RESPONSE OF *Acacia tortilis* AND *Acacia xanthophloea* SEEDLINGS TO REPEATED SOIL DROUGHT STRESS

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DECLARATION

This is my original work and has not been presented for a degree in any other University.

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DEDICATION

This thesis is dedicated to Fr. Thomas McDonald C.S.sp., the chaplain, catholic students community, University of Nairobi.

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EC	Electrolyte conductivity
In	Natural logarithm
LA	Total leaf area
LDW	Leaf dry weight
m	Matrix potential
MPa	Megapascal
p	Turgor potential
pV	Pressure - volume
R	Mean RGR
RDW	Root dry weight
RGR	Relative growth rate
R*	Relative water content (%)
SDW	Shoot dry weight
t	Time
TDW	Total dry weight
tlp	Turgor loss point (%)
w	Dry weight
ψ	Xylem water potential
Π	Osmotic potential
ε	Modulus of elasticity

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ABSTRACT.

In an attempt to understand how trees overcome drought stress and to establish mechanisms of stress tolerance in tropical aridland species, seedlings of *Acacia tortilis* (Forks) Hyne and *Acacia xanthophloea* Benth. were raised under controlled glass house conditions in polythene pots of size 11cm in diameter and 18cm high at The Kenya Forestry Research Institute (KEFRI) Muguga. The controls were watered daily while the other three treatments involved withholding water for 2, 4 and 6 days respectively with one day re-hydration to container capacity after every subsequent drought for each species.

Growth measurements showed relatively higher growth rates and total leaf area in *A. xanthophloea* compared to *A. tortilis* under favourable moisture conditions. *A. tortilis* responded to increased water stress by shifting carbon allocation to the roots leading to root:shoot (r:s) ratio of about 1.5 compared to 0.5 for the controls, unlike in *A. xanthophloea*, where r:s ratio remained at 0.5 in both repeatedly stressed and control seedlings.

A. xanthophloea seedlings, consistently had lower shoot xylem water potentials than *A. tortilis* for all the treatments but exhibited adjustment in cell wall elasticity when repeatedly stressed. *A. tortilis*, with repeated stress, showed osmotic adjustment. Adjustments in cell wall elasticity as observed for *A. xanthophloea* resulted in improved membrane integrity. *A. tortilis* however, showed higher membrane integrity at all levels of water stress likely due to inherent drought tolerance and adjustments during stress.

The study concluded that pre-conditioning could improve drought tolerance in seedlings and that growth reduction as a result of water stress is likely due to integration of several drought tolerance mechanisms, which are detrimental to growth.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1.0. INTRODUCTION

In East Africa, areas that receive erratic and inadequate rainfall are usually associated with sparse tree growth and are classified as arid and semi-arid lands (ASAL). In Kenya, ASAL constitute about 80% of the total land area (IUCN/ODA 1995). Water is the single most important, limiting factor in these arid ecosystems hindering growth and survival of plants (Kramer 1980). Water stress influences a wide variety of morphological and physiological processes such as leaf expansion, stomatal opening, membrane functioning and photosynthetic carbon assimilation and partitioning.

1.1.1. SELECTION AND DESCRIPTION OF EXPERIMENTAL SPECIES

In the last 70 years, most trials on selection of trees for arid and semi-arid lands of Kenya have been on exotic fast-growing species (Milimo 1989, Oballa *et al.* 1997). Indigenous species have been neglected due to their slow growth. Currently, exotic species mainly found as monoculture, are threatened by diseases, hence, it is imperative that indigenous species be promoted (Oballa *et al.* 1997).

Most of tree species of semi-arid East Africa could be selected based on a range of genetic traits. Therefore, selection of tree species in the ASAL could include not only the typical forestry related characteristics such as better growth rates and stem form but also, more importantly, for traits such as resistance to drought and salinity. Members of the genus *Acacia* form a significant portion of indigenous xeric species of Kenya, possessing some of these qualities. They are quite distinct in the woody vegetation and cover large areas either in pure stands or in mixture with other shrubs and trees (Akech 1987). Their multiple use value and relative drought resistance, make them an important resource in these marginal lands. A better understanding of the ecophysiology of this genus is, therefore, necessary especially in their natural environments. For a variety of reasons, such as limited funds, past studies have been restricted to a few species at any one given time. This study was set to investigate two indigenous *Acacia* species, namely *A. tortilis* (Forsk) Hyne and *A . xanthophloea* Benth.

Acacia tortilis **(Forsk) Hyne (Leguminoseae: Mimosoideae)**

Commonly known as the "umbrella thorn", *A. tortilis* is one of the most distinctive and wide spread African *Acacias.* It is a slow growing dryland species, with deep rooting habits and wide spreading umbrella shaped crown growing up to 4 -20m high. The foliage is feathery and typically *Acacia* like (Noad and Birnie 1989).

Although the species is found where annual rainfall is 1,000 mm it is also drought resistant and can survive in climates with less than 100 mm annual rainfall and long erratic dry seasons. Specimens are sometimes found isolated in otherwise treeless arid environments (Anon 1980). In Southern Sudan, as the drought extends Northwards, *A. tortilis* is the last surviving woody plant to form stands on sandy soils adjoining the desert. The plant forms a dense spreading flat crown that provides shade almost all year round. The species also occur in the well drained highland regions to poorly drained river banks (Dale and Greenway 1961). This gives an interestingly wide range of adaptations that calls for its study to understand the ecology and physiology of this species.

Acacia xanthophloea Benth. (Leguminoseae: Mimosoideae)

This is a tree reaching 25 m or more, gregarious in high ground water areas, besides lakes and rivers from 600 to 2,000 m above sea level. It often grows on black cotton soil, which has poor water drainage. In the recent past, its density has increased around the highland areas of Nairobi where it is grown as ornamental. A good population is also grown in the dry parts of the country for wood (Noad and Birnie 1989).

A. xanthophloea is a fast-growing tree that is widely distributed in dry parts of the country. High concentrations occur in the Rift Valley especially in Naivasha, Eastern Province around Wambaa, North Eastern Province, Central Nyanza around

Kano plains and Coastal Province along the Tana delta plains, where it provides good wood for fuel and fencing. The leaves and pods are also very nutritious providing fodder for herbivores in these otherwise dry areas (Akech 1987).

Although some trees have been found to grow in arid regions, it is not clear yet, whether their occurrence in such environments is subject to an existing high water table or because they posses drought tolerance mechanisms. Recent work has also shown that fast growing species are much more susceptible to drought than the slow growing ones (Lediges 1974). This makes *A. xanthophloea* an interesting indigenous tree species to study especially how it responds to drought stress.

1.2.0. LITERATURE REVIEW

1.2.1. Defining drought stress

Drought is an environmental factor, which according to Kramer (1980), refers to a period without rain, long enough to affect the normal physiological functioning of a plant. Any factor that disturbs the normal functioning of an organism is referred to as stress (Levitt 1980). Drought stress can, therefore, be defined as an environmental stress of sufficient duration to produce a plant water deficit, which in turn causes disturbance of physiological processes.

1.2.2. Effects of drought stress on plants

Water deficits influence all phases of growth and are probably responsible for more growth loss than all other causes combined (Kramer 1980). Growth is reduced directly through effects on cell turgor and indirectly through mediation of photosynthesis, respiration, mineral nutrition, enzymatic activity, hormone relations and nitrogen metabolism (Hsiao 1973). At cellular level, drought stress can inhibit enzyme activity and affect membrane functioning. At the tree level, drought affects increases in diameter and height, affects carbon partitioning pattern, influences timing and rates of a wide variety of physiological processes and reduces the ability of the tree to resist other biotic and abiotic stresses (Kramer 1983). In general, biological functioning is completely dependent on water availability (Salisbury and Ross 1969).

1.2.3. Causes and development of water deficits

Water deficits in plants develop during periods when transpirational water loss exceeds absorption. Although water deficit occurs on a daily basis especially on hot sunny days, it becomes more severe when absorption is limited by drying, salty, cold or poorly aerated soil (Turner and Begg 1980). Almost on a daily basis, water loss usually exceeds absorption as transpirational demand exerts pressure on water flux from the soil through the xylem into the leaves. Loss of water from the transpiring surfaces reduces turgor of the leaf tissues. Generally, by noon on a sunny day, the bulk leaf water potential is so low that in some cases leaves loose turgor. Once the stomata close, as occurs in the afternoon, the plant begins to recover from the stress since transpiration is reduced while root absorption continues at a relatively rapid rate.

Continuous daily water deficits without replenishment of soil water leading to long term water deficits make recovery of the plant from the water stress less possible. Permanent wilting will occur when the soil water potential decreases to the point where wilting occurs, and leaves do not recover at night (Slatyer 1967).

Seedling transplantation is another factor that often leads to development of severe water deficits (Kozlowski 1975, Lemcoff *et al.* 1994). Bare root seedlings often lead to physiological shock following desiccation of roots and injury to absorbing root surfaces. Exposure of bare rooted seedlings to drying, for even short periods, may rapidly induce water stress, resulting in reduced growth and increased mortality (Kozlowski 1982). This calls for attention on seedling pre-conditioning before transplantation in an attempt to reduce shock.

1.2.4. Parameters indicating plant water status

Water potential

Traditionally, water potential has been used as a fundamental measure of plant water status. Water potential (ψ), describes the state of water by its chemical potential within the system, relative to that of pure free water, that is water containing no solutes and bound by no forces (Kramer 1980). This gives a measure of the capacity of the water at any point to perform work compared to the work capacity of pure free water (Kramer 1983). Water potential (ψ), is the algebraic sum of the component potentials arising from the effect of pressure (P), solutes (π) and matrix (*m*) (Hsiao 1973). In many plant tissues, *m* does not become significant until much of the tissue water (about 50%) is lost (Weibe 1972). Water potential (ψ) is, therefore, defined by the equation (Slatyer 1967, Blake and Tschaplinski 1992):

$$\psi = P + \pi$$

Water potential has the advantage of providing a common unit (Pascal (Pa) or Megapascal (MPa)) for defining water status either within a tree or within the soil-plant-atmosphere continuum and allows for quantitative comparisons (Kramer 1988). According to Buxton *et al.* (1985), ψ is the most sensitive indicator of moisture stress. However, caution must be taken to avoid the use of absolute values of ψ as indicators of physiological water stress since evolutionary and physiological adaptation to environment could markedly influence the level of ψ at which stress sets in, in different plant species (Hsiao 1973).

Relative water content

This is another parameter that has been effectively used in the determination and description of plant water status. Relative water content (R*) is the water content (percentage) relative to the water content of the same tissue at full turgor (Hsiao 1973). Relative water content is given by the equation (Kramer 1980):

$$R^* = \text{Tissue fresh weight (wt.) - Dry wt. /Saturated wt.- dry wt.}$$

R* has been used severally to determine plant water status (For example Kramer, 1980, Blake *et al.* 1991, Lemcoff *et al.* 1994, Johnson *et al.* 1996). Methods of measuring R* are described elsewhere (Barrs 1968). However, basically, the methods measure R* by rehydrating plant tissues to full saturation overnight before recording changes in ψ and tissue weight as the plant tissue looses water. The use of R* has led to the strong interest in the role of turgor maintenance in drought tolerance and growth maintenance during drought (Osunubi and Davies 1978).

1.2.5. ADAPTATIONS TO WATER STRESS

A wide range of mechanisms of adaptation to water deficits exists among trees (Turner 1969, Kramer 1980). The two main mechanisms by which plants survive drought include drought tolerance and drought avoidance (Kramer 1980). Drought avoidance is achieved through rapid growth and plants are able to complete their life cycles before drought sets in. It is, therefore, clear that trees cannot avoid drought since they are perennials and the only mechanism by which they can survive is through tolerance.

Drought tolerance is used to describe physiological, morphological and biochemical features, which aid in plant survival, growth and reproduction under conditions of limited water supply (Jones *et al.* 1981), tolerance in trees is achieved through two main strategies, namely, dehydration postponement and dehydration tolerance (Kramer 1980, Levitt 1980, Turner 1986).

Variations occur among and within species in their ability to either postpone or tolerate dehydration (Lediges 1974, Turner 1979, Kramer 1980, Martin *et al.* 1987, Tan and Blake 1991). However, the relative contribution of tolerance and postponement to tree survival and growth is not well understood (Blake and Tschaplinski 1992). Dehydration postponement strategies such as stomatal closure or reduced leaf area, inevitably leads to growth reduction as they are bound to reduce the photosynthetic capacity of the plant. By comparison, dehydration tolerance has important implications for productivity because it confers drought tolerance without loosing photosynthates and hence growth (Kozlowski 1982). Munns (1988), however, points out that some of the processes associated with dehydration tolerance such as osmotic adjustment are also associated with reduced cell division and expansion.

Dehydration postponement

This can be achieved either through increased water uptake or reduced water loss or both (Kramer 1980). Adaptations that lead to dehydration postponement therefore include early leaf abscission, low leaf area, thick cuticle, hairy and waxy leaves and stems, sunken stomata, stomatal sensitivity and extensive rooting system. These adaptations help the plant to endure periods without significant rainfall whilst maintaining high plant water status (Kramer 1980).

An increase in depth and density of roots is considered a major mechanism in improving water uptake (Turner 1986). During drought, African locust bean seedlings (*Parkia biglobosa*) were shown to increase the rate of root extension (Osonubi and Fasehun 1987). Deep rooting habits or fibrous roots were considered important in conferring drought tolerance in *Robinia pseudoacacia*

(Ranney *et al.* 1996). It is expected that root growth is accelerated in compensation for the reduced shoot growth hence no reduction in total biomass accumulation should occur in species which posses such morphological adaptations.

Factors that contribute in dehydration postponement by reducing water loss, such as decreasing stomatal conductance, leaf rolling and decrease in leaf area are all processes that are likely to lead to decrease in growth (Turner 1979). A reduction in stomatal conductance is perhaps the most important means of postponing dehydration (Blake *et al.* 1984). Maintenance of low stomatal conductance is an important dehydration postponement mechanism in several species (for example, Quarashi and Kramer 1970, Blake and Filho 1980, Ranney *et al.* 1990). Leaf rolling appears to likewise reduce midday water loss and the degree of rolling appears to depend on the turgor of the leaf bulliform cells (Begg 1980). The reduction in water loss at midday when vapour pressure is low will likely postpone dehydration of leaf tissues (Turner *et al.* 1985).

Reduction of leaf area through leaf abscission leads to reduction of transpiration surface, hence, water loss. A reduction in leaf area appears to be largely affected by soil water status and root hydration. According to Turner (1986), reduction in leaf area is therefore a response rather than an adaptation to water stress. However, a reduction in leaf area was considered an adaptation to water stress (Kozlowski 1982, Osunubi and Fasehun 1987). A clear understanding of the role of leaf abscission in dehydration postponement is therefore necessary.

Dehydration tolerance

Dehydration tolerance refers to the ability of a plant to endure drought and low tissue water status (Kramer 1980). The main two mechanisms involved are turgor maintenance and desiccation tolerance.

Decrease in cell growth must be the most sensitive response of the plant to water stress since cell growth is quantitatively related to cell turgor (Levitt 1980). The maintenance of turgor during a change in plant water status is thought to maintain plant metabolic processes and aid in its growth and survival during drought (Osunubi and Davies 1978, Ranney *et al.* 1990, White *et al.* 1996). Plants that maintain turgor in their leaves will therefore grow and photosynthesise faster when exposed to drought than those that do not (Hsiao 1973). Turgor maintenance at lower tissue water potentials can be achieved through changes in osmotic potential (osmotic adjustment) or tissue elasticity (elastic adjustment) (Tyree and Jarvis 1982).

Osmotic adjustment

As a plant experiences prolonged drought, the osmotic potential at a particular relative water content (R*) becomes more negative, thereby, maintaining higher turgor pressures at the same water potential (Hinckley *et al.* 1978, Tyree and Jarvis 1978). This process is referred to as osmotic adjustment. Osmotic adjustment has been cited as a major drought tolerance mechanism (Kramer 1980, Levitt 1980, Koslowski 1982). It has been demonstrated in many species (Turner and Jones 1980) and has been observed to maintain stomatal opening and photosynthesis to lower water potentials (Ludlow *et al.* 1985, Turner and Jones 1980). Osmotic adjustment, therefore, has important implication for productivity because it confers drought tolerance without necessarily reducing photosynthesis and growth (Koslowski 1982). Tan *et al.* (1992), associated osmotic adjustment with increased growth rate in dehydrated black spruce. An increase in growth rate was observed in *Eucalyptus* species, which adjusted osmotically during drought (Lemcoff *et al.* 1994).

However, osmotic adjustment will only be effective in maintaining growth processes of the shoot during drought only if it also occurs in the roots (Turner 1986). Munns (1988) has questioned the role of osmotic adjustment in maintaining turgor and growth under drought. It is thought that solute accumulation shifts carbon away from growth towards turgor regulation (Blum 1988, Munns 1988). There is therefore no consensus on the role of osmotic adjustment in growth maintenance under drought.

Elastic adjustment

Turgor maintenance can also be achieved through changes in tissue elasticity (Tyree and Jarvis 1982). An increase in cell wall elasticity results in smaller changes in turgor pressure for a given change in relative water content (R*) (White *et al.* 1996). High cell wall elasticity in *E. globulus* facilitated maintenance of turgor over a wide range of R* and allowed it to tolerate moderate water stress (Lemcoff *et al.* 1994, White *et al.* 1996).

The ability of preconditioned seedlings to increase apparent elasticity in response to a major drought stress could represent a significant saving in energy and allow for greater carbon allocations for cell repair process during the drought and for more rapid growth after the stress is alleviated (Blake *et al.* 1991). However, the role of increasing or decreasing elasticity as drought increases has been questioned (Blake *et al.* 1991). It is not clear whether an increase or decrease of cell elasticity plays an important role as a survival mechanism under drought. Similar works in this area have found different results. For example, *Ginko biloba* L. had a higher elasticity than *Salix lasindra* and was judged to be more drought tolerant of the two (Tyree 1976).

Knowledge is lacking on the relative importance of osmotic adjustment and tissue elasticity on turgor maintenance in different plant species (Fan *et al.* 1993). Osmotic adjustment could help explain genetic differences in drought tolerance (Abrams 1988). However, elastic shrinkage may be more important for drought resistance than osmotic adjustment (Joly and Zaerr 1987), particularly in stressed woody plants (Blake *et al.* 1991).

A number of studies have suggested the pre-eminence of osmotic adjustment for turgor regulation in stressed plants (Tyree and Jarvis 1982, Tschaplinski and Blake 1989). Others have shown cell elasticity to be superior (Blake *et al.* 1991, Fan *et al.* 1993) over osmotic adjustment. However, few studies have systematically compared osmotic adjustment, cell wall elasticity and turgor changes in woody plants subjected to water stress. Osmotic potential in *Robinia pseudoacacia* L., declined under drought, however, cell relaxation increased in proportion to the decline in turgor potential (Parker and Pallardy 1988). Studies on *Tsuga heterophylla* revealed that, although osmotic potential declined, it was balanced with an increase in turgor potentials induced by a decline in cell volume, but the elastic modulus remained constant (Kondiko 1980).

In contrast, with lack of any detectable osmotic adjustment, the observed tissue elasticity changes in repeatedly stressed black spruce seedlings, accounted for the observed changes in turgor (Blake *et al.* 1991). Increased tissue elasticity during stress (for example, low ε) facilitated the maintenance of turgor over a wide range of relative water content (R*) and allowed tolerance of water stress in *E. globulus* (White *et al.* 1996).

However, some researchers have proposed lack of relationship between turgor and cell expansion (Turner 1986, Munns 1988). Turgor of leaf apex of wheat actually increased at a time when leaves stopped growing (Barlow 1986). Although leaves remained turgid, cell expansion, stomatal conductance and growth were actually observed to reduce. Munns (1988) indicated that growth is controlled by something other than turgor. Turgor maintenance, and its role in growth, is therefore complex and poorly understood (Blake *et al.* 1991).

Desiccation tolerance

Tolerance to desiccation depends on the ability of cells to withstand injury during severe drought stress (Turner 1986). Dehydration tolerance has been thought to occur in concert with dehydration postponement abilities (Blum and Ebercon 1984). The role of desiccation tolerance in species survival is cited by Martin *et al.* (1987), Tan and Blake (1993). However, Levitt (1980), contended that dehydration tolerance is rarely a significant factor in successful drought endurance by higher plants. Majority of trees exhibit little variation in desiccation tolerance (Hinckley *et al.* 1979).

1.3.0. STATEMENT OF RESEARCH PROBLEM

Differences in drought resistance mechanisms are likely to account for the differential survival and growth of species in arid and semi-arid environments. Improved survival and growth of trees in these environments will depend on breeding to identify specific traits that confer drought hardiness within the existing genome. This process can be significantly facilitated by identifying early physiological and morphological markers (traits) of superior performance of genotypes even at seedling stage. Initial studies on indigenous species have mainly concentrated on provenance testing (Kiplimo 1993). However, this examination is likely to be more successful at clonal rather than provenance level since it is through this that we can establish an existing relationship between performance and survival of different species under drought stress. Success would depend on the capacity to use juvenile tissues to predict the response of mature trees to drought conditions. This valuable information is lacking for our indigenous species.

1.4.0. HYPOTHESES

The major hypotheses of this study were:

1 Slow growth in xeric species are due to drought tolerance mechanisms which favour survival to growth, and

2 Selection for species dehydration postponement strategies is a more superior selection criterion for arid lands than species dehydration tolerance strategies.

1.5.0. OBJECTIVES

The major objectives of this study were:

1 To determine the various physiological and morphological drought tolerance mechanisms in *Acacia tortilis* and *Acacia xanthophloea* seedlings.

2 To examine how the magnitudes of drought tolerance mechanisms vary at various degrees of soil drought, and

3 To determine the relationship between physiological and morphological traits and how they contribute to species growth and survival.

CHAPTER TWO

GROWTH FEATURES OF *Acacia tortilis* AND *Acacia xanthophloea* SEEDLINGS AND HOW THEY RESPOND TO CYCLIC DROUGHT STRESS

2.1.0. INTRODUCTION

In the past, arid and semi-arid lands (ASAL), have been regarded as waste lands. However, currently there is a considerable interest in such marginal tropical dry lands and efforts are being made for their rehabilitation. Although this has started with planting of trees, climatic and edaphic factors prevailing in such environments have challenged the process. Of all these factors, drought is the single most important factor limiting tree establishment. For this reason, limited success in dry land tree establishment has been realised.

Past attempts to select genotypes for arid lands have been based on identifying most vigorous (for example, fast growing) genotypes, and discarding the less vigorous genotypes. Field testing of genotypes are quite expensive, inaccurate and time consuming. For this reason, procedures are necessary that will accurately predict superior field performance in juvenile tissues. Studies now reveal that species that are capable of withstanding drought, do so either through dehydration tolerance or dehydration postponement (Turner 1986, Osonubi and Fasehun 1987, Tschaplinski *et al.* 1993, Johnson *et al.* 1996, White *et al.* 1996). Those that tolerate drought have tissues that can withstand prolonged periods without, water with minimal damage (Martin *et al.* 1987). Other species are capable of postponing drought through a number of morphological adaptations such as deep and dense rooting system (high r:s ratio) or controlling water loss during the hottest part of the day by stomatal closure. Such plants may also drop their leaves in order to reduce transpiration (Turner 1986) or orientate their leaves to avoid high heat loads (Grace 1983) hence reduce excessive water loss. A better understanding in this direction may give us a basis of designing a more convenient and suitable criteria for selecting seedlings for out planting in the marginal arid lands.

2.2.0. Specific objectives:

The specific objectives of the study were:

1. To identify some morphological characters present in the two indigenous *Acacia* species and examine how they are affected by varying intensities of cyclic water regimes, and
2. To investigate how these morphological characteristics relate to species performance in terms of growth and species survival under drought.

2.3.0. MATERIALS AND METHODS

2.3.1. Seedling culture

On 19th October 1996, seeds of *Acacia tortilis* (Forsk) Hyne and *Acacia xanthoploea* Benth which had been collected in August 1996 from dry land areas of Wambaa and Kibwezi respectively in the Eastern Province of Kenya were obtained form the National Seed Centre, Kenya Forestry Research Institute (KEFRI) Muguga, Kenya. To initiate germination, seeds were soaked in water boiled up to 90°C and immediately left to cool overnight before germinating them in petri-dishes. The germinating seeds were incubated at 30°C for 10 days in soaked cotton wool to allow complete germination in the two species.

The germinated seedlings were then transplanted into polythene pots of size 18 cm high by 11 cm in diameter which had been previously potted with soil from a dry land site at Karai, 20 Km South of KEFRI. This was to try to mimick as much as possible, the soil conditions in the dry land areas. The planted pots were then transferred to a green house bench. To facilitate maximum seedling establishment, the seedlings were watered daily to container capacity. Direct heat and light from the sun were reduced by 70% using a muslin cloth which was pulled over the glass house roof, for a period of one month prior to subjecting them to the respective drought treatments.

2.3.2. Experimental design and treatment

The experiment was a two way factorial (Two species x 4 water regimes replicated two times) arranged in four blocks. Each block had 70 pots, per species, each pot planted with one seedling..

The treatments were as below:

Treatment.	Description
1.	control - daily watering
2.	2 day drought cycle
3.	4 day drought cycle
4.	6 day drought cycle

During drought, water was withheld, while re-watering was done to container capacity at the end of every drought cycle.

2.3.3. Physical parameters measured

Light intensity, humidity and temperature within the glass house were measured. Light intensity was determined by averaging three daily readings taken at 9.00, 12.00 and 16.00 hours for four months, using a light meter (LI-COR Model LI-189). During the entire experimental period, light intensity averaged 1200 μmol $m^{-2} s^{-1}$.

The mean daily minimum and maximum temperatures were 15°C and 35°C respectively. Humidity measurements as an indicator of evaporative demand within the glasshouse were also carried using wet and dry bulb thermometers. The average daily humidity was 56.6%.

Analysis was done on soil samples used for potting, for example, soil obtained from Karai, to determine its physical properties using core samplers. The moisture retention capacity was 55% while the bulk density was 0.70gcm^{-3}.

2.3.4. Morphological measurements

Beginning one month after subjecting the seedlings to the various treatments, three seedlings per species per treatment were randomly selected and destructively harvested. This was repeated after every two weeks for three months. After every harvest, leaf area (LA) was measured using automatic leaf area meter (AAC-400, Hayashi Denkho, Tokyo, Japan). The roots were recovered by washing off soil using running tap water and soil sieves of pore sizes 2mm and 4.5 μm. The various plant parts (roots, stems and leaves) were then oven dried at 70°C for 48 hours before determining root dry weight (RDW), stem dry weight (SDW) and leaf dry weight (LDW). The above weights were then used to determine root:shoot (r:s) ratio, LDW/RDW ratio, total dry weight (TDW) and relative growth rate (RGR). All weight measurements were done using electronic balance (Model AND FX-200 A&D Co. Ltd, Tokyo, Japan, sensitive to a milligram).

Ratio of LA to that of total root dry weight was determined for the two species. This gave an indication of a balance between transpiration and absorption.

Mean relative growth rates were determined by the equation:

$$R = In\, W_2 - In\, W_1 / t_2 - t_1$$

Where : W_1 = dry wt. (g) at time t_1 and W_2 = dry wt. (g) at time t_2.

2.3.5. Data analysis

Statistical differences between treatments in LA, LDW, RDW and s:r ratio were tested for each harvest using Turkeys Honestly significant difference (HSD) Multiple range test through a computer software package (SPSS 1995 version) at 0.05 significance level.

2.4.0. RESULTS

2.4.1. Growth rate and total dry weight accumulation

Water stress treatments caused large differences in growth rate leading to significant differences in dry matter accumulated after five months for *A. tortilis* and *A. xanthophloea* seedlings (Fig. 1a and b). Mean total dry weight accumulated by individual seedlings of *A. tortilis* after 4 months of growth were 4.20, 3.50, 1.86 and 1.84 g for the treatments 1,2,3 and 4 respectively (Fig. 1a). Under similar conditions, individual seedlings of *A. xanthophloea* accumulated mean total dry weight (TDW) of 5.42, 3.66, 2.27 and 1.84 g for treatments 1,2,3 and 4 respectively (Fig. 1b).

Under sufficient water supply conditions, *A. xanthophloea* had a higher rate of total dry weight accumulation than *A. tortilis* seedlings (Fig. 1). However, seedlings of *A. xanthophloea* experienced greater decline in TDW when subjected to water stress compared to *A. tortilis* seedlings (Fig. 2). Two-day repetitive water stress did not show any significant effect on the seedlings of *A. tortilis*, however, increasing stress to 4-day cyclic drought had a significant effect on TDW for both species. Six-day repetitive drought did not show any significant difference in TDW accumulation from 4-day drought. Since there were no differences between treatments 1 and 2, which were in turn different from treatments 3 and 4, growth data could be divided into two groups for *A. tortilis* seedlings, namely droughted (treatments 3 and 4) and non droughted (treatments 1and 2).

Mean relative growth rate (R) was reduced from 0.4mg/day in the controls to about half (0.19mg/day) in stressed seedlings of *A. tortilis*, while in *A. xanthophloea*, mean growth rate was reduced by about 33% in 2 day drought cycle, 58% in 4-day drought and about 66% in 6-day drought seedlings compared to their controls which had an R of 0.57mg/day.

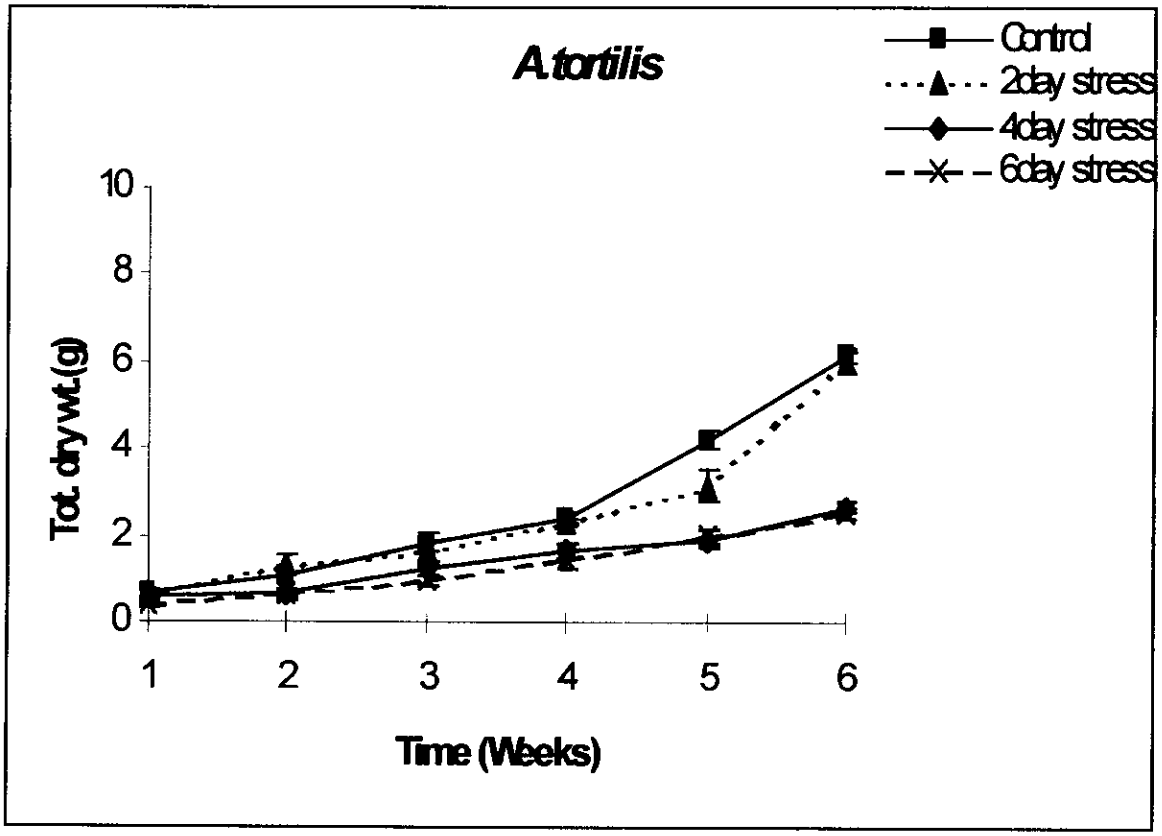


Fig. 1a. Mean total dry weight (g) accumulation in seedlings of *A. tortilis* grown under different levels of water stress, after 5 months of growth.

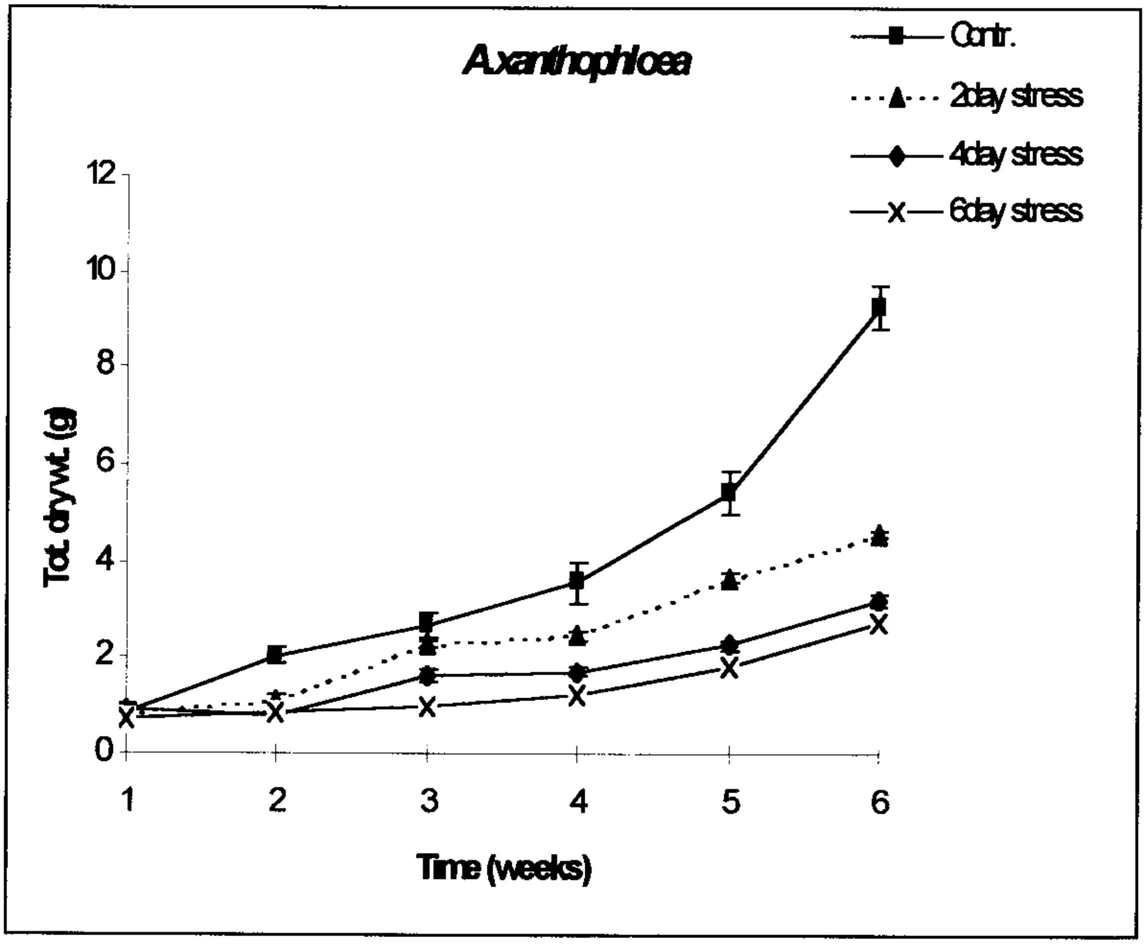


Fig. 1b. Mean total dry weight (g) accumulation in seedlings of *A. xanthophloea* grown under different levels of water stress for a period of five months.

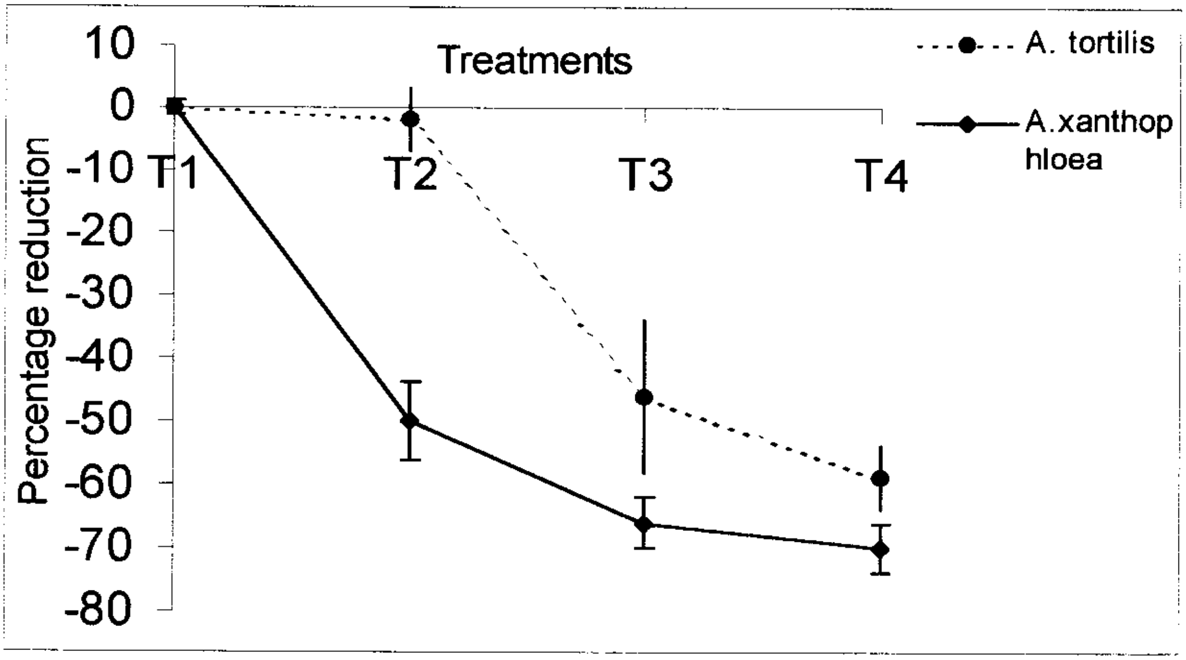


Fig. 2: Percentage (%) change in dry matter (TDW) production between the controls and the stressed seedlings of *A. tortilis* and *A. xanthophloea* after 5 months of growth. T1 represents the controls, while T2, T3 and T4 represent 2, 4 and 6-day repetitive droughts respectively.

2.4.2. Total leaf area and leaf morphology

The pattern of development of total leaf area (LA) over time was similar to that of the total dry matter (Fig 3). After 5 months of growth, LA in *A. tortilis* seedlings were 44.3, 33.9, 16.0 and 12.7cm^2 for treatments 1,2,3 and 4 respectively. LA accumulated by *A. xanthophloea* after the same period of growth under similar drought treatments were 56.17, 38.13, 28.66 and 23.15cm^2 for treatments 1,2,3 and 4 respectively. Total leaf area was strongly reduced by water stress. Compared with the controls, mild water stress (2-day drought) resulted in a 13% reduction in total leaf area in *A. tortilis* while for the same level of water stress, total leaf area was reduced by about 32% in *A. xanthophloea* seedlings. Four and 6-day repetitive soil drought reduced total leaf area, by about 64 and 71% respectively in *A. tortilis* while in *A. xanthophloea* 4 and 6-day repetitive drought caused a 33.3 and 60% reduction in LA. respectively. There was no significant difference in LA between 4 and 6-day droughted seedlings of *A. tortilis* while LA was reduced by about half between 4 and 6 -day repetitive stress in seedlings of *A. xanthophloea*. Individual leaf sizes were also reduced to about half in 6-day stressed seedlings compared to the controls in *A. xanthophloea* while 6-day drought did not show any significant effect on seedlings of *A. tortilis* (Fig.4).

There was no consistency in LA: RDW ratios with regard to treatments in the two species. However, *A. tortilis* had a lower LA:RDW ratio (22.3 cm^{2}g^{-1}) than *A. xanthophloea* (42.4 cm^{2}g^{-1}) under 4 and 6-day repetitive stress.

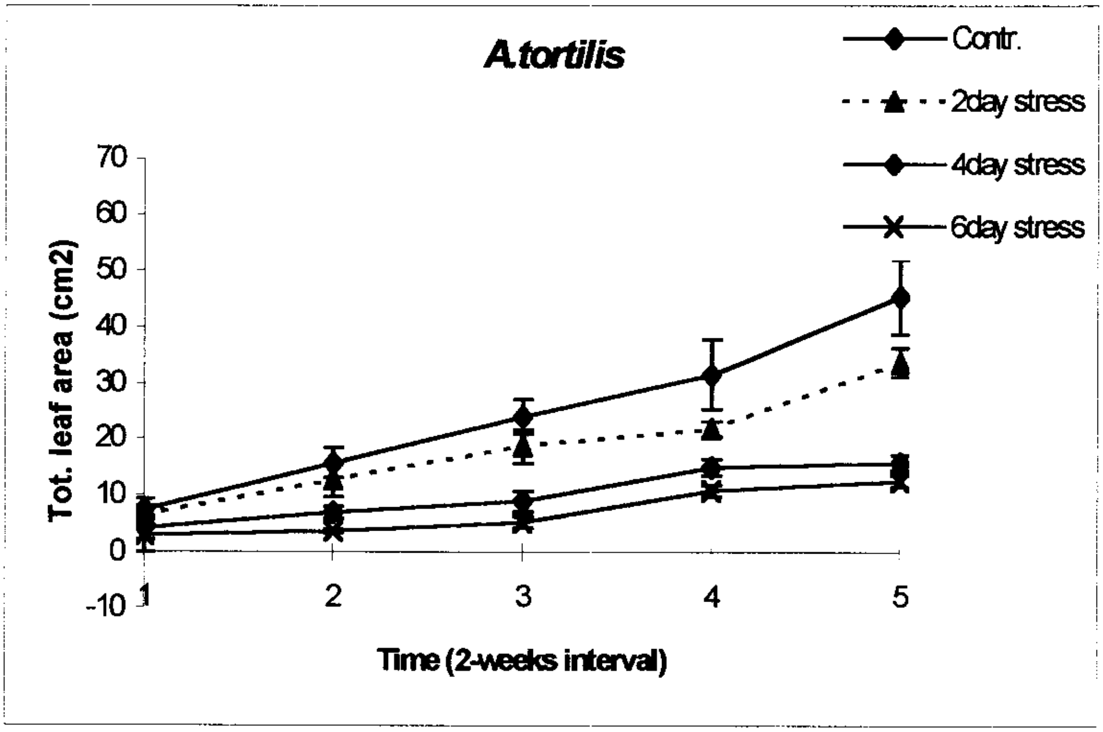


Fig. 3b. Time course increase in total leaf area (mean LA) of *A. tortilis* seedlings subjected to different levels of water stress for 4 months. The controls were watered daily while stress treatments were effected by withholding water for 2, 4 and 6 days for treatments with 1-day full re-hydration

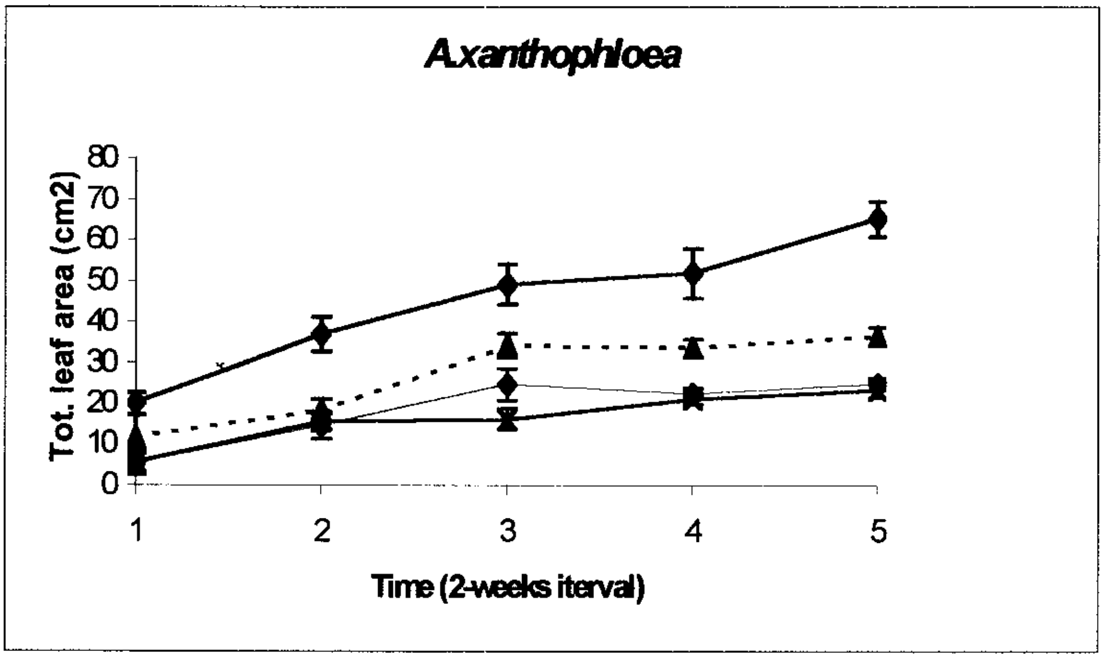


Fig. 3a. Time course increase in total leaf area (mean LA) of *A. xanthophloea* seedlings subjected to different levels of water stress for 4 months. The controls were watered daily while stress treatments were effected by withholding water for 2, 4 and 6 days respectively with 1-day full re-hydration.

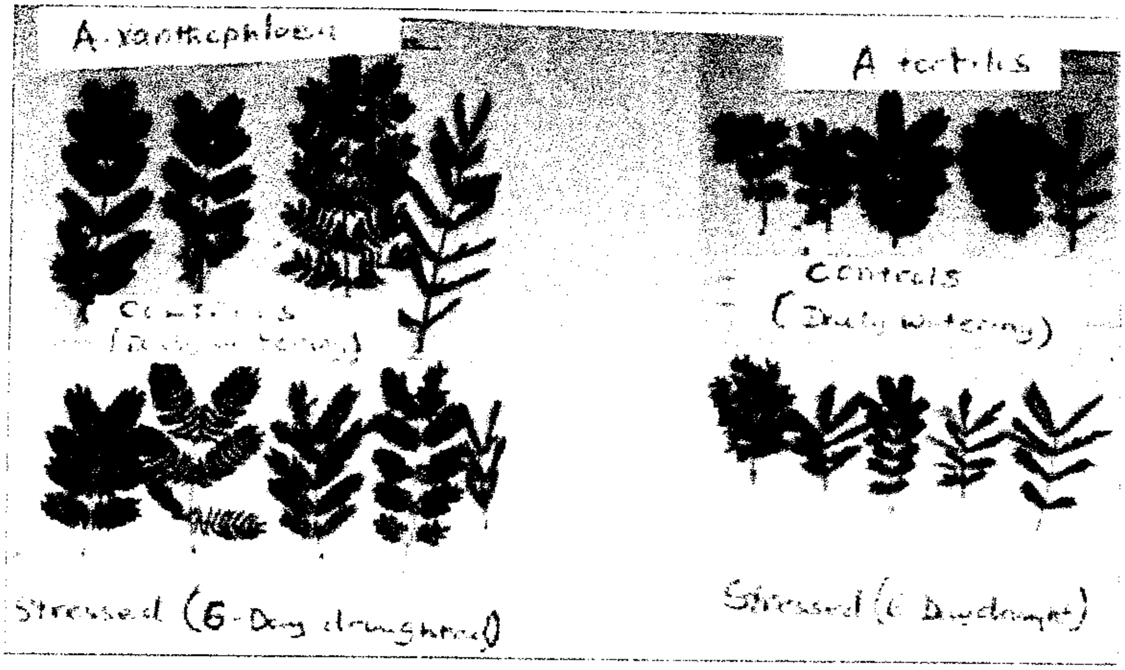


Figure 4. Leaves of *A. xanthophloea* and *A. tortilis* seedlings. The controls were watered daily while the stressed seedlings received six day cyclic drought with one day full rehydration to container capacity. Leaf sizes in *A. xanthophloea* were reduced by repetitive drought while no significant effect occurred in *A. tortilis*.

2.4.3. Carbon partitioning

Carbon allocation to both root and shoot was also analysed by obtaining a ratio of root dry weight to that of shoot dry weight for all the treatments (Table 1) over the study period. Repetitive 4 and 6 day drought caused a significant ($p< 0.05$) increase in carbon allocation to the roots leading to high root:shoot ratios in stressed compared to the control seedlings.

After 16 weeks of growth, 6-day droughted seedlings of *A. tortilis* had r:s ratio of 1.5 compared to 0.52 observed for the non-water stressed. The high r:s ratio in water stressed seedlings was largely due to reduced leaf dry weight. Shoot growth was highly reduced in *A. tortilis* seedlings that were subjected to repetitive stress.

Repetitive water stress, however, did not affect carbon partitioning in *A. xanthophloea* seedlings in which root to shoot ratio was about 0.5 for both repeatedly stressed and unstressed seedlings, indicating equal partitioning to both shoot and root. There was a significant increase in r:s ratio in *A. tortilis* seedlings over time. This was not observed in *A. xanthophloea.*

Table.1. Carbon partitioning to both root and shoot (r:s ratio) in individual seedlings of *A. tortilis* and *A. xanthophloea* grown under four different cyclic water regimes. Harvests (H*i*= harvest *i*) were made after every one month. T represents treatment (T*i* = treatment *i*) for every species. Statistical significance differences (p=0.05) between means as tested by Turkey's Multiple Range Test (SPSS 1995) are indicated by stars.

Acacia tortilis					*Acacia xanthophloea*			
	T1	**T2**	**T3**	**T4**	**T1**	**T2**	**3**	**T4**
H1	0.49	0.40	0.42	0.70	0.29	0.35	0.54	0.40
H2	0.54	0.64	0.79	0.85*	0.45	0.47	0.42	0.48
H3	0.46	0.53	0.56	1.02*	0.62	0.55	0.50	0.62
H4	0.57	0.64	0.79	1.14*	0.57	0.52	0.49	0.48
H5	0.74	0.76	1.04*	1.50*	0.65	0.53	0.60	0.67
H6	0.56	0.62	1.23*	1.50*	0.56	0.52	0.62	0.56

2.5.0. DISCUSSION

Repetitive soil drought reduced growth, total dry weight accumulation and total leaf area in seedlings of the two *Acacia* species. Under adequate water supply, 5 months old seedlings of *A. xanthophloea* showed higher (22%) total dry weight accumulation and total leaf area than *A. tortilis* (Fig. 1). In this respect *A. xanthophloea* appeared to take better advantage of water availability to increase biomass accumulation hence showing greater inherent abilities to grow faster than *A. tortilis*. Kiplimo (1993), found that progenies of *A. tortilis* seedlings from humid environments grew faster under adequate water supply than those from more xeric habitats, but were less tolerant to water stress. A similar observation was made by Cannell *et al.* (1978). They found that, loblolly pine seedlings from humid coastal North Carolina grew faster under mild stress than the seedlings from the dry Arkansas state. These observations compare favourably with the response observed for *A. xanthophloea* seedlings, suggesting that the species could be better adapted to grow in mesic environments.

The observed reduction in total leaf area in *A. xanthophloea* as a result of mild-water stress, could be attributed to leaf shedding and reduction in leaf size while that of *A. tortilis* was more likely due to reduced leaf emergence and expansion rate and to a lesser extent to reduction in leaf size (Fig. 4). However, further studies are still required to confirm this observation.

Reduction in total leaf area as a result of mild water stress, however, did not show any significant effect on total dry weight accumulation in *A. tortilis* seedlings (Fig. 1a). This suggests that photosynthetic efficiency of the leaves was not affected by mild water stress. In this study, leaves of *A. tortilis* appear to be less sensitive to mild water stress compared to *A. xanthophloea,* and therefore, effectively maintained growth rates under mild soil drought than *A. xanthophloea.* The small reduction in LA in *A. tortilis* could be attributed to the species' inherent ability to balance transpirational water loss to available moisture. Although absolute total dry weight accumulation was higher in *A. xanthophloea,* dry matter production per unit leaf area was much higher in *A. tortilis* than *A. xanthophloea,* further suggesting higher photosynthetic capacity and growth potentials in *A. tortilis* under mild water stress. Osonubi and Davies (1978) attributed the survival of oak seedlings during drought to low stomatal sensitivity to water stress enabling carbon assimilation, hence physiological functions of the plant during drought. *A. tortilis* seedlings from drier sources produced more biomass than those from less dry sources, suggesting higher adaptability to photosynthesise and grow under mild water stress in seedlings from drier provenances (Kiplimo 1993).

Intensive (4 and 6-day) repetitive drought reduced total dry weight by about 50% compared to the controls in *A. tortilis* seedlings, while that of *A. xanthophloea* was reduced by about 58% (4-day) and 66% (6-day) (Fig. 2). Under similar conditions, leaf area was reduced by about 60% for the 4-day repetitive drought regime and 70% for 6-day repetitive drought respectively in *A. tortilis* and about 50 and 60% respectively in *A. xanthophloea.* According to Boyer (1968,1970), leaf growth is one of the first physiological processes to be affected by drought and leaf enlargement is severely inhibited by even small decline in water potential well before photosynthesis and respiration are affected. The reduction in total leaf area observed for the two species could strongly be attributed to soil drought. The pattern of total dry weight accumulation resembled that of total leaf area which was a factor of soil water availability. Although the reduction in total leaf area was much higher than reduction in total dry weight accumulation, this could mainly be attributed to sensitivity of leaves to water stress than the other plant tissues, resulting in early inhibition of leaf expansion and emergence and also leaf shedding before the stress could be experienced elsewhere, for example, in roots.

According to Passioura (1976), it is the control of leaf area and leaf morphology which is often the most powerful means a mesophytic plant has for influencing its fate when subjected to long term water stress in the field. Under water stress, plants that are adapted to water stress, therefore, reduce leaf area by accelerating the rate of senescence of physiologically older leaves and leaf shedding (Turner 1979). The reduction in leaf area as a result of intense (4 and 6-day) water stress observed in *A. tortilis* seedlings compared to *A. xanthophloea* could therefore be attributed to higher inherent ability to survive water stress in *A. tortilis,* as it was associated with greater reduction transpiration rates (Fig. 6). *A. tortilis,* however, experienced less reductions in dry weight as a result of water stress compared to *A. xanthophloea* seedlings. This could be attributed to maintained photosynthesis in the remaining leaves of *A. tortilis*, while carbon accumulation was adversely affected by intense water stress in *A. xanthophloea.*

With increasing water stress, a large root biomass can provide a large absorptive surface that increases the rate of water uptake (Kozlowski 1982). Accompanied with reduced leaf area, plant species that shift growth to the roots are better adapted to survive drought (Osonubi and Davies 1978). This pattern of response was pronounced in *A. tortilis* seedlings in which, as soil drought increased, a large reduction in total leaf area was observed (Fig.3) while root biomass increased, leading to reduced leaf area to root dry weight ratio (LA:RDW) (thus, 22.4cm^2g^{-1}). For *A. xanthophloea,* LA:RDW remained high (42.4cm^2g^{-1}) and was apparently not affected by drought. The high LA:RDW observed in *A. xanthophloea* is indicative of high transpiration rate and low absorptive capacity, leading to a much lower tissue water potential, limiting physiological processes and growth (Myers and Landsberg 1989). *A. tortilis,* however, is likely to experience higher tissue water potentials under water stress compared to *A. xanthophloea,* due to low transpiratory leaf surface compared to the large absorptive root biomass. Although *A. xanthophloea* could avoid excessive water loss through leaf shedding, this would mean limited growth under water stress conditions.

In contrast to the slower- growing *A. tortilis*, seedlings of faster-growing

A. xanthophloea were unable to alter carbon partitioning to both roots and shoots under conditions of limited soil moisture. An increase in root:shoot ratio as water stress increases is an adaptation for dehydration postponement as it enhances the ability of species to absorb more water from the drying soil (Kozlowski 1982). Several workers have reported an increase in the ratio of root:shoot (r:s) in plants subjected to water stress (Lediges 1974, Osonubi and Davies 1978, Osonubi and Fasehun 1989). In some cases, water stress appears to enhance root growth in absolute terms. Hsiao and Acevedo (1974) proposed that this increased growth of roots under stress may be due to their capacity to adjust osmotically. Shoot growth under mild stress may be reduced as a result of decrease in shoot turgor while stomata may remain open so that photosynthesis continues. The increased supply of assimilates made available by the reduced strength of the sink in the shoot, then permits osmotic adjustment and extra growth and thus constitute an adaptive mechanism in that more water will become available to the plant (Osonubi and Davies 1978). Similar results were obtained for studies conducted on *Parkia biglobosa* in which 14-day cyclic drought resulted in an increase in r:s from 0.45 in the controls to 2.0 after 16 weeks of growth under stress (Osonubi and Fasehun 1989). The results obtained in *A. tortilis* compare well with those obtained by Osonubi and Fasehun (1989) on *Parkia biglobosa*. After 16 weeks of growth under repetitive stress, 6-day drought resulted in an increase in r:s from about 0.5 in the controls to about 1.5 (Table 1). It can be argued that the observed large root dry weight in *A. tortilis* in comparison to the shoot as a result of stress could be due to large sink for assimilates created at the roots as a result of osmotic adjustment.

The fact that shoot growth almost ceased at the expense of root growth in 4 and 6-day stressed seedlings of *A. tortilis* supports the argument above. The large root biomass could lead to increased water absorption, hence, maintaining growth and survival under water stress.

Lediges (1974) observed that *Eucalyptus grandis* seedlings that had higher (0.51) root:shoot ratios (r:s) were able to survive moderate water stress better than those with lower (0.45) root:shoot. The observed low r:s for *E. grandis* compares favourably with that of *A. xanthophloea* in which r:s ratio remained at 0.5 for both water stressed and unstressed seedlings. Low root:shoot ratio has been found to correlate with dry matter production in conifers growing in more mesic environments (Tan *et al.* 1992). A lower root:shoot ratio, as observed for *A.xanthophloea,* would increase photosynthetic capacity under adequate water supply but might increase mortality under drought. This is because the large leaf surface area would increase transpirational water loss under drought.

A. xanthophloea was also unable to alter r:s ratio even when repeatedly stressed (Table 1) and further more there was a large reduction in total dry weight production when subjected to 4 and 6-day repetitive water stress. This may explain why *A. xanthophloea* could be better adapted to grow in mesic environments.

2.6.0. CONCLUSIONS

Previous studies (Kozlowski 1982) have considered various mechanisms by which plants avoid or tolerate drought stress such as high root:shoot ratio, low LA:RDW ratio, growth rate, and leaf characteristics. However, despite the extensive research, the relative importance of each mechanism in conferring drought resistance remains uncertain (Rieger and Duemmel 1992).

From this study, it could be concluded that the pattern of carbon allocation and leaf characteristics could act as better indicators of drought stress resistance in tropical arid land species than growth parameters such as growth rates. This conclusion is based on the observation that although *A. xanthophloea* showed faster growth rates and TDW accumulation through allocation of more assimilates to the leaves when adequately supplied with water, it could not alter this pattern of allocation with the onset of water stress, hence, the species suffered more water stress compared to the slow growing *A. tortilis* seedlings which could effectively alter the pattern of carbon allocation as water stress set in (Table.1). The more water stress resistant *A. tortilis* increased root:shoot ratios over time and had its r:s ratios above 0.5 even under mild water stress. This could be used as an indication of inherent stress tolerance in such species and could act as selection criterion for arid environments. Leaf characteristics such as the rate of leaf emergence and leaf expansion could also provide a suitable basis for determining drought tolerance in tree species. However, more studies in this direction are suggested for a more decisive conclusion.

CHAPTER THREE

WATER RELATIONS OF *Acacia tortilis* AND *Acacia xanthophloea* SEEDLINGS SUBJECTED TO REPETITIVE SOIL DROUGHTS.

3.1.0. INTRODUCTION

Water stress is one of the most important factors limiting plant growth. Water deficits influence changes in turgor pressure, a principal means through which small changes in plant water status are transduced into changes in metabolism and turgor driven cell expansion (Hsaio *et al.* 1976). Processes that lead to turgor loss, may trigger in some plant species, physiological and biochemical responses that will enable maintenance of turgor at lower xylem water potentials in such species.

The two known mechanisms promoting turgor maintenance in higher plants involve changes in tissue elastic and osmotic properties (Tyree and Jarvis 1982).

A decrease in osmotic potential results in greater turgor pressure at a given leaf water potential, whereas an increase in cell-wall elasticity results in smaller changes in turgor pressure for a given change in relative water content (White *et al.* 1996). In higher plants, decrease in osmotic potential is achieved through active accumulation of organic solutes, thus, osmotic adjustment (Blake *et al.* 1991), while elastic adjustment is as a result of cell wall modifications which make them more elastic (Blake and Tschaplinski 1992).

A deeper understanding into the two mechanisms involved in turgor maintenance in tree species is necessary as they could help determine and explain the differences in drought tolerance in trees. The current study investigated changes that occur in tissue elastic and osmotic properties of two *Acacia* species seedlings subjected to repetitive drought stress.

3.2.0. MATERIALS AND METHODS

3.2.1. Experimental design and treatment

The experiment was a two way factorial (Two species x 4 water regimes replicated two times) arranged in four blocks. Each block had 70 pots, per species, and each pot planted with one seedling. The treatments were as below:

Treatment	Description
1.	control - daily watering
2.	2 day drought cycle
3.	4 day drought cycle
4.	6 day drought cycle

During drought water was withheld, while re-watering was done to container capacity at the end of every drought cycle.

3.2.2. Plant water relations

Xylem water potentials

Shoot xylem water potentials were determined at mid-day on the eve of every re-hydration cycle for each treatment per species on 5-month old seedlings. Because of the small leaf size of the two species, 10cm terminal shoots containing 6-

8 pairs of leaves were used. At every sampling time, four seedlings per species per treatment were used. All water potential determinations were done using a pressure chamber (DIK-700 DAIKI RIKI Co. Ltd. Tokyo, Japan).

Pressure volume (pV) measurements

Pressure volume (pV) analysis were performed on 6 months old seedlings. Three seedlings per treatment per species were collected from the glass house early in the morning. On average, 10cm of apical shoot bearing 6-8 pairs of leaves were excised, re-cut under distilled, de-ionised water and leaving them to re-saturate in a dark chamber for 24 hours. Polythene sheets were used to prevent any evaporative loss and ensure full tissue saturation during the re-hydration process by covering the beaker tops.

The shoots were then removed from water and their fresh saturated weights determined. Shoot water potential (ψ_w) was also determined and the shoot re - weighed. Shoots were allowed to transpire freely under ambient conditions on a laboratory bench. At periodic intervals (approximately 1 hr.), weights and ψ_w of each shoot were measured. On each occasion, the pressure chamber was pressurised slowly (to prevent tissue damage) until an air bubble appeared at the distal end of cut shoot (Tryree and Jarvis 1982). After each pressure chamber measurement, two weight measurements were taken, one immediately preceding and one immediately following the ψ_w determination. The mean of the two weights were used in the subsequent calculation of relative water content (R*).

Measurements were carried out from 7 a.m. in the morning up to 6 p.m. in the evening, at which point the shoots were far beyond their wilting points. Seven (7) pressure chamber and 14 weight measurements per shoot were obtained. The shoots were oven dried at 70°C for 48 hours before determining their dry weights.

The relationship between the reciprocal of tissue water potential ($^1/_{\psi w}$) was plotted against relative water content (100-R*%) for each seedling per treatment per species.

R* was determined by the equation:

(Fresh wt-dry wt) ÷ (saturated wt - dry wt) x 100 (Kramer 1980)

Estimation of tissue water relations variables

Osmotic potential at full turgor (Πs) and at turgor loss point (Πp) and relative water content at turgor loss point (R*tlp), were derived from pV curves by considering a regression line between the inverse of the final balancing pressure points and relative water content (R*) (Tyree and Hammel 1972). The last four points of the plot were considered as they had a clear representation of the linear part of the curve. Mean values for each treatment were then statistically tested to see whether there were any significant differences between treatments. Turgor potentials (P), were estimated as the difference between Ψ and Π i.e. $P = \Psi - \Pi$. Bulk modulus of elasticity (ε) was calculated as the change in P per unit change in R*

Thus $\varepsilon = dP/dR^*/R^*$. Where ε = modulus of elasticity

dP = change in P

dR*= change in R* over the same interval.

R* = mean R*

Where R* is the relative water content.

3.3.0. RESULTS.

Xylem water potentials

Results of mean shoot xylem water potentials measured at mid-day on 5 -month old seedlings of *A. tortilis* and *A. xanthophloea* subjected to four levels of repetitive drought stress cycles are shown in Fig. 5. Mid-day water potentials were used to monitor the level of stress attained by each species at the end of every drying cycle. There was significant ($p>0.05$) difference between water potential values obtained for the controls (daily watering) of the two species with *A. xanthophloea* seedlings showing lower water potential values. Two day repetitive water stress had no significant ($p >0.05$) effect on the xylem water potential values of both *A. tortilis* and *A. xanthophloea* .

Subjecting the two species to higher (4 and 6 day) repetitive drought stress effectively lowered their *. However, *A. xanthophloea* showed significantly lower values of ψ compared to *A. tortilis*. Water potential (ψ) values obtained at the end of every 6-day drought showed ψ of *A. xanthophloea* well below -4MPa while mean values obtained at the same level of stress for *A. tortilis* was -3.5MPa (Fig. 5).

In the three repetitive water stress cycles, *A. tortilis* showed higher xylem tissue water potentials compared to *A. xanthophloea*.

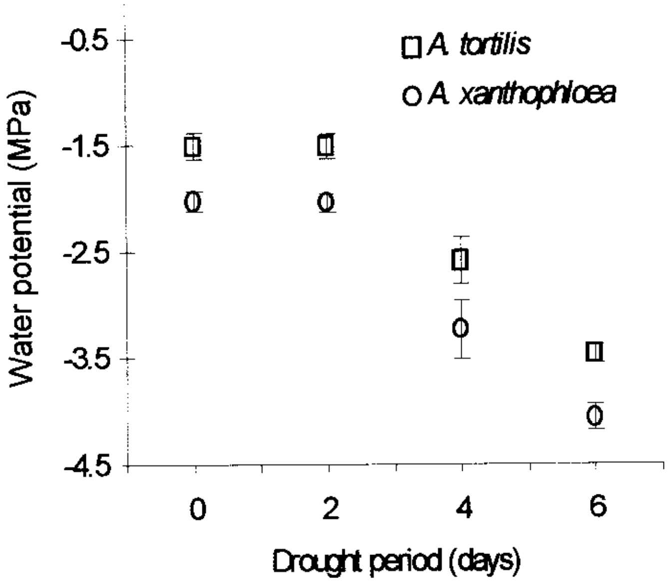


Fig. 5: Comparison of mean shoot xylem water potentials of seedlings of *A. tortilis* and *A. xanthophloea* grown under full irrigation (0) and 2, 4, and 6 day drought stress with 1 day full re-hydration for 5 months. Statistical differences between species and treatments are indicated by error bars.

Pressure - volume (pV) analysis

Inherent differences in tissue water relations in stressed and unstressed seedlings in the two species as compared by pV analysis are shown in Fig.6 pV curves constructed for the two species gave the usual curvilinear then linear relation as observed for most woody species, however for *A. tortilis*, the relation deviated from linear at high pressures greater than -3 MPa (i.e. R*<55-66%), although the linear trend was adequately defined long before this occurred. Similar observations were made by Wilson *et al.* (1979), on leguminous tree species.

Controls of *A. xanthophloea* lost turgor at a relative water content (R*tlp) of 70%. This was a higher value compared to those of *A. tortilis* which lost turgor at R*tlp of 65%. Under similar conditions, osmotic potential at full turgor (Πs) and at turgor loss point (Πp) were also estimated for the two species. Πs and Πp were -1.00MPa and -1.67MPa respectively for *A. tortilis* while the respective values for *A. xanthophloea* were -1.25MPa and -1.67MPa. Analysis of variance revealed no significant ($p>0.05$) differences in Πs and Πp respectively for the controls of the two species.

Seedlings of *A. tortilis* subjected to 6 day repetitive drought stress cycles adjusted their tissue water relations resulting into a decline in R*tlp, Πs and Πp to 53%, -1.67MPa and -2.50MPa respectively. *A. xanthophloea* under the same conditions did not show any significant difference in R*tlp, Πs and Πp from the controls with the respective values of 80% ,-1.00MPa and -1.45MPa (Table 2)

Turgor potential (P) at full saturation increased in *A. tortilis* with increase in water stress. Turgor potential at full saturation for the control seedlings was 1MPa while P at full saturation for 6-day droughted seedlings was 1.67MPa. However, in *A. xanthophloea*, P decreased from 1.25MPa in the controls to 1MPa in the 6-day droughted seedlings. This decrease, however, was not significant ($p>0.05$).

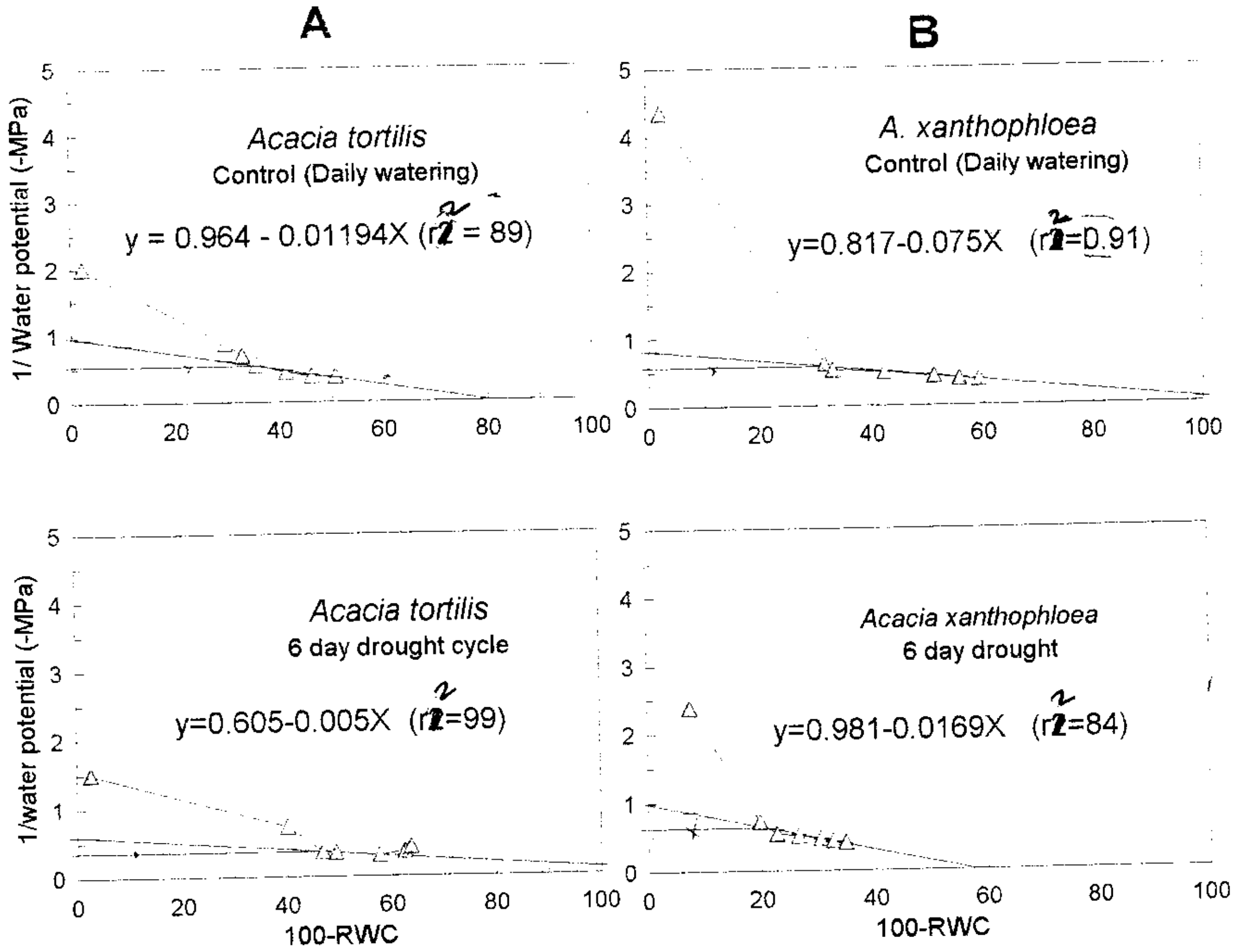


Fig. 6. Pressure-Volume (pV) curves of 6 months old seedlings of *A. tortilis* (A) and *A. xanthophloea* (B). Controls were watered daily while stressed seedlings were subjected to 6 day cyclic soil drought. Extrapolation of the straight section of the curve to a relative water content (R*) of 100% estimates osmotic potential at full turgor. The extrapolation was obtained by regressing the last four points of the curve. Regression equation for each curve is shown.

(44.4MPa) for *A. xanthophloea*, while ε for controls of *A. tortilis* was 8.3MPa. There was significant ($p<0.05$) difference in ε values for the controls of the two species. Subjecting the species to 6-day repetitive stress decreased ε for *A. xanthophloea* seedlings to 20.4MPa while ε for *A. tortilis* seedlings was not significantly ($p<0.05$) affected. Intermediate values between the two extremes were obtained for 2 and 4 day repetitive droughts.

Analysis of variance showed significant ($p<0.05$) differences in the pV parameters (R*tlp, Πs, Πp and ε) considered between the two species in response to increasing drought stress. In *A. tortilis,* there was no significant difference ($p>0.05$) between the response of controls and 2-day droughted seedlings and a similar situation was also observed between 4 and 6 day droughted seedlings . *A. xanthphloea* seedlings showed no significant adjustment in pV parameters with increasing water stress, except modulus of elasticity (ε) (Table 2).

Table. 2: Tissue water relations parameters measured by pV analysis for *A. tortilis* and *A. xanthophloea*. Measurements were made of plants grown under adequate water supply(0 day cycle),6-day dehydration and 1-day re-hydration (6 day cycle) for 4 months. Osmotic potentials (Mpa) at full turgor (Πs) and at turgor loss point (Πp), modulus of elasticity (ε Mpa) and relative water content (%) at turgor loss point(R*tlp). Significant differences between means are indicated by * at ($p<0.05$). Adj.= Adjustment, TRT.= Treatment.

Species	**TRT**	**Πs**	**Πp**	**R*tlp**	ε
A. tortilis	0 day cycle	- 1.00	- 1.67	65	8.3
	4 day cycle	-1.67*	- 2.50*	53*	10.5
	Adj.	+0.67*	+0.83*	-12%*	-2.17
A. xanthphloea	0 day cycle	1.25	1.67	70*	44.4*
	4 day cycle	1.00	1.43	80*	20.4*
	Adj.	- 0.25	- 0.24	+10	+24.0*

3.4.0. DISCUSSION

Maintenance of a positive turgor is critical for cell growth because cell expansion is highly sensitive to dehydration (Hsiao 1973). The role of elastic and osmotic adjustments in the maintenance of turgor above a critical threshold in stressed tree species has been suggested (Blake and Tschaplinski 1992). During this study, *A. tortilis* maintained turgor to the lowest relative water content (R*tlp) of 53 - 65% and water potential. *A. xanthophloea* however, lost turgor at quite high R* (70 -80%) and water potential. Turgor was also observed to decline more rapidly in *A. xanthophloea* compared to *A. tortilis* during the dehydration process. The results also agree with the low ε (8.3MPa) obtained for *A. tortilis* compared to *A. xanthophloea* (44.4mpa) under conditions when water was not limiting (controls).

The low ε value obtained for the control seedlings of *A. tortilis* indicated that the species had inherently more elastic tissues compared to *A. xanthophloea* which had higher ε. A more elastic tissue as denoted by a lower modulus of elasticity (ε) indicates that turgor potential declines less rapidly per unit loss of cell water (Blake *et al.* 1991), hence, maintaining positive turgor at lower water potentials. However, both higher and lower elasticity have been suggested as promoting turgor maintenance. For example, a higher ε value, thus, less elastic tissue, was suggested to increase water absorption in drying soil by increasing the soil-plant water potential gradient (Cheung *et al.* 1975). However, modelling studies of Schulte

(1992) have shown that less elastic tissue does not increase water uptake over a full 24-hour period. Instead lower ε values maintained turgor since elastic tissues can reduce the rate of water potential reduction in a dehydrating plant (Zimmerman and Steudle 1978), hence maintaining positive turgor over a large range of xylem water potentials. This could help explain the slow decline in tissue water potential observed for dehydrating seedlings of *A. tortilis* (Fig. 5). Because *A. xanthophloea* had high ε, (low tissue elasticity), the rapid decline in xylem water potentials during water stress could be detrimental and lead to rapid tissue death unlike in *A. tortilis*.

Repeated drought stress resulted into a decline in εof *A. xanthophloea* by about half, while this had no significant effect on ε for *A. tortilis*. This could imply an improved tissue elasticity in *A. xanthophloea* seedlings induced by repetitive stress (Blake *et al.* 1991, Fan *et al.* 1993). Repeated water stress, however, did not significantly affect tissue elasticity in *A. tortilis*. The decline in ε as observed for *A. xanthophloea* was expected to result into a lower R*tlp associated with the observed decline in ε. Instead, there was an increase in R*tlp by 10%. A similar observation was made by Meinzer *et al.* (1986). This phenomena was attributed to re-hydration. They suggested that excessive turgor pressure developed during re-hydration could cause irreversible stretching to cell walls and decrease ε. However, in the current study, that could not have been the case as the observed decline in ε, that is, increased tissue elasticity, was associated with a 30% decline in electrolyte leakage observed for repeatedly stressed seedlings of *A. xanthophloea* (Table 4). This suggests that the low ε was an indication of improved tissue elasticity which improved species tolerance to water stress. It is suggested here that an irreversible cell wall extension could have resulted into an increase in or no effect at all on electrolyte leakage in repeatedly stressed seedlings of *A. xanthophloea.* This could be so since cell walls of in-elastic cells could buckle and rapture when subjected to drought (Dale and Scutliffe 1986) or otherwise have no effect at all on the membranes since they only over stretched but no breakage occurred on them.

According to Schulte and Hinckley (1985), the relationship between ε and relative water content is not a simple one as it varies from species to species. However, most studies conducted on the behaviour of modulus of elasticity (ε) in stressed tree species have always obtained values below or around 20MPa (Blake *et al.* 1991, Fan *et al.* 1993). The observed ε value for *A. xanthophloea* seemed to be quite high and the value was lowered to 20MPa when the seedlings were repeatedly stressed. However, this decline did not show any significant change on R*tlp of the repeatedly stressed seedlings as was expected. From the study, it is suggested that a threshold exists for ε, which has to be reached before any significant change on R*tlp is observed. Further studies in this direction are still necessary for a conclusive decision.

Repetitive drought stress had no significant effect on cell wall elasticity (ε) of *A. tortilis,* however, significant decline in R*tlp, Πs and Πp were observed for the repeatedly stressed seedlings. R*tlp, Πs and Πp were lowered by 12%, 0.67 MPa and 0.83MPa respectively. Repetitive drought stress, however, did not show any significant effect on Πs and Πp of *A. xanthophloea* seedlings. A decline in Πs or (Πp) as observed in *A. tortilis* is an indication of osmotic adjustment (White *et al.* 1996), through active solute accumulation in the dehydrating tissues (Hinckley *et al.* 1979). A number of studies have indicated the relevance of osmotic adjustment in turgor regulation (Hinckley *et al.* 1979, Tyree and Jarvis 1982, Tschaplinski and Blake 1989, White *et al.* 1996). The observed decline in R*tlp in *A. tortilis* seedlings under water stress correlates with the decline in Πs or (Πp). It could, therefore, be strongly suggested that this decline in R*tlp in repeatedly stressed seedlings of *A. tortilis* compared to the controls could be due to osmotic adjustment induced by repetitive drought stress and could be vital for survival under drought conditions. *A. tortilis* seedlings which were repeatedly stressed maintained positive turgor at lower water potentials a response which was not observed in *A. xanthophloea* seedlings. The absence of osmotic adjustment in *A. xanthophloea* is further confirmed by the lack of shift on the R*tlp under drought conditions.

It is clear from the above observations that cyclic drought impacted differently in the two species. Repetitive drought stress resulted in osmotic adjustment in *A. tortilis* but did not change its tissue elastic properties. On the other hand, *A. xanthophloea* responded to drought stress by adjusting its cell wall elasticity resulting into a decline in ε. In *A. xanthophloea*, the lack of shift in R*tlp, despite changes in ε may suggest that cell wall elasticity may not be important in this species as a means of turgor maintenance during water stress. Osmotic adjustment as exhibited by seedlings of *A. tortilis* could help in turgor maintenance as it could be the reason for the reduction in turgor loss point (R*tlp) observed in repeatedly stressed seedlings of *A. tortilis* compared to the controls. This is further supported by LD_{50} (Chapter 4). *A. tortilis* also appeared to survive drought longer than *A. xanthophloea* even when both controls were subjected to prolonged water stress. It could be suggested that inherent high tissue elasticity (i.e. low ε) observed for non-stressed seedlings of *A. tortilis* compared to those of *A. xanthophloea* could have contributed to the prolonged survival of *A. tortilis* observed during the LD_{50} experiment.

CHAPTER FOUR

MEMBRANE INTEGRITY AND PLANT SURVIVAL IN *Acacia tortilis* AND *Acacia xathophloea* SEEDLINGS SUBJECTED TO REPETITIVE SOIL DROUGHTS

4.1.0. INTRODUCTION

During drought, a plant may be subjected to severe injury or death, unless it possesses adequate dehydration tolerance (Sullivan and Ross 1979). Tolerance to desiccation includes the ability of the cells to withstand mechanical injury, the ability of the membranes to withstand degradation and the ability of the membrane and cytoplasm to withstand denaturation of proteins (Gaff 1980).

Drought induced loss of cell membrane integrity is associated with an efflux of solutes (Levitt 1980), since it is expected that healthy tissues will have solutes filtered by the cell membranes and remain in the cells (Zwiazek and Blake 1990). The use of electrolyte leakage in demonstrating membrane damage hence the stress level attained by plant tissues under drought is well documented (Martin *et al.* 1987, Zwiazek and Blake 1990, Tan and Blake 1993).

Although dehydration postponement seem more desirable since it would permit continued plant growth in concert with water deficits (Martin *et al.* 1987), plants with poorly developed dehydration postponement characteristics are likely to have greatest dehydration tolerance (Blum and Ebercon 1981). Levitt (1980) indicated that dehydration tolerance is rarely a significant factor in successful drought endurance by higher plants. Hinckley *et al.*(1981), observed no significant variation among temperate trees in their tolerance abilities. However, drought tolerance has been demonstrated to determine species distribution and survival in the temperate regions (Martin *et al.* 1987, Tan and Blake. 1993) and is a potential selection criterion for arid land species(Martin *et. al.* 1987). Previous studies on *Acacia tortilis* and *A. xanthophloea* have indicated significant differences in morphological adaptations for dehydration postponement.

In the current study, electrolyte leakage was used as a measure of the level of membrane damage, as a result of drought stress in the two *Acacia* species.

Specific objectives

The specific objectives of the study were:

i) To determine electrolyte leakage intensities at four levels of drought stress,

ii) To establish the relationship between electrolyte leakage, stress intensity and plant survival and

iii) To establish the effects of seedling preconditioning on electrolyte leakage and species survival under drought.

4.2.0. MATERIALS AND METHODS

4.2.1. Seedling culture

On 19th October 1996, seeds of *Acacia tortilis* (Forsk) Hyne and *Acacia xanthoploea* Benth, which had been previously (August 1996) collected from dryland areas of Wambaa and Kibwezi respectively in the Eastern Province of Kenya were obtained form the National Seed Centre, Kenya Forestry Research Institute (KEFRI) Muguga, Kenya. To initiate germination, seeds were soaked in hot water (boiled up to 90°C) overnight, before germinating them in petri -dishes. The germinating seeds were incubated at 30°C for 10 days in soaked cotton wool to allow complete germination in the two species. Germination rates were typically high. However, A. *xanthophloea* had a higher germination rate of up to 60% over *A. tortilis.*

The seedlings were then transplanted into polythene pots of size 18 high by 11 cm in diameter which had been previously potted with soil from a dryland site at Karai, 20 Km South of KEFRI. The planted pots were then transferred to a green house bench at KEFRI. To facilitate maximum seedling establishment, the seedlings were watered daily to container capacity. Direct heat and light from the sun were reduced by 70% using a muslin cloth which was pulled over the glass house roof, for a period of one month prior to subjecting them to the respective drought treatments.

4.2.2. Experimental design and treatment

The experiment was a two way factorial (Two species x 4 water regimes replicated two times) arranged in four blocks. Each block had 70 pots, per species, each pot planted with one seedling.

The treatments were as below:

Treatment.	**Description**
1.	Control - daily watering
2.	2 day drought cycle
3.	4 day drought cycle
4.	6 day drought cycle

During drought water was withheld, while re-watering was done to container capacity at the end of every drought cycle.

4.2.3. Membrane integrity (electrolyte leakage)

After seven months of growth, two seedlings per treatment per species were randomly selected, taken to the laboratory and kept in a dark chamber overnight. The following morning, the seedlings were cut, and re-cut under water leaving the

10 cm distal shoot to re-saturate overnight in the dark chamber. Precautions were taken to ensure full saturation (Martin *et al.* 1987). The shoots were then removed from water, four middle leaves removed from each shoot per species per treatment. Thereafter the shoot water potentials were obtained as described elsewhere (Chapter 3).

Procedure at each sampling

Out of the four leaves per plant per treatment, 2 leaves were left to air dry (dehydrate) on a laboratory bench for 6 hours, while the other two leaflets (unstressed) were immediately examined after re-saturation. The leaves were washed in three rounds of distilled, de-ionised water and then cut laterally into two equal pieces. The pieces from the individual shoots were each placed into vials containing 4ml distilled de-ionised water. Similar procedure was followed with the six-hour dehydrated (stressed) leaves. This was done for all the treatments per species. The vials were then shaken at room temperature for 24 hours in a reciprocating shaker. Conductivity of the effusate was then measured with an electronic conductivity (EC) meter (Model CM 20S TOA Electronics LTD, Tokyo, Japan).

The samples were then autoclaved for 20 minutes to completely lyse the cells, allowed to cool at room temperature and a second conductivity reading taken. Membrane leakage was quantified by working out the ratio(%) of electrolyte leakage in fresh and lysed leaf tissues. This was done for both stressed and unstressed leaves.

4.2.4. Transpiration rates under drying soil conditions

From each treatment per species, four seedlings of almost equal sizes were selected and left to freely transpire under normal glass house conditions. Water content of the soil was brought almost to container capacity and each pot sealed to prevent direct loss of water from the soil. After this, water was withheld from all the pots until all the seedlings withered and dried.

Daily transpiration rates of all the seedlings were measured by weighing the pots each morning between 8.00a.m. and 8.30a.m. using electronic balance (Model AND FX 200, A&D Company Ltd. Tokyo, Japan). Transpiration rates were expressed as grams/day (gd^{-1}). The daily water loss were plotted against time (days).

4.2.5. Drought Tolerance

To measure survival under drought, 20 seedlings per treatment per species were left un-watered. The time taken by half of the seedlings to die was recorded (LD_{50}). That is number of days from onset of withholding water for 50% of seedlings to die. This was used as a measure of drought tolerance.

4.3.0. RESULTS

4.3.1. Leaf electrolyte leakage

Results of electrolyte leakage for *A. tortilis* and *A. xanthophloea* seedlings previously subjected to four levels of water stress i.e. 0,2,4 and 6-day repetitive drought stress with 1-day re-hydration are shown in Table 3. The percentages indicate the level of leakage in relation to the total electrolyte content of the tissue cells.

Electrolyte leakage increased with increasing stress duration for both *A. tortilis* and *A. xanthophloea* seedlings. The highest cell leakage was observed in seedlings that had been previously subjected to 6-day repetitive drought i.e. 27.7% and 26.7% leakage for *A. tortilis* and *A. xanthophloea* respectively, while the least leakage was observed for the controls, 16.7% and 17.9% for *A. tortilis* and *A. xanthophloea* respectively. There was no significant ($p>0.05$) difference in electrolyte leakage between the controls of the two species. Intermediate values of electrolyte leakage were obtained for the 2 and 4-day droughted seedlings as shown in table 3.

Subjecting the leaves to 6-hour air-drying (dehydration) resulted in significant increase in electrolyte leakage in the controls of the two species while the preconditioned seedlings were not significantly affected (Table 4). A general decrease in cell leakage was observed in the two *Acacia* species, with increasing previous drought exposure (hardening) period. With 6-hour dehydration process, control seedlings had the highest cell leakage in both species 27.8% and 33.3% for *A. tortilis* and *A. xanthophloea* respectively. *A. xanthophloea*, however showed significantly ($p<0.05$) higher cell leakage than *A. tortilis*.

Table. 3. Electrolyte conductivity (electrolyte leakage) as a measure of the level of membrane damage due to drought in *A. tortilis* (A) and *A. xanthophloea* (B) seedlings. The controls were grown under adequate water supply while the other treatments were effected by repetitive withholding of water for 2, 4 and 6 days and 1- day re-watering to container capacity for 6-months. The percentages (%) indicate the level of electrolyte leakage in relation to the total electrolyte content of the tissue cells. trt= treatment, where 1= control, while, 2, 3 and 4 represent 2, 4 and 6 day repetitive droughts respectively.

	EC (μscm^{-1}) readings before autoclave		**EC (μscm^{1}) readings after autoclave**		**% electrolyte leakage**	
Treatment	***A***	***B***	***A***	***B***	***A***	***B***
1	0.03	0.12	0.18	0.67	16.7	17.9
2	0.02	0.1	0.26	0.48	17.7	20.8
3	0.06	0.13	0.35	0.64	17.1	20.3
4	0.1	0.16	0.36	0.6	27.7	26.7

Table.4. Results of electrolyte leakage (Electrolyte Conductivity) of 6-hour air-dried (dehydrated) leaves of *A. tortilis* (A) and *A. xanthophloea* (B) seedlings at various stress levels. trt= treatment, where 1= control, while, 2, 3 and 4 represent 2, 4 and 6 day repetitive droughts respectively.

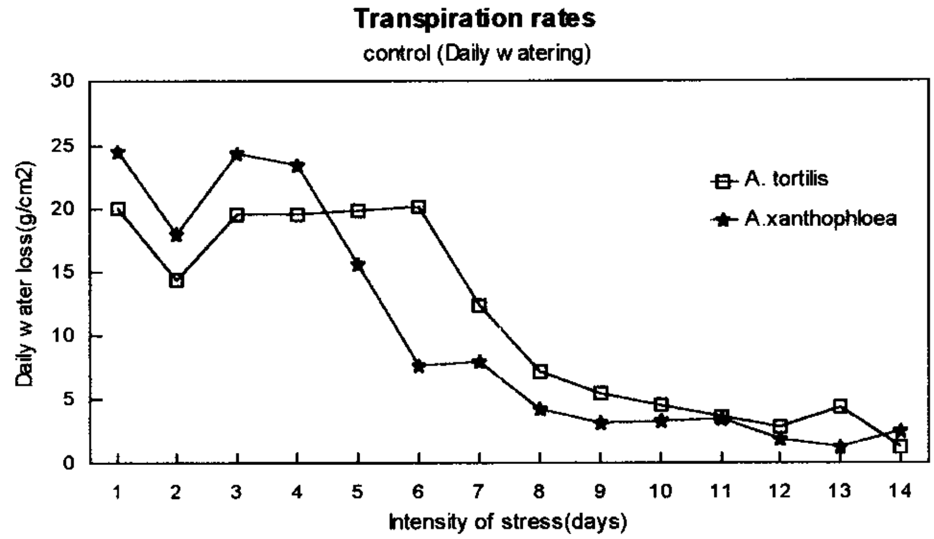
	EC(µscm^{-1})readings before autoclave		EC readings(µscm^{-1} after autoclave		% electrolyte leakage	
treatment.	*A*	*B*	*A*	*B*	*A*	*B*
1	0.05	0.3	0.18	0.9	27.8	33.3
2	0.12	0.18	0.45	0.52	26.7	34.6
3	0.08	0.2	0.48	0.82	16.7	24.4
4	0.03	0.18	0.21	0.77	14.2	23.4

4.3.2. Transpiration rates with decreasing soil moisture content.

Plots for mean daily transpiration rates for the controls and 6-day droughted seedlings of *A. tortilis* and *A. xanthophloea*, are shown in Fig. 7. Under non-limiting soil water conditions, the two species showed relatively higher transpiration rates. Transpiration rates were, however, higher in the controls compared to seedlings that had been previously subjected to 4 and 6 day repetitive drought.

In all the treatments, *A. xanthophloea* showed significantly ($p<0.05$) higher transpiration rates during the first four days compared to *A. tortilis*. However, *A. tortilis* maintained relatively uniform transpiration rates over longer periods of time than *A. xanthophloea*. A sharp decline in daily transpiration rates was observed between day 4 and 10 for *A. xanthophloea* in all the treatments while this started two days later in *A. tortilis*. After day 14 both drought conditioned and the controls showed similar low transpiration rates for the two species.

LD_{50} (i.e. the number of days taken by half the number of seedlings subjected to water stress by completely withholding water to die) was used to monitor plant tolerance to drought stress. Table 5, shows the results obtained when both *A. tortilis* and *A. xanthophloea* seedlings previously subjected to varying water regimes were stressed. The figures show the number of days taken by half (10 seedlings) the total number of seedlings in each treatment to die off. *A. tortilis* seedlings in all the treatments survived significantly ($p<0.05$) longer than seedlings of *A. xanthophloea*, suggesting that *A. tortilis* could be more drought tolerant than *A. xanthophloea*. There was no significant difference (p>0.05) between the seedlings of *A. tortilis* that were watered daily and those conditioned to two-day cyclic drought. Intense cyclic stress, however, showed a significant effect on seedling survival as the seedlings survived for longer days (31) without water. Conditioning seedlings for 4 and 6 days without water, however, improved survival in *A. xanthophloea* by only two days, suggesting that drought conditioning had greater effects on seedlings of *A. tortilis* than those of *A. xanthophloea*.



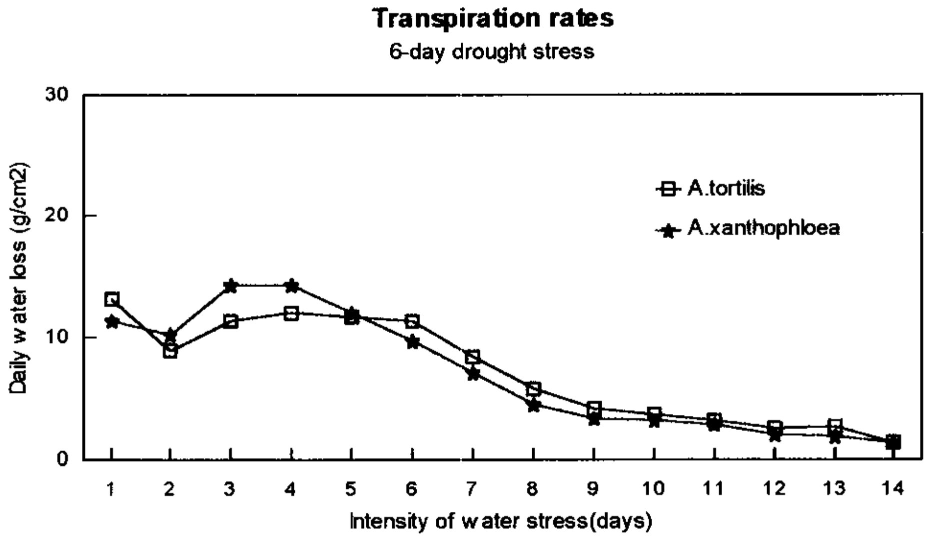


Fig.7. Mean daily transpiration rates (g/cm^2) of 7-month old seedlings of *A. tortilis* and *A. xanthophloea*. The controls were watered daily while the stressed seedlings received 6-day soil drought with 1 day re-hydration to container capacity prior to finally watering all the seedlings to container capacity and completely withholding water as daily weight loss of the dehydrating potted seedlings were taken between 8.00 a.m. and 8.30 a.m.

Table:5 LD$_{50}$ as an index of drought tolerance in *A. tortilis* and *A. xanthophloea.* Control seedlings were grown under full irrigation then water withheld while drought treated seedlings received 2, 4 and 6 day dehydration and 1-day re-hydration cycles prior to finally withholding water. 1= control, while, 2, 3 and 4 represent 2, 4 and 6 day repetitive droughts respectively.

	LD$_{50}$	
Treatments	*Acacia tortilis*	*Acacia xanthophloea*
1	27	22
2	27	23
3	31	24
4	31	24

4.4.0. DISCUSSION

During drought, changes in membrane structure and function occur (Levitt 1980, Zwiazek and Blake 1990a), resulting into efflux of solutes including electrolytes from the cytoplasm (Levitt 1980). The level of electrolyte leakage is proportional to the level of injury (Palta *et al.* 1986), and can be used to detect the level of tissue membrane damage by drought stress (Zwiazek and Blake 1990b). In this study, a positive correlation was established in both species, a correlation between stress intensity and electrolyte leakage, which indicates that membrane damage was as a result of water stress.

Desiccation tolerant plant species are likely to experience less membrane damage hence loose less electrolytes during drought compared to less tolerant ones (Martin *et al*. 1987). For example, Tan and Blake 1993 found that fast growing black spruce progenies experienced less electrolyte leakage during drought and were considered more desiccation tolerant than the slow growing ones. In the current study, there was no significant difference ($p<0.05$) in electrolyte leakage between the two *Acacia* species when re-hydrated tissues were tested for membrane damage at all the four levels of water stress cycles to which the seedlings were previously subjected. This could suggest that the two species either inherently had the same level of membrane integrity, that is, same ability to tolerate water stress or that they developed tolerance abilities during the previous dehydration cycles (preconditioning) which enabled them to more or less cope with the stress levels.

Subjecting the leaves to 6-hour air-drying (dehydration), however, revealed a significant variation in electrolyte leakage between the two species. The high electrolyte leakage experienced by the controls could be attributed to severe damage to the membranes as a result of drought. Compared to the controls, the insignificant change in the electrolyte leakage in the four and six-day droughted seedlings when the leaves were subjected to 6-hour drying, strongly suggested that preconditioning improved desiccation tolerance and species survival during drought in the two *Acacia* species. Other studies, for example. Zwiazek and Blake (1990a), found out that preconditioned seedlings of *Picea marina* were drought tolerant and hence leaked less electrolytes than the non-conditioned ones.

Past studies on drought tolerance and species growth rate have revealed contradicting results. For example, fast growing black spruce progenies experienced less electrolyte leakage during dehydration and were regarded to be more drought tolerant than slow growing ones (Tan and Blake 1993). A similar conclusion was reached by Lemcoff *et al.*(1994) on fast and slow growing *Eucalyptus* progenies. However, Lediges (1974) found that fast growing progenies of *Eucalyptus* species were more prone to death during drought compared to slow growing ones. In the present study, the fast growing *A. xanthophloea* experienced high electrolyte leakage during the six hour dehydration at all stress levels than the slow growing *A. tortilis* and was considered less desiccation tolerant. This was also supported by the

LD_{50} results (Table 5) in which *A. tortilis* survived longer periods without water than *A. xanthophloea* despite the later having much higher absolute transpirational water loss at all levels of water stress (Fig.7).

Previous studies have suggested the role of turgor maintenance (osmotic adjustment and elastic adjustment) in desiccation tolerance and growth under drought (Blake *et al.* 1991, Dale and Scutliffe 1986). Cell walls of inelastic cells may rapture when subjected to drought if the protoplast is unable to separate from the cell wall (Dale and Scutliffe 1986), leading to electrolyte leakage out of the cells. More elastic tissues, however, can shrink under drought to maintain turgor pressure, which helps to prevent membrane damages (Tyree and Jarvis 1982). Studies on cell sap concentration changes in stressed tissues have found that accumulation of water compatible solutes in osmoregulating tissues tend to stabilise the native structure of macromolecules during dehydration (Leopold and Vertucci 1986). This would reduce dehydration damage to the membranes and promote survival under drought (Tan and Blake 1991). Preconditioning resulted into osmotic adjustment in *A. tortilis* while *A. xanthophloea* exhibited cell wall elasticity (Chapter 3). The two turgor maintenance processes could have contributed to the improved drought tolerance with increasing stress cycles. The magnitude of electrolyte leakage in stressed leaves of *A. tortilis* was much less compared to *A. xanthophloea* and could be attributed to the combined processes of inherently elastic tissues in *A. tortilis* and its ability to osmotically adjust when subjected to severe water stress. However, the reduced electrolyte leakage in preconditioned seedlings of *A. xanthophloea* could be attributed to the small tissue elastic adjustment observed during conditioning.

According to Henckel (1964), protoplasmic elasticity plays a positive role in endurance to dehydration. He suggested that rapid dehydration probably causes mechanical injuries to the protoplasm when cells lose water leading to cell and whole plant death and that the higher the protoplasmic elasticity the less sensitive are plants to such effects. The inherently inelastic *A. xanthophloea* (Chapter 3) experienced early death as a result of intense drought as shown by LD_{50} (Table 5). Drought conditioning which resulted into smaller elastic adjustment (Table 1, Chapter 1) could help explain the extended survival of the conditioned seedlings of *A.xanthophloea* by two days.

Early seedling death (i.e. low LD_{50}) observed in some provenance of *A. tortilis* was attributed to high transpiration rates (Kiplimo 1993). Although *A. xanthophloea* experienced higher initial absolute transpiration rates compared to *A. tortilis*, the species was capable of avoiding excessive water loss through leaf shedding. This could have led to the sharp decline in the rate of transpirational water loss after the fourth day of stress as opposed to *A. tortilis* in which this decline occurred after six days of water stress (Fig.7). Differences in transpiration rates could therefore be only a peripheral factor in determining survival in the two *Acacia* species.

4.5.0. CONCLUSIONS

The more tolerant *Acacia tortilis* survived longer periods of intense water stress compared to *A. xanthophloea* (Table 5). This suggested that this could be partly attributed to its inherent tissue elasticity. This is in addition to osmotic adjustment which occurred during conditioning (Chapter 3) and high root:shoot ratio. *A. tortilis* also maintained higher transpiration rates over extended periods of moisture stress than *A. xanthophloea* (Fig.7). This could be important for its survival, as it was capable of growing under moderate stress.

From this study the linear relationship that existed between species survival under drought (LD_{50}), dehydration tolerance (membrane leakage) and dehydration cycles (stress level), strongly suggest that preconditioning improved the ability of seedlings to tolerate drought and could improve seedling establishment during transplanting in the arid environments. The study also demonstrated that slow growing tropical *Acacias* could be more drought tolerant than the fast growing ones. Both tissue elasticity and osmotic adjustment could be more important for survival under drought.

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSIONS.

The study showed that under adequate water supply, *A. xanthophloea* grew faster than *A. tortilis*, suggesting that *A. xanthophloea* is a fast growing genotype compared to *A. tortilis*. With increasing water stress, *A. xanthophloea* showed increased leaf senescence and shedding compared to *A. tortilis*, however, root:shoot ratio remained constant at 0.5 even in repeatedly water stressed seedlings suggesting that *A. xanthophloea* had no ability to adjust root:shoot ratio when repeatedly stressed. Root:shoot ratio, however, increased to 1.50 in the water stressed seedlings of *A. tortilis* compared to 0.5 observed in the non-stressed seedlings. It was also observed that both stressed and non-stressed seedlings of *A. xanthophloea* consistently had higher leaf area:root dry weight ratio compared to *A. tortilis* in which the ratio declined with increasing water stress.

With repeated water stress, *A. tortilis* seedlings showed osmotic adjustment. Osmotic adjustment, however, was not observed in repeatedly stressed seedlings of *A. xanthophloea* but they showed some small adjustment in cell wall elasticity. Screening seedlings for drought tolerance by completely withholding water showed *A. tortilis* surviving longer periods without water than *A. xanthophloea* and that repeatedly stressed seedlings had longer life than non-stressed seedlings when water supply was completely withheld.

From this study, the observed fast growth and high total dry weight accumulation in *A. xanthophloea* could be attributed to water availability, large total leaf area and high stomatal conductance. Most of the biomass was also apportioned to the shoot which led to the high shoot dry weight accumulation due to high photosynthetic capacity as a result of large leaf area. These characteristics can, however, be detrimental to plants during water stress (Turner 1979). For example, the low LA:RDW observed for *Robinia Pseudoaccacia* and *Ulmus pervifolia* were important for their survival under drought (Ranney *et al.* 1990). Leaf abscission greatly reduces transpiration and can be an important mechanism in avoiding the development of extreme water loss leading to low shoot xylem water potential (Kozlowsky 1976). The high transpiration rates in *A. xanthophloea* were most likely associated with high stomatal conductance and could have led to high carbon assimilation hence high dry mass accumulation and fast growth rate under adequate water supply. Similar conclusions, have been reached by Kozlowski (1982). However, the inability of this species to alter carbon allocation with increased water stress could likely make it more vulnerable to drought. As observed, *Eucalyptus* clones which apportioned more dry mass to the shoots were most affected under water stress (Leroux *et al.* 1996). The low root and high shoot biomass would mean less water absorption and high transpirational water loss (Kozlowski 1982). However, *A. xanthophloea* could survive severe water deficit through excessive leaf shedding. The observed leaf shedding in *A. xanthophloea* is likely to be an adaptation for dehydration postponement during water stress, reducing the rate of transpirational water loss and maintaining xylem water potentials and turgor over longer periods during water stress, which is a vital strategy for plant growth during water stress.

Elastic adjustment as observed in *A. xanthophloea* could be important in cell recovery after alleviation of water stress. Similar observations, were made by Blake *et al.* (1991). It could therefore be suggested that adjustments in cell wall elasticity played a role in withstanding drought in *A. xanthophloea* and could be the reason for its rapid recovery after alleviation of water stress as the species was observed to generate new leaves just a few days after re-watering.

The slow growth rate in *A. tortilis* could be attributed to the possession of inherent dehydration postponement strategies such as low LA., high r:s, and low transpiration rate (Turner 1979). All these features are bound to reduce the photosynthetic capacity of the plant. The slow growth could be further compounded by dehydration tolerance characteristics of osmotic adjustment which appeared to divert photosynthates from growth processes to turgor maintenance during declining soil water (Munns 1988). Despite slow growth, possession of these qualities could be the reason why this species was able to survive longer periods without water than *A. xanthophloea.* It may be concluded that most adaptations that help plants to tolerate drought are likely to lead to reduced growth. However, it is suggested that tissue elasticity could have contributed to the observed limited tolerance in repeatedly stressed seedlings of *A. xanthophloea*, and their quick recovery from water stress, especially after re-watering. However, the adjustment was limited, resulting into large reduction in growth as a result of water stress compared to *A. tortilis.*

Several authors have reported increased growth rates during drought in osmotically adjusting tissues (Kozlowski 1982, Lemcoff *et al.* 1994, Tan and Blake 1992, White *et al.* 1996). Tan and Blake (1992), suggested that osmotic adjustment promote growth and survival during and after water stress. In their view, fast growing plant progenies are more likely to osmotically adjust under drought, hence, can withstand high levels of dehydration during drought than the slow growing progenies. Munns (1988) however, indicated that solute accumulation that occur during osmotic adjustment are metabolites that are derived from photosynthesis. It is, therefore, questionable how a process that diverts metabolites from growth process can maintain growth especially under water stress. The current study indicated that *A. tortilis* had a slow growth rate, low leaf area (Chapter 2) and was able to osmotically adjust under repeated stress (Chapter 3). The slow growth rate observed in *A. tortilis* could be as a result of its inherent adaptations to overcome water stress. The species appeared to be predetermined to survive in severe environments where water is limiting.

This study, therefore, strongly suggests that processes which aid in survival under drought are detrimental to growth and are likely to lead to reduced growth rates in plant species. Since most mechanisms that resulted into improved survival under drought likely resulted into reduced growth and dry weight accumulation, it appeared that slow growing arid land *Acacia* species, could be more drought tolerant and capable of surviving severe stresses that are associated with such environments. The results agree with previous findings in which osmotic adjustment appeared to be an adaptation for surviving drought stress rather than promoting growth during drought (Munns 1988). Certain solutes that are associated with osmotic adjustment such as proline (Munns 1988), could foster the recovery of the plant when the soil is re-hydrated and only help to maintain turgor in a dehydrated plant tissue.

Drought induced reduction on growth rate in the two *Acacia* species was probably as a result of morphological and physiological adaptations and their effect on growth. However, it appears that survival and growth under drought are determined by the integration of several drought tolerance characteristics. It could therefore be suggested that slower-growing tropical *Acacias* are likely to be more drought tolerant than the faster-growing species.

Pre-conditioning the seedlings contributed to improved drought tolerance in the two species. For example, preconditioned seedlings of *A. tortilis* survived four days longer compared to unconditioned seedlings, when water was completely withheld. Conditioned *A. xanthophloea* seedlings, however, survived only two days longer compared to the controls. Although osmotic adjustment, high cell elasticity

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6.0.0. CHAPTER SIX

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(low ✱) and the ability to increase cell wall elasticity appears important for drought tolerance under low tissue water potentials, osmotic adjustment seems to be an overriding factor in turgor maintenance in drought tolerance compared to elastic adjustments. However, inherent drought tolerance ability seems to play a major role in long-term survival and growth in the two species than mere adjustments to short-term repeated stress.

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