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**THE EFFECT OF SUN AND SHADE  
ON THE LEAVES OF FOUR COASTAL  
TREE SPECIES**

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## **PREFACE**

The experimental work described in this thesis was carried out in the Department of Biology, University of Natal, Durban, from January 1990 to January 1992, under the supervision of Professor Norman Pammenter.

The studies represent original work by the author and have not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text

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### **ABSTRACT**

*Mimusops caffra*, *Euclea natalensis*, *Olea woodiana* and *Peddiea africana* are tree species associated with different successional stages in a coastal dune forest. Saplings of these tree species were established in four different light intensities. These were full sun, 40% shade, 70% shade and 90% shade. The hypothesis proposed that the species from different successional stages are preadapted for a particular light environment and are disadvantaged in other light environments. Growth, morphological, biochemical and physiological aspects of the four species in the four light environments were determined.

Growth rates showed no consistent pattern with respect to light intensity. However, most species, irrespective of their successional status, had the best growth response in either 40% or 70% shade treatments. All the species showed typical sun and shade responses for morphological, anatomical and some biochemical characteristics. Photosynthetic responses were complex and showed no relationship between the successional status of the species and the light conditions in which they were grown.

There appears to be very little relationship between the growth responses, the measured biochemical and morphological aspects, assimilation rates and the successional status of the species.

Light intensity is therefore not the sole driving force of forest succession but one of the many factors that contribute to the overall process.

## TABLE OF CONTENTS

	PAGE
<b>1. INTRODUCTION</b>	
1.1. Aim of the Project	1
1.2. Succession	2
1.3. The Light Environment	7
1.4. Adaptation to a Changing Light Environment	8
1.5. Details of this Investigation	15
1.5.1. The tree species	15
1.5.2. Methods common to all experiments	16
 <b>2. GROWTH, ARCHITECTURE AND LEAF MORPHOLOGY</b>	
2.1. Introduction	18
2.2. Methods	26
2.3. Results	28
2.3.1. Growth	28
2.3.2. Plant architecture	35
2.3.3. Leaf morphology	38
2.4. Discussion	44
 <b>3. BIOCHEMISTRY AND PHYSIOLOGY</b>	
3.1. Introduction	53
3.2. Methods	67
3.3. Results	71
3.3.1 Chlorophyll analysis	71
3.3.2. Nitrogen analysis	73
3.3.3. Photosynthetic light response	80
3.3.4. The response of assimilation to intercellular CO <sub>2</sub> concentration	86
3.4. Discussion	89
 <b>4. CONCLUSIONS</b>	97
<b>5. REFERENCES</b>	103

## CHAPTER ONE

### INTRODUCTION

#### 1.1. AIM OF THE PROJECT

The aim of this project was to determine the effect of growth under sun and shade conditions on the saplings of trees from various successional stages within a coastal dune forest.

The following hypothesis was proposed: saplings of species from different seral stages of forest succession show a gradient in light requirement from the sun adapted forest pioneers to the shade adapted "climax" canopy and understorey species. The species from a particular seral stage are preadapted to that light environment and perform less well in any other light environment.

The hypothesis was tested by laboratory studies on species from forest pioneer, mid succession and climax stages.

The following questions were investigated:

- (i) What are the effects of sun and shade on growth, plant architecture and leaf morphological characteristics?
- (ii) What are the effects of sun and shade on biochemical characteristics such as the chlorophyll content and leaf nitrogen content?
- (iii) What are the responses of carbon assimilation to light and intercellular CO<sub>2</sub> concentrations?

## 1.2. SUCCESSION

The term succession is used to describe many types of vegetation changes in both space and time. Understanding the process of succession is important for two reasons - the value of the concept in the development of ecology, and its potential for the development of programmes for rehabilitation. However, succession is a highly controversial issue, and although recent work has clarified the variety of patterns and mechanisms involved in succession (e.g. Connell and Slatyer, 1977), no general theory based on processes common to all successions exists (Finegan, 1984). Successional theories are further complicated by inadequate field data which rarely provides insight into the mechanism of successional change.

Before the existing successional theories can be discussed, it is necessary to consider the successional strategies used by plants.

### Successional Strategies

One of the oldest and most widely accepted generalisations in plant ecology is the set of characteristics used to separate early from late successional species. The critical features are the traits which confer competitive success in early succession compared with those which confer success in late succession.

Generally early successional plants are small, short lived and reach reproductive maturity quickly. These plants produce many small seeds which are dispersed over large distances by wind, bats and birds. Photosynthesis by these plants shows saturation at high light intensities, high light compensation points and high rates of CO<sub>2</sub> assimilation, and low efficiency under low light intensities. Early successional plants are able to recover rapidly from nutrient stress (Huston and Smith, 1987).

Late successional plants grow more slowly and live longer. Mature plants produce a few large seeds which are dispersed over short distances by gravity or mammals. Recovery from nutrient stress is slow. Shade tolerant species function efficiently in low light intensities where rates of photosynthesis, light saturation intensities and light compensation points are lower than in high light intensities (Huston and Smith, 1987).

As succession progresses, so the environment is altered. Primary successional plants establish in environments with limited interspecific competition, but which have high light intensities and high temperatures produced by short wave radiation on exposed sites (Huston and Smith, 1987), low soil nutrient availability particularly nitrogen and phosphorous, low water availability and little organic matter (Finegan, 1984). Tolerance or avoidance of these environmental conditions is a critical factor in the establishment of pioneer species (Levitt, 1972).

Pioneer species cause environmental changes which may alleviate constraints on the establishment of later successional species. An often overlooked effect of the presence of a pioneer species is their role in seed dispersal. The seeds of pioneer trees are either wind dispersed e.g. pines, maple, birch and willow (Schopmery, 1974), or bird dispersed e.g. pioneer tropical trees (Hartshawn, 1980). Seed dispersal of later successional species, on the other hand, is often vector specific e.g. acorns, a major component of the diet of jays, are buried by the birds in forests (Bossem, 1979) or the fleshy fruit of *Prunus* and *Ceropia* which are dispersed by frugivorous birds (Schopmery, 1974). Pioneer trees provide suitable habitats in which these dispersal vectors may roost.

Other environmental effects include shading, and an increase in nitrogen and organic matter, the factors often considered to facilitate the establishment of other species (Finegan, 1984). Shading enables the establishment of the shade tolerant species, while organic matter



accumulation on the soil surface, increases the retention of moisture in those zones affecting seeds and seedlings (Finegan, 1984).

Although later successional plants establish in less "adverse" environments, competition between individuals for resources, including light, soil nutrients and water, exists. Each species has its own requirements and optima for these factors, which will determine how well it performs under any set of environmental conditions. Plants alter the biotic environment in such a way that the relative availability of these resources changes, and the criteria for competitive success is altered. In order to survive, an individual must have a competitive advantage, normally costly physiological and morphological adaptations, which prevents any species from being optimally adapted to all conditions (Huston and Smith, 1987). Therefore there is no such thing as an absolute competitive advantage. For example an opportunistic species that produces abundant seeds and which grows rapidly, suppressing and shading a "superior" competitor, is the superior competitor in that bout of competition (Grime, 1973). However, as succession normally involves more than one species and as the environment is altered, the dominant species will be replaced in time.

### Theories of Succession

Two extreme theories of plant succession exist, namely holism and reductionism (Finegan, 1984).

i) The holism theory, developed by Clements (1916), considers succession to occur in developmental stages through which the vegetation passes, until it reaches the ultimate state of equilibrium with the climate and major geological factors in the area. Autogenic change, caused by the presence of vegetation, is the driving force of holism succession, as it makes the environment suitable for the next group of species and therefore facilitates succession. This occurs in stages through wavelike invasions by groups of species into an altered environment and is termed the facilitation

hypothesis. The holism theory emphasises the development of "biological control" of nutrient cycling in such a successional process. Succession is the process of development of an ecosystem of maximum stability (by the yardstick of resistance to disturbance) and of maximum efficiency in the utilisation of resources. Successional change is considered orderly and predictable (Finegan, 1984).

ii) Criticisms about the lack of applicability of the holism theory has led to the development of a reductionist theory, based on Gleason's (1939) interpretation of the plant community as an assemblage of species populations each with a unique behaviour. Several alternative successional pathways within a given vegetation type are possible. Succession is therefore the sequential dominance of the site by species with different life histories, growth rates and sizes at maturity. The facilitation hypothesis is rejected and autogenic changes are seen as neutral or inhibitory rather than the driving force of succession. Resource availability decreases as species densities increase. Pioneer species are opportunistic, exploiting environments free of competition, while later successional species are conservative and efficient in their use of resources (Finegan, 1984).

Although the reductionist approach has given great impetus to plant ecology, the theory is based on a reinterpretation of existing data. The theory neglects the nature of seed dispersal and tends to generalise about environmental change. There is a need to develop a third approach based on observational and experimental techniques of plant population biology (Finegan, 1984).

A third model developed by Huston and Smith (1987), is based on three main premises: i) competition between individuals for resources exists in all plant communities but these may change with time, ii) plants alter the availability of resources and therefore the criteria for competitive success

and iii) physiological and energetic constraints prevent any species from maximising competitive ability in all environmental conditions.

Huston and Smith (1987) view succession as plant by plant replacement where interactions occur amongst individuals not populations. Each individual has life history parameters identical to all other plants of that species, but its growth in any particular environment is determined by the conditions it experiences as a result of competition with other individuals. Changes in resource levels (light and nutrients) that lead to changes in species dominance are autogenic. Finally, competition is considered to be non equilibrium. Light availability, light response curves (based on shade tolerance), mortality rates and life history traits are modelled. The authors feel that such models, based on competition amongst individuals, various combinations of life history traits and physiological traits, can produce the great variety of population dynamics found in natural successions and will lead to a better understanding of the phenomena.

### Forest Succession

Succession is normally described as a directional change with time of the species composition of a single site were the climate remains relatively constant (Finegan, 1984).

One of the driving forces in forest succession is the changing light environment caused by shading by the forest canopy (Bazzaz, 1979; Bazzaz and Pickett, 1980; Finegan, 1984; Huston and Smith, 1987). Forest trees can be broadly divided into pioneer and forest species based on shade tolerance. Saplings of pioneer species normally establish in open sites and are shade intolerant. Saplings of forest trees which establish under the forest canopy are shade tolerant, but those trees which mature into canopy species will have to develop a sun tolerance during their life cycle (Finegan, 1984).

Light conditions in a forest understorey are highly variable. Sunflecks punctuate the low levels of diffuse light and although they may occur at any one spot for a small fraction of the day they can contribute up to 80% of the total daily photosynthetic photon flux density (PPFD) (Björkman and Ludlow, 1972). On a more widespread scale, occasional tree fall exposes understorey species to high light intensity and thus promotes "gap phase" succession in which certain species respond and develop into canopy trees. The ability of understorey species to respond to canopy openings depends on their ability to endure sudden exposure and to take advantage of increased water, nutrients and space (McGee and Hooper, 1970).

Huston and Smith (1987) list maximum growth rate, maximum size, maximum longevity, maximum rate of sapling establishment and shade tolerance as the important traits in competition amongst trees. Bazzaz (1979) considers the degree of shade tolerance and the arrangement of the foliage and branching patterns to be important in determining successional sequences in a forest. These concepts are discussed in more detail in subsequent chapters.

### **1.3. THE LIGHT ENVIRONMENT**

Plants convert radiant energy into stored chemical energy by the process of photosynthesis. Radiation between 400 and 700nm is photosynthetically active radiation.

Irradiance varies seasonally, diurnally and spatially, but two extremes for the light environment exist. These are the deep shade conditions of tropical forest understoreys and the full sunlight conditions of open areas and tops of canopies. The light environment within a forest is discussed below.

## The Forest Light Environment

Within a mature forest a light gradient exists with respect not only to the light intensity but also the spectral composition of the light.

The shadow cast by the canopy trees causes a decline in the intensity of radiation reaching the forest floor. This radiation is of two types; continuous diffuse, low flux radiation and intermittent bursts of direct radiation which are termed sunflecks (Björkman and Ludlow, 1972). This diffuse low flux radiation may have values for radiant energy as low as 0.1-1.9% of the total radiation (Ashton, 1958; Evans, 1939; Leigh, 1975).

As the light passes through the forest canopy different wavelengths are preferentially absorbed. This results in light with a high proportion of far-red and near infra-red as well as a higher proportion of green light relative to blue and red reaching the forest floor (Bazzaz and Pickett, 1980; Evans, 1939; Jordan, 1969).

### **1.4. ADAPTATIONS OF PLANTS TO A CHANGING LIGHT ENVIRONMENT**

Photosynthetic energy capture, which provides plants with chemical energy and is therefore central to their ability to compete and reproduce, is influenced by the amount of light striking the leaves of a plant.

Much research has been conducted on the effect of different levels of irradiance on photosynthesis and how the leaf and plant traits which develop in these conditions influence a plant's photosynthetic response to that light level. Comparative studies on the photosynthetic responses and leaf characteristics of plants grown under high and low levels of irradiance have provided insight into the significance of several leaf traits seen in plants adapted to sunny or shady conditions (Björkman, 1981; Boardman, 1977).

Most of these studies have been conducted on individual leaves, yet whole plant energy capture depends not only on the photosynthetic potential of an individual leaf but also on the geometry and dynamics of the plant canopy and the pattern of energy allocation among all the organs (Givnish, 1988). Many traits characteristic of sun versus shade leaves entail energetic costs involving non-photosynthetic organs or influence the physical environment experienced by other leaves and the significance of such traits is difficult to understand if energy capture is considered at the leaf level only.

It is necessary to consider sun and shade traits and attempt to determine in what way these traits may be considered to be adaptive.

### Sun and Shade Adaptations

Plants are classified into sun and shade plants depending on their adaptability to a particular light intensity (Björkman, 1968a). This adaptability is inherited and results from genetic adaptation to the light environment prevailing in the native habitat. Several features of plant architecture, physiology and resource allocation vary between sun and shade plants. These are summarised in Table 1.1.

Differences in the morphology, anatomy, biochemistry and light responses of sun and shade plants, can be attributed to adaptations for efficient light utilisation in the different light intensities.

The thick leaves of sun plants are considered to have three functions: (i) to protect the lower cell layers from the damaging effects (photoinhibition and chlorophyll bleaching) of high light intensity (Björkman and Holmgren, 1963). (ii) To increase the amount of photosynthetic apparatus (Rubisco and electron carriers) per unit leaf area (Björkman *et al.*, 1972; Goodchild *et al.*, 1972). As rubisco is considered a rate limiting enzyme of photosynthesis,

sun plants need high levels in order to attain high assimilation rates. (iii) To increase the mesophyll cell surface to leaf area ratio (Nobel *et al.*, 1975). This would increase the surface area available for the diffusive transfer of CO<sub>2</sub> from the intercellular spaces to acceptor sites in the mesophyll cells (Nobel *et al.*, 1975). Gaastra (1959), attributed the high photosynthetic

Table 1.1. Characteristic differences between plants adapted to sunny versus shaded habitats. (Derived from Boardman, 1977; Björkman, 1981; Givnish 1987).

LEVEL	SUN PLANTS	SHADE PLANTS
1. PLANT LEVEL	High reproductive effort	Low reproductive effort
2. CANOPY LEVEL	Erect twigs High to low leaf area index	Horizontal twigs Low leaf area index
3. MORPHOLOGY	Small leaves Erect leaves Thick leaves: more palisade, longer cells Thick cuticle, often pigmented Many small stomata	Large leaves Horizontal leaves Thin, broad leaves: less palisade, shorter cells Thin cuticle Few large stomata
4. ANATOMY	Small chloroplast's Few small grana High mesophyll cell surface:leaf area ratio	Large chloroplast's More grana Low mesophyll cell surface:leaf area ratio
5. BIOCHEMISTRY	Less chlorophyll High chlorophyll a:b More NADP High RUBISCO activity High nitrogen content	More chlorophyll More chlorophyll b Less NADP Low RUBISCO activity Low nitrogen content
6. LIGHT RESPONSE	High dark respiration rates High light compensation point High assimilation at saturation High saturation irradiance	Low dark respiration rates Low light compensation point Low assimilation at saturation Low saturation irradiance

rates of sun plants at light saturation, to a low resistance to  $\text{CO}_2$  diffusion from the external air to the chloroplast and to an increased activity in the enzymes involved in the biochemical process.

The factors which limit the photosynthetic rate in a low light environment are different to those that limit the rate in a high light environment. Plants growing in a low light environment must trap the available light and convert it to chemical energy with the highest possible efficiency. Respiratory losses and the cost of maintaining the photosynthetic system relative to the gain in photosynthate production must be kept as low as possible. This is achieved in a number of ways: Shade plants have large, horizontal leaves which are able to present a larger surface area for the capture of light. The thin leaves of shade plants ensures that the light is better able to penetrate the leaf and thus be captured and utilised by the chloroplasts. The efficiency by which light is absorbed by the leaves depends on the chlorophyll content. The high chlorophyll content in the leaves of shade plants confers a significant advantage in low light conditions (Björkman, 1968b). Shade leaf chloroplasts have a greater development of grana and a smaller proportion of stroma thylakoids than sun leaf chloroplasts (Björkman *et al.*, 1972; Goodchild *et al.*, 1972). The grana in shade plant chloroplasts are irregularly arranged, not in a single plane as they usually are in sun plant chloroplasts. This is thought to be a means for these plants to attain a high chlorophyll content per leaf area despite a low ratio of chloroplast volume to leaf area (Boardman *et al.*, 1975; Goodchild *et al.*, 1972). Chlorophyll b is considered to belong to the light harvesting Chl<sub>a</sub>-protein (LHChl) complex (Thornber, 1975), which is primarily associated with Photosystem (PS) II (Butler, 1977). Shade plants may therefore have a higher ratio of PS II to PS I reaction centres than sun plants. This is thought to provide a more balanced energy distribution between the two photosystems in shaded habitats, which, because of the filtering effect of the leaves, have a high proportion of far red light which is



only effective in exciting PS I (Björkman, 1981). A low dark respiration rate helps to maintain a positive carbon balance in a shaded environment (Björkman, 1981). Low light saturation and low light compensation point ensure that shade plants are able to utilise low light for efficient photosynthesis.

### Methods of Identifying Adaptations

Three methods have been used to identify variations between sun and shade plants as adaptations to a particular irradiance level, based on convergence, correlation with photosynthetic impact and detailed cost-benefit analysis (Givnish, 1988).

Convergence of a trait amongst species from different families or orders restricted to growth under a particular level of irradiance, is considered to be evidence that such a trait is the result of natural selection. This can be applied to fixed or plastic traits. Arguments based solely on convergence are limited because they cannot identify how or why a given trait contributes to competitive ability.

A more mechanistic approach is a detailed study of the photosynthetic responses of leaves acclimated to different light levels, together with an analysis of how various features of their morphology and physiology contribute to their photosynthetic performance under those levels (e.g. Ludlow and Björkman, 1984; Nobel, 1976). This approach involves the assumptions that (i) the photosynthetic rates of leaves acclimated to a particular irradiance are greater at that level than the photosynthetic rate of leaves grown under other irradiance levels and (ii) if variation in a given trait enhances leaf photosynthesis at a specific irradiance level then it is an adaptation to that level. The first assumption is valid in extreme cases: leaves of plants grown under high irradiance levels have higher photosynthetic rates per unit area than do leaves of plants grown under low

irradiance levels (Björkman, 1981). The second assumption is harder to prove.

A third approach to identifying adaptations to irradiance levels is a cost-benefit analysis. This involves the assessment of the net effect of a trait on energy capture, balancing the impact on energy gain against the energetic costs of producing it, and then analysing which trait will maximise leaf energy capture (Givnish, 1988). Cost-benefit analysis assumes that competition in a given environment favours plants whose form and physiology maximise their net carbon gain (Givnish, 1982, 1986; Horn, 1971). A cost-benefit analysis quantifies the impact of a trait on photosynthesis. However, traits which respond to irradiance are also known to respond to other environmental factors, many of which may be correlated with irradiance levels. Clough *et al.* (1979) raised the question of whether the traits seen in sun or shade plants are adaptations to irradiance, factors correlated with irradiance or to both. It is therefore important to determine how a plant will respond if only one environmental factor were to vary.

Most of cost-benefit analyses focus on leaf photosynthesis, not whole plant energy gain, and are unable to solve the problem of optimal total investment. Investments in leaf traits and their cost involving non photosynthetic organs involve energetic trade-offs at the whole plant level. Such analyses are complex and out of the scope of this thesis, but some cost-benefits will be proposed.

#### Energetic Tradeoffs at the Whole Plant Level

Givnish (1988) outlined three basic energetic tradeoffs at the whole plant level which shape the evolution of adaptations for energy capture and the distribution of species. These involve the economics of gas exchange, the economics of support and the economics of biotic interaction.

(i) The economics of gas exchange (Givnish, 1986) arise from the link between carbon gain and water loss: any passive structure that permits the passage of large, slow moving  $\text{CO}_2$  molecules will allow the diffusion of smaller, faster moving water molecules. Therefore the photosynthetic benefit of any trait that increases the rate of  $\text{CO}_2$  diffusion into the leaf must be weighed against the energetic cost associated with increased water loss.

Tradeoffs involving the economics of gas exchange which influence both photosynthesis and transpiration include effective leaf size (Givnish and Vermeij, 1976), stomatal conductance (Cowan, 1977, 1986), leaf absorbance (Ehleringer and Mooney, 1978), leaf orientation (Ehleringer and Forsyth, 1980; Nobel 1986), leaf nitrogen content (Mooney and Gulmon, 1979) chlorophyll a/chlorophyll b ratio (Björkman, 1981; Björkman *et al.*, 1972), internal leaf architecture (Parkhurst, 1986) and leaf area index (Horn, 1971).

(ii) The economics of support arise due to the different efficiency with which leaves can be mechanically supported (Givnish, 1986). Such differences imply tradeoffs between photosynthetic benefits and mechanical costs and include aspects of leaf shape (Givnish, 1984), stem branching angles (Honda and Fischer, 1978), leaf arrangement (Givnish, 1984) and compound versus simple leaves (Givnish, 1984).

(iii) The economics of biotic interactions arise because many characteristics that enhance a plant's potential growth rate may also increase its potential attractiveness to herbivores. This implies a tradeoff between photosynthetic benefits and biotic costs (Givnish, 1986; Gulmon and Mooney, 1986).

Traits affected by the economics of gas exchange and support will be discussed in subsequent chapters.

## 1.5. DETAILS OF THIS INVESTIGATION

### 1.5.1. THE TREE SPECIES

The coastal dune forest in the Mlalazi Nature Reserve, situated on the Natal north coast at 28°58'S and 31°47'E, provides a good example of dune forest succession. Within the Mlalazi Nature Reserve there is a marked succession from bare beach, through open dune vegetation to coastal dune forest (Pammenter *et al.*, 1985). The rate of succession has been estimated as three years for the formation of a new ridge colonised by *Scaevola thumbergii*, 13 years for the establishment of open dune scrub, 70 years for the development of closed dune scrub and about 120 years for the succession from open beach to mature dune forest (Weisser and Backer, 1983).

Four tree species from different successional stages within this coastal dune forest were selected for the study.

The species were:

*Mimusops caffra* (E. May. ex. A. DC.): saplings of this species establish on the dunes on the seaward side of the forest margin. Saplings therefore establish in sandy soils with low organic matter and are resistant to salt spray and high wind speed. As a pioneer forest species the saplings must also tolerate the high light intensity associated with the open habitat. As *Mimusops caffra* is long lived it occurs in established forest as a canopy species.

*Euclea natalensis* (A. DC.): this is a canopy species with a very variable morphology which is able to establish and grow in a wide range of light conditions ranging from full sun to shade.

*Olea woodiana* (Kobl.): saplings of this species establish in shade but mature into canopy trees. This species therefore exhibits both sun and shade tolerances at different stages of development.

*Peddiea africana* (Harv.): this is a forest understorey species which establishes in shaded habitats. Plants of this species may be exposed to high light intensity as a consequence of canopy gaps or sunflecks, and therefore some degree of sun tolerance must be present. This species is characteristic of the understorey of the mature forest.

### 1.5.2. METHODS COMMON TO ALL EXPERIMENTS

#### Sapling Establishment

One year old saplings of each of the species were obtained from the farm Twinstreams (Mtumzini). Saplings were transferred to the University of Natal, Biology Department gardens. The saplings were not planted in the gardens, but remained, for the duration of the study in pots. The soil was sandy with a high humus content, but no detailed soil analysis was performed.

The saplings were established in one of four light treatments. Shade cloth was used to create uniform shade and the following light treatments were established: 0% shade (full sun), 40% and 70% shade (medium light intensity) and 90% shade (low light intensity). Five saplings of each species were randomly assigned to sun or one of the three shade treatments. Saplings were watered daily but no additional nutrients were supplied during the course of the study.

Once the saplings had been established in the four light treatments, the apical tip was marked and only new growth beyond that mark was used for experimental purposes.

## Methods and Statistical Analysis

The methods particular to each experiment are discussed in the relevant chapters and only the methods common to all experiments are discussed here.

All the results were analysed using the computer package Statgraphics (Graphic Software System, GSS \* CGI version 2.15). All data was tested for normality and one way analysis of variance (Anova) and Tukey multiple range tests were conducted within species between treatments. No statistical tests were conducted between species. Throughout the text different letters associated with values in graphs and tables indicate significant differences between the treatments at  $P \leq 0.05$ .

## CHAPTER TWO

### GROWTH, ARCHITECTURE AND LEAF MORPHOLOGY

Investigations into the effect of different light intensities during development on aspects of growth, architecture and leaf morphology are reported in this chapter. Growth and architectural effects were investigated at the whole plant level above ground, while morphology was investigated at the leaf level.

A study of the growth of the four species in the different light treatments aids in quantifying the overall effect of the light on the plants. Plant architecture deals with changes to the growth form in response to a particular light intensity. Leaf morphology is of interest as the leaves are responsible for the interception of light, which has consequences for photosynthesis and transpiration.

#### 2.1. INTRODUCTION

Growth analysis is the procedure of analysing plant growth by expressing it as algebraic product of a series of factors (Hardwick, 1984). Two distinct treatments for growth analysis have traditionally been used. The first was developed by Briggs *et al.* in 1920, who combined the concepts of net assimilation rate per unit leaf weight (Gregory, 1918), and relative growth rate per unit leaf weight (Blackman, 1919). This form of growth analysis is normally used for single plants which are widely spaced, with little competition between the individuals. The second method of growth analysis, used for closed stands, was developed by Watson in 1958, who pointed out that crop growth was the product of the leaf area index and the net assimilation rate.

Since the development of growth analysis several attempts have been made to improve the two techniques (Emecz, 1962; Hunt *et al.*, 1984;

Warren Wilson, 1981). The latter two papers have attempted to combine the above mentioned techniques, as crops and developing vegetation normally start as open stands, and gradually develop into a more or less closed stand. These proposed new methods however, have not addressed the central problems of growth analysis, and it is doubtful whether they are more useful than the traditional techniques (Hardwick, 1984). The problems facing any growth analysis study are discussed below.

Classical growth studies have been criticised as being empirical, with the derived quantities revealing little about the mechanisms controlling growth. Complex mechanistic models of growth, on the other hand, have been criticised as being cumbersome (Warren Wilson, 1981). Such approaches are inappropriate if one considers that "plants rarely behave as simple machines" (Hunt, 1979). Currently there is a continuum from the more empirical approach, in which arbitrary regression equations are fitted to the primary data, through to the more complex mechanistic models. Depending on the objective, the material and the measurement methods available for a particular study, different points within this continuum will be appropriate. Despite these criticisms, growth analysis has often been applied to solve agronomic or ecological problems with valuable results.

Plant architecture deals with patterns of above ground branching and leaf display, which affect the capacity of a plant to intercept solar radiation.

Growth form and branching patterns show considerable variation within forests (Ashton, 1978; Halle *et al.*, 1978). Some aspects of the growth form might influence the competitive ability of a species and should therefore be considered. However, quantification of the geometric, mechanical, aerodynamic and optical properties of plant canopies is a complex task and as a result very little data on the form and properties of tree crowns of tropical forests exist. Some of these are discussed below.



### Tree Architecture at the Canopy Level

Profile diagrams suggest that trees from different levels in the forest have different shapes. Trees whose crowns emerge above the general canopy level (emergents) have broad spreading crowns, trees of the continuous canopy have rounded crowns and subcanopy trees have elongate crowns (Givnish, 1984). However, it is difficult to determine whether these differences are genetic or whether they are a result of environmental differences. For example, the relative increase in crown width with height, may be the result of populational thinning, where more space is made available to those trees which survive later into the thinning process.

A simple argument, based on water availability, can be used to try to determine the photosynthetic and transpirational implications of differences in crown shape. In regions where the sun passes close to the zenith, leaves in shaded portions of trees with hemispherical crowns should experience low light intensities and temperatures, and therefore low photosynthetic rates and transpirational demand. In contrast the leaves of the upper portion of the crown, exposed directly to the sun, will experience high light intensities and high temperatures, with resultant higher evaporative demand and photosynthetic rates. The high evaporative demand, due to the high temperatures, will increase the transpirational costs in the upper portions of the tree.

Horn (1971) and Givnish (1976) hypothesised that as moisture availability in an environment increases and the cost of transpiration decreases, crown shape should shift from cylindrical, self shading forms toward flat topped canopies with maximal exposure. Givnish (1976) showed that along an increasing moisture gradient in the central USA, crown shapes of the dominant tree genera do shift from cylindrical forms in *Carya* spp. and *Quercus* spp. of the semi-arid woodlands, to hemispherical forms in *Acer*,

*Fagus* and *Tilia* of the mixed mesophytic forests, to the flat topped forms in *Fraxinus* and *Ulmus* in mesic swamp forest.

Although the above argument is based on the single constraint of water availability, it represents at least one of the selective pressures that may shape crown form.

### Tree Architecture at the Leaf Level

#### i) Vertical distribution of foliage

Horn (1971, 1975), has analysed the photosynthetic costs and benefits in two kinds of leaf arrangement in trees, which he calls monolayers and multilayers. Monolayers have their leaves in a single shell, whereas multilayers scatter their leaves within several layers, each which acts as a density filter. This, together with the nonlinear response of photosynthesis to light, implies that a multilayered tree can hold a more productive leaf surface than the ground area that it covers (Horn, 1971).

If one considers photosynthetic acclimatisation to different levels of irradiance, the following hypothesis can be proposed (Horn 1971): the scattered leaves of the upper layers are able to photosynthesise at their full potential, and still transmit enough light for maximum rates of photosynthesis to the layers below. Total plant growth will be maximised by continuing this process until light levels reach the light compensation point, where the cost of adding a new leaf just balances the energetic profit it earns (Horn, 1971). However, the light compensation point, although a familiar physiological concept, is a poor measure of the net benefit of the leaf because it only accounts for the balance between photosynthesis and instantaneous leaf respiration. Givnish (1984) suggests five further energetic costs, associated with photosynthesis, that should increase the "ecological compensation point" at which total leaf benefits and costs just balance.

These include: (i) nighttime leaf respiration, (ii) daily cost of leaf construction, (iii) costs of additional roots, (iv) xylem and phloem needed to supply an additional leaf and (v) the mechanical cost of supporting an additional leaf. The opportunity costs of extracting nutrients from a leaf which has low net photosynthesis and placing them in a new well lit leaf (Field, 1981) must be also considered.

Conclusions which can be drawn from Horn's model are: multilayers are more productive under bright light while monolayers are more productive in deep shade because they have less internal shading and no leaves operating below the compensation point. Thus multilayers (with small leaves) should be favoured by trees of early succession, and monolayers by those trees which regenerate in the shade. If Givnish's (1984) energetic costs are incorporated into Horn's model, the following predictions can be made: the optimal number of leaf layers (hence leaf area index (LAI)) should decrease with habitat aridity and inefficiency of nutrient retranslocation. Therefore the ecological compensation point will be lower in moist habitats, in plants or herbs with less expensive mechanical tissue (Raven, 1976) and in plants with efficient nutrient translocation.

Although these predictions have important ecological implications regarding tree form and structure, there has been little work to test them.

## ii) Leaf inclination and reflectivity

Leaves can absorb, reflect and transmit radiation over several ranges of wavelengths, each of which has a difphysiological consequence. There are four bands of interest: long-wave infrared, photosynthetically active radiation (PAR), the remainder of the visible spectrum plus the shorter wave infrared and ultraviolet radiation (Givnish, 1984). At ambient temperatures, leaves re-radiate in the long-wave infrared. Absorption of near infra-red tends to increase the heat load on the leaves (Gates and Benedict, 1963)

and many leaves have low absorption and high reflectance at this wavelength.

Although the effects of leaf inclination and reflectance are similar, there are two respects in which they differ: leaf reflectivity affects the interception of both direct beam and diffuse radiation, whereas leaf inclination has its greatest effect on direct beam radiation. Leaf inclination increases the leaf area which can be held over a given surface, which may result in greater light penetration through the crown. Reflective leaves can be arranged horizontally to cast denser shade and therefore suppress competitors. Leaf reflectivity may be different at different wavebands.

Both leaf inclination and leaf reflectivity affect the receipt of radiation by the leaf, its thermal budget and its gas exchange. These in turn affect the energetic balance between photosynthetic benefits and transpirational costs. The effect of leaf reflectivity and inclination on the physiology of the plant is straightforward: the optimal absorption/reflectance should be that which maximises the difference between photosynthetic benefits and transpirational costs. As reflectivity and inclination increase, so absorption of radiation by the leaf decreases with consequent effects on photosynthetic benefits and transpirational costs.

Although quantitative data are scarce, it is commonly recognised that leaf inclination and reflectivity increases toward dry, sunny, and/or nutrient poor environments. As light absorption increases, photosynthesis increases until factors other than light become limiting. If the air temperature is high, photosynthesis may decline at high absorption, as leaf temperature exceeds the thermal optimum. In a shady environment high absorption is needed for photosynthesis to balance respiration. However, as light absorption increases heat load on the leaf rises with a consequent rise in the transpiration. These costs are higher in sunny or dry environments than in shady or moist environments (Givnish, 1984).

Ashton and Brunig (1975), report that in tropical gap succession, trees with large more or less horizontal leaves are common on moist, fertile sites, whereas trees with smaller steeply inclined leaves occur on drier, less fertile sites.

### iii) Leaf morphology

The leaf, the primary site of photosynthesis, is structurally specialised for its functions of light interception and carbon dioxide absorption. As the leaf is the primary site of interaction with light, it is in this organ that phenotypic responses to changes in light intensity are first observed.

The larger a leaf, the more surface area it has available to intercept light. Plants from shaded habitats, where the light intensity is low and may be limiting, tend to have large individual leaves. Plants from habitats where light is not limiting, are characterised by a smaller leaf size (Björkman, 1981; Boardman, 1977). Brown (1919) observed that leaf size increases from the sunlit canopy to the lower strata of tropical forests. Cain *et al.* (1956) reported an average area of individual leaves for trees in the Mucambo rainforest of Brazil of 56.7 cm<sup>2</sup> for canopy species and 85.8 cm<sup>2</sup> for understorey species. Hall and Swaine (1981) reported that many forest trees have larger leaves when they are seedlings and saplings than they do as adults.

Changes in leaf size are often coincident with changes in leaf shape. The effect of light intensity on the leaf shape is difficult to quantify and two opposing views exist. Nobel (1976) showed that the leaf shape of *Hyptis emoryi* remains fairly constant with changes in light intensity despite changes to the length i.e. any changes that occur to leaf length are balanced by changes in the leaf width. Givnish (1984) however, reported that leaf width decreases toward dry, sunny or nutrient poor habitats. Leaf width can affect leaf energy budget and hence rates of gas exchange. As leaf width

increases so does the average thickness of the boundary layer around the leaf, thus increasing the resistance to diffusion and heat exchange and affecting leaf temperature, transpiration and photosynthesis (Givnish, 1984). Gates and his colleagues (Gates, 1965; Gates, 1980; Gates and Papian, 1971; Taylor, 1975), have summarised the principal effects of leaf size on transpiration and leaf temperature. Under sunny conditions and moderate to high stomatal resistances, large leaves are warmer and transpire at a higher rate per unit leaf area than small ones. In shaded conditions, where the heat load is less, large leaves have higher boundary layer resistances and therefore lower transpiration rates than smaller leaves.

Three models have been developed for the evaluation of the significance of leaf size: Parkhurst and Loucks (1972) proposed that leaf size is adjusted to maximise water use efficiency and thus maximises carbon gain for a given amount of water loss. However, Givnish and Vermeij (1976) showed that natural selection is unlikely to maximise the ratio of photosynthesis to transpiration. A second model similar to that of Parkhurst and Loucks (1972), is that proposed by Givnish and Vermeij (1976) which suggests leaf size is adjusted to maximise whole-plant carbon gain, based on the balance between photosynthetic benefits and transpiration costs at moderate to high stomatal resistances. The third model proposed by Taylor and Sexton (1972) suggests that leaf size is adjusted to maintain leaf temperature near the optimum for photosynthesis and/or to prevent thermal damage. However, the thermal response of photosynthesis is open to selection and tends to peak at high temperatures in warmer environments (Mooney *et al.*, 1977). Therefore, if the leaf size was shaped by some other mechanism, the thermal response of photosynthesis may adapt to the resulting leaf temperature and not vice versa.

The final leaf size and shape are determined by the processes of cell division and enlargement. Milthorpe and Newton (1963) showed that smaller leaves which develop under shaded conditions in *Cucumis sativus* (opposite

to the expected trend) had fewer cells than the large leaves which formed under high light intensity. Schoch (1972) on the other hand found in *Capsicum annuum*, that the great expansion in the shade is accompanied by both more cells and by larger cell size.

Not only is leaf size affected by light intensity, but plants grown in high light intensities have a different leaf anatomy from those grown at low light intensities (Nobel *et al.*, 1975; Turrel, 1936; Wylie, 1951). Generally, shade leaves are larger with a thinner lamina than sun leaves which are smaller and thicker due to the strong development of the palisade and spongy mesophyll tissues (Cormack and Gorham, 1953; Hanson, 1917; Jackson, 1967; Shields, 1950; Turrel, 1936).

As high light intensities are often associated with high evaporative demand, the leaves of sun plants tend to have xerophytic characteristics like higher specific leaf weights (SLW) and dry weights and lower water contents (Björkman and Holmgren, 1963; Clough *et al.*, 1979; Dengler, 1980; Lichtenhaler *et al.*, 1981).

The SLW, is a useful measurement because it removes some of the large variation in area and weight of individual leaves. The SLW is the result of the relative rates of lamina expansion and increase in weight, therefore changes in either or both these which may result from a treatment, can lead to complex patterns of SLW. Morgan and Smith (1981) found that the response of SLW to light is species specific. The SLW also varies with temperature (Woodward, 1983) and with the age of the plant (Young, 1975).

## **2.2. METHODS**

Total growth could not be measured by harvesting and dry weight determinations as the saplings had to be returned to Twinstreams. However,

parts of the plants (e.g leaves and branches) were removed for the derivation of allometric relationships. Total growth was determined both as the increase in height (cm) and as the above ground biomass accumulated during the 18 month study period. The above ground biomass accumulation was estimated from the following allometric relationship:

$$(\text{No. leaves} * \text{LDW}) + (\text{BL} * \text{BDW/L} * \text{No. branches})$$

where:

No. leaves = number of new leaves produced

LDW = average dry weight of the leaves (g) determined on approximately 10 leaves per plant for three plants per treatment.

BL = average length of the branches produced (cm)

BDW/L = mean dry weight per unit branch length (g/cm) determined on three branches per plant for three plants per treatment

No. branches = number of new branches produced

Fresh weight (FW) of the leaves was measured immediately after picking. The leaf dry weight (DW) was determined once the leaves had been dried to constant weight at 70°C (approximately 48 hours). The FW:DW ratio and specific leaf weight (SLW) was determined from these leaves.

Relative growth rate could not be calculated as the saplings obtained for the experiments were already well established and the initial weight was unknown.

Aspects of architecture measured were the angle of branches to the horizontal, leaf inclination measured relative to the horizontal and length of the internodes between leaves. Branch angle was measured on each branch for 3 saplings of each species in each treatment. Leaf inclination was measured for 10 arbitrarily chosen leaves on three saplings of each species in the different treatments. Replication for internode length was 10 per plant for three plants per treatment.



Non quantifiable observations on architecture were also noted for some of the species. These observations included the shape and growth form of the saplings in the different light treatments.

All morphological work was conducted on fully expanded leaves produced in the experimental conditions. The leaf size and leaf shape (assessed as the length: breadth ratio (L:B)) were determined using the CI 251 Area Meter (CID Incorporated).

The total leaf area produced in the different light intensities was calculated as:

$$Ls * \text{No. leaves}$$

where:

Ls = average leaf size determined on 10 leaves per plant for three plants per treatment

No. leaves = average number of new leaves produced

## 2.3. RESULTS

### 2.3.1. GROWTH

Growth results are presented as the increase in biomass as well as the increase in plant height during the 18 month study period. Each species is discussed separately.

#### *Mimusops caffra*

The results are presented in Fig 2.1.

The biomass increase (Fig 2.1.a) was greatest in the 70% shade plants. This biomass accumulation can be attributed to the high number of leaves produced by the plants in this treatment (Fig 2.1.c). Plants which

grew in 90% shade had a very small biomass increase due to the small number of branches and leaves produced (Fig 2.1.b&c). There was no significant difference between the biomass of plants from the sun and 40% shade (Fig 2.1.a). However, sun plants produced significantly more branches than the 40% shade plants, while the 40% shade plants produced significantly more leaves.

Increase in height (Fig 2.1.d) was lowest in the 90% shade plants and not significantly different between the 40% and 70% shade plants.

### *Euclea natalensis*

The results are presented as Fig 2.2.

The small biomass increase (Fig 2.2.a) of the 90% shade plants was due to the small number of branches and leaves produced by these plants (Fig 2.2.b&c). The biomass increase was not significantly different between the sun, 40% and 70% shade plants. However, the allocation of biomass between the stem and leaves was different in plants from these three treatments. Sun plants had a greater increase in leaf biomass which was attributable to the greater number of leaves produced (Fig 2.2.c). Although there was no significant difference in the number of branches produced by the sun, 40% and 70% shade plants, the branch biomass of the sun plants was significantly less than the 40% and 70% shade plants.

The greatest increase in height was observed in the 40% and 70% shade plants and the least in the 90% shade plants (Fig 2.2.d).

### *Olea woodiana*

The results are presented in Fig 2.3.

The large biomass increase of the 40% shade plants (Fig 2.3.a) was due to the high number of branches and leaves produced in this treatment (Fig 2.3.b&c). There was no significant difference in the biomass increase in the sun and 70% shade plants. The leaf biomass of the plants of these two treatments was not significantly different despite fewer leaves being produced by the sun plants (Fig 2.3.c). Plants in 70% shade produced more stem biomass than the sun plants. Despite the high number of branches produced in 90% shade plants (Fig 2.3.b) these plants had the smallest biomass increase.

The sun plants had the smallest increase in height and the 40% and 70% shade plants the largest (Fig 2.3.d).

### *Peddiea africana*

The results are presented as Fig 2.4.

The small biomass increase of the 90% shade plants was due to these plants producing so few new leaves (Fig 2.4.a&c). Although sun plants produced a high number of leaves (Fig 2.4.c) the leaf biomass was less than the 40% and 70% shade plants. The large branch biomass of the sun plants however, contributed significantly to the total biomass and as a result biomass increase was not significantly different between the sun, 40% and 70% shade plants (Fig 2.4.a). There was no significant difference in the increase in height between the sun and 90% shade plants.

The plants from 40% and 70% shade had the largest increase in height (Fig 2.4.d).

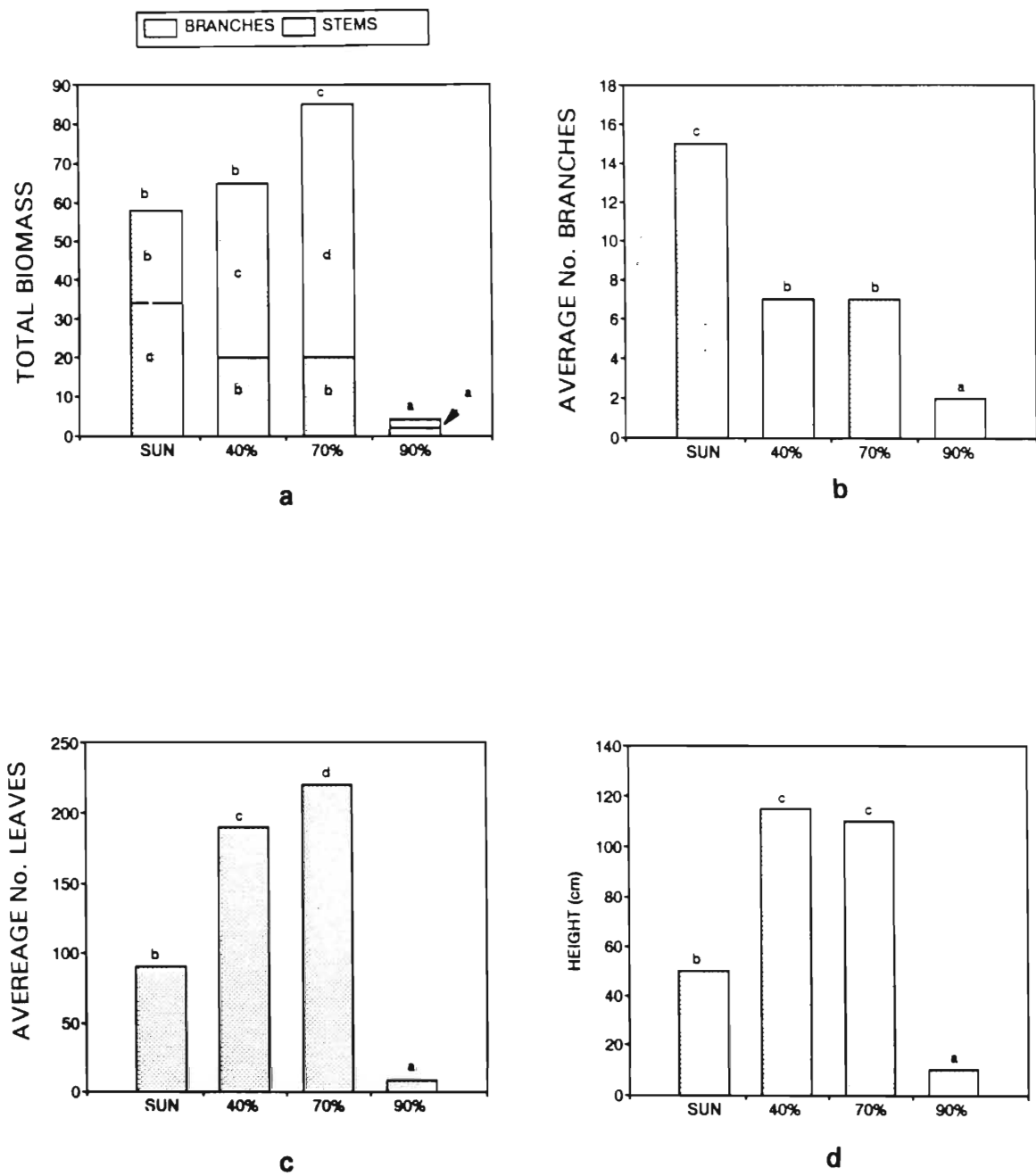


Figure 2.1. The effect of light intensity on the above ground growth of *M. caffra* over a 18 month period: a) Biomass increase b) Average number of branches produced c) Average number of leaves produced d) Increase in height. Bars with different letters are significantly different at  $P \leq 0.05$ .

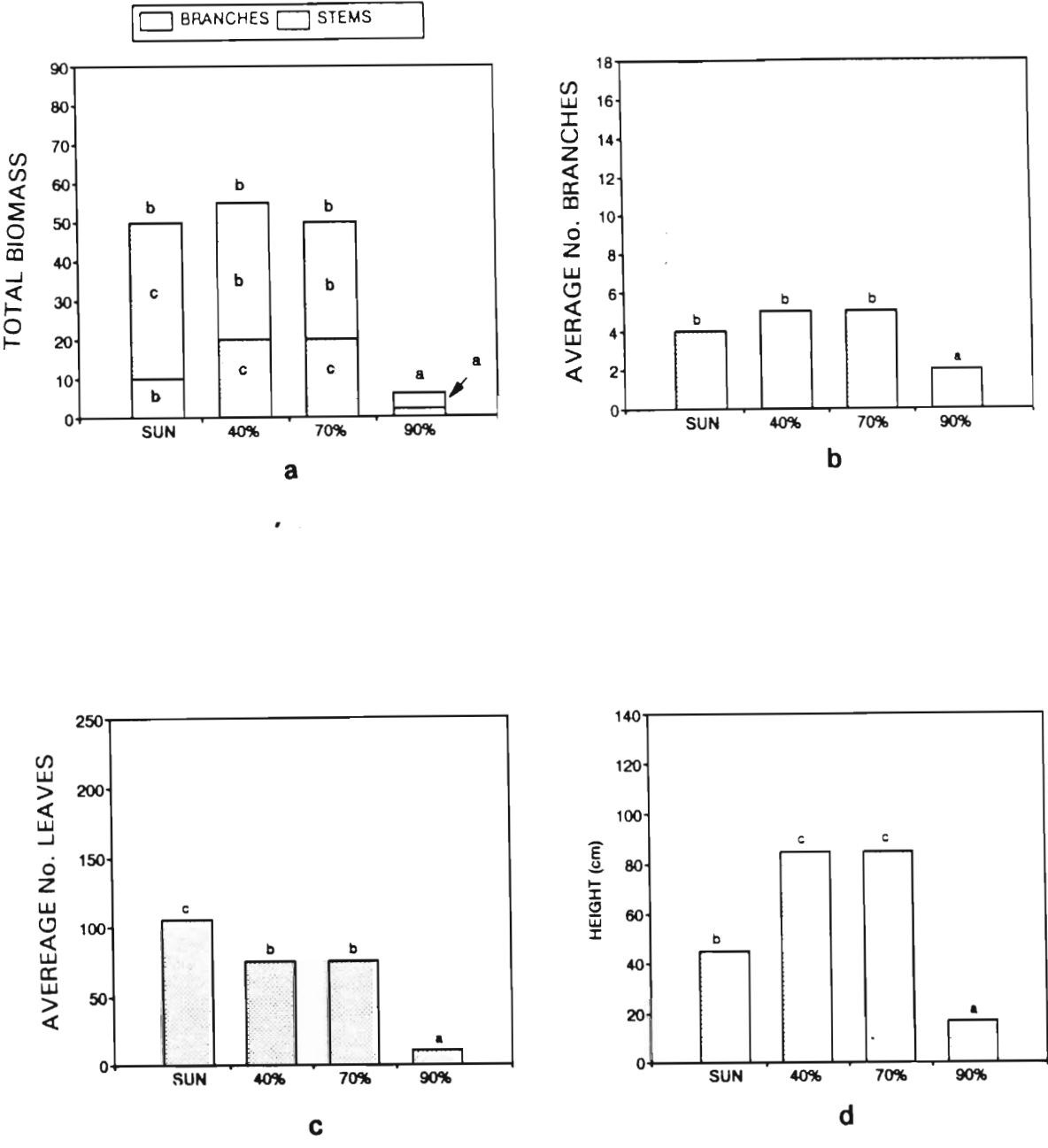


Figure 2.2. The effect of light intensity on the above ground growth of *E. natalensis* over a 18 month period: a) Biomass increase b) Average number of branches produced c) Average number of leaves produced d) Increase in height. Bars with different letters are significantly different at  $P \leq 0.05$ .

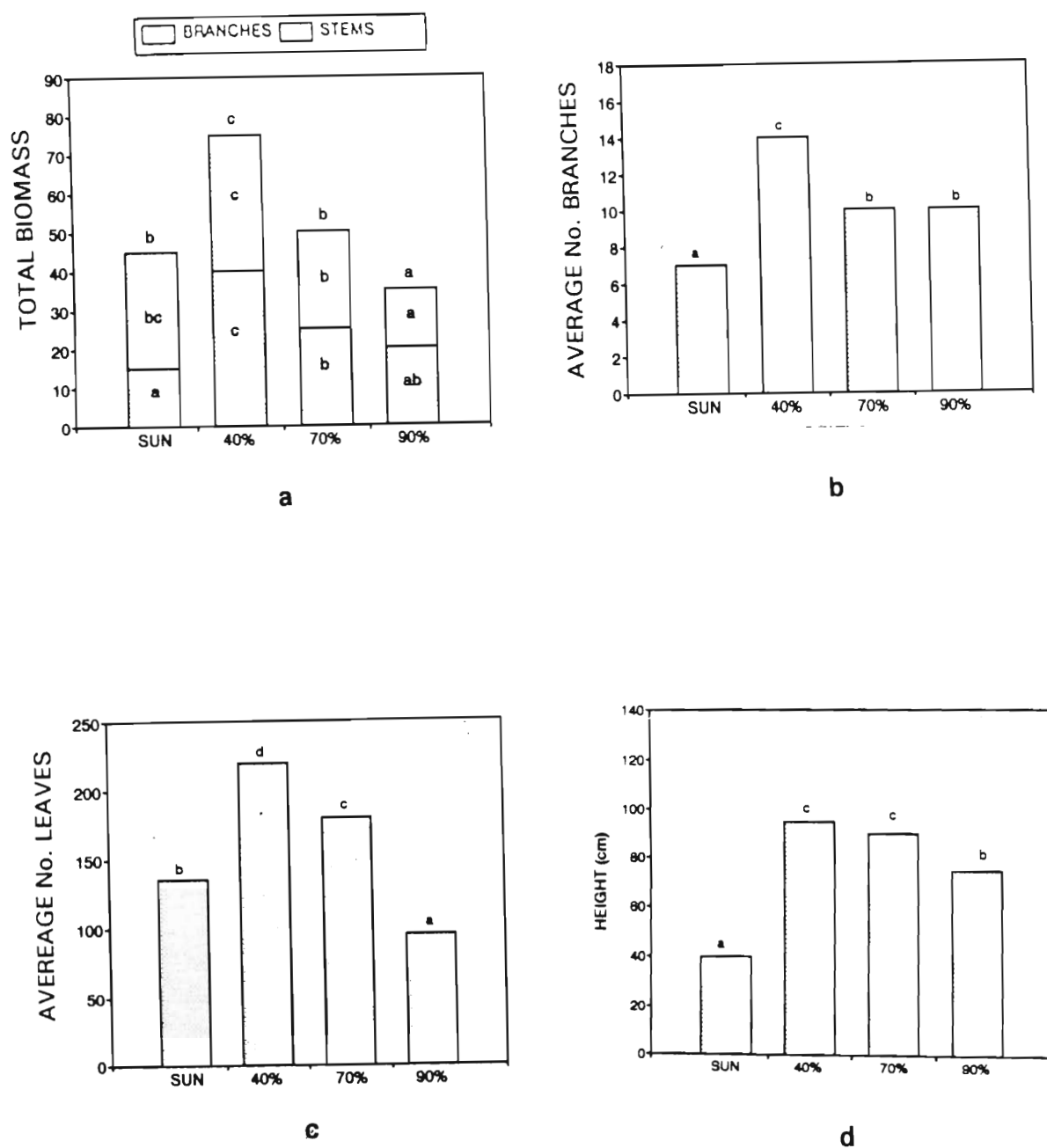


Figure 2.3. The effect of light intensity on the above ground growth of *O. woodiana* over an 18 month period: a) Biomass increase b) Average number of branches produced c) Average number of leaves produced d) Increase in height. Bars with different letters are significantly different at  $P \leq 0.05$ .

### 2.3.2. PLANT ARCHITECTURE

The results for the plant architecture study were of particular interest as each of the species showed different degrees of plasticity to light intensity. The architecture of *M. caffra* was largely unaffected by changes in light intensity, while the architecture of plants of *O. woodiana* was considerably influenced by light.

#### *Mimusops caffra*

Non quantifiable observations of the growth form of *M. caffra* showed the following: the bare stems found on the plants grown in the full sun resulted from the loss of leaves formed prior the start of the experiment. New branches which developed from the top of the main stem were short and thick, and new leaves were formed at the top of the branches in tight clusters. In contrast, the plants from the moderate shade produced new branches along the entire length of the main stem. These branches however, were longer and thinner than those on the sun plants and tended to "droop".

Quantifiable results for the growth form of *M. caffra* are given in Table 2.1.

Table 2.1. The effect of light intensity on the architecture of *M. caffra*. Different letters indicate significant differences at  $P \leq 0.05$ .

TREATMENT	BRANCH INCLINATION ° to horizontal	LEAF INCLINATION ° to horizontal	INTERNODE LENGTH (cm)
SUN	50 b	30 a	0.5 a
40% SHADE	55 b	45 b	2.5 b
70% SHADE	55 b	40 ab	2.5 b
90% SHADE	40 a	30 a	2.5 b

The steeper branch inclinations of the sun, 40% and 70% shade plants were significantly different from the 90% shade plants. Sun and 90% shade leaves had low leaf inclinations. Internode lengths were significantly different between the sun and shade treatments.

### *Euclea natalensis*

The results for the architectural response of *E. natalensis* to light intensity are presented as Table 2.2.

Table 2.2. The effect of light intensity on the architecture of *E. natalensis*. Different letters indicate significant differences at  $P \leq 0.05$ .

TREATMENT	BRANCH INCLINATION ° to horizontal	LEAF INCLINATION ° to horizontal	INTERNODE LENGTH (cm)
SUN	55 b	45 c	1.5 a
40% SHADE	60 b	25 b	1.5 a
70% SHADE	55 b	25 b	3 b
90% SHADE	10 a	10 a	3 b

Both the branch and leaf inclination decreased with increasing shade. Although the branch inclination was not significantly different between the sun, 40% and 70% shade plants, the new branches formed in 90% shade were almost plagiotropic (10°). Leaf inclination was significantly different between the sun, medium shade (40% and 70%) and deep shade (90%) plants. Internode length doubled in the deeper shade treatments.



*Olea woodiana*

The architecture of *O. woodiana* was greatly affected by light intensity. Both non quantifiable and quantifiable characteristics (Table 2.3.) were observed.

Perhaps the most significant non quantifiable characteristic observed was the formation of the new branches in 90% shade. All new branches produced in this treatment, formed from the apex of the main stem at right angles to it. Branches in all other treatments were produced along the entire length of the mainstem.

Table 2.3. The effect of light intensity on the architecture of *O. woodiana*. Different letters indicate significant differences at  $P \leq 0.05$ .

TREATMENT	BRANCH INCLINATION ° to horizontal	LEAF INCLINATION ° to horizontal	INTERNODE LENGTH (cm)
SUN	55 a	70 a	1.0 a
40% SHADE	35 b	55 b	3.0 b
70% SHADE	30 c	15 c	3.5 b
90% SHADE	10 d	5 d	3.5 b

Branch and leaf inclinations were significantly different between all the treatments and decreased in increasing shade. Although branch and leaf inclination showed similar trends, the differences in the leaf inclination were particularly marked: sun and 40% shade leaves had steep leaf inclinations (70° and 55° respectively) while the 70% and 90% shade leaves were almost horizontal (15° and 5° respectively). The internode length increased significantly in the shade.

Peddiea africana

Plants of *P. africana* show a trend towards decreasing branch and leaf inclination in increasing shade. This trend however, is not a great for the branch inclination as for the leaf inclination. The internode length increases with decreasing shade (Table 2.4).

Table 2.4. The effect of light intensity on the architecture of *P. africana*. Different letters indicate significant differences at  $P \leq 0.05$ .

TREATMENT	BRANCH INCLINATION ° to horizontal	LEAF INCLINATION ° to horizontal	INTERNODE LENGTH (cm)
SUN	55 c	65 d	0.75 a
40% SHADE	50 bc	55 c	1.00 ab
70% SHADE	45 b	30 b	1.25 b
90% SHADE	30 a	15 a	1.75 c

2.3.3. LEAF MORPHOLOGY

Leaf morphology varied greatly in response to the light treatments. Leaf size, total leaf area and SLW for the four species are presented in Figures 2.5-2.8. The dimensionless length:breadth ratio and FW:DW ratios are presented in Tables 2.5-2.8.

Mimusops caffra

Average leaf size increased in the shade treatments. Although plants in 70% shade had the largest leaf size, this was not significantly different from the leaf size of the 90% shade plants. However, due to the small number of leaves produced in the 90% shade, the total leaf area of these plants was very small. SLW decreased in the shade (Figure 2.5).

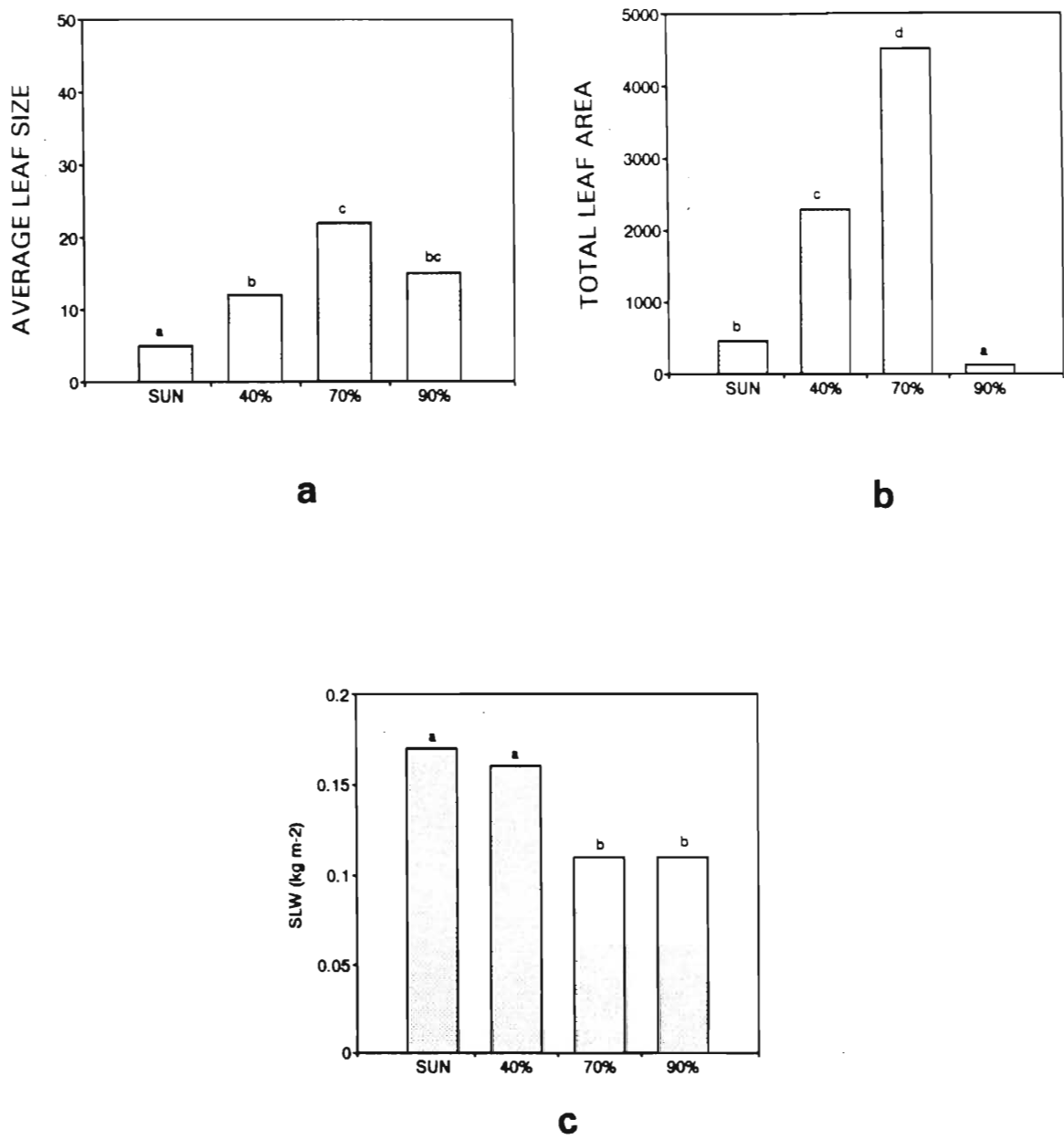


Figure 2.5. The effect of light intensity on a) average leaf size b) total leaf area and c) SLW of *M. caffra*. Bars with different letters are significantly different at  $P \leq 0.05$ .

Shading caused elongation of the leaves. FW:DW ratios increased in the shade and were significantly different between the plants from the sun or 40% shade and those from the 70% or 90% shade (Table 2.5).

Table 2.5. The effect of light intensity on the leaf morphology of *M. caffra*. Different letters indicate significant differences at  $P \leq 0.05$ .

TREATMENT	LENGTH:BREADTH	FW:DW
SUN	1.6 a	2.2 a
40% SHADE	1.8 ab	2.3 a
70% SHADE	1.9 b	2.6 b
90% SHADE	1.9 b	2.6 b

*Euclea natalensis*

Despite the fact that sun leaves were significantly smaller than shade leaves, total leaf area was not significantly different between the sun, 40% and 70% shade leaves. Although the leaves from the 90% shade plants were the same size as those from the other shade treatments, the total leaf area of these plants was significantly less. The SLW decreased significantly in the shade (Figure 2.6).

Sun leaves were more elongate than shade leaves. The FW:DW ratio of the leaves was significantly different only between the sun and 90% shade leaves (Table 2.6).

Table 2.6. The effect of light intensity on the leaf morphology of *E. natalensis*. Different letters indicate significant differences at  $P \leq 0.05$ .

TREATMENT	LENGTH:BREADTH	FW:DW
SUN	2.45 a	2.03 a
40% SHADE	2.04 b	2.11 ab
70% SHADE	2.12 b	2.34 ab
90% SHADE	1.79 b	2.67 b

*Olea woodiana*

Leaf size increased significantly in the shade. Total leaf area was significantly higher in shade plants than in sun plants. Despite the large individual leaf size of the 90% shade plants, they had the smallest total leaf area amongst the plants from the shade treatments. The SLW decreased significantly between the high light intensity (sun), medium light intensity (40% and 70% shade) and low light intensity (90% shade) (Figure 2.7).

A decrease in light intensity resulted in broader leaves but had no effect on the FW:DW ratio of the leaves of *O. woodiana* (Table 2.7).

Table 2.7. The effect of light intensity on the leaf morphology of *O. woodiana*. Different letters indicate significant differences at  $P \leq 0.05$ .

TREATMENT	LENGTH:BREADTH	FW:DW
SUN	3.3 a	2.1 a
40% SHADE	3.3 a	2.3 a
70% SHADE	2.7 b	2.1 a
90% SHADE	2.5 b	2.3 a

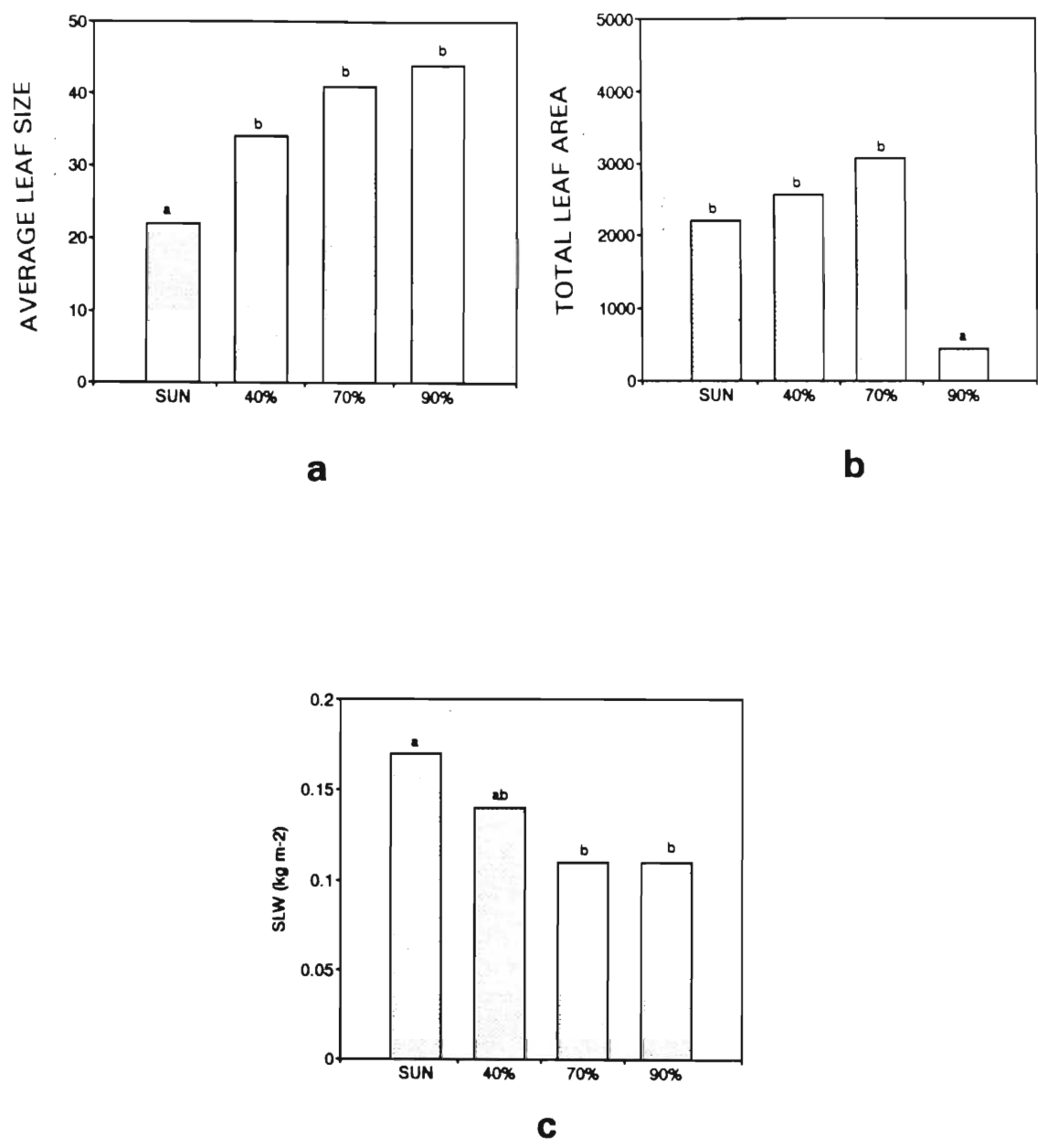


Figure 2.6. The effect of light intensity on a) average leaf size and b) total leaf area and c) SLW of *E. natalensis*. Bars with different letters are significantly different at  $P \leq 0.05$ .

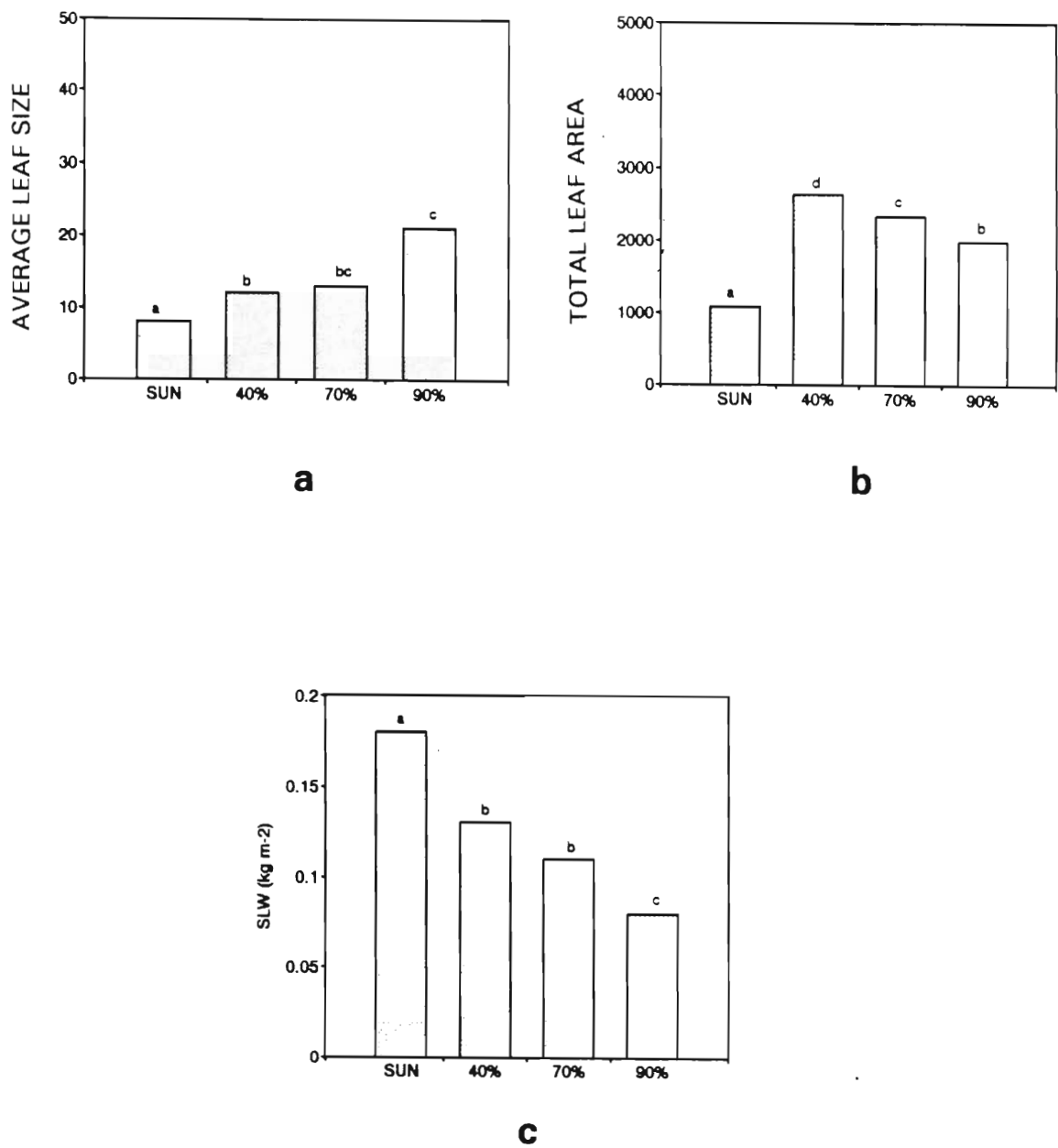


Figure 2.7. The effect of light intensity on a) average leaf size and b) total leaf area and c) SLW of *O. woodiana*. Bars with different letters are significantly different at  $P \leq 0.05$ .

Peddiea africana

Shading caused an increase in leaf size in plants of *P. africana*. However, despite the leaves of the 90% shade plants being significantly larger than all other treatments, these plants had small total leaf areas. The SLW decreased with decreasing light intensity and was largest in the sun and 40% shade and smallest in those plants from 90% shade (Figure 2.8).

Light intensity had no significant effect on either the leaf shape nor the FW:DW ratio, despite the trend towards broader leaves and a higher FW:DW ratio in the shade (Table 2.8).

Table 2.8. The effect of light intensity on the leaf morphology of *P. africana*. Different letters indicate significant differences at  $P \leq 0.05$ .

TREATMENT	LENGTH:BREADTH	FW:DW
SUN	3.04 a	3.31 a
40% SHADE	3.07 a	3.33 a
70% SHADE	3.52 a	3.53 a
90% SHADE	2.88 a	3.77 a

**2.4. DISCUSSION**

Age, size and the number of branches and leaves, varied between the species at the start of the experiment, making across species comparisons difficult. The trends in the growth rates of the four species in the different light treatments appear to varying degrees to be influenced by their successional status. Therefore, *M. caffra*, a pioneer species should have had the greatest growth rate in the sun, while *P. africana*, a subcanopy species, should have had the greatest growth in the deeper shade treatments. The resultant growth rates however where



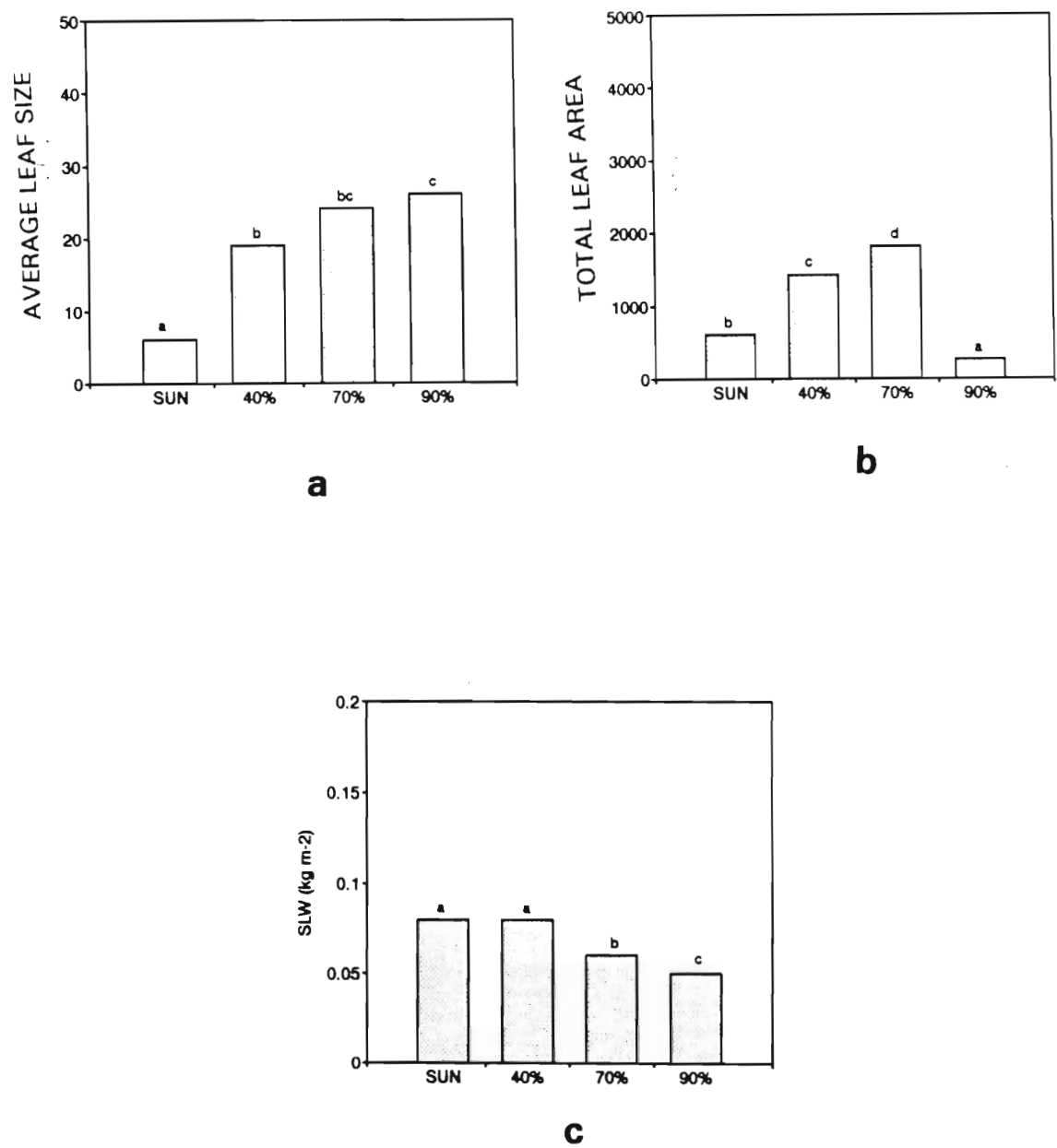


Figure 2.8. The effect of light intensity on a) average leaf size b) total leaf area and c) SLW of *P. africana*. Bars with different letters are significantly different at  $P \leq 0.05$ .

not as expected. Architectural and morphological adaptations to sun and shade were similar for all species. Similar trends were observed for leaf and branch inclination, internode length, leaf size and SLW. Branches and leaves were less steeply inclined, internode lengths and leaf size increased and SLW decreased in the shade plants of all four species. However, the observed trends for leaf shape and FW:DW ratios were not as well defined.

Of all the plants in the sun treatments *M. caffra* did have the highest growth rate and was therefore the best adapted species for growth at high light intensities. In the 90% shade treatment *P. africana* and *O. woodiana*, both shade tolerant species, had the highest growth rates, while *M. caffra* and *E. natalensis* both had very low growth rates. This low growth rate in *M. caffra* and *E. natalensis* however, was attributable to different factors. Although plants of *M. caffra* produced new leaves in the first five months, no new leaves were produced for the remainder of the experimental period. Plants of *E. natalensis*, on the other hand, had a delayed growth response, with the first new leaves being produced approximately ten months after the start of the experiment. Despite this delayed response, during the eight months that the plants produced leaves, a definite increase in growth rate could be observed. Given time this growth rate may have improved further, but it is doubtful that these plants would have achieved growth rates comparable to plants of *E. natalensis* from the other light treatments.

Within *M. caffra*, the largest growth rate was observed in the plants from the 70% shade treatment. A possible explanation for this observed trend is discussed below. The dune front, where saplings of *M. caffra* establish and grow, is characterised by high light intensities, low nutrient availability, loose soils and wind. The saplings of *M. caffra* are adapted for growth in this stressed environment and are therefore able to outcompete the saplings of other species. It is the biomass allocation

patterns which enable these plants to withstand the the adverse wind associated with the dunes. The experimental plants of *M. caffra* showed different biomass allocation between the stems and leaves, depending on the light intensity in which they grew. The bulk of the biomass in the sun plants was attributed to numerous, thick side branches. On the other hand, branches produced in those plants from 70% shade, contributed less than one third of the total biomass, and most of the biomass was contributed by the large number of large leaves. Mechanical support tissue, required in high light and windy habitats, is expensive to produce and will have an adverse effect on the growth rate of the plants. However, interspecific competition is limited on the dune front, and the costs of producing these adaptations are outweighed by the benefits. Expensive mechanical support would not be required in those plants which grew in the shade in the natural habitat and these plants would be able to produce a large leaf biomass. However, the extremely large growth rate in the 70% shade plants is not a likely reflection of what will occur in the natural habitat for two possible reasons: the experimental conditions, in which there was no competition for the available resources, is unlikely in the natural habitat and, as seedlings of *M. caffra* are seldom found in the forest, a germination response (light quality or intensity) could also influence the species distribution.

Another adaptation to high light intensity and the consequent heat load on the leaves of the sun plants of *M. caffra*, was the clustering of the leaves in a tightly packed rosette at the apex of the stem. Each layer reduced the amount of light striking the next layer. Sun plants of *M. caffra* also lost all the old leaves which possibly represented the loss of leaves not adapted to the high light intensity.

The effect of increasing leaf size with decreasing light intensity is two fold: i) leaf size affects heat exchange and transpiration (Gates and Papion, 1971). Under conditions of high light intensity, large leaves are

warmer and transpire at a higher rate than small ones, while in shaded conditions the increased boundary layer associated with increased leaf size will lead to decreased transpiration. As high light intensity is often associated with xeric conditions it is advantageous for plants in such conditions to produce smaller leaves and thereby reduce leaf temperatures and transpiration rates. ii) in shaded conditions where light may be limiting to growth, a large leaf will provide more individual leaf area for the intercept of light. However, the total leaf area that the plant has available for the intercept of light is a product of individual leaf size and the number of leaves on that plant. Givnish and Vermeij (1976) argue that as leaf size increases, the elevated leaf temperature increases the rate of carboxylation and as a result photosynthesis is enhanced. However, in high light intensity larger leaves also have higher transpiration rates (Gates and Papion, 1971), and it is probable that the optimal leaf size is that which maximises the differences between photosynthetic profits and transpirational costs (Givnish, 1984).

In conditions of high light intensity and therefore high evaporative demand, smaller leaves with smaller transpirational costs are more advantageous. However, the potential transpirational benefits of smaller leaves in the sun are debatable if one considers the shape of the leaves of *M. caffra*. Givnish (1984) reports that leaf shape affects leaf temperature and transpiration rates. Gates (1965, 1971, 1980), showed that broad leaves in sunny conditions are warmer and transpire at higher rates than narrower leaves. The fact that the sun leaves of *M. caffra* are broader than the shade leaves and are therefore potentially warmer and may transpire at higher rates than if they were narrower, begs the question of actual benefits of smaller leaves on the transpiration rates if this effect is being counteracted by the effect of the leaf shape.

Amongst the four species, plants of *M. caffra* had the highest SLW which was probably related to their successional status and the need for

thick schlerophyllous leaves in the habitat in which they naturally occur. Despite the trend towards smaller SLW in the shade, 90% shade plants of *M. caffra* had considerably higher SLW than the other three species.

There were no large differences between the growth rates in the sun, 40% and 70% shade plants for *E. natalensis*. The total leaf area of the sun, 40% and 70% shade plants of were similar. However, sun plants produced a large number of small leaves while the shade plants produced fewer, larger leaves. Although there was no significant difference between the number of side branches produced in the three treatments, the shade plants had higher stem biomasses, due to slightly thicker branches. Stem:leaf ratios therefore differed between the three treatments. In the 90% shade plants however, the benefits of producing large leaves for light interception, would be outweighed by the costs as so few leaves are produced and the total leaf area available for light interception was small. This will have implications for the growth of these plants.

Although the branch inclination did not change between the sun, 40% and 70% shade plants in *E. natalensis*, the leaf inclination was significantly different among the sun, medium shade and deep shade plants. Changes to branch inclination will indirectly affect light absorption but, in *E. natalensis* it appears as if leaf orientation is primarily responsible for the control of light intensity. The steeply inclined leaves of sun plants greatly reduce the amount of light intercepted by these leaves and therefore minimises the potential damaging effects of high light intensity. Light interception is increased in the 40% and 70% shade plants with the reduction in the leaf inclination. Plants in 90% shade had horizontal branches and leaves which maximised light interception. Plants of *E. natalensis* are able to exploit the light environment by slightly adapting the growth form, and morphological adaptations which are expensive to produce are minimised.

The SLW of *E. natalensis* varied significantly between treatments. Sun plants had a high SLW and were therefore better adapted for the high light intensity and evaporative demand. The leaves of the 90% shade plants on the other hand were larger and thinner and therefore better suited for that particular environment. Sun plants of *E. natalensis* also had a higher dry weight content than the shade plants which was possibly related to the more xeric conditions associated with a sunny habitat (Björkman and Holmgren, 1963; Clough *et al.*, 1979; Dengler, 1980; Lichtenhaler *et al.*, 1981).

Saplings of *O. woodiana* appear to be well adapted for growth in the different light treatments as is evident in the morphological and architectural adaptations observed. However, leaves of *O. woodiana* in the sun treatment did show signs of photobleaching (see Chapter 3). Plants of *O. woodiana* in all the treatments showed adaptations at both the canopy and leaf level. These adaptations minimised the effect of high light intensity in the sun plants, and maximised the amount of light that was intercepted by the shade plants. The canopy profile of sun plants was elongated and thus self shading. Light interception at the individual leaf level was minimised by numerous, steeply inclined and densely packed leaves on steeply inclined side branches. However, as no mature plants in the natural habitat were studied, it could not be determined whether this was the strategy used in the mature canopy trees of *O. woodiana*.

Plants of *O. woodiana* in 90% shade produced horizontal side branches from the apex of the main stem. Leaves, produced at right angles to these side branches, were horizontal. Etiolation of the stem was also visible. Therefore, 90% shade plants minimised self shading and thus maximised light interception in low light intensity. Plants therefore increased the amount of incident radiation absorbed without a significant

increase in the metabolic costs of producing additional branches to support leaves.

The high growth rate of the 40% shade plants of *O. woodiana* coincided with a large number of large leaves which significantly increased the total leaf area. These plants therefore had a large area available for the interception of light which was effectively utilised for the production of branches and leaves. The total growth was not significantly different between the sun and 70% shade plants. However plants from these two treatments differed in all aspects of architecture and morphology. Plants from these two treatments grew in very different light conditions and altered the leaf inclination of their leaves accordingly.

Although the 70% shade plants grew in low light conditions, the low leaf inclination maximised the amount of light intercepted by individual leaves. Sun plants produced fewer branches, fewer, smaller leaves and a significantly smaller total leaf area than the 70% shade plants. One would expect such differences to have an influence on the growth potential of the sun plants but this does not seem to occur.

The trend observed in *P. africana* of no significant difference in the growth rate between the sun, 40% and 70% shade plants was surprising in view of the trends observed for the number of branches and the number and size of leaves for plants grown in these three light treatments. The large growth rate of the sun plants was attributable to a high stem biomass as the result of more, slightly thicker side branches. However, the leaves are the organs responsible for the interception of the light necessary for photosynthesis and therefore indirectly for growth. Sun plants of *P. africana* produced a large number of small leaves, and consequently they had a very small total leaf area available for the interception of light. This would have an influence on overall production of photosynthates of these plants in the long term. The production of

mechanical support tissue (branches) is expensive and this raises the question of where the sun plants of *P. africana* obtained the necessary energy for the high growth rates observed. An increase in allocation to stems at the expense of roots could have occurred.

Evans and Hughs (1961) reported that typical shade plants e.g. *Impatiens parviflora* adapt to low light intensities by being able to increase the leaf size along with only a slight reduction in photosynthetic rate per unit area. Leaf size increased significantly in low light intensities for *P. africana*, however the effect of this on the photosynthetic potential is examined in more detail in Chapter 3.

Amongst the four species, the SLW of *P. africana* in all treatments was exceptionally small. For *P. africana*, a shade tolerant sub-canopy species, large thin leaves would be more advantageous in the natural habitat.

An interesting observation to come out of this study was the lack of significance that light intensity had on the FW:DW ratios of the "shade species", *O. woodiana* and *P. africana*. The significance of this trend remains to be answered.

High light intensity stunted the plants of all four species. Trends in the height increase did not tend to reflect trends in growth. With the exception of plants of *P. africana*, plants in the 40% and 70% shade treatments had the greatest increase in height.



## CHAPTER THREE

### BIOCHEMISTRY AND PHYSIOLOGY

The effect of different light intensities during growth on biochemical and physiological aspects of the four species was investigated. The biochemical effects were the chlorophyll and nitrogen content of the leaves, and the physiological effects were the photosynthetic light response and the response of assimilation to intercellular  $\text{CO}_2$  ( $A:c_i$  curve).  $A:c_i$  curves were used as an indication of ribulose 1-5 biphosphate carboxylase (Rubisco) activity and potential ribulose biphosphate (RuBP) regeneration rates in the leaf. Chlorophyll content, leaf nitrogen content, Rubisco activity and RuBP regeneration, are all directly involved in the photosynthetic process and therefore have a direct effect on the photosynthetic light response of a plant.

#### 3.1. INTRODUCTION

Chlorophyll is the major light absorbing pigment found in green plants. The efficiency with which light may be absorbed by the leaf therefore depends on the chlorophyll content per unit leaf area. The higher the chlorophyll content the greater is the proportion of incident light that is absorbed by the leaves. At any given wavelength ( $\lambda$ ), the relationship between fractional absorbance ( $a$ ) and pigment concentration is given by

$$a_\lambda = 1 - \exp(-k_\lambda l_\lambda c)$$

where  $k_\lambda$  is a constant,  $l_\lambda$  is the effective optical pathway of light in the leaf and  $c$  is the pigment concentration (Björkman, 1981). This relationship however, is valid only for a homogeneous solution e.g. extracted chlorophyll in an acetone solution. The leaf is a heterogeneous system in which the chlorophyll protein complexes *in vivo* have different light absorption characteristics than extracted chlorophyll. Therefore, in a leaf, the fractional absorptance of light is less than proportional to the increase in pigment content (Björkman, 1981). However, as a result of multiple light reflection

and scattering within a leaf, a leaf is a more efficient absorber than a chlorophyll solution of the same chlorophyll concentration.

Glabrous leaves with a chlorophyll content of 400-600 mg chl m<sup>-2</sup> are able to absorb approximately 80 to 85% of the daylight in the waveband 400 to 700nm (Björkman, 1968). A threefold increase in chlorophyll content from 250 to 750 mg m<sup>-2</sup> causes a small (2-3%) increase in absorptance in the blue and red wavelengths (the region where chlorophyll has a high specific absorption), while the main effect of an increased chlorophyll content is in the green and far red regions where chlorophyll has a low absorption coefficient. For example, a light green leaf with 250 mg chl m<sup>-2</sup> absorbs about 60% of the light at the absorptance minimum of 550 nm, while a dark green leaf with 750 mg chl m<sup>-2</sup>, absorbs 82% at this wavelength. The average absorptances over the waveband 400-700 nm for the light green and dark green leaf are approximately 73% and 87% respectively. Although the increase in the chlorophyll content in this range does not result in a proportional increase in absorptance, it may still confer a significant advantage under conditions where the quantum flux density is low and thus severely limits the photosynthetic rate (Björkman, 1981).

### Chlorophyll Content in Sun and Shade Plants

It is generally stated that the leaves of shade plants are thinner and their chloroplasts larger and richer in chlorophyll than the leaves of sun plants. Shade plants in their native habitats tend to have a higher content of total chlorophyll expressed on a weight basis than sun plants. As shade plants tend to have large thin leaves, the chlorophyll content per unit leaf area is often lower than in sun plants (Björkman, 1968; Goodchild *et al.*, 1972; Wild and Wolf, 1980). However, thin leaves are not always characteristic of shade plants and many rainforest species e.g. *Cordyline rubra*, have a higher chlorophyll content per unit area than sun plants (Goodchild *et al.*, 1972).

Growth under low light levels tends to result in an enrichment of chlorophyll b ( $\text{chl}_b$ ) relative to chlorophyll a ( $\text{chl}_a$ ) and shade plants grown in deep shade, tend to have a lower  $\text{chl}_a/\text{chl}_b$  ratio than sun plants grown under high light levels (Björkman, 1981). Thornber (1975) showed that the two major chlorophyll proteins in sodium dodecyl sulphate (SDS) extracts of angiosperm chloroplasts were complex I, an altered form of the P700  $\text{Chl}_a$ -protein, and the major light harvesting complex consisting of  $\text{chl}_a$ ,  $\text{chl}_b$  and protein. Since  $\text{chl}_b$  is associated with only the light-harvesting  $\text{chl}_{a/b}$  protein complex of the photosynthetic unit, whereas  $\text{chl}_a$  is found in this and other complexes which do not change their size in response to environmental conditions (Thornber, 1975; Alberte *et al.*, 1976), it is not surprising that the  $\text{chl}_a/\text{chl}_b$  ratio is altered by light intensity. The differences in  $\text{chl}_a/\text{chl}_b$  ratio probably reflects differences in the proportion of the light-harvesting complex to the total chlorophyll complement of the chloroplast (Björkman, 1981). Butler (1977) associated this light-harvesting complex with photosystem (PS) II, which suggests that shade plants have a higher ratio of PS II to PS I reaction centres than sun plants. A possible function of this increased PSII/PSI ratio in shade plants is to provide a more balanced energy distribution between the two photosystems in shaded habitats like forest floors which, because of the filtering effect of the canopy have a high proportion of far-red light, effective only in the excitation of PSI. Such changes in the photosystem ratio could also explain the tendency of shade plants to have a slightly higher ratio of total chlorophyll to P700 (Björkman, 1981).

#### Chlorophyll Content of Sun and Shade Plants Grown Under Different Light Intensities

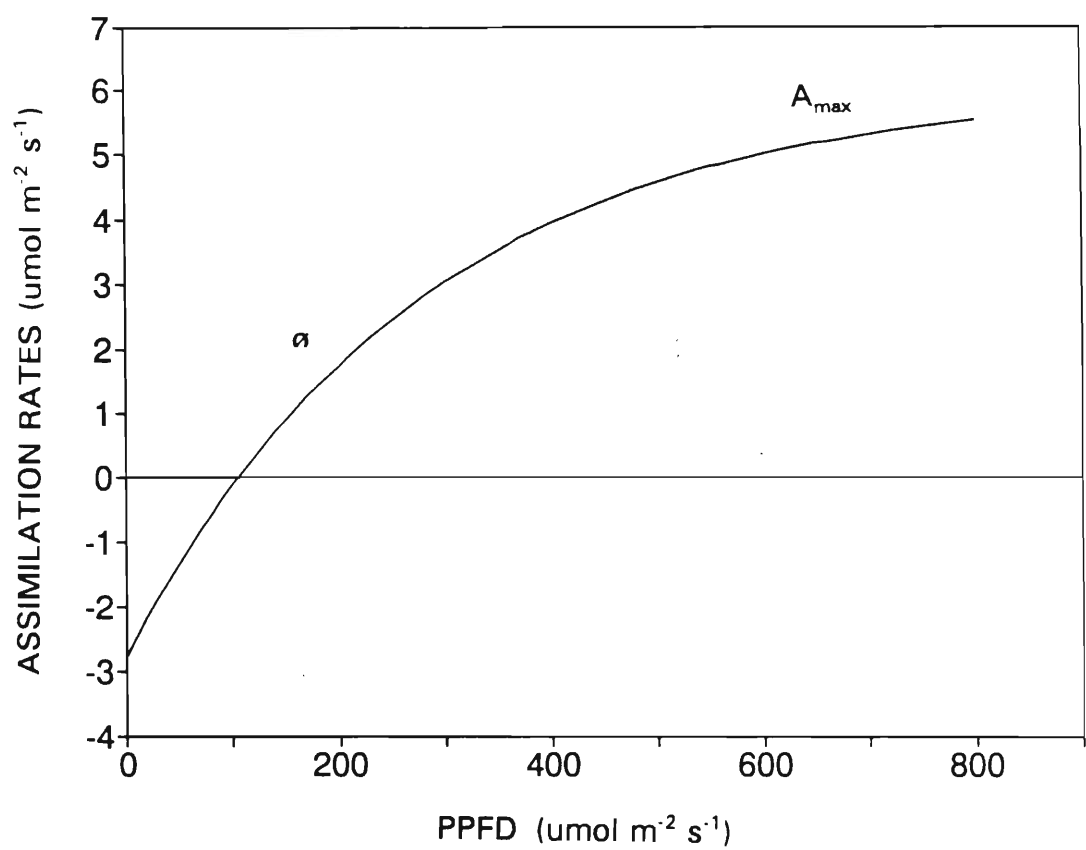
Light intensity affects the pigment composition of sun and shade plants differently. For a sun plant chlorophyll content per leaf area tends to remain relatively constant over a wide range of light intensities, but severe shading may cause a significant decrease in the chlorophyll content (e.g.

*Solanum dulcamara* (Clough *et al.*, 1979). High light intensity has been shown to destroy the chlorophyll of shade plants (Björkman, 1981).

Total leaf nitrogen content, which is often a limiting resource for plant growth, is greatly affected by light intensity (Field and Mooney, 1986). As a large proportion of the leaf nitrogen is required for the proteins involved in photosynthesis (Stocking and Ongun, 1962), a significant proportion of the change in the leaf nitrogen content is a result of change in the concentration of rubisco. This enzyme represents approximately 20% of total nitrogen in the leaves of well fertilised C<sub>3</sub> plants (Evans and Seeman, 1984). However, the effect of the leaf nitrogen on the photosynthetic capacity of the leaf is debatable. On the one hand, the photosynthetic capacity of the plant has been shown to be generally proportional to the leaf nitrogen content (Rawson and Hackett, 1974; Bolton and Brown, 1980; Gulmon and Chu, 1981; Mooney *et al.*, 1983). However, if a wide range of leaf nitrogen contents are examined the relationship between leaf nitrogen and CO<sub>2</sub> assimilation rate is nonlinear, and the slope declines as the nitrogen content increases (e.g Wong, 1979). Thomas and Thorne (1975) showed that the addition of 200 kg N.ha<sup>-1</sup> to a wheat crop increased the protein and chlorophyll content per unit leaf area by 27% and 15% respectively, without any measurable increase in the assimilation rate per unit leaf area.

The photosynthetic response of individual leaves to light intensity has been extensively studied as it is fundamental to our understanding of adaptations of plants to sun and shade (Björkman, 1981). The photosynthetic response is plotted as a light response curve where assimilation rate is a function of light intensity.

Such a response provides useful information on the utilisation of light energy by the leaf (Fig 3.1).



**Figure 3.1.** A generalised response of CO<sub>2</sub> assimilation rate to photon flux density (after Long and Hallgren, 1985). See text for explanation.

At low light intensities the light response curve shows a linear relationship between the assimilation rate and light intensity. This initial slope represents the apparent maximum quantum yield ( $\phi$ ) based on incident (not absorbed) light. If reflected and transmitted light are taken into account then the true maximum quantum yield can be obtained (Long and Hallgren, 1985). Quantum yield represents the maximum efficiency of light conversion by the photochemical processes (Björkman and Holmgren, 1963).

Above this initial slope and below light saturation there is a non-linear portion of the curve, where both light absorption and the distribution of light within the leaf can affect rates of photosynthesis (Leverenz, 1988).

Individual leaves of most  $C_3$  plants are unable to use additional light above a photosynthetic photon flux density (PPFD) of approximately  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  or 25% of full sunlight, while some  $C_4$  plants fail to saturate, even in full sunlight. The light saturated assimilation rate ( $A_{\text{max}}$ ), is a measure of the photosynthetic capacity of the leaf and therefore the rates of process other than photochemical (Björkman and Holmgren, 1963). The assimilation rate varies with almost all environmental variables as well as with leaf age and ontogeny (Long and Hallgren, 1985).

The light compensation point ( $L_c$ ) is that irradiance at which the instantaneous leaf rates of photosynthesis and respiration just balance.

#### Photosynthetic Light Response of Sun and Shade Plants

Although large differences exist between the photosynthetic light responses of sun and shade plants, much more research has been conducted on the response of individual species to different light intensities.

A study by Björkman (1981) on the light response of typical sun and shade plants grown under the light regime of their respective habitats shows that shade plants e.g. *Cordyline rubra* have lower dark respiration rates than sun plants e.g. *Encelia californica* and *Nerium oleander*. As a result the light compensation point for shade plants occurs at much lower quantum flux densities and the rate of photosynthesis at low light levels is considerably higher in shade plants than sun plants. However, light saturation is reached early in shade plants at approximately  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  (approximately 5% full sunlight). At this level the sun plants still operate in the linear portion of the curve and have a higher net photosynthesis than shade plants. Björkman

(1981) also reported that sustained exposure to quantum flux densities in excess of that required to saturate photosynthesis may lead to photoinhibition.

Such light responses are clearly adaptive because they allow shade plants to function efficiently at low light intensities that prevail in their habitats and enable the sun plants to make effective use of moderate and high quantum flux densities (Björkman, 1981).

### The Photosynthetic Response of Sun and Shade Plants to Growth Under Different Light Intensities

It is well documented that the photosynthetic characteristics of many species of plants, both  $C_3$  and  $C_4$ , are influenced by the light intensity under which the plants is grown.

i) Sun Plants. The classic study by Björkman *et al.* (1972) on acclimation of the photosynthetic light response to irradiance of *Atriplex patula*, a species which occupies sunny beaches, provides a good illustration of the characteristic differences in the response of sun plants to growth under sunny versus shaded habitats.

Björkman *et al.* (1972), grew seedlings of *Atriplex patula* at high ( $920 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), intermediate ( $290 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and low ( $92 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) light intensities. When grown under a high light intensity, leaves of this species have high light-saturated photosynthetic rates and relatively high rates of dark respiration. Both of these rates showed a strong decline with decreasing light intensity during growth. However, the minimum daily radiation required for significant growth in *A. patula* is at least an order of magnitude higher than for shade plants such as *C. rubra*, and the dark respiration rates and light compensation points of these shade species,

determined in their native habitat (Björkman, Ludlow and Morrow, 1972) are lower than in *A. patula* grown near its shade tolerance limit.

ii) Shade Plants. It is widely recognised that shade plants may suffer damage to their leaves, grow poorly or even die, when attempts are made to grow them in high light intensities. There is strong evidence to suggest that shade plants have an intrinsically low potential for photosynthetic light acclimation, and it seems likely that their susceptibility to high light injury is a consequence of their inherent low ability to increase their capacity for effective utilisation of high quantum flux densities for photosynthesis (Björkman, 1981).

In a comparative study on the potential for photosynthetic light acclimation for sun and shade ecotypes, Björkman and Holmgren (1963) grew plants from populations of *Solidago virgaurea* from exposed and shaded habitats either in low light or high light intensities. They found that whereas the response of the sun ecotypes were similar to that generally found in sun plants, the shade ecotypes were unable to acclimate to a high light growth regime. Although the shade ecotypes were capable of rapid growth under the low light regime their growth was severely restricted in the high light regime i.e. growth of the shade ecotypes in the high light intensity did not result in an increased capacity for light saturated photosynthesis. The observed reduction in quantum yield at rate limiting quantum flux densities was attributed to photoinhibition (Björkman and Holmgren, 1963).

Similar results have been reported by Gauhl (1969, 1970, 1976) for sun and shade clones of *Solanum dulcamara*. Hariri and Prioul (1978) found that plants of *Pteris cretica* grown under low light ( $1-3 \text{ mol m}^{-2} \text{ day}^{-1}$ ) had the highest light limited and light saturated rates and that an increase in the daily quantum flux by a factor of four to five caused a decline in the photosynthetic rate. A shade type response was also observed in *Fragaria*



*vesca* (Chabot and Chabot, 1977) and *Fragaria virginianana* (Jurik *et al.*, 1979). In these species an increase in daily flux from 2-3 mol m<sup>-2</sup> to about 10 mol m<sup>-2</sup> day<sup>-1</sup> had little effect but a further increase to about 35 mol m<sup>-2</sup> day<sup>-1</sup> caused a marked decline in the light saturated photosynthetic rate.

The use of sun-shade photosynthetic ecotypes is useful in elucidating photosynthetic differences between plants grown in high or low light habitats. However, recent evidence (Gauhl, 1979) shows that the photosynthetic response to light of the shade ecotype of *S. dulcamara* is modified by water availability. Clones collected from shaded habitats showed no signs of photoinhibition when grown under a high light regime as long as the water supply was ample, but typical symptoms of photoinhibition became evident when the water potential of the root medium was reduced even slightly. Similar reductions in water potential had no effect on these shade clones under a low light regime, and *Solanum* clones from sunny habitats were unaffected irrespective of the light regime. This raises the question of the interpretation of the results in previous sun-shade ecotype studies since growth conditions in all these studies were designed to differ only in available light and not other environmental factors known to influence photosynthetic responses.

### The Photosynthetic Light Response of Tree Species

Although photosynthetic responses of herbaceous species and shrubs to different photon flux densities during growth have been well characterised, the photosynthetic responses of forest trees from different successional stages has not. Two of the more recent studies on the effect of sun and shade on the photosynthesis of trees from different successional stages are discussed below.

Thompson *et al.* (1988) attempted to determine whether the leaves of shade grown seedlings have increased quantum yield and if the leaves of

sun grown seedlings have higher light saturated rates of photosynthesis than shade grown leaves. The hypothesis that a plant acclimates to a given irradiance and nutrient availability by physiological adjustments that serve to increase carbon gain (Mooney and Gulmon, 1979) was central to their work. *Flindersia brayleyana*, a rain forest tree species with a broad tolerance to irradiance, was chosen as their test species. Plants were established in low, medium and high light intensity and low or high nutrient supply.

The results showed increased light saturated photosynthesis, increased saturation irradiance, increased dark respiration rates and increased light compensation points with increasing irradiance during growth. Quantum yield was more affected by nutrients than by the irradiance levels. Thompson *et al.* (1988) concluded that while the apparent quantum yield was not higher under low irradiance, reduced respiration lowered the light compensation point and that such acclimation to deep shade maintained a positive carbon budget in the leaves. Growth irradiance exerted a predominating influence on dark respiration, light compensation and light saturation. Such effects have been widely reported for annual and perennial plants (Björkman *et al.*, 1972).

The more recent work by Ramos and Grace (1990) determined the effect of sun and shade on the gas exchange of four tropical tree species with contrasting distribution patterns in forest succession. They found that all species grown in the shade reached light saturation in the range 250-500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and displayed maximum photosynthetic rates of 4.6-5.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Plants from the sun treatment however were not yet light saturated at 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and two species never reached light saturation. Photosynthetic rates were 6.2-8.8  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The apparent quantum efficiency was insensitive to light and the climax species did not have higher quantum efficiencies than the early stage species. Dark respiration rates were always higher in the sun treatments. Shade grown plants always had lower light compensation points than sun grown plants, but the shade

tolerant species did not always have lower compensation points than the pioneer species.

Sun and shade plants may possess the capacity to undergo photosynthetic adaptations in response to different light intensities. Such adaptations involve changes in the levels of carbon reduction cycle enzymes, electron transport components and proteins and pigments associated with light harvesting.

### Enzymes of Photosynthetic Carbon Metabolism

Enzymes involved in the fixation and reduction of  $\text{CO}_2$  make up the bulk of the protein content of the chloroplast stroma. A major fraction of this protein is the enzyme, ribulose-1,5 biphosphate (RuBP) carboxylase oxygenase or rubisco. Because rubisco has a low affinity for  $\text{CO}_2$  and also functions as RuBP oxygenase, the  $\text{CO}_2$ -fixing activity of the enzyme at normal atmospheric  $\text{CO}_2$  concentrations, appears in many cases to be just sufficient to support the light saturated rate of  $\text{CO}_2$  fixation by intact leaves, despite the large amounts of the enzymes being present in the chloroplasts. For this reason Rubisco has been implicated as a potential rate limiting enzyme (Björkman, 1981).

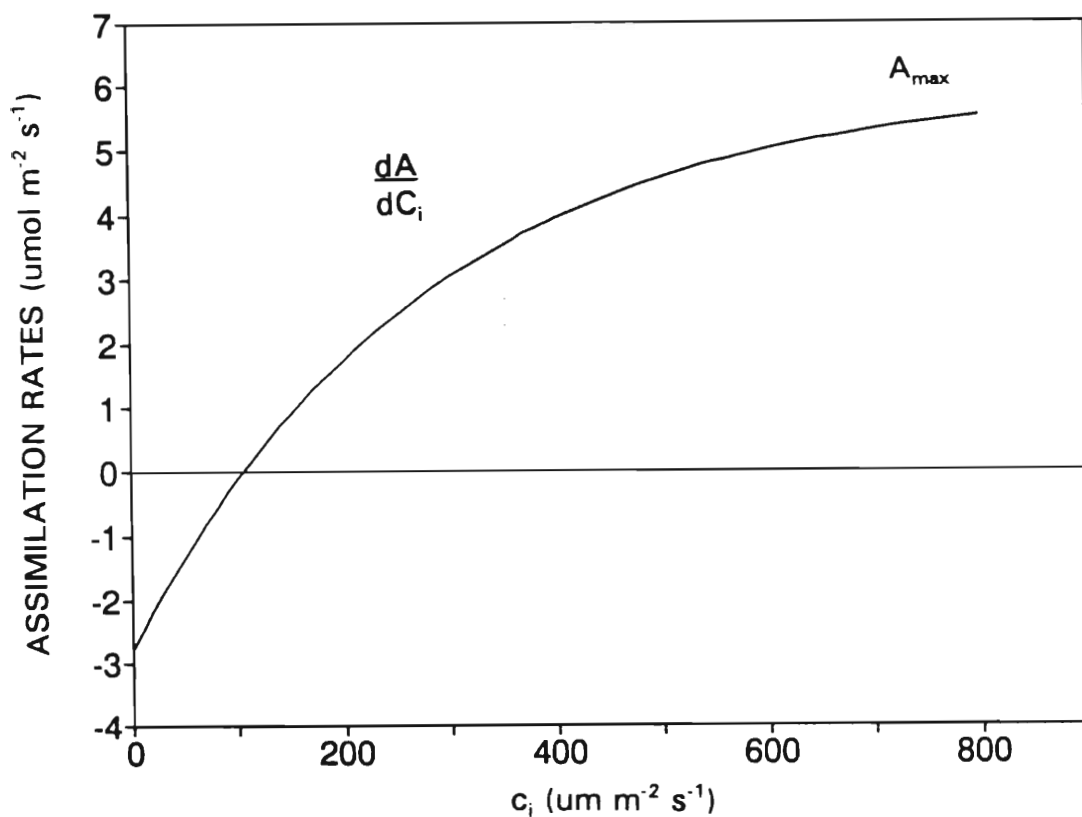
Rubisco activity is light dependent, both because the regeneration of the substrate RuBP is dependent upon ATP and NADPH produced by the light reactions and because the mechanisms for the control of the activity of the enzyme are linked to the PPFD (Seemann *et al.*, 1988).

### Generalised A:c<sub>i</sub> Curves

A plot of assimilation (A) as a function of internal  $\text{CO}_2$  concentration ( $c_i$ ) yields a typical saturation curve (Fig 3.2). Such a curve is known as the demand function as it describes the biochemical processes of

photosynthesis which occur in the mesophyll and thus generate the demand for  $\text{CO}_2$ .

If the internal concentration of  $\text{CO}_2$  ( $c_i$ ) at the sites of carboxylation is low, rubisco is saturated with respect to RuBP. The initial effect of increasing  $c_i$  from zero, is the activation of rubisco. Subsequently there is an approximately linear response of assimilation to the  $\text{CO}_2$  concentration. During the initial linear portion of the curve, assimilation is limited by rubisco activity. The slope of the curve is a measure of the efficiency of carboxylation and is proportional to the amount and activity of rubisco in the leaf.



**Figure 3.2.** A generalised response of  $\text{CO}_2$  assimilation rate ( $A$ ) to leaf internal  $\text{CO}_2$  mole fraction ( $c_i$ ) (after Farquhar and Sharkey 1982); see text for explanation.

Numerous authors have found a correlation between the initial slope of the curve, rubisco levels and extractable rubisco (e.g. Collatz, 1977; von Caemmerer and Farquhar, 1981). At higher  $c_i$  concentrations, if the rate of RuBP carboxylation is increased sufficiently, the ability to regenerate RuBP (in the Calvin Cycle), becomes limiting. This ability depends inter alia on the capacity for electron transport at light saturation. The rates of electron transport, and the regeneration of ATP, then become independent of  $c_i$ . Saturation, is therefore the maximum rate of RuBP regeneration (Farquhar and Sharkey, 1982). In this way A:C<sub>i</sub> curves can be used to separate carboxylation and electron transport limitations.

As this experiment was aimed at comparing trends in rubisco carboxylation efficiency and RuBP regeneration, rather than exact amounts of rubisco, use of A:C<sub>i</sub> curves was considered to be sufficient.

#### Effects of PPFD on the Carboxylation Efficiency

Recent studies have indicated that the initial slope of the A:c<sub>i</sub> curve is a sensitive indicator of the photosynthetic capacity and is highly correlated to the activity of RuBP carboxylase in the leaf (Evans and Seamann, 1984). Weber *et al.* (1987), conducted a study to determine the response of the initial slope of the A:C<sub>i</sub> curve to varying PPFD. Spinach and soybean were chosen as examples of species with high photosynthetic rates (sun plants), and *Arbutus unedo* as a species with a low photosynthetic rate (shade plants). They found for all species, that the initial slope of the A:C<sub>i</sub> curve was PPFD-independent above a critical PPFD, whereas below this the slope decreased rapidly. In addition the CO<sub>2</sub> compensation point is PPFD independent until a lower PPFD is reached below which it increases rapidly. However, the mechanisms controlling this response were unclear. Possible explanations included: (i) the activation state of rubisco could be reduced by changes in stromal milieu. Mott *et al.* (1984), have shown that when PPFD is reduced, RuBP concentration drops then gradually increases with no

changes in the rates of photosynthesis. These data suggest that initially RuBP limits assimilation, but over time a new lower activation state of Rubisco is established which allows RuBP concentrations to increase. Sharkey *et al.* (1986), have shown that the activation of Rubisco increases and that the inhibition of the active site decreases with increasing PPFD. (ii) the rate of dark respiration may decrease with increasing PPFD.

Weber *et al.* (1987), conclude that PPFD not only affects the photosynthetic rate by limiting the CO<sub>2</sub> saturation rate, but that it can also affect the initial slope of the A:C<sub>i</sub> curve.

#### Rubisco Activity in Sun and Shade

Generally sun leaves have a higher rubisco activity than shade leaves (Boardman, 1977; Björkman, 1981). Givnish (1988) suggests that the optimal levels of rubisco under any given conditions are those which maximise the differences between the benefits and the costs (energetic costs of enzyme synthesis). The lower rubisco levels in shade leaves can be considered to confer important savings on the plants without affecting the photosynthetic rate at low light intensities (Björkman, 1981). At low PPFD, where the capacity of RuBP regeneration limits photosynthesis, the activity of rubisco use is potentially low. Plants which grow at low PPFD might be expected to produce less rubisco per unit leaf area than plants growing at high PPFD's, and regulate the rubisco activity in such a way that it is fully active at lower a PPFD than plants growing at a high PPFD (Seemann, 1989). It has also been suggested (Björkman, 1981), that the failure of obligate shade plants to attain high rates of light saturated photosynthesis may at least in part be attributable to a genetically low capacity to synthesise high levels of rubisco.

### 3.2. METHODS

The procedure for chlorophyll determination was based on work of Arnon (1949) on the absorption of light by aqueous acetone (80%) extracts of chlorophyll.

One cm<sup>2</sup> pieces of known weight, from the mid section of fully expanded leaves, were placed separately in 10 ml of 80% acetone and then ground using an Ultraturax. The ground material was centrifuged at 5000 rpm for 5 minutes and the supernant removed for analysis. The samples were kept in the dark until needed (the longest time period being approximately 1 hour). Light absorption was measured at 645 nm and 663 nm using the Spectrophotometer (Varian DMS 80).

The concentration of chl<sub>a</sub>, chl<sub>b</sub> and total chlorophyll were determined as by Arnon (1949).

A Buchi Nitrogen Determination system, which uses the Kjeldahl method, was used to determine the nitrogen content of the leaves. Glycine standards were used to determine the efficiency of the system and a minimum recovery rate of 97% was observed. Leaves were dried and ground and then digested with concentrated H<sub>2</sub>SO<sub>4</sub> containing selenium catalyst. The resultant digest was distilled with 32% NaOH and the distillate collected in a boric acid receiver. The nitrogen in the sample was quantitatively determined by titrating the distillate with 0.01M HCl (Bremner, 1960). Boric Acid indicator was used, and in order to prevent errors in the end point, all the indicator was made up at the same time and new standards were determined at the start of each run.

Responses of assimilation to incident PPFD were determined using an Infra Red Gas Analyser (LCA2, Analytical Development Corporation Ltd).

Measurements for light response curves were obtained during the course of a morning using three saplings of each species. A natural light response curve was obtained by taking measurements on approximately 10 attached leaves per sapling. Each leaf was used for only a single measurement, as repeated measurements resulted in stomatal closure. Shade plants were moved into the sun in order to get high irradiance necessary for light saturation.

The first readings were taken immediately after sunrise. Leaves were fitted into the cuvette for the period needed to take the readings (30-40 s). Readings continued until the leaves were light saturated at approximately  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The reference  $\text{CO}_2$  concentration, analysis  $\text{CO}_2$  concentration, relative humidity (RH), air temperature and photosynthetic active radiation (PAR) were recorded on the IRGA.

The assimilation rate was determined using the following equation (based on Long and Hallgren, 1985):

$$A = \frac{\Delta\text{CO}_2 * \text{molar air flow rate}}{\text{leaf area}}$$

where:

$A$  = assimilation rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )

$\Delta\text{CO}_2$  = difference between inlet (reference) and outlet (analysis)  $\text{CO}_2$  mole fractions, the outlet mole fraction being corrected for transpirational water vapour added to the air stream (the transpirational correction factor recommended by the manufacturers was applied).

Curves were fitted to the data using non linear regression analysis. However, the hyperbolic fit that has been used (Long and Hallgren, 1985) does not permit an intercept on the assimilation axis at zero PPFD, while a saturation exponential forces the line through the origin (zero assimilation at zero PPFD) and so does not permit a positive value of PPFD at zero



assimilation. Several authors have attempted to model the light response curves (e.g. Jarvis *et al*, 1985; Leverenz, 1988; Zeigler-Jons and Selinger, 1987). The equation that best fitted the data in this study was the Mitscherlich response function often used in agriculture (Potvin and Lechowicz, 1990). The modified equation was:

$$A = A_{\max} (1 - \exp(-n \cdot (\text{PPFD} - L_c)))$$

where:

$A$  = assimilation rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )

$A_{\max}$  = light saturated assimilation ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )

$n$  = factor determining initial curvature

PPFD = incident photosynthetic photon flux density ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )

$L_c$  = light compensation point ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )

Dark respiration ( $R_d$ ) was calculated by substituting PPFD = 0 into this equation.

The quantum efficiency ( $\phi$ ) was calculated as the slope from zero PPFD to light compensation; ie  $R_d/L_c$

In order to determine the  $A:C_i$  curve, the third fully expanded leaf of each species from each treatment, was placed into the cuvette of the portable IRGA and held under saturated light ( $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Saturating light intensity was produced by a halogen lamp. The IRGA was attached to a gas diluter which regulated the amount of  $\text{CO}_2$  reaching the leaf. The  $\text{CO}_2$  source was a gas cylinder containing  $\text{CO}_2$  concentrations in excess of  $1200 \mu\text{molmol}$ . Moist,  $\text{CO}_2$  free air (RH approximately 70%) was passed over the leaf for 30 minutes until steady state was reached. Thereafter dry,  $\text{CO}_2$  free air was passed over the leaf for 3 minutes. This was the time calculated to dry the leaf chamber. Measurements, as for the light response curves, were recorded. The  $\text{CO}_2$  concentration was increased, using the gas diluter, the air stream humidified and was passed over the leaf for 30 minutes until

steady state was reached. Dry air of the same CO<sub>2</sub> concentration was used to obtain measurements. The CO<sub>2</sub> concentration was increased steadily in steps until saturation was reached (1000 μmolmol<sup>-1</sup> CO<sub>2</sub>). Three replicates were conducted per species per treatment.

Assimilation was calculated as for light response curves. Transpiration was calculated as:

$$\frac{\Delta H_2O * \text{Molar Air Flow Rate}}{\text{Leaf Area}}$$

Internal CO<sub>2</sub> was calculated according to the equation of Long and Hallgren (1985):

$$c_i = \frac{(g_c - E/2) * c_a - A}{(g_c + E/2)}$$

where:

$c_i$  = internal CO<sub>2</sub> concentration (μmol mol)

$g_c$  = stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>)

$E$  = transpiration rate (mol m<sup>-2</sup> s<sup>-1</sup>)

$c_a$  = ambient CO<sub>2</sub> concentration (μmol mol)

$A$  = assimilation rate (μmol m<sup>-2</sup> s<sup>-1</sup>)

The A: $c_i$  curves were used to assess carboxylation and electron transport limitations. The slope of the initial linear portion of the A: $C_i$  curve was taken as the carboxylation rate. The maximum rate of RuBP regeneration was assessed as the maximum assimilation rate (μmol m<sup>-2</sup> s<sup>-1</sup>).

### 3.3. RESULTS

#### 3.3.1. CHLOROPHYLL ANALYSIS

The results of the chlorophyll analysis in this thesis are presented as both the chlorophyll content per unit area and the chlorophyll content per unit dry weight for each of the species (Figures 3.3. - 3.6). Total chlorophyll expressed on a leaf weight basis gives an indication of how much of the leaf tissue has been allocated to chlorophyll. The results obtained in this method of expression however, can be greatly affected by the leaf thickness and SLW. Total chlorophyll expressed on a leaf area basis indicates the amount of chlorophyll per unit area available for the absorption of light, and is perhaps a better method of expressing the results.

Chl<sub>a</sub>/chl<sub>b</sub> ratios for all the species in all the treatments are presented in Table 3.1.

**Table 3.1.** The effect of light intensity on the chlorophyll a:b ratio of the leaves of the four experimental species. Different letters indicate significant differences at  $P \leq 0.05$ .

	<i>Mimusops caffra</i>	<i>Euclea natalensis</i>	<i>Olea woodiana</i>	<i>Peddiea africana</i>
SUN	2.1 a	1.48 a	1.8 a	1.99 b
40% SHADE	2.2 a	1.95 b	1.72 a	1.6 a
70% SHADE	2.2 a	1.86 b	1.9 a	1.32 a
90% SHADE	2.2 a	1.99 b	1.94 a	1.54 a

*Mimusops caffra*

Chlorophyll contents on a dry weight and leaf area basis are presented in Figure 3.3. Total chlorophyll increased significantly in the shade when expressed either on the basis of leaf weight or leaf area (Fig 3.3). On a leaf weight basis the sun and 40% shade plants were significantly different from the 70% and 90% shade plants for chl<sub>a</sub>, chl<sub>b</sub> and total chlorophyll. On a leaf area basis the chl<sub>a</sub>, chl<sub>b</sub> and total chlorophyll content of the leaves differed between the sun, 40% shade and 70% and 90% shade plants. Chlorophyll a:b ratios increased in the shade but these trends were not significant (Table 3.1).

*Euclea natalensis*

Total chlorophyll increased in the shade (Fig 3.4). On a leaf weight basis there were significant differences between all the treatments but on a leaf area basis, the chlorophyll content of the sun and 40% shade leaves were not significantly different. Patterns in chl<sub>a</sub> and chl<sub>b</sub> content of the leaves varied depending on the method of expression: chl<sub>a</sub> content increased in the shade treatments, but on a leaf weight basis such a trend was significant between all the treatments, while on a leaf area basis the chl<sub>a</sub> content of the 40% shade plants is not significantly different from either the sun or 70% shade plants. When expressed on a leaf weight basis, chl<sub>b</sub> increased significantly in the 70% and 90% shade treatments, but there was no significant increase in the chl<sub>b</sub> content in the leaves when expressed on a leaf area basis.

The increased chl<sub>a</sub>/chl<sub>b</sub> ratios (Table 3.1) in the shade plants was contrary to the trend of chl<sub>b</sub> enrichment in the shade as reported by Bjorkman (1981).

### *Olea woodiana*

The total chlorophyll content was low in the leaves of sun plants, and increased significantly in the shade leaves (Fig 3.5). This trend was particularly evident when expressed on a leaf weight basis where the chlorophyll content of the 90% shade plants was approximately double that of the 70% shade plants.  $\text{Chl}_a$  and  $\text{chl}_b$  content of the leaves followed the same trends as the total chlorophyll content. Although  $\text{chl}_a/\text{chl}_b$  ratios increased in the shade this was not significant (Table 3.1).

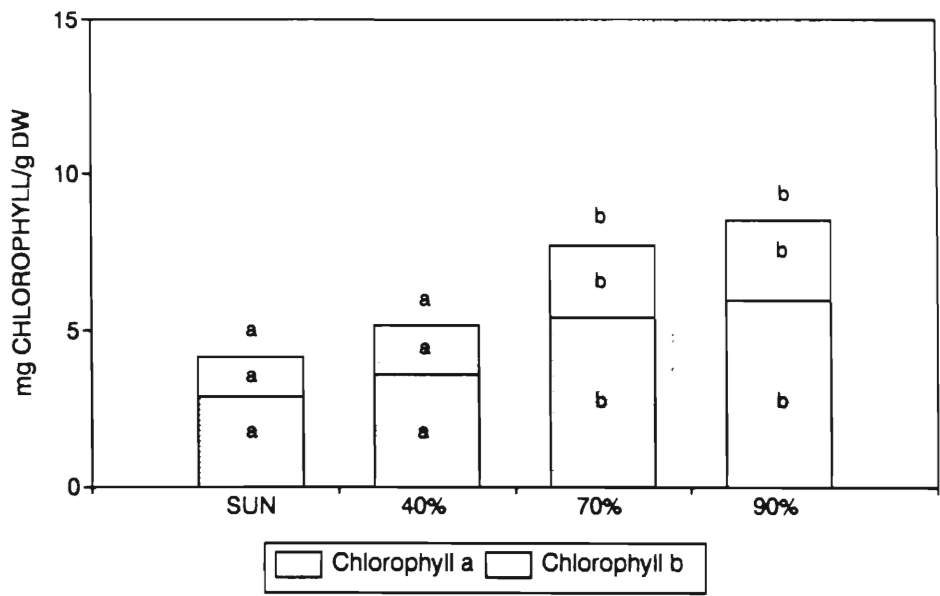
### *Peddiea africana*

Total chlorophyll increased in the shade, and was significantly different between the sun, medium shade (40% and 70%) and 90% shade plants (Fig 3.6). These trends were the same when expressed either on a leaf area or on a leaf weight basis.  $\text{Chl}_a$  and  $\text{chl}_b$  contents showed the same trend as the total chlorophyll content of the leaves. The  $\text{chl}_a/\text{chl}_b$  ratio significantly decreased in the shade (Table 3.1).

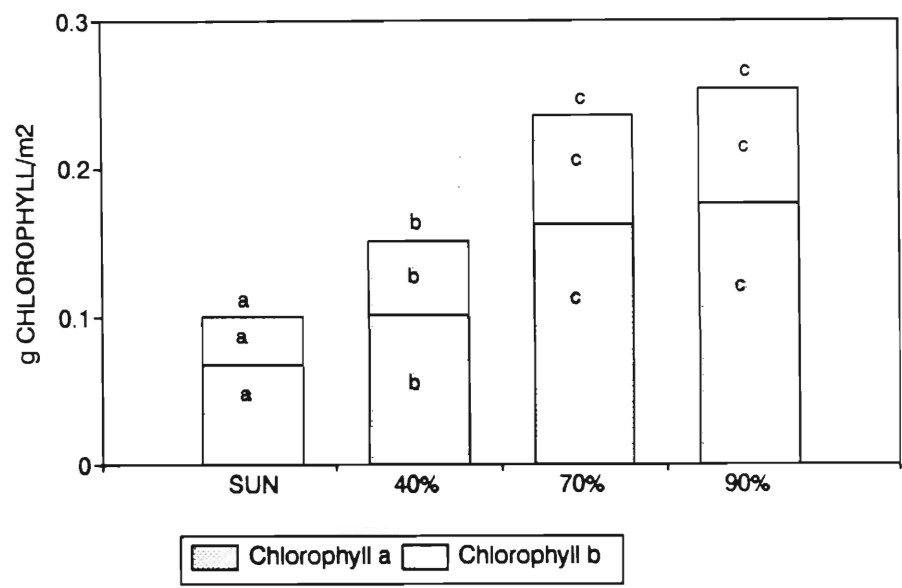
## **3.3.2. NITROGEN ANALYSIS**

The results of the nitrogen analysis are presented as the nitrogen content per gram DW and as the nitrogen content per unit leaf area (Figure 3.7). A comparison of the leaf nitrogen levels between the species in each of the treatment is discussed below.

On a leaf weight basis, all the species showed an increase in nitrogen content in the shade. However, the significance of this increase differed between the species. For *M. caffra* this increase was not significant. In *E. natalensis* the increase was significant only between 90% shade leaves and the other treatments. In *O. woodiana* leaf nitrogen content was significantly different between the sun and 40% shade leaves and the 70% shade leaves

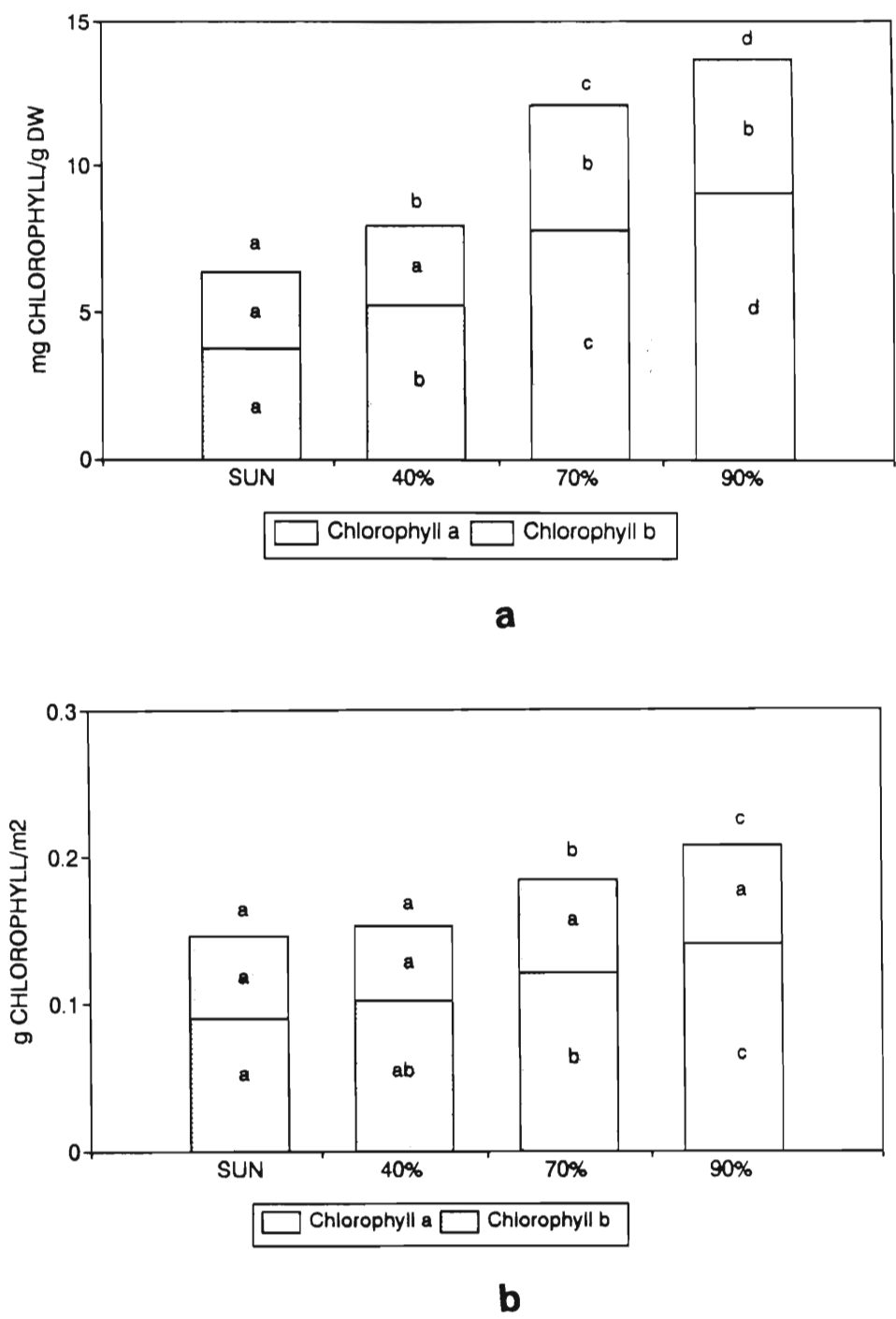


**a**

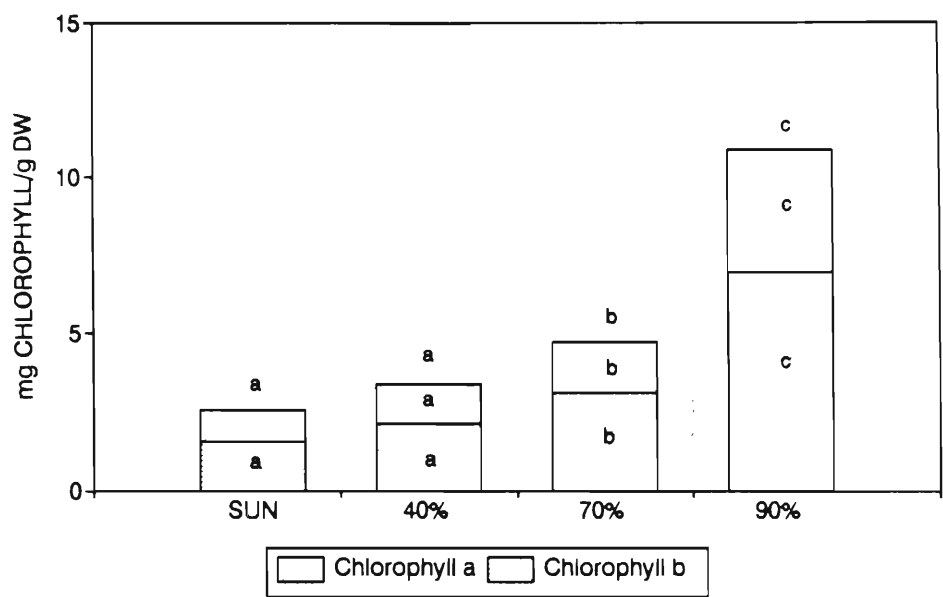


**b**

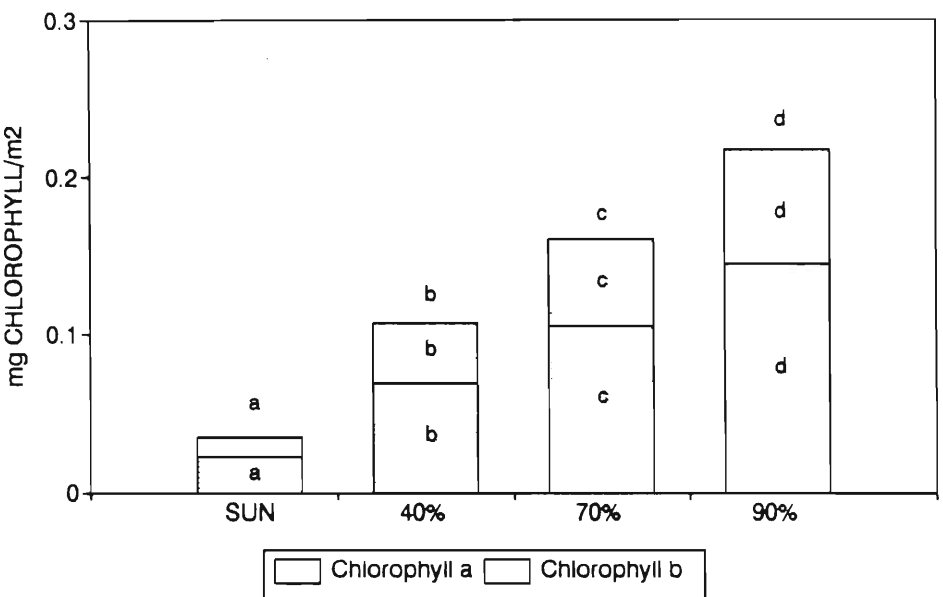
Figure 3.3. The effect of light intensity on the chlorophyll content of leaves of *M. caffra*: a) Chlorophyll content expressed on a leaf dry weight basis b) Chlorophyll content expressed on a leaf area basis. Different letters indicate significant differences at  $P \leq 0.05$ .



**Figure 3.4.** The effect of light intensity on the chlorophyll content of leaves of *E. natalensis*: a) Chlorophyll content expressed on a leaf dry weight basis b) Chlorophyll content expressed on a leaf area basis. Different letters indicate significant differences at  $P \leq 0.05$ .



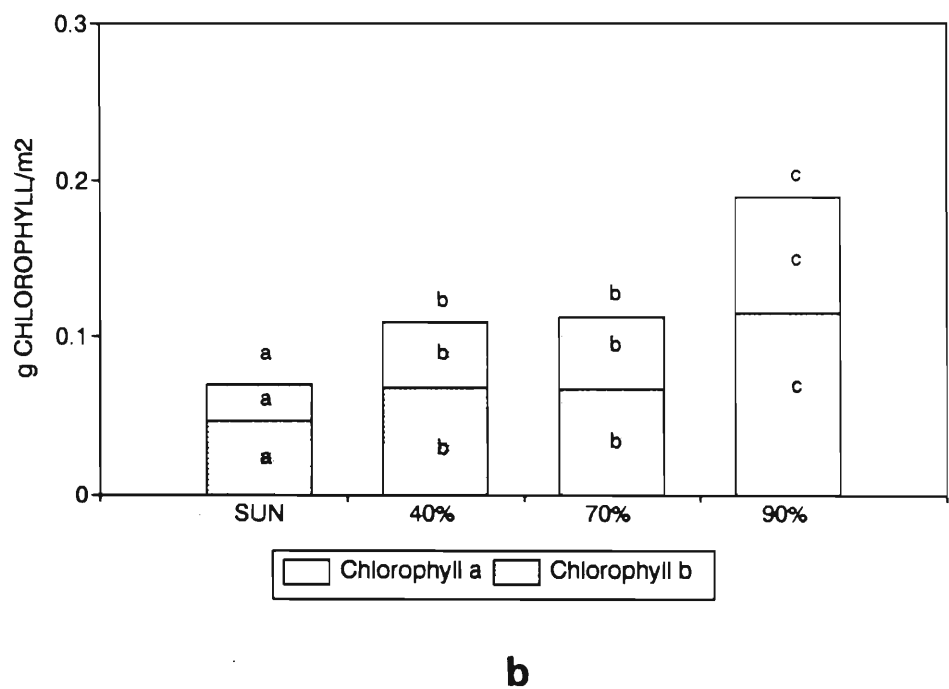
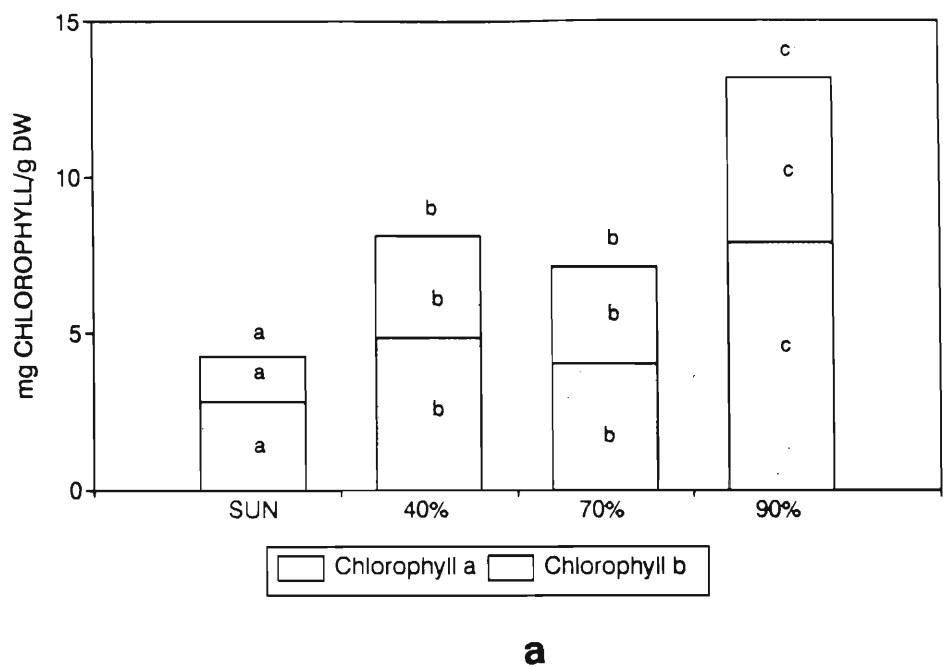
a



b

**Figure 3.5.** The effect of light intensity on the chlorophyll content of leaves of *O. woodiana*: a) Chlorophyll content expressed on a leaf dry weight basis b) Chlorophyll content expressed on a leaf area basis. Different letters indicate significant differences at  $P \leq 0.05$ .





**Figure 3.6.** The effect of light intensity on the chlorophyll content of leaves of *P. africana*: a) Chlorophyll content expressed on a leaf dry weight basis b) Chlorophyll content expressed on a leaf area basis. Different letters indicate significant differences at  $P \leq 0.05$ .

and the 90% shade leaves, with the sun leaves having approximately half the nitrogen content of the 90% shade leaves. In *P. africana* the sun leaves had significantly less nitrogen than the 70% and 90% shade leaves while the nitrogen content of the 40% shade leaves was significantly less than the 90% shade leaves.

On a leaf area basis all species, with the exception of *P. africana*, showed a decrease in the leaf nitrogen content in the shade. Such trends however were not always significant. In plants of *M. caffra* and *O. woodiana* the nitrogen content was significantly different between the sun and shade plants. The trends however were not significant for plants of *E. natalensis*. In plants of *P. africana* the leaf nitrogen content increased in the shade treatments and was significantly different between the sun and shade treatments.

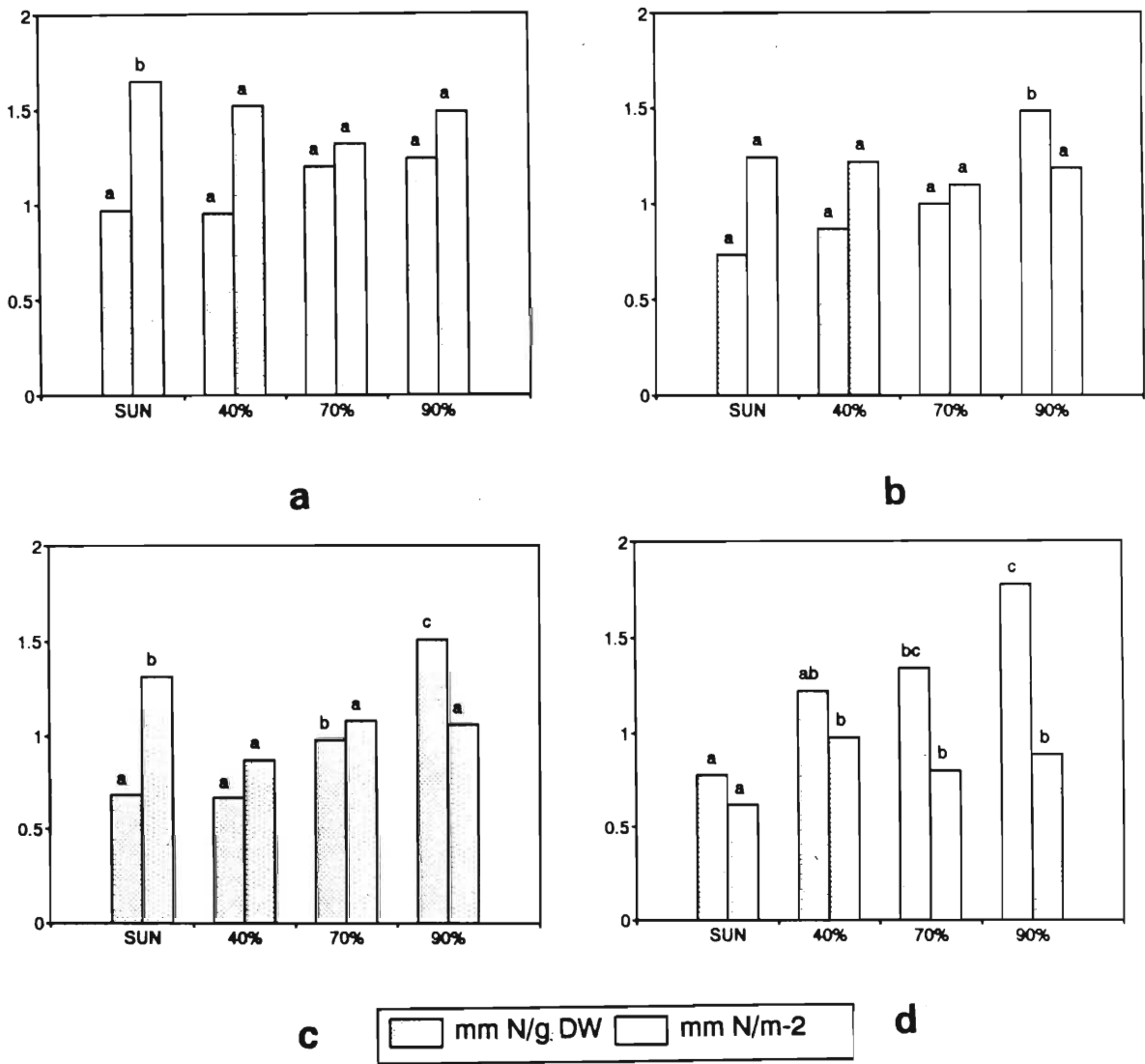
A comparison that was not statistically tested to show the relative quantities of nitrogen on both a leaf area and leaf weight basis for each species in the four treatments is discussed:

i) Comparing the nitrogen content on a leaf weight basis: in the sun treatment it is evident that the highest nitrogen content occurred in the leaves of *M. caffra*, while the lowest nitrogen content was in sun leaves of *O. woodiana*. The sun leaves of *P. africana* had surprisingly high nitrogen contents considering it is a sub-canopy species. In the 40% shade treatment *P. africana* leaves had the highest nitrogen content, while the leaves of *O. woodiana* had the least. The nitrogen content of the leaves of *M. caffra* and *E. natalensis* was similar. In the 70% shade treatment there was very little difference between the nitrogen contents of *E. natalensis* leaves and those of *O. woodiana*, which had the lowest nitrogen contents among the species in this treatment. Leaves of *P. africana* had the highest nitrogen content in the 70% shade. In 90% shade treatment, the leaves of *M. caffra* had the least nitrogen. Again the leaves of *E. natalensis* and *O. woodiana* had similar

nitrogen contents while leaves of *P. africana* had the highest nitrogen content.

ii) Comparing nitrogen content on a leaf area basis:

Among the species plants of *M. caffra* had the highest nitrogen content in all treatments. The nitrogen content of *E. natalensis* and *O. woodiana* were similar in all treatments. *P. africana* had the lowest nitrogen content of the plants in the sun treatment, but in the shade treatments there was little difference between the nitrogen content of *P. africana* and *O. woodiana* or *E. natalensis*.



**Figure 3.7.** The effect of light intensity on the nitrogen content of the leaves of a) *M. caffra* b) *E. natalensis* c) *O. woodiana* d) *P. africana*. Bars with different letters are significantly different at  $P < 0.05$ .

3.3.3. PHOTOSYNTHETIC LIGHT RESPONSE

A complete data set together with the line of best fit to the modified saturation exponential derived from non-linear regression for two treatments is shown in Figure 3.8.

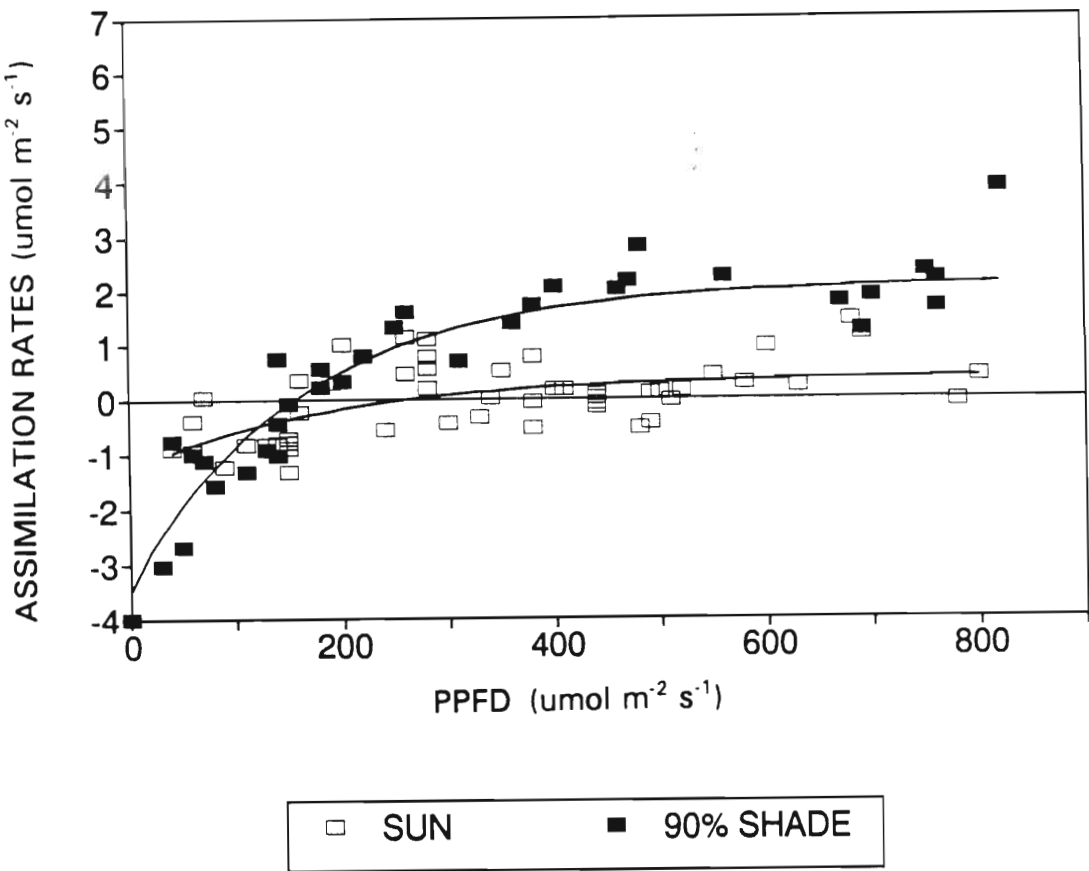
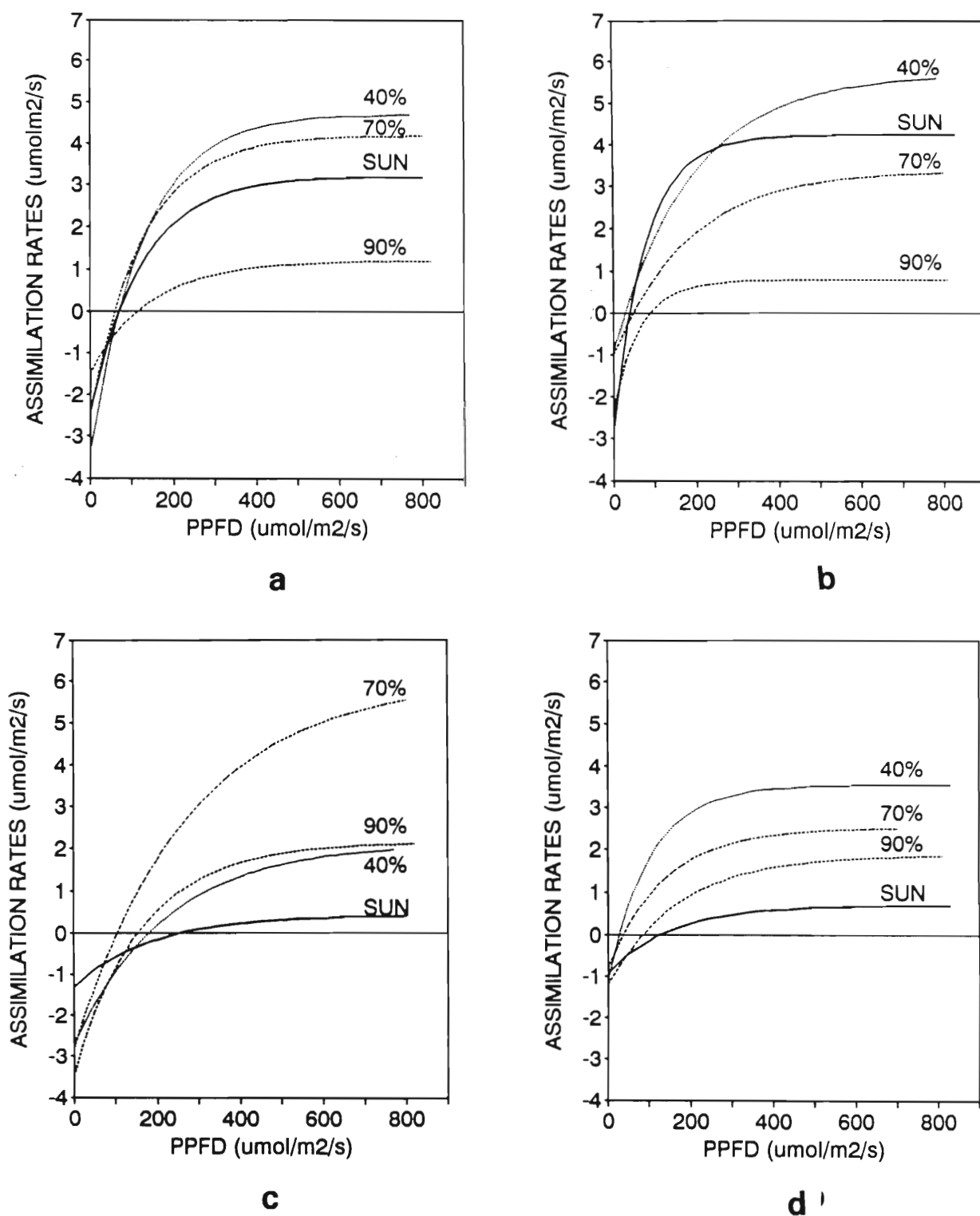


Figure 3.8. A complete data set and best fit line for sun and 90% shade plants of *O. woodiana*.

Figure 3.9. shows the lines of best fit to the light response data for the four species and four growth treatments. The constants for these lines and the derived values of  $R_d$  and  $\phi$  are presented in Tables 3.2. - 3.5.

The photosynthetic responses of the four species to growth in different light intensities was complex. However, *M. caffra* and *E. natalensis* showed some typical sun plant characteristics while *O. woodiana* and *P. africana* showed some typical shade plant responses.



**Figure 3.9.** Photosynthetic light response curves of four tree species grown under full sun, 40% shade, 70% shade and 90% shade. a) *M. caffra* b) *E. natalensis* c) *O. woodiana* d) *P. africana*

*Mimusops caffra*

The effect of growth light intensity on the photosynthetic light response of *M. caffra* was marked, particularly in low light intensity. Plants from all treatments were light saturated at approximately 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 3.9.a).

A more detailed comparison of the effect of light intensity on derived and calculated characteristics is given in Table 3.2.

Table 3.2. The light response curve of photosynthesis. Parameter estimates and their standard errors for the saturated exponential of *M. caffra*.

TREATMENT	$A_{\text{max}}$ $\mu\text{molm}^{-1}\text{s}^{-1}$	$L_c$ $\mu\text{molm}^{-1}\text{s}^{-1}$	n	$R_d$ $\mu\text{molm}^{-1}\text{s}^{-1}$	$\phi$
SUN	3.2 $\pm 0.3$	70 $\pm 12$	-0.081 $\pm 0.001$	2.4	0.034
40% SHADE	4.7 $\pm 0.2$	70 $\pm 10$	-0.078 $\pm 0.003$	3.4	0.048
70% SHADE	4.2 $\pm 0.1$	59 $\pm 6$	-0.078 $\pm 0.003$	2.5	0.041
90% SHADE	1.2 $\pm 0.2$	115 $\pm 14$	-0.069 $\pm 0.002$	1.5	0.012

Plants in 40% shade had the highest  $A_{\text{max}}$  values and the 90% shade plants had very low  $A_{\text{max}}$  values. The light compensation point was low for the 70% shade plants, not different between the sun and 40% shade plants and very high for the 90% shade plants. The curvature of the light response curves was similar for all four treatments. Dark respiration was highest in the 40% shade plants and lowest in the 90% shade plants. The apparent quantum efficiency decreased significantly in the 90% shade.

*Euclea natalensis*

Changes in growth light intensity resulted in four distinct light response curves for *E. natalensis*. Sun, 70% and 90% shade plants were light saturated at approximately 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  respectively, while the 40% shade plants did not reach light saturation until 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 3.9.b). A more detailed comparison of the factors determining the light response curves is found in Table 3.3.

Table 3.3. The light response curve of photosynthesis. Parameter estimates and their standard errors for the saturated exponential of *E. natalensis*.

TREATMENT	$A_{\text{max}}$ $\mu\text{molm}^{-1}\text{s}^{-1}$	$L_c$ $\mu\text{molm}^{-1}\text{s}^{-1}$	n	$R_d$ $\mu\text{molm}^{-1}\text{s}^{-1}$	$\phi$
SUN	4.2 ± 0.2	40 ± 8.92	-0.012 ± 0.002	2.8	0.069
40% SHADE	5.7 ± 0.8	28 ± 5.31	-0.005 ± 0.001	0.9	0.033
70% SHADE	3.4 ± 0.2	49 ± 11.39	-0.005 ± 0.001	1.0	0.021
90% SHADE	0.8 ± 0.1	89 ± 10.42	-0.015 ± 0.003	2.3	0.026

The light saturated assimilation rates were greatest in 40% shade and lowest in 90% shade. The light compensation point was low in the 40% shade plants. The curvature of the graph was similar for the 40% and 70% shade plants and for the sun and 90% shade plants. Dark respiration rates were highest for the sun and 90% shade plants. Plants from 40% shade had very low dark respiration rates. Apparent quantum efficiency was greatest in the sun plants and did not differ considerably among the shade plants.

Olea woodiana

High light intensity growth conditions caused photoinhibition in plants of *O. woodiana*. Plants from all treatments reached light saturation above 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , but plants from 70% shade never reached light saturation. All plants had high light compensation points (Figure 3.9.c). These observations are shown in more detail in Table 3.4.

Table 3.4. The light response curve of photosynthesis. Parameter estimates and their standard errors for the saturated exponential of *O. woodiana*

TREATMENT	$A_{\text{max}}$ $\mu\text{molm}^{-1}\text{s}^{-1}$	$L_c$ $\mu\text{molm}^{-1}\text{s}^{-1}$	n	$R_d$ $\mu\text{molm}^{-1}\text{s}^{-1}$	$\phi$
SUN	0.4 ± 0.2	258 ± 51.11	-0.005 ± 0.003	1.3	0.005
40% SHADE	2.1 ± 0.5	180 ± 18.37	-0.004 ± 0.001	2.7	0.015
70% SHADE	6.0 ± 2.2	105 ± 22.67	-0.003 ± 0.001	2.8	0.026
90% SHADE	2.3 ± 0.2	154 ± 12.34	-0.006 ± 0.001	3.5	0.021

High light intensity significantly reduced the light saturated assimilation rate. Plants from 70% shade had very high  $A_{\text{max}}$  values. Light compensation points were high for all the treatments, especially for the sun and 40% shade plants. Dark respiration rates increased with decreasing light intensity. The apparent quantum efficiency increased in the shade.



*Peddiea africana*

The light response curves of *P. africana* were distinct for each treatment. Growth under high light intensity caused photoinhibition and the highest assimilation rates were recorded in the 40% shade plants. Plants from all treatments were light saturated at approximately 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 3.9.d). These observations are shown in more detail in Table 3.5.

Table 3.5. The light response curve of photosynthesis. Parameter estimates and their standard errors for the saturation exponential of *P. africana*.

TREATMENT	$A_{\text{max}}$ $\mu\text{molm}^{-1}\text{s}^{-1}$	$L_c$ $\mu\text{molm}^{-1}\text{s}^{-1}$	$n$	$R_d$ $\mu\text{molm}^{-1}\text{s}^{-1}$	$\phi$
SUN	0.7 $\pm 0.3$	128 $\pm 9.2$	-0.007 $\pm 0.002$	0.9	0.007
40% SHADE	3.5 $\pm 0.2$	28 $\pm 8.6$	-0.009 $\pm 0.002$	1.1	0.038
70% SHADE	2.5 $\pm 0.3$	38 $\pm 15.5$	-0.007 $\pm 0.002$	0.8	0.021
90% SHADE	1.9 $\pm 0.1$	85 $\pm 7.8$	-0.005 $\pm 0.001$	1.2	0.014

Plants of *P. africana* in 40% shade had the highest light saturated assimilation rates, lowest light compensation points and the highest apparent quantum efficiency. Sun plants on the other hand, had low light saturated assimilation rates, a high light compensation point, a low dark respiration rate and a low apparent quantum efficiency. High light intensity appears to destroy the photosynthetic potential of plants of *P. africana*.

4.3.4. THE RESPONSE OF ASSIMILATION TO INTERCELLULAR CO<sub>2</sub> CONCENTRATION

A complete A:C<sub>i</sub> curve for two treatments is shown in Figure 3.10. Feedback inhibition at high CO<sub>2</sub> concentrations was observed in all the plants studied.

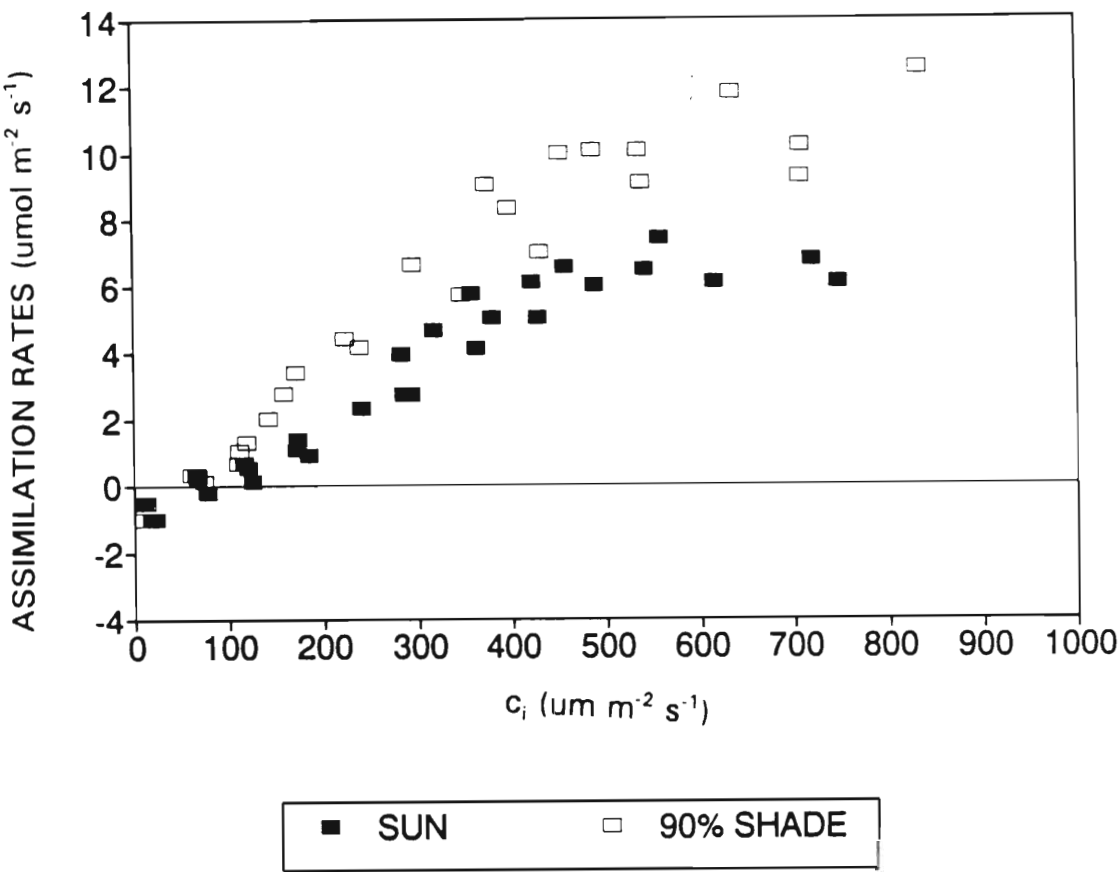


Figure 3.10. A complete A:c<sub>i</sub> curve of sun and 90% shade plants of *E. natalensis*.

The mean initial slopes and maximum (saturated) assimilation values for three curves per treatment for the three species are presented in Tables 3.6 - 3.9. The initial slope is the carboxylation efficiency and is taken as a measure of the rubisco activity. The maximum assimilation value is a measure of the capacity of RuBP regeneration.

Mimusops caffra

The initial slope of the  $A:C_i$  curves were significantly different among the treatments. Rubisco activity in the 40% shade plants was approximately three times greater than in the sun plants. Although rubisco activity in the 90% shade plants was considerably less than the 40% shade plants, it was still double the activity of the sun plants (Table 3.6).

$A_{max}$  (RuBP regeneration capacity) followed similar trends. However, the rapid regeneration in 40% shade was not significantly different from that in the 70% shade. Regeneration capacity was significantly different between the 70% and 90% shade plants. The RuBP regeneration in the sun plants was approximately half of that in the other treatments (Table 3.6).

Table 3.6 Carboxylation efficiency and maximum RuBP regeneration rate for plants of *M. caffra* grown under different light regimes.

TREATMENT	RUBISCO ACTIVITY (mmol m <sup>-2</sup> s <sup>-1</sup> )	RUBP REGENERATION (μmol m <sup>-2</sup> s <sup>-1</sup> )
SUN	8.2 a	5.1 a
40% SHADE	27.5 d	12.0 c
70% SHADE	20.5 c	11.8 c
90% SHADE	16.8 b	10.2 b

Euclea natalensis

The initial slopes were not significantly different between the 40% and 70% shade plants. Rubisco activity in 90% shade was significantly lower, and the lowest activity occurred in the sun plants (Table 3.7).

$A_{max}$  was significantly higher in the 40% shade plants than the plants from the remaining treatments. There was no significantly difference

in RuBP regeneration capacity between the sun, 70% and 90% shade plants (Table 3.7).

Table 3.7. Carboxylation efficiency and maximum RuBP regeneration rate for plants of *E. natalensis* grown under different light regimes.

TREATMENT	RUBISCO ACTIVITY (mmol m <sup>-2</sup> s <sup>-1</sup> )	RuBP REGENERATION (μmol m <sup>-2</sup> s <sup>-1</sup> )
SUN	16.8 a	7.8 a
40% SHADE	26.5 c	12.8 b
70% SHADE	27.2 c	8.1 a
90% SHADE	20.0 b	8.9 a

*Olea woodiana*

The initial slope was significantly different between the sun and shade treatments. The rubisco activity of the shade plants was approximately double that of the sun plants (Table 3.8).

RuBP regeneration rate was not significantly different between the shade plants and was double that of the sun plants (Table 3.8).

Table 3.8. Carboxylation efficiency and maximum RuBP regeneration rate for plants of *O. woodiana* grown under different light regimes.

TREATMENT	RUBISCO ACTIVITY (mmol m <sup>-2</sup> s <sup>-1</sup> )	RuBP REGENERATION (μmol m <sup>-2</sup> s <sup>-1</sup> )
SUN	9.8 a	7.8 a
40% SHADE	19.5 b	10.2 b
70% SHADE	21.4 b	9.8 b
90% SHADE	20.0 b	9.8 b

Peddiea africana

The initial slope was low and not significantly different between the sun and 90% shade plants. The greatest rubisco activity occurred in the 70% shade plants, and this was approximately one and a half times that of the sun and 90% shade plants (Table 3.9).

The highest rate of RuBP regeneration occurred in the 40% and 70% shade plants. Regeneration in these treatments was double that occurred in 90% shade, and approximately one and a half times greater than that of the sun plants (Table 3.9).

Table 3.9. Carboxylation efficiency and maximum RuBP regeneration rate for the plants of *P. africana* grown under different light regimes.

TREATMENT	RUBISCO ACTIVITY (mmol m <sup>-2</sup> s <sup>-1</sup> )	RuBP REGENERATION (μmol m <sup>-2</sup> s <sup>-1</sup> )
SUN	8.9 a	5.8 b
40% SHADE	10.5 b	9.8 c
70% SHADE	14.5 c	9.8 c
90% SHADE	9.0 a	4.2 a

**3.4. DISCUSSION**

Before the relationships among the photosynthetic potential and the chlorophyll content, leaf nitrogen content, rubisco activity and RuBP regeneration are discussed, it is necessary to first consider the effect of light intensity on each of these factors individually.

Although no statistical tests were conducted between the species, a simple comparison of the chlorophyll content of the plants of the four species in the different light treatments shows the following:

- a) All species show an increase in the chlorophyll content of the leaves in the shade. Although Clough *et al.* (1979), reported that shading may cause a significant decrease in the chlorophyll content of sun plants, this was not observed in *M. caffra* or *E. natalensis*.
- b) Although *P. africana* is a sub-canopy species, high light intensity caused less photobleaching of the chlorophyll than it did to the leaves of *O. woodiana* which matures into a canopy tree.
- c) When expressed on a leaf weight basis, plants of *E. natalensis* have considerably higher chlorophyll contents than all other species, and therefore higher chlorophyll contents in the sun than the pioneer species (*M. caffra*) and higher chlorophyll contents in the shade than the shade species (*O. woodiana* and *P. africana*).
- d) When expressed on a leaf area basis, the shade species have lower chlorophyll contents than the sun species, even in the lower light intensities.
- e) Plants of *M. caffra* had higher  $\text{chl}_a/\text{chl}_b$  ratios than the other three species. Trends in  $\text{chl}_a/\text{chl}_b$  ratios were significant only in *E. natalensis* and *P. africana*. However, the reported trend of  $\text{chl}_b$  enrichment in the shade (Björkman, 1981), occurred only in *P. africana*, and this species was therefore the only one which was able to balance the energy distribution between PSI and PSII in the shade.

It is interesting to note that in conditions of high light intensity plants of *M. caffra* had the highest nitrogen content when expressed on either a leaf weight or area basis. However, in 90% shade these plants had the lowest nitrogen content of all the species when expressed on a leaf weight basis but the highest when expressed on a leaf area basis. The high nitrogen content associated with high light intensity will be an adaptation for the natural habitat of this pioneer species. In order to utilise high light intensity there is a need for nitrogen for photosynthetic enzymes. Although on a leaf weight basis the 90% shade plants had the lowest nitrogen content among the species, it was not significantly different from the nitrogen content of sun plants. The 90% shade plants therefore accumulated large quantities of

nitrogen, which they are unable to use efficiently for photosynthesis and growth (Chapter 2).

Low light intensity increased the nitrogen content of the leaves of *E. natalensis*. However, plants in 90% shade have very low growth rates (Chapter 2) so this high nitrogen may represent "luxury" consumption, over and above the growth requirements of the plants.

High light intensity greatly reduces the nitrogen content on a leaf weight basis in *O. woodiana*.

Plants of *P. africana* from low light intensities have very high nitrogen contents in their leaves. As *P. africana* is a sub-canopy species which grows best in shade this is not surprising as in the natural habitat these plants will be able to utilise this nitrogen for photosynthesis and growth. The trend of increased nitrogen content on a leaf area basis in the shade is linked to the increased SLW of this species in shade treatments (Chapter 2).

Although assimilation rates were low when compared to other species, the response of the assimilation rate to changes in light intensity appears to be related to the successional status of the species. Jurik *et al.* (1988) found that assimilation rate were highest for early successional tree species and that they decreased as succession proceeded. High assimilation rates were recorded in *M. caffra* and *E. natalensis*. Although the 70% shade plants of *O. woodiana* had very high assimilation rates these appear to be the exception, and on the whole the assimilation rates recorded in the shade species were considerably lower than those for the sun species.

High light intensity inhibited the photosynthetic process of the shade species, *O. woodiana* and *P. africana*. This process was first described by Montfort (1941) and termed "photolability". Montfort (1941), demonstrated that the first damage caused by strong light is done at the centres of

primary photoreactions which is manifested in an increased quantum requirement for CO<sub>2</sub> uptake. Photolability was particularly evident in the sun grown plants of *O. woodiana*. The sun plants of *P. africana* also showed some degree of photolability.

Low light intensity (90% shade) decreased the assimilation rates of *M. caffra* and *E. natalensis*.

Typically shade plants have low dark respiration rates, low light saturation intensities and low light compensation points, which are considered to be adaptations to the environment in which they are found. Sun plants by comparison have high dark respiration rates and light compensation points (Björkman, 1981). Although no statistical tests were conducted between species, plants of the shade species, *P. africana*, did appear to have lower dark respiration rates than all other species in the four light treatments. High dark respiration rates were recorded in both *M. caffra* and *O. woodiana*. This was typical of a pioneer species but the response of *O. woodiana* was surprising.

Comparisons of the light compensation points and the light saturation intensities however, were not as straight forward. Light compensation points are of particular ecological interest. Ramos and Grace (1990) observed that shade grown plants always have lower light compensation points than sun grown plants, but that the light compensation point of shade species was not necessarily lower than sun species.

Lower light compensation points were observed in the shade plants of *O. woodiana* and *P. africana*. However, with the exception of 40% shade *E. natalensis* plants, shade plants of *M. caffra* and *E. natalensis* had higher light compensation points than the sun plants. It is necessary to point out the very high light compensation points of the plants of *O. woodiana* even in the deep shade treatments. The reasons for this are unclear.



The light saturation intensity indicates the light intensity above which additional light can no longer be utilised. Plants of *M. caffra* were saturated at light intensities between 300 and 500  $\mu\text{molm}^{-2}\text{s}^{-1}$ . *E. natalensis* saplings were light saturated between 150 and 700  $\mu\text{molm}^{-2}\text{s}^{-1}$ .

*O. woodiana* saplings from 70% shade never reached light saturation, while saplings from the other light treatments were light saturated above 500  $\mu\text{molm}^{-2}\text{s}^{-1}$ . Light saturation intensities for *P. africana* ranged from 200-400  $\mu\text{molm}^{-2}\text{s}^{-1}$ . These results were surprising, as shade plants were expected to have low saturating intensities and sun plants high saturating intensities. The saturating light intensities observed in *O. woodiana* may be due to damage to photosystems I and II.

Ramos and Grace (1990) found that the apparent quantum efficiency was insensitive to light and that climax species did not necessarily have higher quantum efficiencies than early stage species. This observation was applicable to these results as there appeared to be no relationship to a species successional status and the observed trends for quantum efficiency. In the sun, plants of *E. natalensis* had the highest quantum efficiency. The quantum efficiency of *M. caffra* in 40% and 70% shade treatments were higher than, while that of *E. natalensis* was similar to, *O. woodiana* and *P. africana*. In the 90% shade treatment the plants of *M. caffra* had the lowest quantum efficiency, but this was similar to that of *P. africana* in the same treatment.

Work on either sun or shade plants has shown that, in general, sun plants have higher rubisco activity than shade plants. The lack of statistical tests between the species and the large number of treatments in this study, made it difficult to determine whether this observation was true for the species in this study. However, if one looks at general trends, then it is evident that the high rubisco activity in 40% shade plants of *M. caffra* and 70% shade plants of *E. natalensis* is approximately double that of the highest rubisco activity observed in *P. africana*. A very general statement that sun

plants have higher rubisco activity than the shade plants can be made from this study.

Very little (if any) literature exists on the effect of different light intensities during growth of sun and shade plants, on rubisco activity and RuBP regeneration. Therefore, it was difficult to compare the trends observed in this study with other work.

All species in this study had the lowest rubisco activity in the sun plants and the highest in either the 40% or 70% shade plants. The amount of rubisco present in the leaves should suffice to maximise the benefits against the costs. The benefits of high amounts of rubisco include potentially increased carboxylation rates, which in turn have positive effects on assimilation of carbon, while the costs involve the costs of production of the enzyme. High carboxylation rates will depend on the presence of sufficient substrate (RuBP), for the reaction. In order to understand the observed trend, it is necessary to consider not only the rubisco activity, but also the capacity for the regeneration of RuBP, the substrate for rubisco activity. In this study, the maximum RuBP regeneration rate was low in all the sun grown plants, while regeneration rate was high in the shade grown plants.

Photosynthetic activity in low light conditions is proportional to the light absorbed by the photosynthetically active pigment (Gabrielsen, 1948). Although all the species showed an increase in leaf chlorophyll content in the shade, this was not necessarily associated with an increase in the photosynthetic activity of shade plants. Björkman and Holmgren (1963) have shown that variations in chlorophyll content in this range have little effect on the light absorption and differences in photosynthetic activity are therefore attributable to properties other than light absorption. The low photosynthetic rates of the 90% shade plants of *M. caffra* and *E. natalensis*

are therefore a consequence of other factors as well as that of light absorption.

The photosynthetic apparatus of shade species is typically unable to tolerate high light intensities. This is indicated by photobleaching and the loss of reaction centres on exposure to bright light (Boardman, 1977). The typical bleaching and deformation of chloroplasts of shade plants grown in strong light, termed photolability (Montfort, 1941) is associated with a decrease in the photosynthetic efficiency. The decline in photosynthetic activity evident in the sun grown plants of *O. woodiana* and *P. africana*, may be evidence of photolability.

Björkman (1981) found that light saturation rates of photosynthesis showed little relationship to chlorophyll content but a strong relationship between factors likely to limit dark respiration e.g. rubisco or total soluble protein. Field and Mooney (1986) suggested that photosynthetic assimilation rate is limited by both biochemical factors and diffusion factors, but that the majority is biochemical. Biochemical limitations are imposed by the nitrogen containing compounds e.g. rubisco. Numerous authors have found a direct relationship between the leaf nitrogen content and the photosynthetic rate (Rawson and Hackett, 1974; Gulmon and Chu, 1981). However, in these experiments the only plants that showed a direct relationship between the nitrogen content (on a DW basis) and assimilation rates were the sun and 40% and 70% shade plants of *O. woodiana* and sun plants of *P. africana*. The low nitrogen content of sun and 40% shade plants of *O. woodiana* and sun plants of *P. africana* was associated with the low assimilation rate. The high nitrogen content of the 70% shade plants of *O. woodiana* was associated with a high assimilation rate. Wallace and Dunn (1980) also reported no relationship between the total leaf nitrogen and the photosynthetic rates for three gap phase successional species.

Analyses by Björkman (1981) and Farquhar and Sharkey (1982) indicated that the higher photosynthetic rates of sun leaves are a result of both higher stomatal conductance, and higher intrinsic photosynthetic capacity of the mesophyll at a given conductance. This higher mesophyll capacity reflects in part the higher concentration of rubisco and other photosynthetic enzymes in sun leaves. However, there appeared to be no relationship between the photosynthetic activity of sun leaves and the rubisco content of *M. caffra* and *E. natalensis*. The low assimilation rates of sun plants of *O. woodiana* and *P. africana* were related to the low rubisco activity in this light regime.

A direct relationship between rubisco activity and assimilation rates was also observed in the 40% and 70% shade plants of *M. caffra*, 40% shade plants of *E. natalensis*, 70% shade plants of *O. woodiana* and 40% and 70% shade plants of *P. africana*.

In conclusion the relationship between the chlorophyll content, nitrogen content, rubisco activity and maximum RuBP regeneration rate and photosynthetic activity under normal conditions of the species is a complex one that is difficult to define without further detailed experimentation.

## CHAPTER FOUR

### CONCLUSIONS

As plant primary production is ultimately dependent on photosynthetic  $\text{CO}_2$  uptake, it is necessary to determine whether there is any association between the growth rates and the photosynthetic potential of the species and whether this is in any way associated with their successional status. Photosynthate production also depends on the amount of photosynthetically active tissue constructed by the plants and on aspects of carbon use including expenses and investments. The relationships between, and the effect of, carbon use efficiency and photosynthetic uptake on the growth of a species are complicated and very difficult to quantify and any associations discussed here are purely hypothetical.

Before these relationships can be discussed, it is necessary to highlight some of the limitations of the experimental procedure. The aim of the project was essentially to determine the effect of light intensity on aspects of growth, morphology and photosynthesis of the four species and whether this was any way related to their successional status. However, as the experimental light regimes were created by the use of shade cloth they in no way resemble the light regime in the natural habitat; shade cloth effectively alters the density of light but has no effect on the red:far red ratio. In the natural habitat as light passes through the canopy the blue and red wavebands are preferentially absorbed which effectively increases the proportion of far red radiation in shaded habitats. Red:far red ratios have been shown to have some effect on etiolation and flowering responses of certain species, and this is important to consider when analyzing the results. A second potential problem was the nutrient status of the experimental plants. It was decided not to supply additional mineral nutrients to the experimental material as in the natural habitat the plants do grow on nutrient poor dune soils and subsequently have a relatively slow growth rate. This slow growth rate in the natural habitat, may be necessary for the survival

of the plants; rapid expansion of the leaves due to the addition of nutrients, lowers a plants tolerance to the detrimental effect of salt spray (H. Logan, unpublished honours thesis, 1979). As a consequence of not adding additional nutrients, the experimental material may have suffered some degree of nutrient deficiency. Although this may have occurred, all the plants of each species will have been nutrient stressed to a similar extent in all treatments. Some species however, are better adapted for coping with nutrient limitations and this must also be considered. Thirdly the lack of competition between plants for the available resources (light and water) might have affected the growth response and thus affected the results. The data presented here might have limited applicability to plants growing in the natural habitat, but they do aid in elucidating some understanding of the probable light related responses of the plants in the natural habitat.

The results of the project show that the light requirement of the four successional species is not simply related to their successional status. In fact all four species showed good growth in light regimes other than those that they are found in the natural habitat. Particularly surprising results were the fast growth rate of *M. caffra* and *P. africana* saplings in the 70% shade and sun treatments respectively, and the low growth rate of *O. woodiana* in the sun treatment. There also appeared to be little relationship between the growth rates and the photosynthetic potential in all species.

Another unexpected trend in the study was that all species showed sun and shade characteristics irrespective of their successional status. In all species the leaf size, chlorophyll and nitrogen contents of the leaves increased and leaf and branch inclination and SLW decreased in the shade treatments. It is noteworthy that there was very little difference in the response among the species of different successional status.  $Chl_a:chl_b$  ratios however, did not follow similar trends and *P. africana* was the only species that showed characteristic shade responses and so was effectively

able to balance the energy distribution between the PSI and PSII in the shade.

General observations and possible explanations for some of the observed trends are discussed below:

The pioneer species *M. caffra* was well adapted to its the environment which was associated with a high light regime in the natural habitat. The branches were thickened for strength against the high wind speed that is associated with high light intensity in the natural environment. The dune forefront is also subject to salt spray and low nutrient conditions, but the effect of these on saplings of *M. caffra* were not determined. Although saplings of *M. caffra* had the best growth amongst the species in the high light treatment, the best growth within this species was found in the 70% shade treatment. It is suggested that this scenario is unlikely to occur in the natural habitat as seedlings of *M. caffra* are not found in low light regimes. It is possible that this is a consequence of the seed germination characteristics, but studies on the light response of germination need to be undertaken. One must also consider that in the shaded natural habitat, plants will be competing for resources while in the experimental conditions this did not occur. As a pioneer species *M. caffra* will be able to tolerate low nutrient conditions and it is probable that the high growth rate in the 70% shade may be a combination of the ability to tolerate low nutrient and the lack of competition. One must also consider that plants of *M. caffra* are adapted for growth in low nutrient situations and this may give these plants a competitive advantage over the other three species. The high nitrogen content in the sun plants was not accompanied by an increase in growth rate. The low growth rate of the sun plants may be associated with the costs involved in producing thickened branches. Plants of *M. caffra* were unable to successfully grow in 90% shade which indicates some minimum requirement for light for successful sapling establishment. There appeared to be no relationship between the growth rates and the photosynthetic potential for the sun, 40% and 70% shade plants of *M. caffra*. The low

growth rate in the 90% shade and the photosynthetic potential however are related.

Although there were no differences in the growth rates of the sun, 40% and 70% shade plants of *E. natalensis*, there were very marked differences in the assimilation rates and the nitrogen and chlorophyll content of the leaves, all factors which affect the photosynthetic potential of these plants. Although the individual assimilation rates of the leaves of sun plants of *E. natalensis* were lower than the 40% and 70% shade plants, it is possible that the assimilation rate of the sun plants will be offset by the large number of leaves. Despite the similarity between growth in these three treatments, rubisco activity and RuBP regeneration rates were significantly different. The adaptive significance of the high quantum efficiency and low light compensation points of the sun plants is also unknown.

The low growth rate and apparent damage to leaves of the sun saplings of *O. woodiana* poses the question of the stage of development at which saplings of this species acquire the ability to adapt to high light intensities. One possible explanation is that the outer layer of leaves on the canopy are "sacrificial" and although they are unable to contribute significantly to the overall carbon assimilation, they are able to shade the leaf layers below and therefore create a more suitable light environment for photosynthesis. There was no relationship between assimilation rates of *O. woodiana* plants and growth rates. The fairly large growth rates in sun plants was surprising in view of the low chlorophyll content and assimilation rates associated with these plants. The 70% shade plants which did not reach light saturation and which had high chlorophyll and nitrogen contents, had the same above ground growth rates as did the sun plants. The reasons for this are not clear. Possible further work involving *O. woodiana*, would be a detailed study on the effect of light intensity on biochemical processes and chloroplast structure.



There appears to be no relationship between the successional status of *P. africana* and the reported growth response, as there was no significant difference in the growth rate between the sun, 40% and 70% shade plants. Assimilation rates did not follow the same trend however, and are in fact more in keeping with the successional status of *P. africana*, with the plants from the shade treatments having higher assimilation rates than the sun plants. Shade plants (40% and 70%) also had high quantum efficiencies and low light compensation points which are typical shade plant adaptations. Another question which remains to be answered is how the sun plants of *P. africana* are capable of such high growth rates when the assimilation rates are so low and the bulk of their biomass can be attributed to branches. An observation which was not reported in the results was the flowering response observed in the sun plants of *P. africana*. Ephemerals are known to change their growth strategy to reproduction during periods of stress, but this is not a common response of perennials. Rather than a stress response, this flowering response may in fact be a R:FR response. It is therefore necessary to determine whether in *P. africana* flowering is in fact controlled by R:FR ratio. It would also be interesting to determine whether the seeds that are produced under conditions of high light intensity are in fact viable.

Although the results of the project have helped to highlight some of the strategies involved in the successional process, many questions remain to be answered. The natural light environment is not static and areas of high light intensity are created by tree fall, and shade is created by plant growth. Therefore species are probably adapted to a wider light regime than that in which they are found at any point in time. However, germination studies to determine whether seeds of the four species are able to germinate in the four light regimes are necessary. Other work which needs to be conducted includes detailed biochemical work to determine the levels of rubisco in the leaves, as the  $A:c_i$  curves are merely an indication of possible amounts. Detailed microscopical work might show anatomical consequences of light intensity on the four species.

The proposed hypothesis that species from different successional stages are preadapted for a particular light environment and are disadvantaged in other light environments was not supported. Light therefore is probably not the only driving force of forest succession but one of many factors that will contribute to the overall process. As a result it is difficult to make assumptions about the successional process if only one potential factor being studied. In the natural habitat there is an interaction not only between light, nutrients, water availability and predation, but also competition amongst plants for the available resources. Although this project has helped in some respects to understand the response of species to light, further studies on the interactions between some of the other factors driving succession (water and nutrient availability) are necessary. Another point to consider is that no studies were conducted in the natural habitat, and in order to get a better understanding of the whole successional process such studies are essential.

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