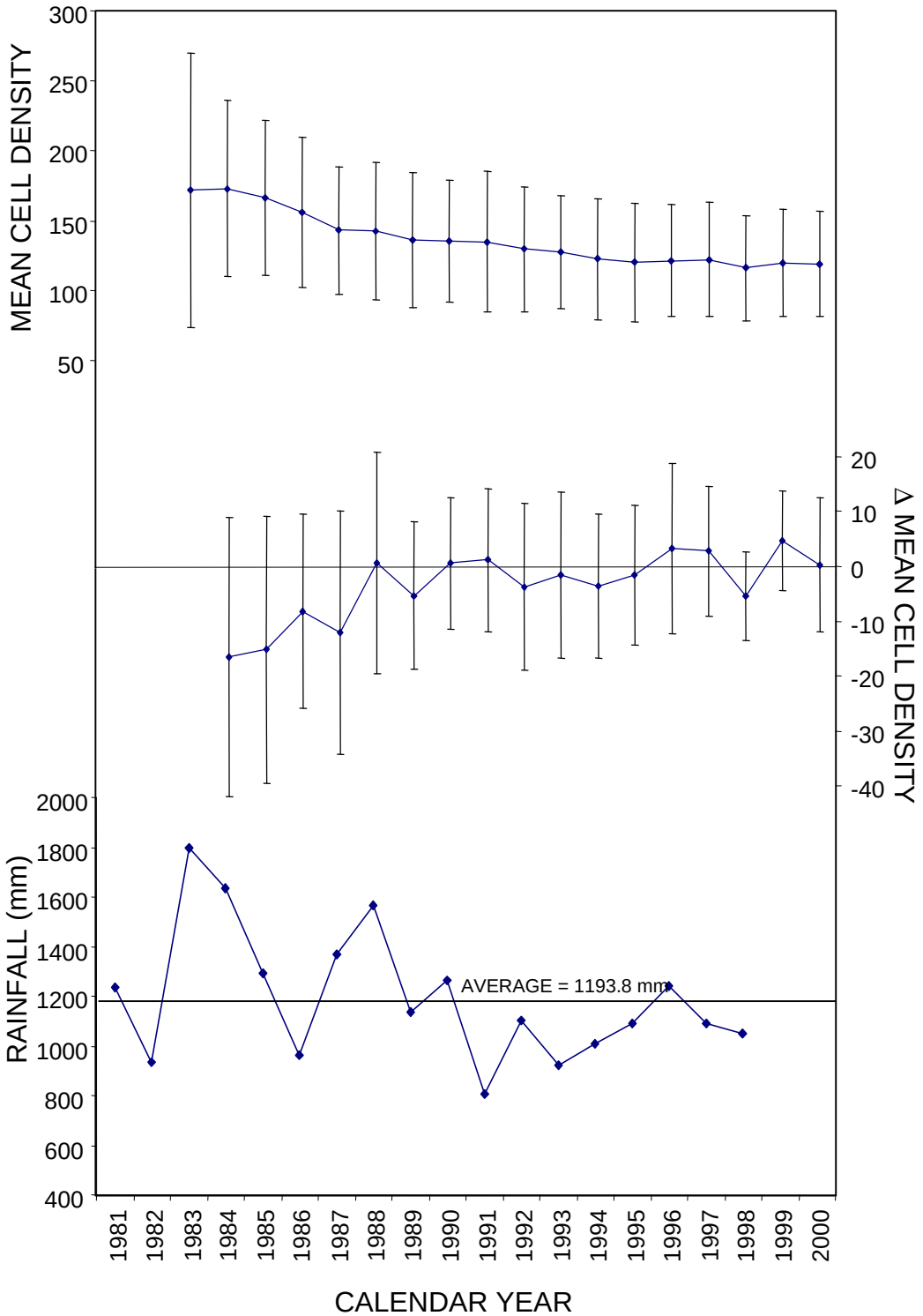

Figure 12. Tracheid cell density (average cell count in a 310x620 µm grid repeated in three areas of the earlywood fraction) of *Pinus elliottii* rings from transect 2, Usutu Forest, Swaziland (upper illustration). The mean and standard deviation values reflect the variability in corresponding growth ring in all the trees in the transect. In order to test if there is common forcing of the tracheid cell density the year-to-year difference in tracheid density is plotted (middle illustration). These are compared to the local rainfall record (lower illustration) to determine if the trees have a physiological response to rainfall.

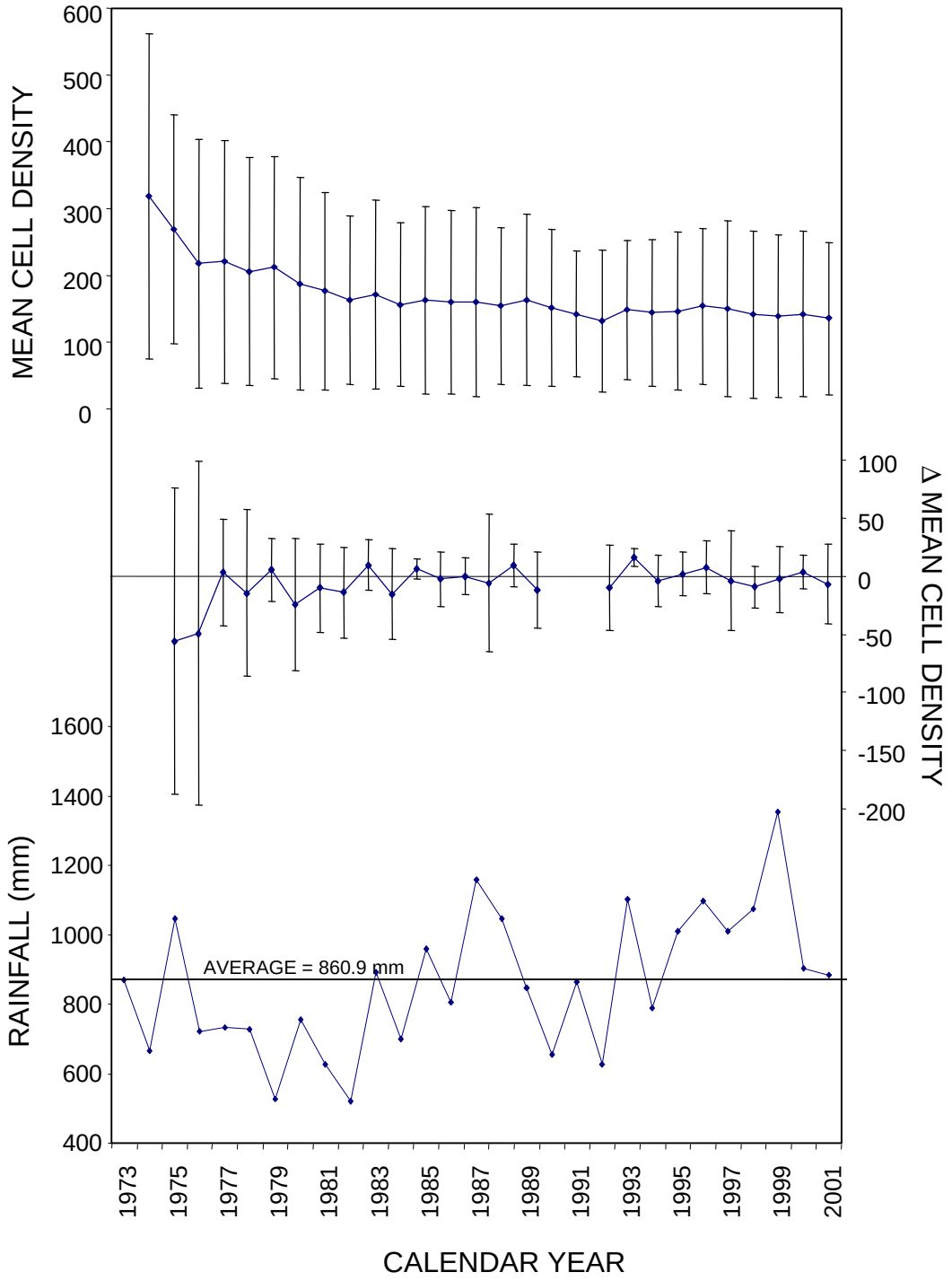

Figure 13. Tracheid cell density (average cell count in a 310x620 µm grid repeated in three areas of the earlywood fraction) of *Pinus patula* rings from transect 3, Riverside Forest, Maclear (upper illustration). The mean and standard deviation values reflect the variability in corresponding growth ring in all the trees in the transect. In order to test if there is common forcing of the tracheid cell density the year-to-year difference in tracheid density is plotted (middle illustration). These are compared to the local rainfall record (lower illustration) to determine if the trees have a physiological response to rainfall. The data gap corresponding to the year 1990 represents a marginal growth ring that was not initially identified.

4.3 Spatial changes in tree physiology

4.3.1 Stable isotope analyses

The spatial trend in the carbon isotope values is summarised for transects 1 and 2 from Usutu in figure 14 and for transect 3 from Riverside in figure 15. The uppermost plot presents the "isotopic profile" of the transect from stream to hilltop, with each line representing the profile from a different year. The average isotope value for each tree is also presented, and it is clear that the average plot gives a very similar spatial trend to any individual year. Since the average precision on each measurement is <0.2‰ the differences between the different trees in any year are significant. In the case of the *Pinus elliottii* transects 1 and 2 from Usutu, there is no spatial trend in $\delta^{13}C$ values that may be linked to riparian water-use. The spatial arrangement of carbon isotopes from the *Pinus patula* transects at Riverside is expected, on the basis of the diachronic analysis of individual trees, to reflect relative water stress and there is clearly a trend from the stream to the hilltop. This trend however has the most negative isotope values associated with the trees that were felled from the top of the hill, and visa versa. Within any individual specimen the negative values are associated with high rainfall, and so it appears as if the trees in the riparian area are more water stressed on average than those on the adjacent hill.

4.3.2 Anatomical analyses

The spatial variability in the anatomical indices for transects 1 and 2 from Usutu are presented in figure 14. Since these indices are not sensitive to diachronic changes in rainfall it was not anticipated that they would show any spatial sensitivity. This expectation is met with the caveat that in both transects the tree that was located in the stream has a distinctly lower tracheid density when compared with the trees that were immediately adjacent to it. This low density is associated with bigger tracheids relative to the adjacent trees as well as a positive shift in the carbon isotope values. Although the anatomical indices for transect 3 from Riverside (figure 15) also show no meaningful spatial differentiation, they do not have the same distinct signature for the stream-side tree. This may be the result of saturation of the roots of the lowermost trees at the Usutu transects, whereas the lowermost Riverside trees were not in the stream.

4.4 Discussion

The dataset that has been determined in this research includes the measurement of carbon isotopes, which is a proxy for carbon assimilation and stomatal performance, tracheid size and density, which combine to give a measure of the water conveyance capability of the plant (the inverse of hydraulic resistance). These are the mechanisms by which the tree maintains the water potential in the leaf and the evaporative flux. When considered in the context of the equation given above, E (evaporative flux) and Ψ_{LEAF} (leaf water potential) are assumed to remain at a more-or-less constant level and so changes in R_{PLANT} (hydraulic resistance of the tree) should reflect changes in Ψ_{SOIL} (soil moisture potential). Variations in Ψ_{SOIL} are typically in response to variation in rainfall through time and variations in riparian water availability.

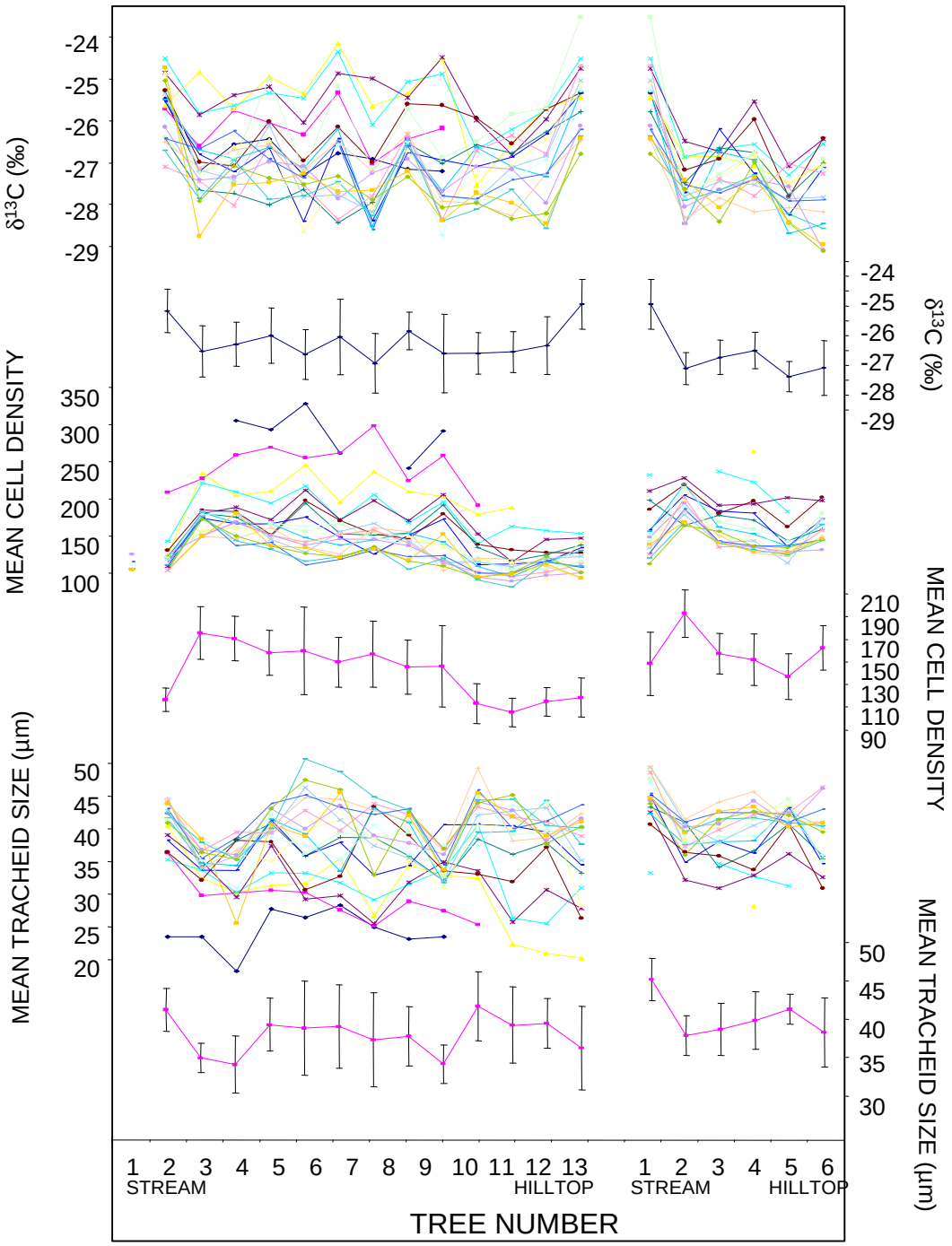

Figure 14. Spatial representation of the isotope (upper), tracheid cell density (middle) and tracheid cell size (lower) measurements for *Pinus elliottii* trees from transects 1 and 2, Usutu Forest, Swaziland. Transect 1 is plotted on the left and transect 2 on the right. Each line represents the spatial profile for a year without any attempt to distinguish individual years. The average values for each of the trees is also plotted, which yields a spatial profile that correlates well with the profile for any individual year. In the case of tracheid size and density measurements the first two years are excluded from the average calculation. There is no spatial trend that may indicate differential access to riparian water.

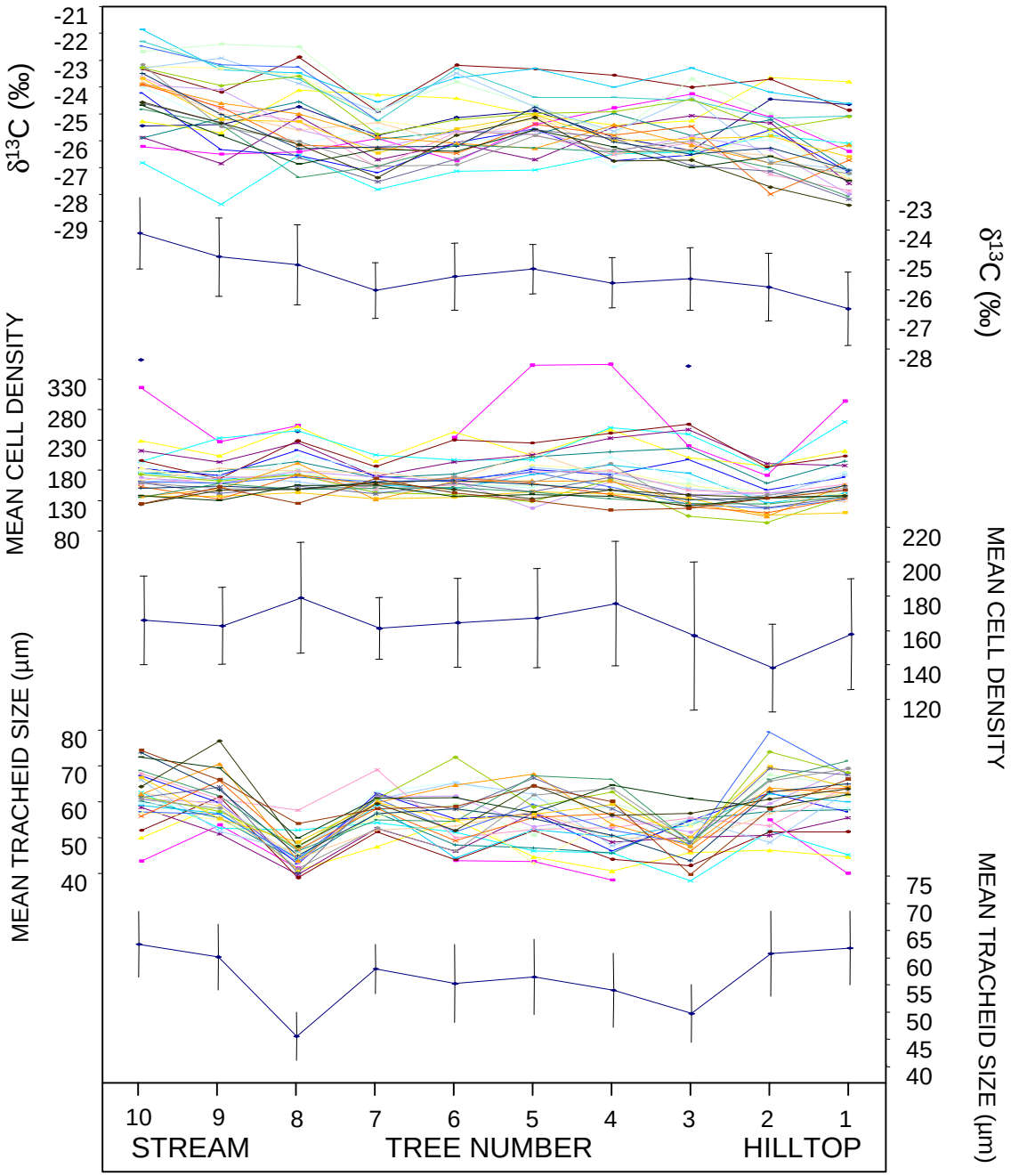

Figure 15. Spatial representation of the isotope (upper), tracheid cell density (middle) and tracheid cell size (lower) measurements for *Pinus patula* trees from transect 3, Riverside Forest, Maclear. Each line represents the spatial profile for a year without any attempt to distinguish individual years. The average values for each of the trees is also plotted, which yields a spatial profile that correlates well with the profile for any individual year. In the case of tracheid size and density measurements the first two years are excluded from the average calculation. There is no spatial trend that may indicate differential access to riparian water.

Variability in tracheid size and density showed no spatial trend or correspondence with rainfall. These characteristics show an ontogenic effect that dominates any environmental response. It may be possible to detrend the ontogenic effect, but as trees get older the ontogenic effect decreases and at the same time the maturation of the roots may lead to decreased dependence on vadose water. The highest environmental sensitivity will be prior to root maturation when the uncertainty in detrending is high. To some extent this is achieved in the analysis of year-to-year variability, and the results still do not appear to be sensitive to variations in soil moisture potential.

Variability in $\delta^{13}C$ values did not correspond with rainfall at Usutu but it did at Riverside. There is insufficient control in this dataset to determine if this difference is the result of the lower rainfall at Riverside, or if it is the result of the different genetic adaptation strategies of the different tree species that were sampled. The temporal variability at the Riverside sample site suggested that high soil moisture potential would be associated with more negative $\delta^{13}C$ values. When the spatial variation was considered the more negative $\delta^{13}C$ values were found at the hilltop. In the light of the theoretical and empirical evidence this result should imply that soil water potential is greatest at the hilltop, and not in the riparian habitat. This is untenable, and further consideration has to be given to other factors that affect $\delta^{13}C$ values.

The positive shift in the $\delta^{13}C$ of the lowermost trees from the Usutu transects also contradict the tenuous link between similar shifts and the lowest rainfall year. While this conclusion appears incongruous, similar results have been observed in flooded sites (Brendel *et al.* 2002). The precise mechanism is unclear, but the adaptive ability that the tree has to vary the hydraulic resistance is incapable of accommodating water saturated conditions. If soil saturation drives this contradictory response in the stomatal control of the trees, then it may also emerge that the high rainfall in the region results in high soil moisture levels and periodic saturation. Under such circumstances the physiological response of the trees to water scarcity may be indistinguishable from their response to water abundance. The definition of the riparian habitat in such high rainfall areas would not be possible using this approach, but it would also be mute as the whole area would effectively be a wetland. The low rainfall at Riverside would seldom lead to saturation, and a different mechanism must be invoked to explain the spatial trends in $\delta^{13}C$ at this site.

In considering the spatial variability of the water-use indices in this study, it should be noted that the summary plots in figures 14 and 15 have deceptively large “error” bars. These reflect the variability of the indices over the life of each individual tree (n=the number of rings in each tree). They cannot be used as an estimate of statistical uncertainty when making comparisons between trees. It is apparent when all the data points are plotted together that any year that yields a below-average value in one tree will also yield below-average value in almost all the trees. Likewise an above-average data point in any tree will be associated with above-average data points in almost every tree for that year. In most instances the spatial profile in any index for any year is very similar to the average profile, but with a shift in the absolute values. The spatial data therefore presents very real and consistent differences in water use physiology from one tree to the next, even if this does not reflect the availability of riparian water. This variability may be related to microenvironmental differences, but these should not have a more substantial effect than the difference in

microenvironment between the riparian and non-riparian habitats. Since there appears to be a tree-specific response, an alternative reason for the observed data may be the genetically controlled response of the trees. While all the trees in each transect are a single species, they are not genetic clones and there is scope for genetic differences in physiological response. Genetic related variation in $\delta^{13}C$ between clones have previous been noted (Comstock and Ehleringer 1992, Brendel 2002). In general these variations are though to be quite small, in the order of 1‰ for $\delta^{13}C$ (Schleser *et al.* 1995), but depending on the adaptive strategy such variability can be quite large (Pennington *et al.* 1999). The maximum variation of carbon isotopes within any tree in this study sample is 2.6‰ while between trees from any transect it is 2.45‰. The maximum variation of tracheid size within a tree, excluding the two juvenile years where measurement is difficult, is 15.2μm while between trees it is 11.1μm. The maximum variation of tracheid density, also excluding the two juvenile years, is 95.7 while between trees it is 87.6. This suggests that, if genetically controlled adaptation is responsible for the variability, it is almost as significant in determining the differences in water uptake and use between trees as the environment is in determining the inter-annual response within trees. It is not possible to test this suggestion with the existing data, but if genetic variability accounts for the observations that have been presented, then it suggests that its impact on water-use by these trees is more profound than the microenvironmental differences between the riparian and non-riparian habitat. This is clearly a subject for future research as greater benefit in terms of water-use would be achieved by managing the genetic stock of seedlings rather than where they are planted on the landscape.

4.5 Conclusions

This research project has not yielded any spatial patterns in the anatomy and carbon isotopes of *Pinus elliottii* or *Pinus patula* trees that may be linked to riparian water-use. Similarly there is little evidence in the physiology of the trees to support the idea that these forest tree species have a more profound impact on the hydrology of the riparian habitat than they do on the overall hydrological regime of a catchment. The possibility that sub-optimal growth conditions brought about by too much water or too little water have similar physiological signatures, or the possibility that the physiological response of the trees to varying water availability is dominated by the genetic makeup of the tree, accounts for the observed data is not the focus of this research, and may be taken up as future research subjects.

Chapter 5

Further Research

5.1 Is the anatomical control of water transpiration coherent?

This project has produced a multi-dimensional data matrix that comprises a spatial component, a time component, and a tree physiology component. For each ring (point in time) of each tree (point in space) there are three anatomical components namely the tracheid size, cell density and ring width measurements, as well as the isotopic characteristic that is a proxy for stomatal conductance. This matrix allows the physiological response of any single tree to be assessed through time and correlated with environmental changes. Different physiological responses between trees were presumed to reflect different edaphic conditions in the vicinity of the roots. Despite the theoretical expectation that this would be a useful delimiting tool, the foregoing analysis indicates that these parameters either do not portray differential water access between trees in riparian and non-riparian habitats, or they lack the sensitivity to demonstrate the distinction. This result brings the fundamental assumptions of the tree physiology into question, and it is necessary to test these assumptions using the existing data.

5.2 An anatomic package of water-use structures

The previous analysis tested the correlation of rainfall and the physiological indices *between* trees. The focus is now on the coherence between the physiological indices *within* any tree. In order to test if the water conductance parameters co-vary, the coefficient of determination (r^2) values were calculated for the relationships between $\delta^{13}C$, tracheid cell size, tracheid cell density, ring widths, and rainfall for each tree. These are presented in table 8 for the Usutu trees, and table 9 for the Riverside trees.

The results confirm that the variation in rainfall through the growth of the Usutu trees does not correlate with changes in the anatomical structures including ring width and carbon isotopes. The resulting r^2 values are generally very low. In contrast the physical structures: ring widths, tracheid size and tracheid density are all very well correlated. Where low r^2 values are obtained for the physical structures the data are generally very closely clustered and the poor correlation relates to lack of variability in the parameters rather than a lack of interdependence.

While the physical structures of each tree in the Usutu transects are well correlated, each relationship is specific to the tree from which it is derived. Combining the datasets yields a coefficient of correlation that is worse that that of any individual tree. While all the trees appear to share a similar adaptive strategy, the extent and magnitude of the response is specific to each individual. This supports the previous suggestion that the genetic influence over the response must be considered when comparing the structures between specimens.

Table 8. Coefficient of variation (r^2) values for best-fit curves relating the different parameters that were measured on the *Pinus elliottii* specimens felled in transects 1 and 2 from the Usutu Forest, Swaziland. In each case a linear fit was found to give the best correlation, with the exception of the relationship between tracheid size and density. Here a "power curve" of the form $y=ax^b$ was used.

Transect 1	Rainfall vs.				Ring width vs.			Tracheid size vs.	
Tree number	$\delta^{13}C$	Tracheid size	Tracheid Density	Ring widths	$\delta^{13}C$	Tracheid size	Tracheid Density	$\delta^{13}C$	Tracheid Density
1	0.12	0.02	0.12	0.46	0.19	0.47	0.44	0.13	0.40
2	0.37	0.05	0.47	0.39	0.54	0.52	0.54	0.13	0.56
3	0.13	0.12	0.30	0.22	0.19	0.06	0.58	0.13	0.62
4	0.02	0.10	0.58	0.32	0.42	0.49	0.70	0.19	0.77
5	0.17	0.11	0.49	0.31	0.48	0.75	0.90	0.27	0.88
6	0.16	0.05	0.15	0.23	0.45	0.81	0.73	0.35	0.77
7	0.13	0.21	0.34	0.14	0.03	0.09	0.07	0.44	0.58
8	0.10	0.11	0.49	0.27	0.51	0.55	0.55	0.03	0.73
9	0.23	0.05	0.40	0.31	0.65	0.00	0.72	0.03	0.42
10	0.00	0.02	0.39	0.00	0.61	0.72	0.75	0.38	0.72
11	0.00	0.39	0.52	0.01	0.18	0.54	0.37	0.26	0.73
12	0.11	0.55	0.32	0.05	0.25	0.19	0.46	0.21	0.61
13	0.02	0.27	0.27	0.05	0.10	0.27	0.39	0.15	0.67
Transect 2									
1	0.02	0.30	0.36	0.00	0.26	0.24	0.78	0.01	0.52
2	0.24	0.08	0.01	0.01	0.11	0.03	0.07	0.22	0.34
3	0.34	0.10	0.45	0.00	0.58	0.63	0.69	0.58	0.56
4	0.01	0.45	0.53	0.00	0.28	0,63	0.63	0.40	0.95
5	0.05	0.34	0.24	0.00	0.04	0.12	0.65	0.27	0.55
6	0.09	0.06	0.00	0.00	0.44	0.76	0.75	0.36	0.70
ALL	0.05	0.11	0.13	0.09	0.20	0.13	0.13	0.06	0.48

In contrast to the *Pinus elliotti* trees in the Usutu Forest, the carbon isotope profiles of the *Pinus patula* trees in the Riverside forest show a very good correlation with rainfall. It has already been suggested that there is a shift from the dependence on water in the vadose zone to the use of phreatic water as the roots mature. This would suggest that the coefficient of correlation (r^2) would become less significant after a certain point in time. On the basis of the visual covariance of $\delta^{13}C$, and the inter-annual change of $\delta^{13}C$ versus rainfall, the coefficient was calculated for the period 1977 to 1990, and from 1991 to 2001 as well as the entire dataset. The results do not support the notion that the trees begin to tap groundwater as the best correlations are for the entire dataset.

In the *Pinus elliottii* trees from the Usutu Forest and *Pinus patula* trees from the Riverside Forest large annual growth rings are associated with higher density of tracheid cells that are smaller in size and more positive $\delta^{13}C$ values (figure 16, 17). For any give tree the relationship between the physical structures and carbon isotopes is reasonable, but seldom as strong as the correlation between the physical characteristics. To the extent that a relationship exists, large rings are associated with relatively positive carbon isotope values. This is contradictory to the theoretical understanding of carbon assimilation through the stomata. Larger annual growth

Table 9. Coefficient of variation (r^2) values for best-fit curves relating the different parameters that were measured on the *Pinus patula* specimens felled in transect 3 from the Riverside Forest, Maclear. In each case a linear fit was found to give the best correlation, with the exception of the relationship between tracheid size and density. Here a "power curve" of the form $y=ax^b$ was used.

Transect 3	Rainfall vs.									Ring width vs.			Tracheid size vs.	
Tree number	$\delta^{13}C$	$\delta^{13}C$ '77-'90	$\delta^{13}C$ '91-'01	$\delta^{13}C$ Derivative	$\delta^{13}C$ Derivative '77-'90	$\delta^{13}C$ Derivative '91-'01	Tracheid size	Cell Density	Ring width	$\delta^{13}C$	Tracheid size	Cell Density	$\delta^{13}C$	Cell Density
1	0.60	0.65	0.40	0.34	0.24	0.21	0.15	0.10	0.19	0.00	0.25	0.17	0.27	0.77
2	0.70	0.69	0.09	0.28	0.12	0.04	0.00	0.05	0.18	0.40	0.33	0.69	0.09	0.62
3	0.32	0.33	0.23	0.39	0.31	0.03	0.01	0.16	0.20	0.11	0.04	0.74	0.00	0.01
4	0.38	0.41	0.30	0.27	0.21	0.03	0.15	0.16	0.29	0.15	0.40	0.31	0.03	0.60
5	0.22	0.21	0.09	0.38	0.29	0.00	0.16	0.13	0.29	0.00	0.13	0.45	0.00	0.36
6	0.17	0.18	0.14	0.40	0.33	0.33	0.12	0.02	0.27	0.01	0.05	0.04	0.06	0.30
7	0.08	0.09	0.29	0.32	0.17	0.05	0.01	0.05	0.19	0.02	0.05	0.45	0.08	0.26
8	0.07	0.13	0.55	0.29	0.28	0.33	0.00	0.10	0.21	0.09	0.11	0.17	0.05	0.21
9	0.00	0.00	0.29	0.40	0.32	0.04	0.23	0.08	0.02	0.01	0.21	0.34	0.01	0.37
10	0.01	0.01	0.18	0.36	0.35	005	0.12	0.08	0.04	0.00	0.09	.010	0.08	0.65
ALL	**0.15**	**0.12**	**0.12**	**0.15**	**0.26**	**0.02**	**0.04**	**0.08**	**0.15**	**0.03**	**0.05**	**0.24**	**0.00**	**0.30**

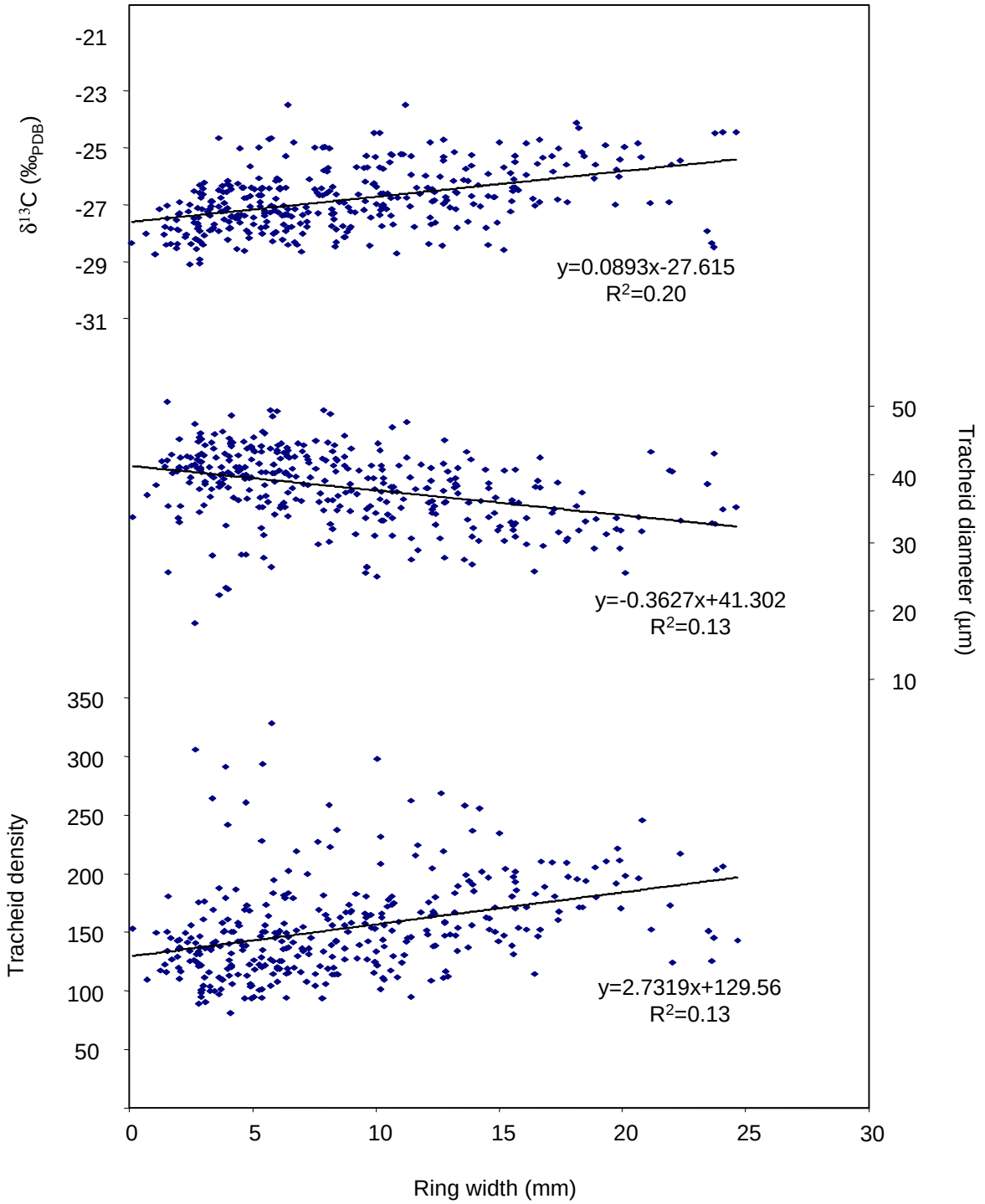

Figure 16. The amount of carbon assimilation, measured by the width of the annual growth rings, is correlated with carbon isotopes (top), tracheid diameters (middle) and tracheid density (bottom) for *Pinus elliottii* trees from the Usutu Forest, Swaziland. The relationship obtained for any individual tree is statistically better than that obtained for the entire dataset, suggesting a genetic influence on the relationship.

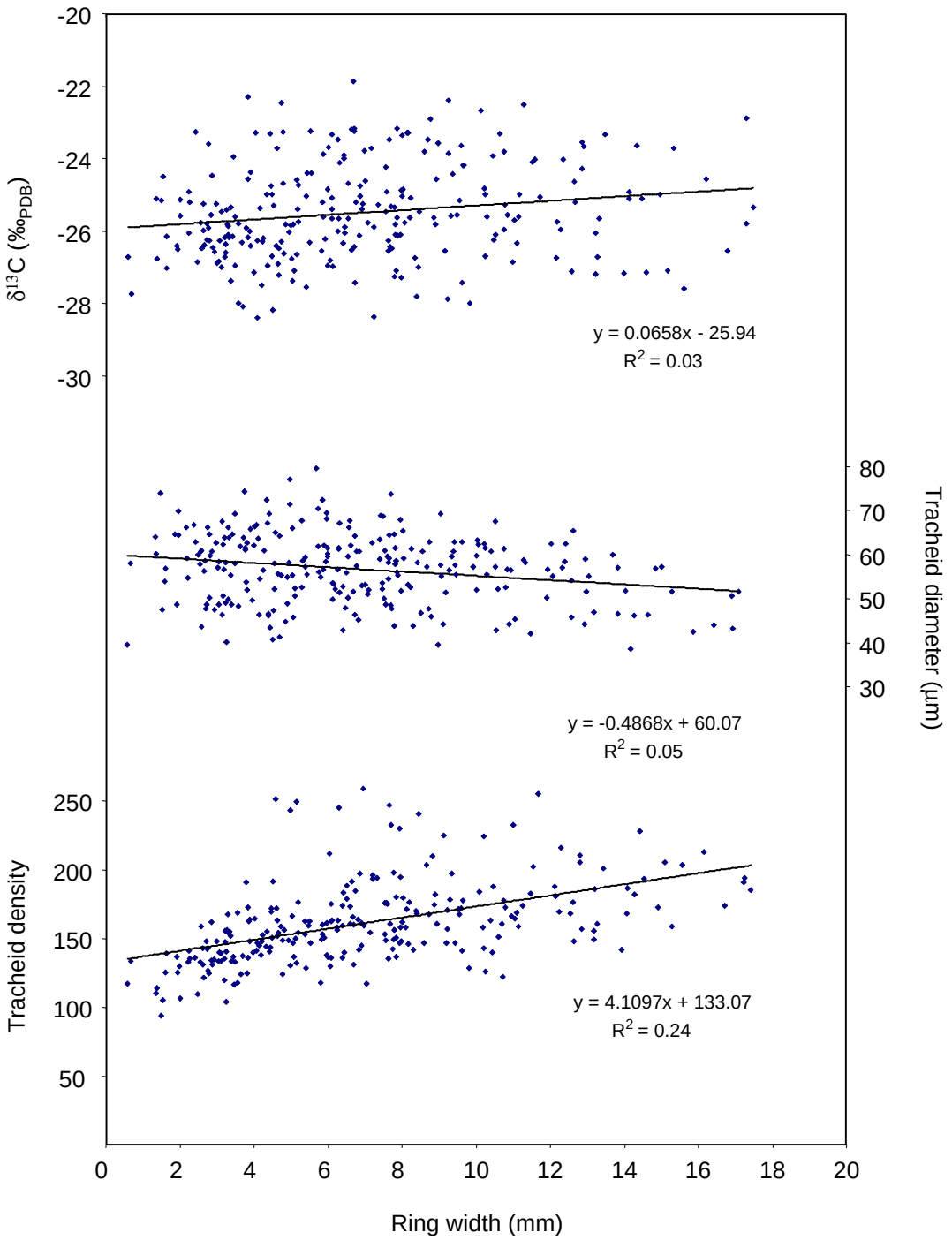

Figure 17. The amount of carbon assimilation, measured by the width of the annual growth rings, is correlated with carbon isotopes (top), tracheid diameters (middle) and tracheid density (bottom) for *Pinus patula* trees from the Usutu Forest, Swaziland. The relationship obtained for any individual tree is statistically better than that obtained for the entire dataset, suggesting a genetic influence on the relationship.

rings assumes greater carbon assimilation which can only happen if the average carbon concentration in the stomata is high in comparison with years that yield smaller annual growth rings. High stomatal carbon concentrations are associated with more negative isotope values.

Although there remain aspects of this data that needs further explanation, there appears to be a coherent anatomical interrelationship between the parameters that were studied. The theoretical position that informed the hypothesised relationship with riparian water is therefore valid. This returns the focus onto the other factors that influence the water conductance of the trees, and the possibility that genetics produces such disparate responses from different trees is further supported by the better correlations found *within* individual trees relative to those found *between* trees.

5.3 Species specific water adaptive strategies

Figure 18 shows a strong correlation between tracheid size and density in the Riverside trees that is similar in nature to that found in Usutu (again each individual tree has a unique response that is better correlated than the entire dataset). However the *Pinus patula* tracheids are significantly larger than the *Pinus elliottii* tracheids and the *Pinus patula* $\delta^{13}C$ values are about 1.7‰ more positive than those of *Pinus elliottii*.

The large tracheid diameters at Riverside is an indication that these trees are able to conduct much more water than the trees from Usutu. This is despite the fact that rainfall at Usutu is approximately 50% higher than that at Riverside. While this seems incongruous it is a very good adaptive strategy to water limited environments. For a tree that has occasionally access to high soil water potential and probably for short durations, there is a need to maximize the photosynthetic opportunities. When rain does fall the tree must minimize the water resistance, R_{PLANT}, and this is accomplished by having large tracheid cells in place. However in the intervening periods when soil water potential is low the tree needs to maximize R_{PLANT} to ensure that water loss through the leaves is minimized. This is done by stomatal control, and hence the good correlation between carbon isotopes and rainfall for the *Pinus patula* trees at Riverside and the positive $\delta^{13}C$ values relative to *Pinus elliottii* at Usutu.

In contrast the poor correlation between carbon isotopes and rainfall for the *Pinus elliottii* trees at Usutu, which may in part be the result of similar responses to sub-optimal water availability (abundance and scarcity), may also be the result of reduced stomatal control of transpiration. This may be achieved if the physiological response of the tree that leads to larger growth rings is common to both the tracheids and the stomata. Large annual growth rings of *Pinus elliottii* in Usutu would therefore be associated with higher density of smaller tracheids, but would also be associated with higher density of smaller stomates. Such an adaptive strategy can only work for fast growing species that are able to flush new needles and add woody tissue with the appropriate structure instead of changing the stomatal response in existing needles as water availability changes. This presumes an adaptation to high water availability.

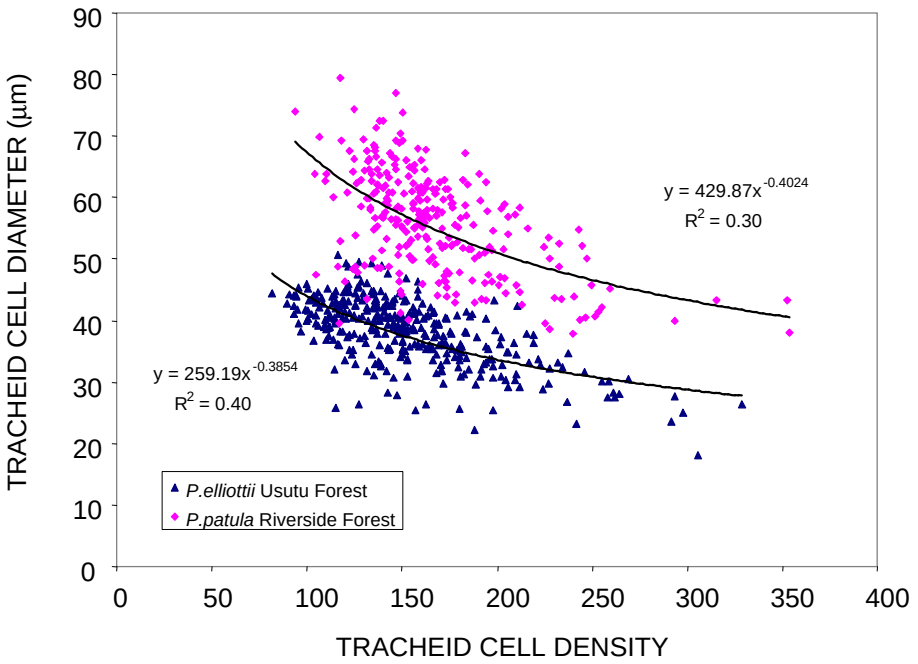

Figure 18. A power curve relationship between tracheid diameter and tracheid density is predicted. However in this study the relationship for *Pinus elliottii* trees differs from that of *Pinus patula* trees. The results indicate that *Pinus patula* trees have enhanced ability to conduct water, even though they grew in a marginal rainfall area.

5.4 Optimal rainfall regimes

The suggestion that *Pinus elliottii* trees have reduced stomatal control relative to *Pinus patula* is supported by the fact that the inter-annual variation in $\delta^{13}C$ values in the former species is normally less than ±2‰, while it is ±3‰ in the latter. This variability can be plotted against rainfall (figure 19a and 19b) to determine the stomatal response. In these plots all of the data are included which increases the variability, presumably as a result of the genetic controls. However in this instance the objective is to determine the optimal rainfall adaptation for the species rather than the individual trees, so while the trends are not statistically significant they do suggest an adaptive strategy for the species.

For *Pinus patula* (figure 19a) rainfall below approximately 800 mm leads to positive shifts in $\delta^{13}C$ values that indicates a low water availability response. When rainfall is above 800 mm the tees all show a negative shift in the $\delta^{13}C$ values indicating low water stress. From another perspective water becomes a limiting factor for growth in regions that receive less than 800 mm of annual rainfall.

For Pinus elliottii (figure 19b) the positive shifts in $\delta^{13}C$ occurs when annual rainfall is below approximately 950mm, and also when it is above approximately 1500mm. This supports the previous suggestion that too much water, and perhaps saturated soil conditions are also detrimental to carbon assimilation. Optimal annual rainfall for this species appears to be between 950 mm and 1500mm.

This suggestion can be tested by analysing trees from different rainfall regimes using the same approach.

5.5 Future Research

The carbon isotope signature that differentiates the Riverside trees from one another is manifest within a few years of germination. An area of research that may be of value to the forestry industry would be to induce water stress in seedlings, and measure the carbon isotope response in a shorter-lived organ such as a needle. In this way trees with similar genetic responses can be managed to yield uniform fibre quality or uniform growth characteristics. In the case of *Pinus elliottii* the marker of genetically controlled water-use strategies is tracheid size and density. These characteristics are not manifest in the first few years of growth, but become more consistent after about 10 years. Although this type of screening is not as quick as carbon isotope analyses, it could still be used as a screen for thinning trees that will yield different fibre quality.

If this approach is to be developed to better understand the impact of commercial forest tree species on the riparian habitat, it will be necessary to filter genetic from environmental controls on water-use. This can be done with controlled greenhouse experiments. In particular it will be possible to test the water stress response of genetic clones (tree specimens that share exactly the same genes), and this can be compared with the water stress response on non-cloned trees of the same species.

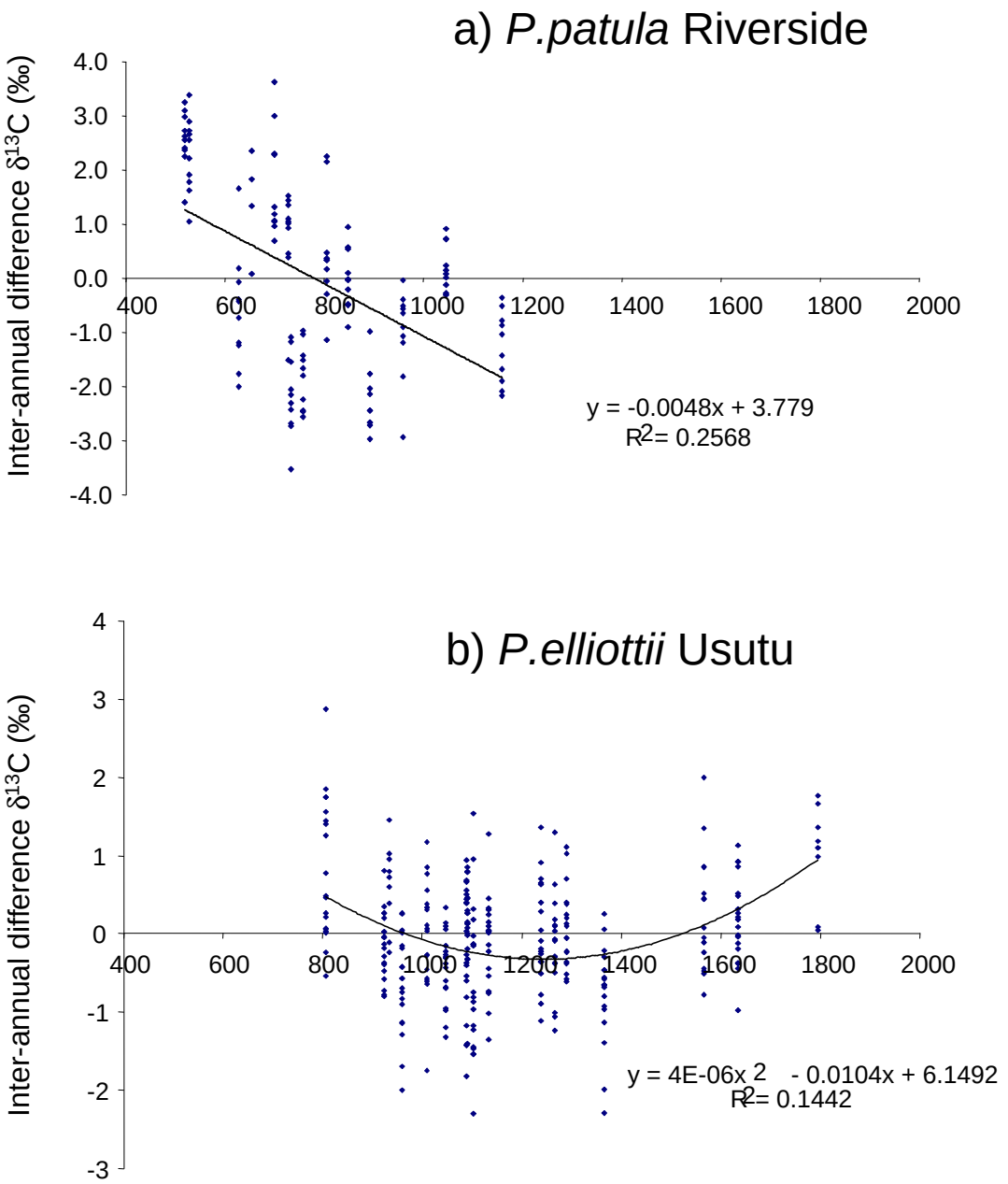

Figure 19a and 19b. The inter-annual changes in carbon isotope values for *Pinus patula* trees from Riverside Forest, Maclear, and *Pinus elliottii* trees from Usutu Forest, Swaziland, may reflect the species specific rainfall optima. Stomatal closure, associated with diminished carbon assimilation, is portrayed by positive value. This appears to occur when rainfall is less than 800mm in *Pinus patula*, and when it is less than 950mm in *Pinus elliottii*. In addition rainfall in excess of 1500mm appears to produce a similar closure of the stomata. This may be associated with waterlogging of the soil. A high degree of variability is noted, and may reflect the inter-tree genetic differences in water-use strategies, as well as localised differences in soil moisture retention.

In order to understand the genetic and environmental control on tree water-use adaptive strategies it is necessary to contrast the response of each species in different environments. This research project focused on two different tree species in two very different environmental settings. In order to verify these preliminary findings it is necessary to expand the sample to include *Pinus elliottii* trees from slightly dryer areas, and *Pinus patula* trees from slightly wetter areas. This project establishes a baseline methodology for expanding the approach.

Chapter 6

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Appendix 1

Results

Anatomical and isotopic measurements for transect 1, 13 *Pinus elliottii* trees from the Usutu Forest, Swaziland; transect 2, 6 *Pinus elliottii* trees from a marginal tributary higher in the same catchment, and transect 3, 10 *Pinus patula* trees from the Riverside Forest near Maclear.

Table 5a. $\delta^{13}C$ (‰) measurements relative to the international PDB standard on annual rings of *Pinus elliottii* from transect 1, Usutu Forest, Swailand. The average precision on duplicate measurements of the same sample is <0.2 ‰.

	Tree Number												
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1981	-25.4	-27.2	-26.5	-26.4	-27.3	-26.8	-26.9	-27.1	-27.2				
1982	-25.7	-26.6	-25.7	-26.0	-26.3	-25.3	-27.0	-26.4	-26.1				
1983	-25.6	-24.8	-25.7	-24.9	-25.3	-24.1	-25.6	-25.3	-24.5	-27.5	-26.5	-25.7	-25.4
1984	-24.5	-25.8	-25.6	-25.3	-25.4	-24.3	-26.1	-25.0	-24.9	-26.7	-26.2	-25.7	-24.5
1985	-24.8	-25.8	-25.4	-25.2	-26.0	-24.8	-25.0	-25.4	-24.5	-26.0	-26.8	-25.9	-24.7
1986	-25.2	-27.0	-27.0	-26.0	-26.9	-26.1	-27.0	-25.6	-25.6	-25.9	-26.5	-25.7	-25.3
1987	-26.4	-27.6	-27.7	-28.0	-27.6	-28.4	-27.9	-26.5	-27.0	-26.6	-26.7	-26.3	-25.8
1988	-25.5	-26.8	-27.2	-26.6	-28.4	-26.4	-28.4	-26.7	-26.9	-27.1	-26.8	-26.3	-25.3
1989	-25.5	-26.7	-26.9	-26.6	-27.1	-26.2	-28.5	-26.6	-27.6	-26.6	-27.1	-27.3	-25.3
1990	-25.8	-27.7	-27.4	-27.8	-27.2	-26.6	-28.6	-26.5	-28.7	-26.7	-27.4	-26.9	-25.2
1991	-26.4	-27.2	-26.9	-25.0	-27.2	-25.2	-27.2	-25.7	-26.8	-26.5	-25.8	-25.7	-23.5
1992	-24.8	-27.4	-26.6	-26.4	-28.6	-27.4	-28.0	-26.7	-28.0	-27.3	-27.0	-26.4	-25.0
1993	-25.3	-27.1	-27.4	-26.1	-27.8	-27.7	-28.2	-26.4	-27.8	-27.9	-27.1	-26.8	-25.0
1994	-27.1	-27.4	-28.0	-26.5	-27.5	-28.3	-27.8	-26.3	-28.3	-26.7	-26.3	-26.8	-24.7
1995	-26.1	-27.4	-27.3	-27.0	-27.1	-27.8	-27.2	-26.9	-27.7	-27.1	-27.1	-27.9	-26.1
1996	-26.5	-27.1	-26.7	-26.5	-27.1	-26.5	-27.2	-26.3	-27.9	-28.0	-28.2	-27.2	-26.4
1997	-26.4	-26.6	-26.2	-26.9	-27.3	-26.5	-28.6	-26.6	-27.8	-27.8	-27.4	-27.2	-26.2
1998	-26.7	-27.8	-26.9	-27.9	-27.8	-27.4	-28.2	-26.4	-28.4	-28.1	-27.6	-28.6	-26.3
1999	-25.0	-27.9	-27.0	-27.3	-27.5	-27.3	-27.9	-27.3	-28.0	-27.9	-28.3	-28.2	-26.8
2000	-24.7	-28.7	-27.5	-27.5	-27.2	-27.7	-27.6	-27.2	-28.4	-27.7	-27.9	-28.4	-26.4

Table 5b. $\delta^{13}C$ (‰) measurements relative to the international PDB standard on annual rings of *Pinus elliottii* from transect 2, Usutu Forest, Swailand. The average precision on duplicate measurements of the same sample is <0.2 ‰.

	Tree Number					
Year	**1**	**2**	**3**	**4**	**5**	**6**
1983	-25.4	-26.8	-26.8	-27.1	-27.5	-27.0
1984	-24.5	-26.8	-26.6	-26.5	-27.3	-26.5
1985	-24.7	-26.5	-26.7	-25.5	-27.1	-26.4
1986	-25.3	-27.1	-26.9	-25.9	-27.8	-26.4
1987	-25.8	-27.4	-26.6	-26.7	-27.8	-27.0
1988	-25.3	-27.7	-26.2	-27.2	-28.2	-27.1
1989	-25.3	-28.4	-26.7	-26.9	-28.7	-28.5
1990	-25.2	-27.8	-27.0	-26.8	-28.5	-27.1
1991	-23.5	-27.8	-27.2	-26.8	-28.4	-26.9
1992	-25.0	-27.6	-27.4	-26.8	-27.5	-27.0
1993	-25.0	-27.8	-27.4	-27.5	-27.8	-27.8
1994	-24.7	-28.4	-27.4	-27.8	-27.0	-27.2
1995	-26.1	-28.0	-27.6	-27.4	-27.5	-29.1
1996	-26.4	-28.3	-27.8	-28.2	-28.0	-28.1
1997	-26.2	-27.5	-27.7	-27.4	-27.9	-27.9
1998	-26.3	-27.9	-27.6	-27.3	-28.2	-28.5
1999	-26.8	-27.6	-28.4	-27.0	-28.4	-29.1
2000	-26.4	-27.4	-28.0	-27.3	-28.4	-28.9

Table 5c. $\delta^{13}C$ (‰) measurements relative to the international PDB standard on annual rings of *Pinus patula* from transect 3, Riverside Forest, Maclear. The average precision on duplicate measurements of the same sample is <0.2 ‰. The ring corresponding to the 1990 growth year was only discernable in the larges specimens.

	Tree Number									
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1973	-25.8			-26.2	-25.4	-26.1		-26.2		-24.4
1974	-24.7	-24.4	-26.4	-25.9	-24.9	-25.1	-25.8	-24.7	-25.4	-25.4
1975	-26.4	-25.1	-24.3	-24.8	-25.4	-26.8	-25.9	-26.4	-26.5	-26.2
1976	-23.8	-23.6	-25.3	-25.4	-25.0	-24.4	-24.3	-24.1	-25.7	-25.3
1977	-26.1	-25.8	-26.4	-26.5	-27.1	-27.1	-27.8	-26.5	-28.4	-26.8
1978	-27.6	-25.3	-25.1	-25.5	-26.7	-26.1	-26.7	-25.1	-26.9	-25.9
1979	-24.9	-23.7	-24.0	-23.6	-23.3	-23.2	-24.9	-22.9	-24.2	-23.3
1980	-27.1	-25.2	-25.8	-25.0	-25.8	-25.6	-26.0	-24.6	-25.2	-25.9
1981	-27.2	-25.6	-26.5	-26.7	-25.6	-26.1	-27.2	-26.6	-26.3	-24.2
1982	-24.6	-24.2	-23.3	-24.0	-23.3	-23.7	-24.6	-23.5	-23.4	-21.9
1983	-27.3	-26.0	-26.0	-27.0	-25.8	-26.1	-25.6	-26.1	-25.4	-24.0
1984	-26.2	-25.0	-23.7	-25.8	-24.7	-23.8	-24.9	-22.5	-22.4	-22.7
1985	-27.4	-25.6	-26.6	-25.8	-25.2	-25.6	-25.3	-23.6	-23.3	-23.2
1986	-27.1	-26.8	-24.4	-25.7	-24.7	-23.5	-24.9	-23.9	-22.9	-23.3
1987	-27.9	-27.3	-26.1	-26.5	-25.8	-25.7	-27.0	-25.3	-24.8	-23.6
1988	-28.0	-26.4	-25.8	-26.4	-25.6	-25.7	-26.3	-25.6	-24.1	-23.9
1989	-27.4	-26.6	-26.3	-26.5	-25.1	-26.1	-25.3	-25.6	-25.0	-23.8
1990							-25.2	-23.3	-23.2	-22.5
1991	-25.1	-25.2	-24.5	-24.4	-24.4	-23.3	-25.3	-23.7	-23.2	-22.3
1992	-25.1	-25.6	-24.5	-24.9	-25.0	-25.2	-25.8	-23.6	-23.9	-23.3
1993	-26.6	-25.8	-25.9	-26.0	-25.0	-25.6	-26.5	-25.3	-25.2	-23.7
1994	-26.1	-26.9	-26.2	-25.4	-26.3	-26.1	-25.9	-25.0	-24.6	-23.9
1995	-26.7	-28.0	-25.5	-25.8	-25.4	-26.5	-26.3	-26.2	-24.7	-23.9
1996	-28.2	-27.1	-26.9	-25.9	-25.6	-26.7	-27.5	-26.6	-25.3	-24.6
1997	-27.2	-26.8	-26.0	-26.4	-25.8	-26.9	-27.0	-26.0	-25.5	-23.2
1998	-27.1	-26.3	-26.5	-26.0	-24.8	-26.2	-26.2	-26.3	-25.0	-23.5
1999	-28.1	-27.0	-26.4	-26.4	-26.3	-26.2	-27.0	-27.4	-25.4	-24.8
2000	-27.5	-26.6	-27.0	-26.2	-25.6	-26.4	-26.3	-26.9	-25.8	-24.7
2001	-28.4	-27.7	-26.7	-26.8	-25.1	-25.8	-27.4	-26.1	-25.3	-24.6

Table 6a. Tracheid diameter (µm) measurements on annual rings of *Pinus elliottii* from transect 1, Usutu Forest, Swaziland.

	Tree Number												
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1981	23.4	23.4	18.2	27.7	26.4	28.3	24.9	23.1	23.5				
1982	36.3	29.7	30.2	30.6	30.2	27.5	25.1	28.9	27.5	25.3			
1983	40.5	32.4	30.2	31.3	31.6	35.4	26.8	34.4	32.8	32.4	22.3	20.9	20.2
1984	35.2	33.6	30.2	33.2	33.2	31.7	29.1	31.4	33.7	40.6	26.4	25.5	31.0
1985	39.0	35.0	29.5	37.4	29.2	29.7	25.5	31.8	34.9	33.6	25.7	30.7	27.8
1986	36.4	32.1	38.2	38.0	30.7	32.7	43.3	39.0	33.5	33.0	31.9	37.1	26.3
1987	44.6	34.2	38.2	40.8	35.8	38.7	38.6	35.8	32.0	38.3	36.0	37.8	33.2
1988	38.2	33.6	33.6	41.3	35.8	37.9	32.9	34.4	40.5	40.7	40.4	39.5	34.4
1989	41.6	34.7	34.4	41.4	39.2	33.4	43.0	40.9	31.7	39.4	39.6	44.3	37.5
1990	44.7	35.2	31.0	38.6	37.5	35.4	40.5	36.1	37.5	37.2	43.5	40.3	35.2
1991	41.3	38.5	35.3	42.1	36.2	39.5	38.5	39.4	32.6	44.9	39.1	39.6	39.8
1992	42.6	36.2	36.2	35.1	39.9	41.3	38.6	35.2	31.1	40.6	40.3	44.0	28.2
1993	42.7	33.9	35.8	39.6	46.4	41.3	37.3	35.5	32.0	42.0	42.7	43.2	35.2
1994	44.1	36.6	39.5	39.3	42.8	39.7	43.9	42.1	36.9	43.3	41.8	41.1	38.9
1995	42.3	36.8	36.0	43.0	40.0	43.5	39.0	37.8	36.1	44.1	42.8	38.2	41.5
1996	44.6	34.1	36.7	40.3	44.7	44.5	42.8	41.1	36.7	49.3	38.1	38.6	42.3
1997	43.0	35.4	38.6	43.8	45.2	43.3	42.2	43.1	34.4	45.9	40.0	41.1	43.6
1998	42.2	37.9	35.3	40.0	50.7	48.7	44.8	42.9	33.6	44.3	44.5	40.5	40.1
1999	40.9	36.4	35.3	43.1	47.4	46.0	33.0	42.5	36.9	43.9	45.1	37.7	40.3
2000	43.8	38.5	25.6	40.7	38.8	45.5		42.0	33.7	45.4	41.9	38.9	41.1
2001				43.6						47.5	38.8	43.6	

Table 6b. Tracheid diameter (µm) measurements on annual rings of *Pinus elliottii* from transect 2, Usutu Forest, Swaziland.

	Tree Number					
Year	**1**	**2**	**3**	**4**	**5**	**6**
1983				28.1		
1984	33.2		34.7	32.7	31.2	
1985	42.4	32.1	30.9	32.9	36.1	32.5
1986	40.7	36.4	35.8	33.7	40.8	30.9
1987	43.3	40.9	34.1	36.6	40.7	35.8
1988	45.1	34.8	38.1	36.2	43.2	34.6
1989	42.2	37.6	37.9	38.1	43.4	35.3
1990	47.0	37.3	38.7	38.8	42.3	36.0
1991	47.6	37.9	37.6	41.4	44.5	35.0
1992	49.0	38.5	40.3	42.4	43.6	40.1
1993	49.4	40.8	37.9	40.5	42.7	40.3
1994	48.6	37.3	39.8	42.1	42.5	46.3
1995	44.3	40.2	40.7	44.3	40.7	46.1
1996	49.5	41.5	44.1	45.6	41.2	40.9
1997	45.4	41.0	42.5	42.4	41.0	42.9
1998	44.8	39.6	41.5	41.7	40.9	40.4
1999	43.8	36.0	41.4	42.6	42.1	39.5
2000	44.8	39.4	42.6	43.4	40.2	40.9

Table 6c. Tracheid diameter (µm) measurements on annual rings of *Pinus patula* from transect 3, Riverside Forest, Maclear.

	Tree number									
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1973								40.2		
1974	39.9	54.9		38.1	43.3	43.5		41.4	53.5	43.4
1975	44.6	46.5	45.8	40.6	44.5	54.9	47.4	40.7	59.0	50.0
1976	45.2	51.8	37.9	45.7	46.3	51.5	54.1	52.2	52.6	62.6
1977	55.6	50.7	50.0	48.7	56.9	46.2	52.5	39.5	50.9	58.4
1978	51.6	51.6	42.1	43.9	51.9	43.7	51.6	38.7	61.4	52.0
1979	57.7	57.3	55.1	45.7	47.0	47.9	57.0	43.2	55.5	60.1
1980	57.1	62.5	54.6	46.3	56.7	55.1	62.4	42.5	59.6	67.2
1981	59.9	62.2	53.5	50.2	52.2	44.3	59.0	43.9	56.7	59.0
1982	58.4	57.6	49.6	58.1	60.7	65.4	62.9	46.8	58.5	59.5
1983	64.7	67.6	53.6	59.6	50.5	59.2	56.4	47.8	57.5	61.4
1984	61.6	63.2	53.2	55.2	42.9	58.2	56.0	45.4	55.0	57.1
1985	62.9	48.6	55.3	47.7	62.1	65.3	60.7	46.0	51.8	66.2
1986	64.4	53.2	55.5	52.7	52.4	49.9	68.9	57.7	60.7	67.9
1987	69.2	59.5	51.5	55.9	52.9	61.6	61.4	42.9	59.8	60.7
1988	62.9	56.7	48.9	50.3	57.3	53.3	52.1	44.2	58.8	62.4
1989	67.6	79.5	48.3	52.1	59.1	51.7	60.9	44.1	56.5	57.2
1990										
1991	68.2	74.0	47.5	62.7	58.6	72.4	60.8	47.3	57.3	61.4
1992	63.9	69.8	50.1	59.2	56.5	54.7	59.9	48.7	55.3	66.7
1993	62.3	63.8	47.7	53.7	67.7	64.6	59.8	43.5	70.5	62.1
1994	63.8	62.8	46.3	56.7	55.7	49.1	58.2	46.5	65.9	56.0
1995	67.5	69.2	48.7	58.0	66.6	57.9	62.0	40.1	64.1	61.3
1996	69.3	65.7	48.8	63.7	61.8	46.2	52.6	41.3	58.2	60.7
1997	64.9	62.7	43.6	50.6	55.3	58.0	56.7	44.9	63.4	73.7
1998	71.4	66.2	48.7	66.2	67.2	54.5	54.9	46.3	61.6	68.6
1999	61.9	58.4	60.8	64.6	57.1	61.1	61.0	49.8	69.4	72.4
2000	63.6	60.7	56.8	56.3	64.5	51.9	59.4	47.6	77.0	64.2
2001	66.3	57.9	39.6	60.1	64.4	58.7	58.1	53.9	66.2	74.4

Table 7a. Cell density measurements on annual rings of *Pinus elliottii* from transect 1, Usutu Forest, Swaziland. The average number of cells counted in a 310 x 620 µm grid (n=3) is presented.

	Tree Number												
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1981			306	293	329	261		242	292				
1982	209	227	259	269	255	262	298	224	258	191			
1983	124	235	204	210	246	195	237	210	203	179	188		
1984	143	222	209	194	217	171	206	168	196	140	163	157	153
1985	109	181	189	172	212	172	198	171	206	153	115	146	147
1986	131	185	183	152	198	171	152	147	180	139	131	128	127
1987	122	180	175	150	194	153	151	154	192	135	117	125	138
1988	118	174	166	166	176	148	126	151	173	111	112	115	134
1989	111	181	168	167	148	137	145	154	142	109	95	114	130
1990	119	179	181	167	163	166	148	138	112	121	102	120	124
1991	120	156	162	165	156	167	147	143	139	115	106	105	115
1992	120	158	161	151	138	154	155	150	135	119	115	93	143
1993	119	184	181	145	128	156	167	143	114	115	108	119	123
1994	104	151	168	151	142	152	158	140	114	101	95	102	112
1995	125	150	169	151	138	146	145	137	116	95	89	97	101
1996	106	149	146	161	135	126	161	156	104	120	119	110	93
1997	115	176	137	141	111	119	131	122	124	100	100	116	108
1998	106	176	144	132	116	122	134	105	120	91	81	114	110
1999	105	173	150	136	127	122	136	117	110	94	101	123	101
2000	105	150	181	137	134	121	132	117	153	95	99	112	93

Table 7b. Cell density measurements on annual rings of *Pinus elliottii* from transect 2, Usutu Forest, Swaziland. The average number of cells counted in a 310 x 620 µm grid (n=2) is presented.

	Tree Number					
Year	**1**	**2**	**3**	**4**	**5**	**6**
1983				264		
1984	232		237	223	183	
1985	211	228	191	193	202	198
1986	186	219	181	197	162	202
1987	199	164	178	171	144	159
1988	158	205	184	181	136	173
1989	156	219	162	152	136	165
1990	152	189	156	160	143	172
1991	142	216	169	159	130	181
1992	139	199	150	145	127	158
1993	134	179	141	143	113	173
1994	125	202	135	136	125	158
1995	149	194	142	135	129	131
1996	127	184	151	127	123	150
1997	127	187	143	137	137	145
1998	120	169	141	131	125	143
1999	113	164	156	136	134	145
2000	139	169	141	129	127	146

Table 7c. Cell density measurements on annual rings of *Pinus patula* from transect 3, Riverside Forest, Maclear. The average number of cells counted in a 310 x 620 µm grid (n=3) is presented.

	Tree number									
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1973			351.3					243.7		361.5
1974	293.3	171	220	354	352.5	233.7		253.3	227	315.7
1975	212	186.7	200.7	246.7	204.7	243	195.3	251.7	203.7	228
1976	259.3	182	239.7	249.7	197.3	197.3	205.7	245	232.7	194
1977	187.7	190.7	247	232.7	205.7	193.7	170	225	193.3	211.7
1978	203.3	185.3	255.3	240.7	224.7	230	186	228	166.3	196
1979	194.7	159	216.3	210.3	201.3	173.3	168	194	178	175.3
1980	168.3	148	197.7	172.7	181	160.7	169.7	213	171.7	183.3
1981	142	126	175	188	172.7	149.7	155.7	173.7	164.7	176
1982	176.7	141.3	164.7	202	151	156.7	182	203.7	148	191.3
1983	147	122.3	158.3	163	154.3	156	147	180.3	161.3	174
1984	140	143.3	154.7	179.7	188.3	147	161.7	173.7	156.7	184
1985	172.3	137	150	174.7	184.7	146	144.7	161	171.3	160
1986	156.3	141.7	146.7	179	176.7	156	148.7	171	167.7	158.3
1987	147	142.3	139.7	161	117.3	162.7	168.3	178	158.3	167.7
1988	128.3	136	130.3	166	209.7	163.3	176	178.7	183	163
1989	131.3	117.7	124	152	176.7	164	161	168.7	158.7	161
1990										
1991	136	94	105	164	128.3	140.3	143.3	172	162.7	173
1992	110	106.3	134.3	141.3	144.3	135.3	133	143	137	136.3
1993	155.7	104	126.7	162.3	162.7	162	131.3	191.7	149.3	137
1994	133.7	109.7	120	140.3	158.7	167.3	156	172.3	132.3	153
1995	135.7	118	139	168.7	145	158.7	141.3	153.7	142	159.7
1996	150.7	139.7	116.3	190.7	160.3	169.3	153.3	149	149.7	159.3
1997	154.3	133.7	131.3	147.7	156.3	163.3	161.3	149	153.3	150.3
1998	136.7	125	125.3	133	143.7	152.3	150.7	148.3	158	135.3
1999	137.7	133.3	121.3	137	140	136.3	155.3	155.3	129.7	138.3
2000	138.3	135	139.3	148	133	148.3	159.7	148.3	146.7	124
2001	147	133.3	117	114	130	142.3	166.3	125.3	152	125

Appendix 2

Capacity building

During the conceptualization of this project in 1999 the anticipated contribution to capacity building was thought to be small. This was based on the limited opportunities that the project appeared to offer within a highly constrained perception of how capacity building is defined. Breen et al. (in press) reviewed a diverse range of literature to provide a broader definition of capacity development specifically in the context of Water Research Commission funded research projects. Here capacity development is defined as 'a process whereby people are enabled to better perform defined functions either as individuals or through improved technical skills and or professional understanding, or as groups aligning their activities to that lead to enhanced performance' (Breen *et al*. in press). In this definition change is a cornerstone of success whether the change is in the 'values, aspirations and behaviour' of people, or in the new ways in which 'research teams, support agencies, [and] strategic partners' adopt new ways of doing things (Breen *et al*. in press). Using this definition the following capacity building can be reported:

1. Alliances

   In the course of this project Dr Edmund February and Rose Newton of the botany department of the University of Cape Town worked very closely with Dr. Stephan Woodborne and Dr. Iain Robertson of Environmentek, CSIR. Although the project was initially considered applied research, the results have challenged this, and there has been a substantial component of fundamental research. Before it is possible to elevate the findings from the fundamental level to an applied level there will necessarily be further collaborative research between the individuals and organizations.

   In addition to the direct interactions involved in this project, the alliance facilitated the sharing of ideas and data on diverse topics.

2. Research infrastructure development

   In order to execute this project it was necessary to establish a bulk processing Soxhlett extraction setup. This was done at QUADRU, CSIR. The bulk processing of isotope analyses was accomplished by coupling an elemental analyzer with a stable light isotope mass spectrometer in continuous flow mode. This system was established at QUADRU, CSIR, by commissioning an elemental analyser that was sponsored by NRF. This instrument was sponsored in an attempt to bridge commercial and academic isotope research applications. The dendrochronology equipment was commissioned at the Department of Botany, University of Cape Town, and dedicated digitising software was purchased and installed for the measurement of tree anatomy.

3. Individual skills development

   All the scientific and technical staff involved in the commissioning of new equipment did so with very little existing technology. In the process the individual skills were greatly enhanced. In particular Ms. Annalie Havenga and Mrs. Gill Collett were responsible for the laboratory work in QUADRU, and

their skills base was significantly enhanced in the bulk processing of isotope samples.