The Ecophysiology of Selected Coastal Dune Pioneer Plants of the Eastern Cape

Thesis

Submitted in fulfilment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY
Rhodes University
Faculty of Science

By

Bradford Sherman Ripley

July 2001

Table of Contents

Chapter 1 – General Introduction

Chapter 2 - Overview of Methods

Chapter 3 -The Study Site and Plants Investigated

Chapter 4 - Plant Productivity

Chapter 5 - Plant Water Relations

Chapter 6 - Plant Nutrient Relations

Chapter 7 - Plant Physiology

Chapter 8 – Habitat Limitations and Plant Performance

Acknowledgements

- Professor Norman Pammenter, who helped design this research, shivered with me while making pre-dawn measurements on the beach in winter, endured long summer days until late in the evening performing similar tasks, endured equipment failure, read drafts of this thesis, co-authored manuscripts, offered advice, friendship and so much more.

- Professor Ted Botha for his care in reading drafts of this thesis and the loan of his colour printer.

- To the many students who have help with various aspects of this research especially Craig Peter who endured many field trips and was ready to arise before the sun to tackle the next set of diurnal measurements. Craig is also thanked for his production of a map of southern Africa showing the various study sites. Irma Knevel is thanked for her involvement in these field trips, her fantastic organisational and catering skills and for the production of a vegetation map of the study site.

- Professors Valdon Smith and Norman Pammenter for their collaborative research on the function of *Arctotheca populifolia* leaf hairs and for their unpublished *Scaevola plumieri* data.

- Dr Paul Cowley for introducing me to the beautiful Eastern Cape Coast and for showing me some of the lesser known coastal beaches.

- Professor Roy Lubke for his vast wealth of information on coastal dune environments and his willingness to share it. Roy is also thanked for his valuable comments on several of the chapters in this thesis.

- Siep Talma, EMATEK, Pretoria who performed the isotopic analyses and advised on data interpretation.

- Dr Sherman Ripley, my father for proof-reading the final manuscript.

- Natalie Ripley for enduring all the trials and tribulations that arise when one's husband tackles a doctoral study on a part-time basis.

- The Joint Research Council, Rhodes University and the EC-INCO-DC program, INVASS for making this research possible by providing financial assistance.

ABSTRACT

Understanding the mechanisms and adaptations that allow only certain species to thrive in the potentially stressful foredune environment requires a knowledge of the basic ecophysiology of foredune species. Ecophysiological measurements were conducted on the foredune pioneer species *Arctotheca populifolia* (Berg.) Norl., *Ipomoea pes-caprae*(L.) R. Br. and *Scaevola plumieri* (L.) Vahl. and showed significant differences among species with respect to the physiology associated with biomass production, water and nutrient relations.

Differences related to CO_2 assimilation included differences in photosynthetic and respiratory rates, susceptibility to light stress and leaf and stem non-structural carbohydrate concentrations. These resulted in differences in primary production rates of shoots. Mechanisms leading to the differences in CO_2 assimilation among species included differences in stomatal behaviour, carboxylation efficiencies, efficiencies of utilisation of incident photosynthetic photon flux density (PPFD) and rates of ribulose-1,6-bisphosphate (RuBP) regeneration. Correlated with differences in photosynthetic capacity were differences in chlorophyll contents but not differences in leaf nitrogen content.

Differences in interspecific stomatal behaviour resulted in significantly different transpiration rates which in combination with differences in assimilation rates resulted in differences in water-use efficiency. The absolute amounts of water transpired, although significantly different among species, were moderate to high in comparison with species from other ecosystems and were typical of mesophytes. Transpiration rates in combination with plant hydraulic conductances and soil water availability resulted in leaf water potentials that were not very negative and none of the investigated species showed evidence of osmotic adjustment.

The volume of water transpired by each of the species per unit land surface area was estimated from the relationship between abiotic factors and plant water loss. These relationships varied among species and had varying degrees of predictability as a

result of differences in stomatal behaviour between the three species. The water requirements of *A. populifolia* and *S. plumieri* were adequately met by the water supplied by rainfall and the water stored in the dune sands. It was therefore not necessary to invoke the utilisation of ground water or the process of internal dew formation to supply sufficient water to meet the requirements. However, *I. pes-caprae* despite its lower transpiration rates and due to its higher biomass, lost greater volumes of water per unit dune surface area than *either A. populifolia* or *S. plumieri.* This resulted in periods of potential water limitation for *I. pes-caprae*.

Incident light was the most important determinant of leaf photosynthetic CO_2 assimilation and transpiration, particularly as a linear relationship between incident PPFD and atmospheric vapour pressure deficit (VPD) could be demonstrated. Whole plant photosynthetic production by *S. plumieri* was shown to be light limited as a result of mutual shading despite high incident and reflected PPFD occurring in the foredune environment. The leaf hair-layer of *A. populifolia* was shown to be important in reducing transmitted UV and hence reducing photoinhibition but it also caused reduced transpiration rates because of the thicker boundary layer and thus increased leaf temperatures.

The nutrient content of above-ground plant parts of the investigated species were typical of higher plants despite the low nutrient content measured for the dune soils. With the possible exception of nitrogen the nutrient demand created by above-ground production was adequately met by the supply of nutrients either from sand-water or from aquifer-water transpired by the plants. Differences in the volumes of water transpired, and hence the quantity of nutrients potentially taken up via the transpiration stream, resulted in interspecific differences in above-ground plant macronutrient content. The reallocation patterns of nutrients differed both between the various nutrients measured and interspecifically.

Standing biomass and the density of plants per unit land area was low in comparison to that of other ecosystems and was different among investigated species. This may be important in maintaining the adequate supply of resources (water, nutrients and light). As a result of the interspecific differences in biomass when production was expressed per unit land surface area the resultant productivity was not dissimilar among species. Productivity was high when comparisons were made with species from other ecosystems.

No single resource (water, nutrients or light) could be identified as the controlling factor in the foredune environment and a combination of both resource stress and environmental disturbance are likely to be involved. Physiology, production, growth and growth characteristics conveyed certain adaptive advantages to these species in respect to both resource stress and environmental disturbance. Interspecific differences in these adaptations can be used to offer explanations for the observed microhabitat preferences of the three investigated species. Furthermore features common to all three species offer some explanations as to why these species and not others are able to inhabit the foredunes.

List of Figures

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 7

CHAPTER 8

APPENDIX

List of Tables

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 7

List of Plates

CHAPTER 3

CHAPTER 5

CHAPTER 7

CHAPTER 1
GENERAL INTRODUCTION

THE KEY QUESTIONS

The beach according to Barbour (1990) includes the strip of sandy substrate from the mean tide line to the top of the foredunes. Other authors although acknowledging considerable interaction have made a distinction between the beach and the dunes; the beach being considered a marine wave-driven ecosystem while the dunes are a terrestrial wind-driven ecosystem (Brown and McLachlan, 1990). Wave and wind action continuously changes the sandy foreshores. Dune plants trap the wind-blown sand and their burial and subsequent simulated growth builds dunes (Tinley, 1985). Plant growth on these dunes fluctuates with sand erosion and accretion resulting in a dynamic system.

The system of vegetation associated with wind-blown sand extends above the mean tide-line inland and the foredunes may be conveniently defined as the entire area affected by wind-blown sand. In accordance with this definition the foredunes of the east coast of southern Africa are inhabited by a limited number of plant species and are dominated by even fewer species (Tinley, 1985; Avis, 1992; Hertling, 1997). Similar conclusions have been reached for foredunes in other parts of the world (Barbour, 1990).

Why do, and what allows, only particular species to flourish in this environment? These fundamental questions form the basis of this research.

THE FOREDUNE ABIOTIC ENVIRONMENT AND RELATED PLANT ADAPTATIONS

An obvious explanation for the limitation of only particular species to the foredunes is that this environment has unique abiotic factors that allow plants with only particular adaptations to exploit.

Although globally distributed (and hence subjected to a wide range of climates) coastal foredunes have various abiotic factors that are common. These abiotic factors prevalent on coastal beaches have been extensively reviewed (Boyce, 1954; Rozema *et al*., 1985; Barbour *et al*., 1985; Hesp, 1991; Davy and Figueroa, 1993) and a synopsis identifies the following as being features characteristic of the coastal foredune environment. Included are plant adaptations that have been associated with these abiotic factors by various authors.

Wind

High winds are characteristic of beach environments and damage to coastal vegetation has been attributed both directly to the mechanical effects of wind and indirectly via desiccation, sand-blasting and salt-spray (Boyce, 1954). Winds of comparable speeds that result in similar evaporation rates have been measured both on beaches and inland. The inland wind resulted in neither the desiccation nor the mechanical damage to vegetation that beach wind did (Boyce, 1954; and authors cited therein) and this led Boyce (1954) to conclude that the sand-blasting and salt-spray that accompanies such beach winds had a greater effect on the vegetation than wind *per se.*

Sand-blasting is largely restricted to a height of about 50cm above the sand surface (Boyce, 1954) and hence may have a differential effect on species of different heights. The fact that many of the foredune species are shorter than 50cm suggests that they are morphologically and anatomically adapted to withstand sand-blasting. The possession of thick leaf cuticles and sclerophylly are considered as adaptations to wind and sand-blasting (Rozema *et al*., 1985).

Wind is an important determinant in sand transport and dune geomorphology with both the wind speed and prevailing direction having an influence on dune formation (Packham and Willis, 1997).

Salt-spray

It has been argued that salt-spray is one of the main determinants of coastal vegetation (Davy and Figueroa, 1993) and the intensity of salt-spray within the vegetated portion of the beach has been correlated with wind speed, height above the ground and site micro-topography. Salt-spray deposition peaks on the beach in the immediate surroundings of the surf breakers and decreases strongly over the next 600-800m distance from the sea

(Barbour *et al.*, 1973; Lubke, 1983). Deposition on foredune species may be as high as 166.1 g NaCl m^{-2} $year^{-1}$ (Rozema *et al.*, 1985). Salt is thought to penetrate leaves through lesions or via stomata (Boyce, 1954; Barbour *et al.*, 1985). The main response to the accumulation of chloride ions in dicotyledonous plants is the development of leaf succulence (hypertrophy) and the ability to hypertrophy appears to be correlated with salt-spray tolerance. Monocotyledonous plants do not exhibit leaf hypertrophy and may be reliant on leaf modifications such as thick cuticles, pubescence and leaf orientations to reduce salt-spray accumulation. Leaf orientation has been demonstrated to markedly affect the salt-spray loading and Barbour (1978) measured intensities of loading of an order of magnitude higher on vertically as opposed to horizontally orientated leaves. Adaptations that have been attributed to salt-spray include: hypertrophy (discussed above); horizontal leaf orientation; leaf pubescence (Barbour *et al.*, 1985); cuticular structure that resists salt entry (Davy and Figueroa, 1993); salt tolerance (Rozema *et al.*, 1985); the development of salt bladders or epidermal trichomes which transfer salt to the leaf surface; and osmotic adjustments (Hesp, 1991). The latter three adaptations are applicable irrespective of whether the salt originates from salt-spray or saline soil solution (see below).

Soil/sand salinity

Salt concentrations measured in coastal dune soils are no higher than values measured for cultivated soils (Kearney, 1904, cited in Boyce, 1954; Harte and Pammenter, 1983; Avis, 1992). Even following storm surges, soil-salt concentrations attain values that are less than 1%, when concentrations are expressed in terms of concentrations at water holding capacity (Barbour *et al.*, 1985; Rozema *et al.*, 1985). More arid beaches may have greater soil-salt accumulation and measurements in California range from 0.35 to 4.55% at water holding capacity (De Jong, 1979; Davis, 1942, cited in Barbour *et al.*, 1985). In tropical and subtropical regions soil-salinity in the root zone of the vegetation at the sea-ward edge of the dunes had values ranging from 0.2 to 0.3% at water holding capacity (Davis, 1942; Johnson, 1977, cited in Barbour *et al.*, 1985).

Salts are readily leached from beach sand (Boyce, 1954) due to the low ion exchange capacity and low organic content of the sand. The continued loading of the sand surface with salt and its subsequent leaching and the percolation of the resultant solution through the soil, means that dune plants are required to cope with moderate salinity in the soil solution. Occasional inundation of foredunes with seawater may also contribute to soil salinity. Despite this little correlation between soil salinity and plant distribution has been found in coastal dunes (Barbour *et al.*, 1985; Avis, 1992).

Adaptations to salt stress are mentioned above but an additional mechanism may be the exclusion of salt uptake. Harte and Pammenter (1983) measured foliar salt concentrations in the dune pioneer species *Scaevola plumieri* and found that values were considerably lower than values measured in the halophyte *Avecinnia marina* [1.1]. They considered the possibility of such an exclusion mechanism in this species.

Water availability

Although many researchers have viewed the beach environment as xeric (Hesp, 1991; Barbour, 1990) and much of the beach research has been published in journals such as the *Journal of Arid Environments*, the xeric nature of this environment has been questioned by some (Salisbury, 1952; Davy and Figueroa, 1993). The main reason for the questioning of the aridity of beaches comes from the physiological measurement of leaf water potentials. Leaf water potentials measured at midday on a wide range of species in various locations (including non-xeric and foredune locations) rarely exceed values of -1.5 MPa (De Jong, 1979; Dubois, 1977, cited in Barbour *et al*., 1985; Pammenter, 1983; Pavlik, 1984, 1985) whereas values encountered in unambiguously xeric species are far lower (Jefferies *et al*., 1977).

Rainfall is the main determinant of water availability. However the coarse texture of the substrate with a low organic content, subject to high irradiation and high wind speed, results in high evapotranspiration and low water retention. Water may also be available

---

[1.1] With the exception of *Arctotheca populifolia*, *Ipomoea pes-caprae* and *Scaevola plumieri* all plant names are given in full due to the vast number of species discussed in this thesis.

from internal dew formation, fog and dune aquifers (See Chapter 5, Water relations). Soil salinity and the resultant reductions in soil water potential may reduce water availability. Adaptations to low water availability include osmotic adjustment (Pavlik, 1984; Smirnoff and Stewart, 1985; Rozema *et al*., 1985), xeromorphic structures (Hesp, 1991) including leaf rolling (Pavlik, 1982; Rozema *et al*., 1985), pubescence (Hesp, 1991; Ripley *et al.*, 1999) and epicuticular waxes (Hesp, 1991; Rozema *et al*., 1985).

Sand-burial

The nature of most beach sand is such that when wind velocities exceed about 4 m s^{-1} the threshold velocity required to move sand has been exceeded (Boyce, 1954). Beaches are frequently subjected to winds in excess of this velocity and the transported sand deposits when wind to surface interactions reduce the wind velocity below this threshold. This often involves wind to vegetation interactions, the nature of which are mainly determined by the type of vegetation. It is not only the amounts of sand associated with burial events but also the rate of sand-burial that is of importance. This is particularly so for annual species that are reliant on the establishment of the new population from buried seed. Burial at depths greater than certain thresholds result in a lack of germination or inability to emerge from the sand (Hesp 1991; and references cited therein). Perennial species must achieve growth rates in excess of rates of burial or survive buried until sand deflation uncovers them.

Adaptations to sand-burial include the ability to enhance CO_2 assimilation (Yaun *et al.*, 1993) and above-ground growth at the expense of stored reserves (Seliskar, 1994; Brown, 1997; Harris and Davy, 1988); high productivity (Deshmukh, 1977; this study); burial tolerance (Martinez and Moreno-Casasola, 1996); and open canopies which reduce sand trapping (*pers. obs.*).

Features associated with plant establishment are also considered adaptive with respect to sand-burial and include the possession of large seeds that germinate from greater depths than do small seeds (Barbour *et al*., 1985) and rhizomes that produce fragments that can emerge from considerable depths (Davy and Figueroa, 1993).

Nutrient deficiencies

The low nutrient content of dune sands in comparison to agricultural soils (Willis, 1965; Barbour *et al*., 1985; Ehrenfeld, 1990; Cain *et al*., 1999) and the positive response of dune plants to nutrient additions both in the greenhouse and in the field have led researchers to conclude that the beach environment is poor in nutrients. Sources of nutrients include swash deposition from the sea, salt-spray, ground-water, mineralization and meteorological inputs (See Chapter 6 for details).

Not only are nutrient concentrations low but nutrients are often heterogeneously spatially distributed (Cain *et al*., 1999). This is particularly so for nitrogen. The other nutrients are distributed more homogeneously as they mainly originate from fog and sea-spray (van der Valk, 1974).

Plant adaptation to low nutrient availability includes competitive growth to exclude other species from the resource (Pemadasa and Lovell, 1974), nutrient reallocation within plants (Harte and Pammenter, 1983), sparse growth habits, adaptive life-histories and adapted rooting patterns (Hesp, 1991).

High temperatures and light intensities

The beach environment has both high incident (Barbour *et al.*, 1985) and high reflected light intensities (Chapman, 1976) and this not only has direct effects on photosynthesis but also affects leaf temperatures and transpiration.

Adaptations to high light intensities include leaf hairiness which increases leaf surface area for heat dissipation, protects the leaf surface from direct sunlight, increases light reflectance from the leaf surface and produces a boundary layer that traps wet air and reduces transpiration (Hesp, 1991; Ripley *et al*., 1999); possession of an epicuticular wax layer that that increases albedo; heat tolerance; leaf orientations that minimise light interception (Hesp, 1991); and a high capacity for temperature acclimation (Mooney, 1980).

GENERALISATIONS ABOUT FOREDUNE PLANTS

Although foredunes do possess many common abiotic factors and the resultant plant adaptations to these factors may offer some explanations about what limits certain species to this environment, no single collection of adaptations appears common to all foredune species. However dune plants with similar adaptations can be grouped into what have synonymously been termed life-forms (Barbour, 1992), life-form syndromes (Hesp, 1991) and life-histories (Davy and Figueroa, 1993). The considerable taxonomic diversity that exists among foredune communities can be resolved into a small number of such groupings. Summer annuals that complete their lifecycles between the spring and autumn equinoxes and clonal perennials, both herbs and grasses, are the two major categories encountered on the foredunes (Hesp, 1991; Barbour, 1992; Davy and Figueroa, 1993). More recently, Garcia-Mora *et al*. (1999) in a survey of 55 dune species from the Gulf of Cadiz (Spain) used seven plant traits (adaptations) to identify three functional types. These are (i) winter annuals with large soft leaves, (ii) perennials with well developed root/rhizome systems and (iii) perennials or summer annuals that are dispersed by seawater and have mechanisms to withstand sand-burial. Such groupings have been used to summarise both a taxonomically diverse range of species from a broad geographic range (Hesp, 1991; Barbour, 1992) and the plants found growing within the dunes at a single geographic location (Garcia-Mora *et al*., 1999). Thus it is apparent that the various groupings have evolved different adaptations that allow them to exploit the factors prevalent on foredunes. There appears to be more than one biotic solution to the same set of abiotic conditions.

Furthermore, the observed variations in adaptations possessed by foredune species arise not only from the evolution of different solutions to similar ecological problems but also because the foredune environment is not uniform and differential selective pressures exist. Variations arise from the following: i) latitudinal differences in climate that result in considerable variation between foredunes of different location about which some general trends in climate and resultant plant adaptations have been proposed (Barbour *et al*., 1985; Hesp, 1991; Davy and Figueroa, 1993) and ii) a gradient in microclimate from the high-tide line inland. Correlated with this is a successional change in plant species

(Cowles, 1899; Olson, 1958; Morrison and Yarrington, 1973, 1974; Avis, 1992; Packham and Willis, 1997; van der Heen, 2000), each species potentially having its own specific adaptations.

Latitudinal trends in plant adaptation as related to climate

A reasonable correlation between coastal dune latitude and climate exists (Hesp, 1991; and references cited therein) and hence latitudinal trends in species richness (Doing, 1985; Hesp, 1991), life-form (Barbour, 1992) and plant adaptation (Hesp, 1991) are apparent. However a lack of relevant information particularly in certain geographic areas has resulted in authors proposing such trends with considerable caution.

Species richness is maximal in Mediterranean ecosystems and decreases at higher or lower latitudes. Hesp (1991) attributes this to a change in temperature, aridity stress and sand movement between locations of higher and lower latitudes. He also notes a decrease in the dominance of annual and an increase in the dominance of perennial species in moving from Mediterranean to arid ecosystems, accompanied by an increase in the number of adaptations attributed to abiotic stresses. The number of foredune C_4 taxa increases with increasing ambient temperature (Barbour *et al*., 1987; Hesp, 1991).

Latitudinal variations within a single species have also been recorded. The photosynthetic temperature optima of *Heliotropium curassavicum*, thermostability and activation energies of NAD-malate dehydrogenase (Simon, 1979) and dark respiration rates (Lechowicz *et al.,* 1980) of *Lathyrus japonicus* have all been shown to vary with latitude.

Plant succession and zonation within the foredunes

A second source of variation was recognised as early as 1899 when Cowles described the zonation of species found on sand dunes associated with shorelines. Clements (1916) used these findings to formulate his theories concerning succession wherein he considered that the community developed during time and ultimately achieved a state of balance with the physical environment after which no further changes occur. These theories have been both elaborated and refuted by subsequent authors. See Avis (1992) for a review of the concepts in vegetational science and successional theories.

Irrespective of the various theories of succession, investigations on coastal sand dunes by various authors have associated changing abiotic and biotic conditions, from the high-tide line inland, with a succession of plant species and communities (Olson, 1958; Morrison and Yarrington, 1973, 1974; Avis, 1992; Packham and Willis, 1997; van der Heen, 2000). Within this succession, distinct zones as characterised by species compositions, may be recognisable although a single species is not necessarily confined to a single zone. Such zonation is recognisable within the coastal dunes of the Eastern Cape Province, Republic of South Africa and Lubke (1983) defines five such zones. Much research has focused on the zone closest to the high-tide line. In South African coastal dunes Burns and Lubke (1996) term this the pioneer zone being inhabited by a pioneer community (Lubke, 1983; Lubke and van Wijk, 1988).

Similar zones and representative plant communities have been recognised in other coastal dunes and the following terms for the pioneer zone and its representative plant community can be considered synonymous: the leading edge habitat (Barbour, 1992); primary dune colonisers (Lubke and de Villiers, 1991); strand plant community (Tinley, 1985); littoral strand vegetation (Lubke *et al.*, 1988); the forebeach community and hummock dunes (Young, 1987; McLachlan *et al*., 1987); and the back shore and incipient foredune zone (Hesp, 1991).

Because it is possible to recognise zones within coastal dunes by representative plant communities the previously stated questions need to be redefined to consider not only why and what limits certain species to the foredunes, but why and what limits certain species to recognisable zones within the foredunes.

The correlation between succession and changing abiotic factors (Donnely and Pammenter, 1983; Tinley, 1985; Lubke and Avis, 1982; Lubke *et al.,* 1997; Hertling, 1997) has been used to explain the limitation of particular species to specific zones. This approach led Avis (1992) to conclude that rainfall and soil moisture were the controlling factors in a dune slack environment at Kleinemonde (Eastern Cape Province, Republic of South Africa). Others consider wind (Boyce, 1954), nutrient build-up, sand-burial (van der Valk, 1974), salt-spray (Barbour and De Jong, 1977; Boyce 1954; Oosting and Billings, 1942), elevation and related flooding frequency and disturbance (van der Veen, 2000) and species interactions (van der Veen, 2000) to be the controlling factors.

Such correlation in identifying specific abiotic factors as being important has led researchers to investigate the response of dune plants to these specific factors.

THE FUNCTION OF PLANT ADAPTATION

Previous discussion has focused on explaining plant distributions as a result of possessing adequate adaptations to exploit and survive the prevailing environmental conditions. However many of these “ecophysiological attributes” or “adaptations” have been inferred from morphological features or are interpolated from research conducted on species from other ecosystems (Barbour *et al.*, 1985), good examples being succulence and leaf pubescence. Barbour (1992), in an analysis of the beach flora of the Gulf of Mexico, divides the flora into those that inhabit the leading edge and those that occur more generally on the rest of the beach. He concluded “that the leading edge should be more suitable for prostrate succulents and pubescent plants with large leaves which tolerate salt spray, soil salinity, or drying winds”. Despite the evidence that shows that these sorts of plants dominate the pioneer zone or “leading edge” environment few ecophysiological studies have been conducted to explain why these features convey tolerance to these particular conditions, or how these inferred adaptations function. The adaptive advantages of many of these morphological features often do not follow preconceived notions (Barbour, 1992). For example prostrate growth forms are not more abundant on beaches of high wind energy nor is increased succulence strongly correlated with high-energy beaches with presumed higher salt-spray loads.

Not all authors have interpolated or assumed the function of dune species adaptations. Many studies have investigated the response of dune and in some instances comparative non-dune species to natural or controlled treatments of the abiotic factors that are prevalent on foredunes. Many of these are discussed at length in subsequent chapters. A few examples illustrate this point and include the work of Boyce (1954) who experimentally demonstrated that leaves from plants dominant in the foredunes were more resistant to the effects of salt-spray than leaves from other species and that such treatments could be shown to result in hypertrophy. The artificial application of salt-spray or alteration of soil salinity reduced growth, productivity and seed production while increasing water use efficiency and cell sap osmolarity of various foredune species (Cheplick and Demetri, 1999). The burial of dune species with sand has been shown to increase plant vigour, affect the allocation of biomass to aerial plant parts (Martinez and Moreno-Casaola, 1996; Harris and Davy, 1988; Brown, 1997) and increase photosynthetic rate (Yaun *et al*., 1993). The role of ethylene in mediating this response has been investigated (Selikar, 1994). The response of dune species to both high and low nutrient treatments has been investigated and shown to affect features such as photosynthesis, growth and nutrient allocation patterns (Willis and Yemm, 1961; Willis, 1965; Ernst, 1983; Pavlik, 1983b).

Although such studies have been useful in identifying features or responses that appear to impart advantages to dune species, in most instances the underlying mechanisms remain elusive. Exceptions exist where the roles of specific adaptations have been investigated and examples include: i) the demonstration that leaf rolling in *Ammophila arenaria,* in response to declining water status markedly reduced transpiration rates (Pavlik, 1982) and ii) osmotic adjustment in *Elymus mollis,* that maintained soil to leaf water potential gradients during seasonal decreases in water availability (Pavlik, 1985). Other studies showed that *Heliotropium curassavicum* originating from locations with differing temperature regimes maintained the capacity for the acclimation of photosynthetic temperature optima (Mooney, 1980). Investigations have shown that the hair layer on the leaves of *A. populifolia* reduce the amount of UV transmitted through to the leaf surface thus reducing photoinhibition with only minimal reduction in the transmittance of the photosynthetically active radiation wavelengths (Ripley *et al*., 1999).

AN ECOPHYSIOLOGICAL APPROACH

The attempts that have been made at answering the question “What limits only particular species to the foredunes or to a particular zone within the foredunes?” have largely been through the description of abiotic factors to which various and mostly presumed adaptations have been correlated. Such correlation has been used to explain dune succession or zonation. Species with particular adaptations can be categorised according to life-form, life-history or functional type. There are a relatively small number of such groupings and the relative proportions of these, changes with latitude. However such correlation does not answer the question asked. Nor does the research in which chosen abiotic factor(s) have been experimentally manipulated because such approaches presuppose that the chosen factor(s) are limiting. However it must be stated that many of these latter experiments were not necessarily intended to address the question as stated above.

A different approach is not to look for correlation nor to subject foredune species to various treatments but to consider the requirements of particular species, their acquisition and use of resources and the plant response to natural environmental stresses. This integrated approach has been adopted in this study. It may be differences in the abilities of the plants to acquire resources and cope with stress that determines their distribution, rather than changes in the availability of resources or the severity of a particular abiotic factor. In reality it is likely to be a combination of both changing abiotic factors and plant adaptation that determines their growth preferences. This approach requires extensive research relating species specific physiology to environmental conditions (ecophysiology).

Ecophysiological studies have been conducted on the dominant coastal foredune species of both Europe and America. Examples include Willis (1965), Dubois (1977, cited in Barbour *et al*., 1985), De Jong (1978), Pavlik (1983 a, b & c, 1984, 1985), Skiba and Wainwright (1984) and Fay and Jeffrey (1992). Such studies have usually focused on plant response to one or two environmental factors and although they do not represent an integrated approach they do offer some explanations of species abilities to grow on the foredunes. The findings of such research are reviewed in subsequent chapters. Despite the potential for discovering causal limitations of plant distributions little research of this nature has been conducted in southern Africa. The ecophysiological studies that have been conducted on southern African coastal plants have usually focused on the single species *Scaevola plumieri* (Donnely and Pammenter, 1983; Harte and Pammenter, 1983; Pammenter, 1983, 1985; Peter and Ripley, 2001) and comparative data from other dune species is largely unavailable.

In order to address the lack of ecophysiological information on coastal species of southern Africa an investigation on species confined to the pioneer zone of the foredunes was undertaken. Research on the species found growing in the pioneer zone has several distinct advantages: i) few species occupy this zone and those that are dominant are found only within this zone and ii) the pioneer zone is characterised by the most extreme environmental conditions (Brown and McLachlan, 1990). Consequently plant adaptations including physiological adaptations are likely to have been emphasised by selection and hence may be more easily recognised and quantified.

The magnitude of the work required to attempt to answer some basic ecophysiological questions meant that not only was the research confined to the pioneer zone but the number of species under investigation was limited to three, namely *Arctotheca populifolia*, *Ipomoea pes-caprae* and *Scaevola plumieri* (a full description of these species follows in Chapter 3). The three species chosen represent some of the dominant species of sub-tropical beaches of the eastern coast of South Africa (Avis, 1992). They are from three different families having anatomical and morphological features that are clearly distinct. Such features have been viewed as adaptations for living in the beach environment. These adaptations include: succulence (*S. plumieri* and *A. populifolia*); leaf pubescence (*A. populifolia*); thick waxy cuticles (*S. plumieri* and *I. pes-caprae*); large underground stems (*S. plumieri* and *I. pes-caprae*); annual or biannual life-histories (*A. populifolia*; Tinley, 1985); and long-lived perennial life-histories (*S.*

plumieri and *I. pes-caprae*; Lubke, *pers. com.*). The differences between the chosen species could be expected to reveal both different and possibly some common physiological characteristics that allow these species to live under beach conditions.

The species chosen are all dicotyledonous and this forms an interesting contrast to the species from northern hemisphere temperate beaches which are dominated by monocotyledonous species (Doing 1985; Barbour *et al.*, 1985; Davy and Figueroa, 1993; Little and Maun, 1996; van der Maarel and van der Maare-Versluys, 1996). These monocotyledonous species have received the most attention in ecophysiological studies (Examples include: Willis, 1965; Pavlik, 1983 a & b, 1984; Skiba and Wainwright, 1984; Fay and Jeffrey, 1992). American foredunes are dominated by a mixture of both monocotyledonous and dicotyledonous plants (Barbour *et al.*, 1985) and both types have been the subject of ecophysiological investigations (De Jong, 1978, 1979; Pavlik, 1983 a, b & c, 1984, 1985; and many more). This study presents the opportunity to compare the physiology of monocotyledonous and/or dicotyledonous species from temperate and subtropical regions.

The species chosen for this study are not endemic to southern Africa. Only a limited number and variety of studies have been undertaken on these species and no comparative studies exist (Table 1.1). Like any initial study, although directed by specific questions, some of the findings are purely descriptive whilst others are explicit. Furthermore from the onset of the study it was acknowledged that more questions were likely to be raised than could be answered by this research.

Outlined below are the ecophysiological questions asked and these form a logical structure upon which this thesis is constructed. Several short chapters introducing the methods used, the study site and the plants investigated are followed by four results chapters. These are focused on four main themes (plant productivity, plant water relations, plant nutrient relations and plant physiology) about which specific questions were asked. The first three results chapters deal with the subject matter that is evident from their titles while the last chapter investigates some specific aspects of physiology pertaining to productivity and plant water and nutrient relations. The thesis is concluded with a chapter called "Habitat Limitations and Plant Performance" that serves to integrate the findings reported in the other chapters and relates those findings to the plant's habitat preferences.

Table 1.1: Type of research and authors who have conducted studies on *A. populifolia*, *I. pes-caprae* and *S. plumieri*.

Type of research	**Location**	**Authors**
	A. populifolia	
Ecophysiology, function of leaf hairs	East Coast, South Africa	Ripley *et al.*, 1999
Mycorrhiza	East Coast, South Africa	Haller, 2000
Ecology, dune formation	East Coast, South Africa	Hesp and MaLachlan, 2000
	I. pes-caprae	
Descriptive ecology	Costa Rica	Wilson, 1977
Sand burial and growth	Gulf of Mexico	Martinez & Moreno-Casasola, 1996
Germination	Gulf of Mexico	Martinez *et al.*, 1992
Mycorrhiza	Gulf of Mexico	Corkidi & Rincon, 1997
Photosynthetic physiology	New South Wales, Australia	Adams *et al.,* 1988
Herbivory	Queensland, Australia	Bach, 1998
Reproductive success	Gulf of Mexico	Devall & Thien,1989
Biochemistry of antioxidant enzymes	Tamil Nadu, India	Venkatesan & Chellappan, 1999
Seedling survivorship	Queensland, Australia	Bach, 2000
Mycorrhiza	East Coast, South Africa	Haller, 2000
	S. plumieri	
Ecophysiology, salt spray effects on vegetation zonation	East Coast, South Africa	Donnely & Pammenter, 1983
Ecophysiology, nutrient relations	East Coast, South Africa	Harte & Pammenter, 1983
Ecophysiology, gas exchange, water relations, nutrient recycling and growth	East Coast, South Africa	Pammenter, 1983
Phenology	East Coast, South Africa	Steinke & Lambert, 1986
Ecophysiology, gas exchange	East Coast, South Africa	Pammenter, 1985
Systematics	Andros Island, Bahamas	Koontz *et al.*, 1996.
Mycorrhiza	East Coast, South Africa	Haller, 2000
Ecophysiology, predicting water loss from ambient conditions	East Coast, South Africa	Peter & Ripley, 2001
Genetics	East Coast, South Africa	Harman, 2000
Ecophysiology, distribution modelling	East Coast, South Africa	Peter *et al.*, in preparation

QUESTIONS ASKED

The approach of this investigation was to ask questions about the acquisition and use of water, nutrients and light and to consider the functioning of associated physiology and the resultant productivity. These questions were asked both in the context of how dune and non-dune species perform and how this performance changes seasonally.

Plant Productivity (Chapter 4)

Production and productivity although affected by other factors are the result of the plants' abilities to acquire and use resources and therefore forms a functional framework for comparing species. Furthermore these parameters may be key to understanding the survival of plants in an environment where wind blown sand and continuous mechanical damage are likely to be important factors. Questions concerning production and productivity were asked both at the leaf and whole plant level.

- **How productive are these species in their natural habitat?**
    1. How productive are the selected species in relation to other species from coastal and non-coastal environments?
    2. How does production and productivity vary seasonally?
    3. What is the relationship between photosynthetic productivity and above ground productivity?
    4. What is the relationship between CO_2 exchange and carbon allocation?

- **What determines productivity?**
    1. Which are the most important abiotic factors determining productivity?
    2. Are these beach plants susceptible to light stress (photoinhibition) and does this affect productivity?
    3. Is productivity water limited?(Question raised in Chapter 5 & continued in Chapter 6)

Plant Water Relations (Chapter 5)

Central to the maintenance of production rates is the maintenance of plant water status. Hence plant water relations both in relation to productivity and to the potentially xeric nature of the foredunes were investigated.

- **How much water do these species require?**
    1. How much water do the selected species require and how does this compare with other species from coastal and non-coastal environments?
    2. How does this change seasonally?
    3. Can the prevailing climate be used to predict water requirements?

- **How do these species use available water?**
    1. How efficiently do the selected species use water and how does this compare with other species from coastal and non-coastal environments?
    2. Is there evidence of water conserving mechanisms?
    3. Is there evidence of strong stomatal regulation of water loss? (Question raised in Chapter 5, but continued in Chapter 7).

- **How available is soil water to the selected dune species?**
    1. Are the selected species required to develop low leaf water potential in order to obtain soil water and does this change seasonally?
    2. How do these leaf water potentials compare among the selected species and to other species from coastal and non-coastal environments?
    3. Do these species develop very negative leaf water potentials as do some xeric species?
    4. How do the components of water potential change in relation to changes in water use?

- **What is the source of the water used by these species?**
    1. Do species use soil water or ground water (aquifer)?
    2. Do relative proportions of soil or ground water used change seasonally or with prevalent climatic conditions?
    3. Is below-ground stem connectivity between plants necessary to maintain plant water status?

Plant Nutrient Relations (Chapter 6)

Mineral nutrients have traditionally been considered limiting in coastal dune environments and this potential limitation as well as the use and re-use of acquired nutrients were investigated.

- **What quantity of essential nutrients do these species use?**
    1. What are the differences in nutrient use between the selected species and how does this compare with other species from coastal and non-coastal environments?
    2. Does nutrient use and requirement vary seasonally?

- **From where are nutrients acquired?**
    1. Does water contained in the sand above the ground-water table contain sufficient nutrients to meet the requirements of these plants?
    2. Does ground-water contain sufficient nutrients to meet the requirements of these plants?
    3. Does the supply of nutrients from these sources change temporally?

- **Do plants remobilize nutrients from older to younger leaves?**
    1. Are there differences in the remobilization of the various nutrients?

2. Are there differences in the remobilization of nutrients between species and how does this compare with nutrient remobilization in other species from coastal and non-coastal environments?

- **Does the utilisation of brak (salty) soil or ground water lead to the foliar accumulation of sodium and chloride?**
  1. Are sodium and chloride ions accumulated in older leaves?
  2. Does salt accumulation determine leaf longevity?
  3. How does salt accumulation and its remobilization in the selected species compare with that of other species growing in saline environments?

Plant Physiology (Chapter 7)

Associated with questions concerning the acquisition and use of resources and the resultant productivity are questions about the underlying mechanisms. These include:

- **What are the characteristics of the photosynthetic physiology of these plants?**
  1. How does leaf photosynthesis respond to increasing photosynthetic photon flux density (PPFD)?
  2. How does leaf photosynthesis respond to increasing ambient CO_2 concentrations?
  3. Can these responses explain the measured differences in gas exchange and primary production between species?

- **To what extent do stomata control gaseous exchange?**
  1. How do leaf conductances compare among the selected species and other coastal and non-coastal species?
  2. Do diurnal stomatal responses differ among selected species?
  3. How much do stomata limit photosynthesis at ambient conditions?
  4. How does stomatal regulation affect the relative rates of CO_2 assimilation, transpiration and the intercellular CO_2 concentration?
  5. Is there evidence of stomatal optimisation?
  6. Does the response of stomatal conductance to PPFD and correlated changes in vapour pressure deficit (VPD) explain the observed response of transpiration (E) to VPD?
- **Are there differences between the gas exchange of leaves of different age?**
  1. Are the leaf age effects different in the selected species?
  2. Is it necessary to account for leaf age effects when whole plant water and nutrient requirements are calculated?

- **How do whole shoots respond to diurnal changes in PPFD?**
  1. Does mutual leaf shading affect the photosynthetic performance of whole shoots?
  2. What is the most important determinant of whole shoot photosynthetic performance?

- **What is the physiological role of *A. populifolia*'s leaf hair layer?**
  1. Does the hair layer reduce potential photoinhibition?
  2. Does the hair layer increase water use efficiencies and affect leaf temperatures?

Habitat Limitation and Plant Performance (Chapter 8)

In an attempt to answer the question "Why and what limits only particular species to the foredune environment" the relationship between plant function and adaptation in relation to resource stress and environmental disturbance was considered. This was done by means of a conceptual model that integrated the findings of the previous chapters and related these findings both to the conditions prevalent on foredunes and to the habitat preferences of the three species investigated. The questions asked include:

- **Is the foredune environment limited by resource availability or are resources present in excess?**
    1. Is there evidence that the acquisition or oversupply of water, light and nutrient limits plant function and productivity?
    2. Are the limitations common to all three species?

- **How does the resource availability affect plant function and do the investigated species respond in the same way?**
    1. Do the investigated species have similar adaptations?
    2. Do the investigated species have unique adaptations in response to different limitations?

- **Is the possession of adaptations linked to acquiring limited or coping with excess resources the key to understanding which plant species can thrive in the foredune environment, or does the key lie with plant adaptations to disturbance?**
    1. If resources limit plant function then are adaptations related to resources what allow only certain species to inhabit the foredune environment?
    2. If disturbance limits plant function then are adaptations related to disturbance what allow only certain species to inhabit the foredune environment?
    3. Are adaptations to both disturbances and resources what allow only certain species to inhabit the foredune environment?

This study arose from a longstanding interest in the beach environment and was encouraged by discussions and collaboration with colleagues Roy Lubke and Ted Avis (Rhodes University) who have studied the ecology of this environment for many years. The questions that this study raises will remain the focus of my own research and the research of my students in subsequent years.

CHAPTER 2
OVERVIEW OF METHODS

INTRODUCTION

In order to answer the questions asked in Chapter 1 and to quantify the physiology associated with productivity, water and nutrient acquisition and use, differences between species and the changes in physiology as related to season were monitored. The overall design was to conduct a series of physiological measurements on the same leaves or same plants at two hourly intervals throughout the day from pre-dawn until after sunset. These measurements were repeated three times a year in spring, summer and winter of 1997 and 1998. Similar experimental protocols have been used to show differences between species during different seasons in temperate dune vegetation (Pavlik, 1983 a & b, 1984, 1985). Due to periods of inclement weather or work commitments, data were not collected in some seasons. Some of the techniques used were destructive and in these cases plants adjacent to study plants were used for measurements. The sampling procedures and experimental techniques are outlined below under the heading "routine experiments and sampling".

The analysis of data from the routine experiments raised some specific questions and specific experiments were designed to address some of these. These experiments are outlined below under the heading "non-routine experiments and sampling". A full description of each method can be found at the beginning of the relevant chapter.

ROUTINE EXPERIMENTS AND SAMPLING

Timing of routine experiments and sampling

The routine measurements were conducted on the following dates (Table 2.1) and the table includes the climatic season within which each of these experimental days fall.

Table 2.1: The dates when routine experiments and sampling was performed. The season within which each of these dates fall is indicated.

Date	Season
30/1/1997	Summer 1997
10/9/1997	Spring 1997
19/2/1998	Summer 1998
23/7/1998	Winter 1998
15/9/1998	Spring 1998

Physiological sampling and measurements

On each of the listed days at approximately two hourly intervals replicate measurements of instantaneous gas exchange (Chapters 4 & 5), leaf water potentials (Chapter 5), and chlorophyll fluorescence (Chapters 4 & 7) were made on three plants or shoots of each species. The distinction between plants and shoots is made because the extent of individual *I. pes-caprae* and *S. plumieri* plants is difficult to ascertain. Hence samples were collected or measurements were made on the shoots of these two species. At the same time intervals as used above, three leaf samples from individual plants or shoots of each of the species were frozen in liquid nitrogen for the analysis of soluble sugars, starch and pigment content (Chapter 4).

In addition to these diurnal measurements several other sampling procedures were conducted routinely at the end of each experimental day. These included the collecting of individual plants of each species for the construction of pressure-volume curves (Chapter 5) and the quantification of ion accumulation in individual leaves of entire plants (Chapter 6). Additional plants were harvested for the determination of average leaf nutrient content (Chapter 6) and the isotopic ratios of water contained in below-ground plant stems (Chapter 5).

Climatic data i.e. wind speed, relative humidity and ambient temperatures were collected at 5-minute intervals during the experimental periods (Chapter 3).

NON-ROUTINE EXPERIMENTS AND SAMPLING

Above-ground productivity and production

Gas exchange measurements showed that photosynthetic carbon assimilation differed between species and between plants of the same species on different experimental days (Chapter 4). The effects of this on above ground production, productivity and biomass were of interest. Hence from September 1997 to October 1998 monthly leaf production and loss were monitored on twenty tagged plants along linear transects within the study area (Chapter 4). In addition the number of shoots per unit dune area and the above-ground biomass were measured on various occasions. In addition to being of intrinsic interest this data in conjunction with plant nutrient content data allowed above-ground nutrient accumulation to be quantified.

Effects of shading on leaf and stem soluble sugars and starch

Gas exchange data and the simple daily carbon budgets that were calculated from these data (Chapter 4) showed that certain species on some experimental days were barely accumulating more carbon than was lost through respiration. This raised questions about the roles of foliar and stem sugars and starch in maintaining carbon balance. Hence on two occasions (19/2/98 and 23/7/98) areas of dune containing all three species were covered with 50% shade cloth. After three days leaf and stem samples were harvested and frozen in liquid nitrogen for the analysis of soluble sugars and starch (Chapter 4). Values measured were compared with unshaded controls.

Light stress effects on productivity

In view of the high light intensities encountered on the beach and the finding that there was a differential reduction in photochemical efficiency between species and on different experimental days (Chapter 4) it was of interest to consider the rate of recovery of this parameter following a light treatment. In an attempt to quantify the effects of light stress (Chapter 4) the dark recovery of fluorescence parameters following the incubation of the leaves of each species under natural illumination were monitored on 9/9/97 (spring), 19/2/98 (summer) and 27/7/98 (winter).

Ground-water use by dune plants

It was apparent from diurnal leaf water potentials, transpiration rates and volumes of water transpired (Chapter 5) that all three species investigated did not behave like typical xerophytes with respect to plant water relations. One possible explanation for this was that plants had access to unlimited dune aquifer water. To investigate this possibility the stable isotopic ratios ($^{16}O/^{18}O$) in *S. plumieri* stem-water, ground-water and rain-water were compared on several occasions during the experimental period (Chapter 5).

Reliance of below-ground connections for the maintenance of plant water status

Both *I. pes-caprae* and *S. plumieri* have extensive clonal below-ground connections. In addition individual shoots have adventitious roots. In view of the finding that plant water relations were more typical of mesic plants than xerophytes (Chapter 5) the role of these clonal connections was considered. On 18/2/98 (summer) stem connections between *S. plumieri* plants and also *I. pes-caprae* plants growing on a small hummock dune and plants on a larger adjacent dune were cut. Prior to this and for the subsequent three days the water relations of the plants on these dunes were monitored in an attempt to ascertain the necessity for underground connectivity in supplying water (Chapter 5).

Nutrient supply from soil (sand) and ground water

Nutrient content of dune sands are very low (Chapter 6) and hence in addition to being a source of water it is possible that ground-water is an important source of nutrients for dune plants. Therefore on several occasions both ground-water and sand-water were sampled for nutrient analyses (Chapter 6).

Leaf age effects

Measurement of leaf nutrients showed that leaves of various ages had different contents of potassium and phosphorus (Chapter 6). Both are macronutrients that are associated with proteins and enzymes and hence the gaseous exchange physiology of leaves of differing ages was investigated. In June 2000 the gaseous exchange parameters of each individual leaf on three entire plants of each species was measured in order to ascertain leaf age effects (Chapter 7). This measurement was also important in assessing the limitations of carbon and water budgets constructed from gas exchange measurements made on single leaves of the same approximate age from replicate plants (Chapter 7).

Physiology

Considerable interspecific variation in the light and gaseous exchange response (Chapter 4 & 5) was apparent from field measurement made at ambient conditions. The quantification of these differences was complicated by the variability resulting from changing ambient conditions. Therefore field CO_2 and light responses were constructed under controlled conditions on the 18/5/00 & 6/1/01 (Chapter 7).

As the three species were compared and comparisons were repeated in various seasons over a number of years the answers or part answers to the questions asked were seen to vary both interspecifically and temporally making the presentation of the results complex. In order to simplify the presentation comparisons were made between species for each season and seasons are presented in a chronological sequence.

CHAPTER 3
THE STUDY SITE AND PLANTS INVESTIGATED

This chapter gives a brief description of the location, topography, vegetation, climate and weather of the study site. The species studied are then introduced including details of their nomenclature, distributions and vegetative characteristics.

STUDY SITE

This study was undertaken on the foredunes to the west of the Old Woman's River mouth ($27^o08'49''$E; $33^o28'59''$S), Eastern Cape Province, Republic of South Africa (Figure 3.1). In addition some of the data in Chapter 7 was collected at Mtunzini ($31^o47'$E; $28^o58'$S), 130 km north-east of Durban, KwaZulu Natal Province by Pammenter and Smith (unpublished).

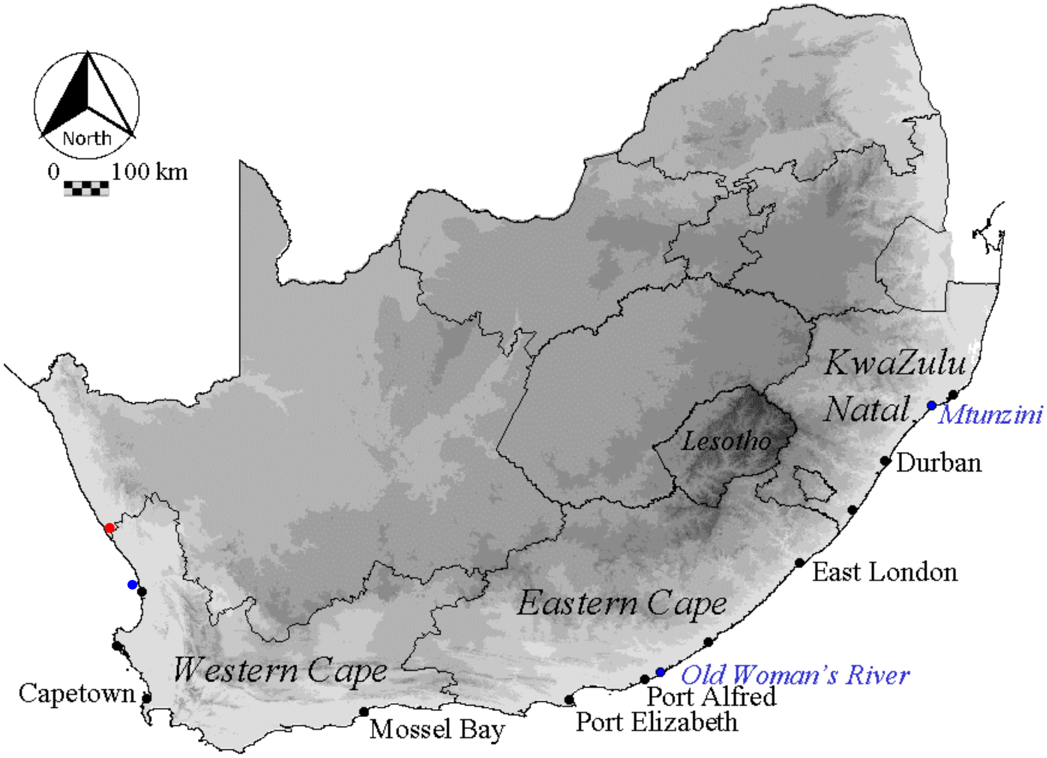


Figure 3.1: A map of the positions of various coastal sites including Old Woman's River mouth and Mtunzini, in relation to some of the major centres (Map by C. Peter).

Topography and orientation of the foredunes at Old Woman's River

Plate 3.1 and Figure 3.2 give the orientation and topography of the foredunes on the south west bank of the mouth of Old Woman's River. The foredune ridge runs approximately parallel to the sea and is flanked on the landward side by a saline blind estuary. The estuary mouth opens sporadically about once every two years depending on sea and rainfall events.

Plate 3.1: View of the foredunes on the west bank of Old Woman's River. The topography and vegetation of the area circled are shown below. Note the different perspectives.

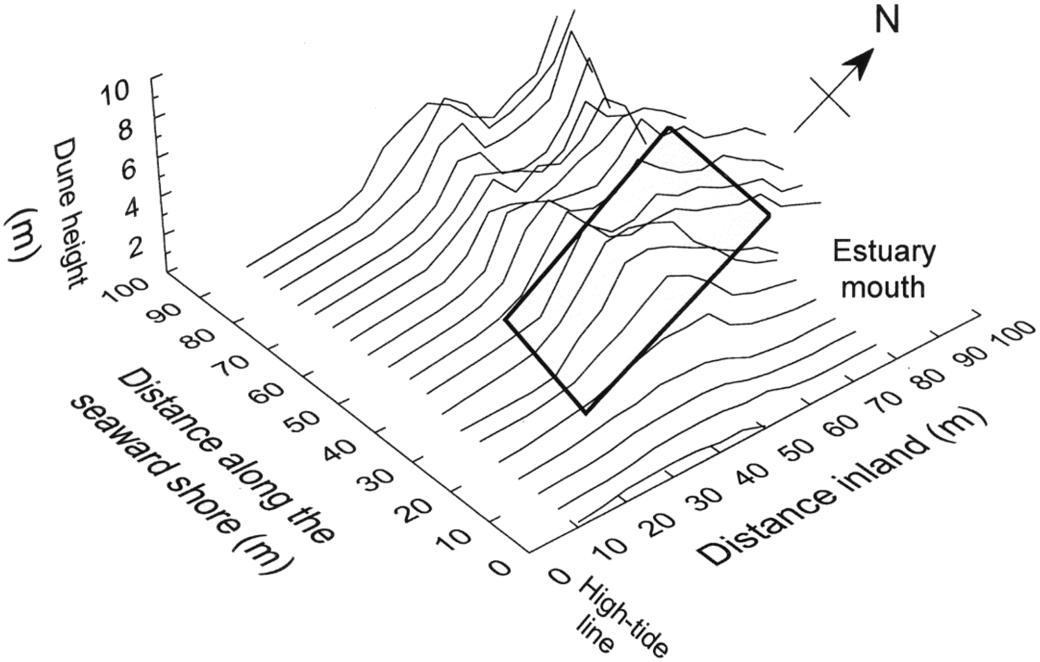


Figure 3.2: Topography of the foredunes on the west bank of Old Woman's River. The area within the rectangle indicates the location of the plants measured or sampled for the routine analyses.

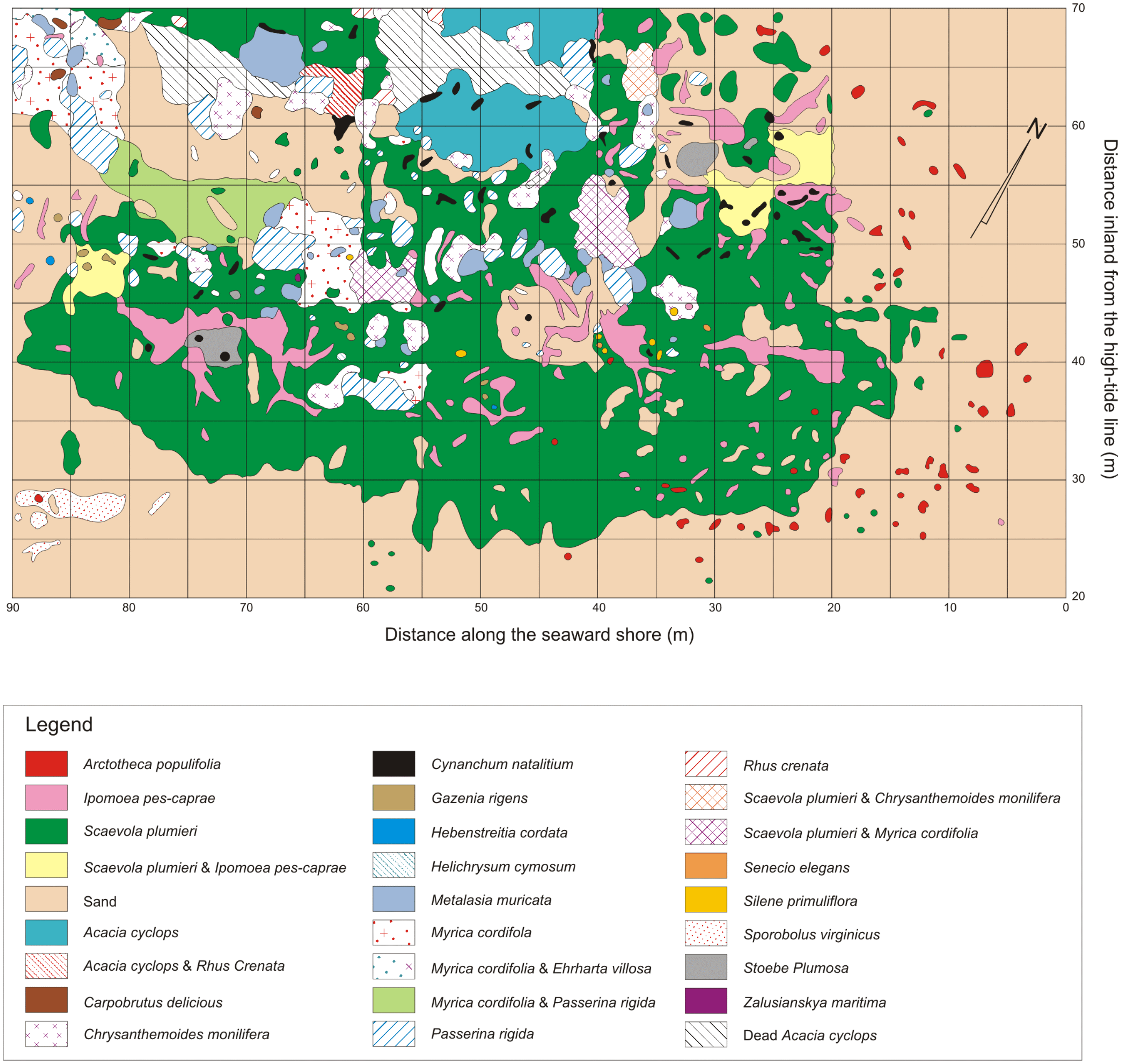


Figure 3.3: Vegetation diagram of the foredunes at Old Woman's River. Note the axis differences to Figure 3.2 (Diagram by I. Knevel).

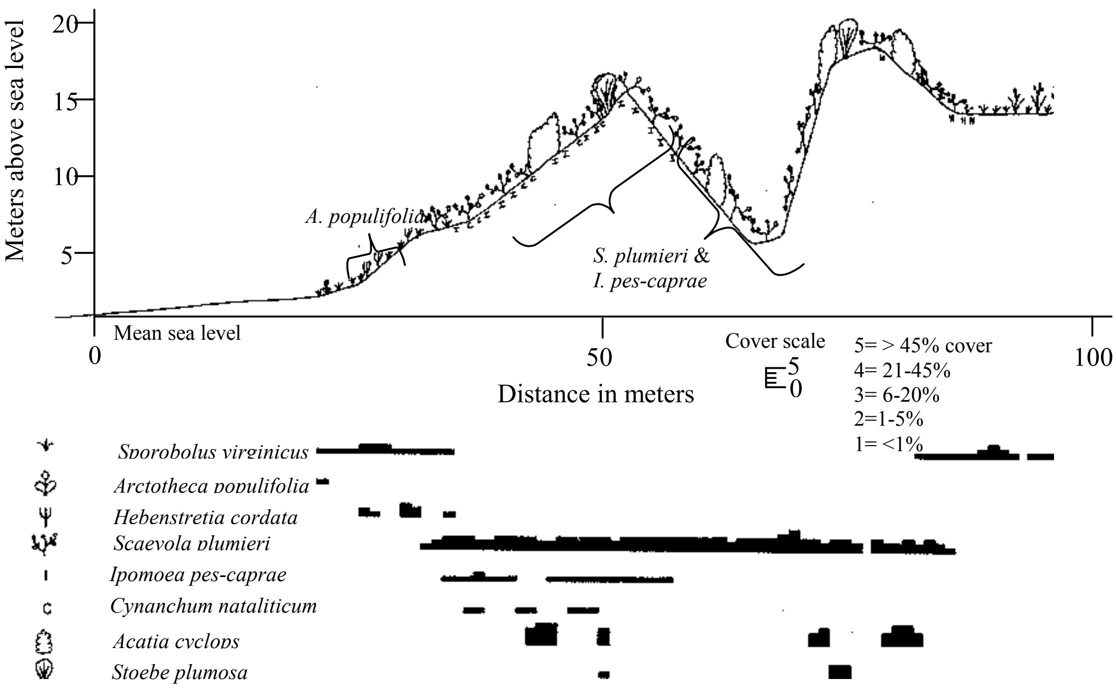


Figure 3.4: A profile diagram of the dunes at Kleinemonde Point, Eastern Cape. Species abundance is indicated in the lower part of the diagram as aerial cover in each m^2 along the transect. Diagram modified from Lubke (1983). Note the location of *A. populifolia*, *I. pes-caprae* and *S. plumieri* on the foredune.

Dune vegetation and growth preferences within the foredunes

The distribution of vegetation in the foredunes is shown in Figure 3.3 and a change in the species composition in a seaward to landward direction is evident. This is typical of the zonation noted for sandy beaches of the Eastern Cape. Species diversity and abundance (aerial cover) increases with distance inland (Lubke, 1983). The three species (*A. populifolia*, *I. pes-caprae* and *S. plumieri*) investigated in this study are confined to the foredune pioneer zone where they are by far the most dominant species. Although the three species studied co-exist in the same local areas (zones) even within these zones particular positional growth preferences have been recognised. Lubke (1983) and Avis (1992) after constructing dune profiles (Figure 3.4) noted that *A. populifolia* is frequently found just above the high-tide line whereas both *I. pes-caprae* and *S. plumieri* are found further inland on the foredunes. These positional differences were also clearly evident on the vegetation map of the study site (Figure 3.3) which shows the distribution of *A. populifolia* above the high-tide line with *S. plumieri* and *I. pes-caprae* having a more landward position. Avis

(1992) in a study of the successional processes at Kleinemonde, Eastern Cape noted that *A. populifolia* clumps increased from 1981 to 1983 and then subsequently died out as the secondary colonisers of dune slacks became more abundant.

CLIMATE AND WEATHER

All aspects of plant response are directly or indirectly dependent on the prevailing and historic abiotic conditions to which they have been subjected. Seasonal and stochastic changes in abiotic factors markedly influence plant response and hence when monitoring "snapshots" (individual experimental days) of plant response (physiology) in different seasons and chronology it is necessary to consider both the abiotic conditions on the days on which experiments were conducted and longer-term changes (climate).

Climate at Old Woman's River

Climatic data are recorded by the South African Weather Bureau (SAWB) at Great Fish Point approximately 5 km to the south west of the Old Woman's River study site.

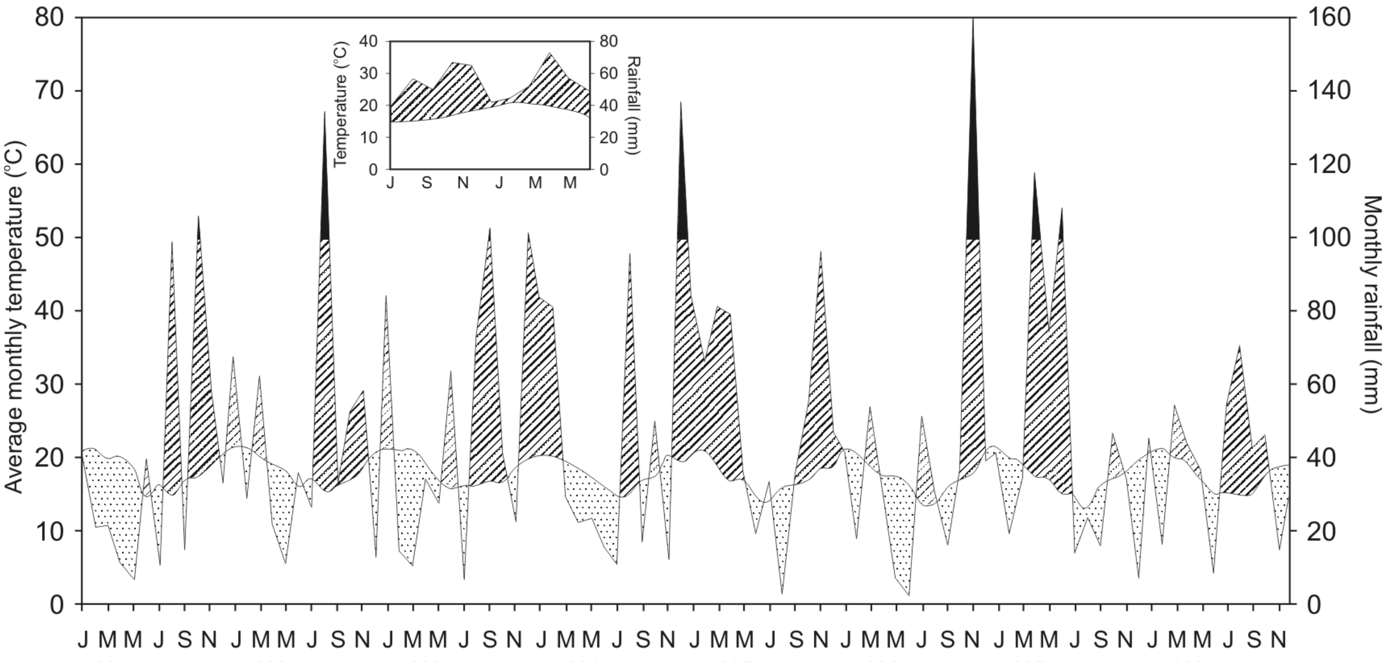


Figure 3.5: Climate data for the Great Fish Point Lighthouse weather station for the period 1991 to1999. The inset summarises the average values for the period 1960 to 1998 in the form of a climate diagram. Angled lines indicate humid periods, dotted areas indicate arid periods, and solid areas indicate rainfall higher than 100mm.

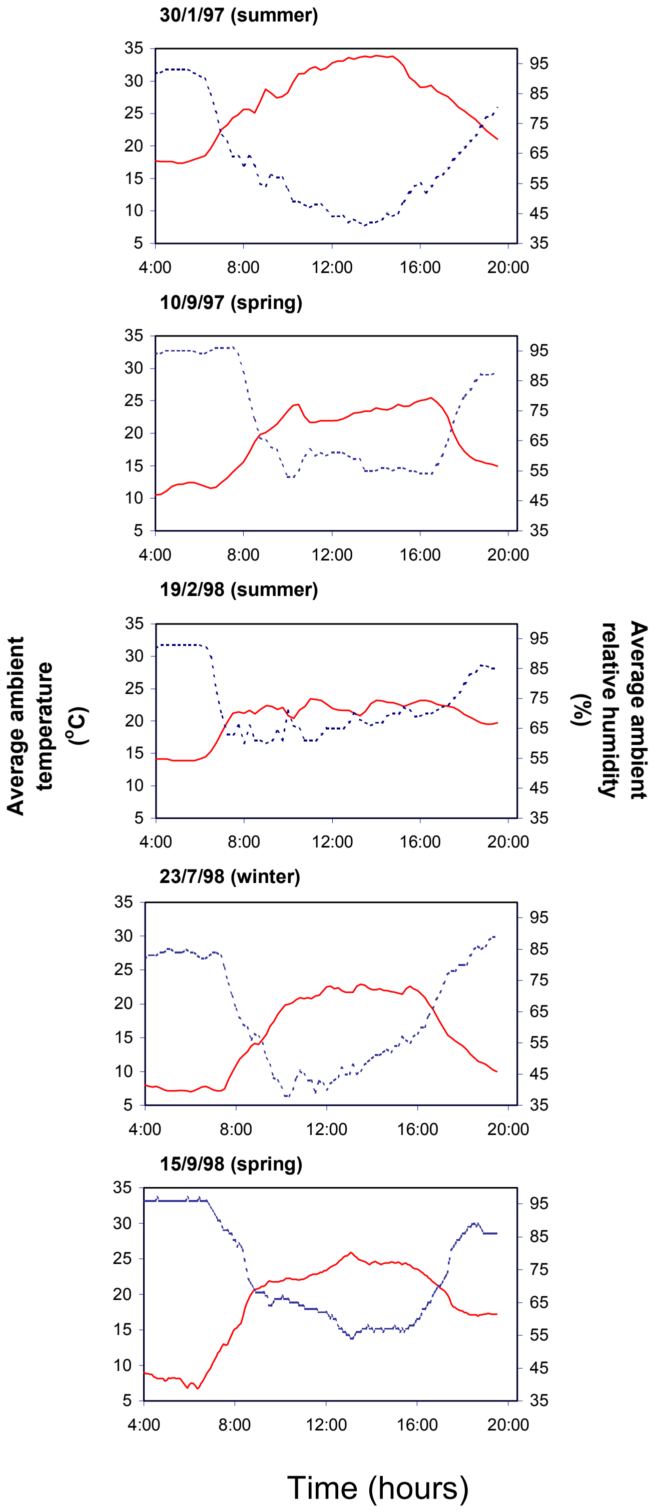


Figure 3.6: Diurnal variations in average ambient temperature (solid line) and relative humidity (broken line) on the indicated experimental days.

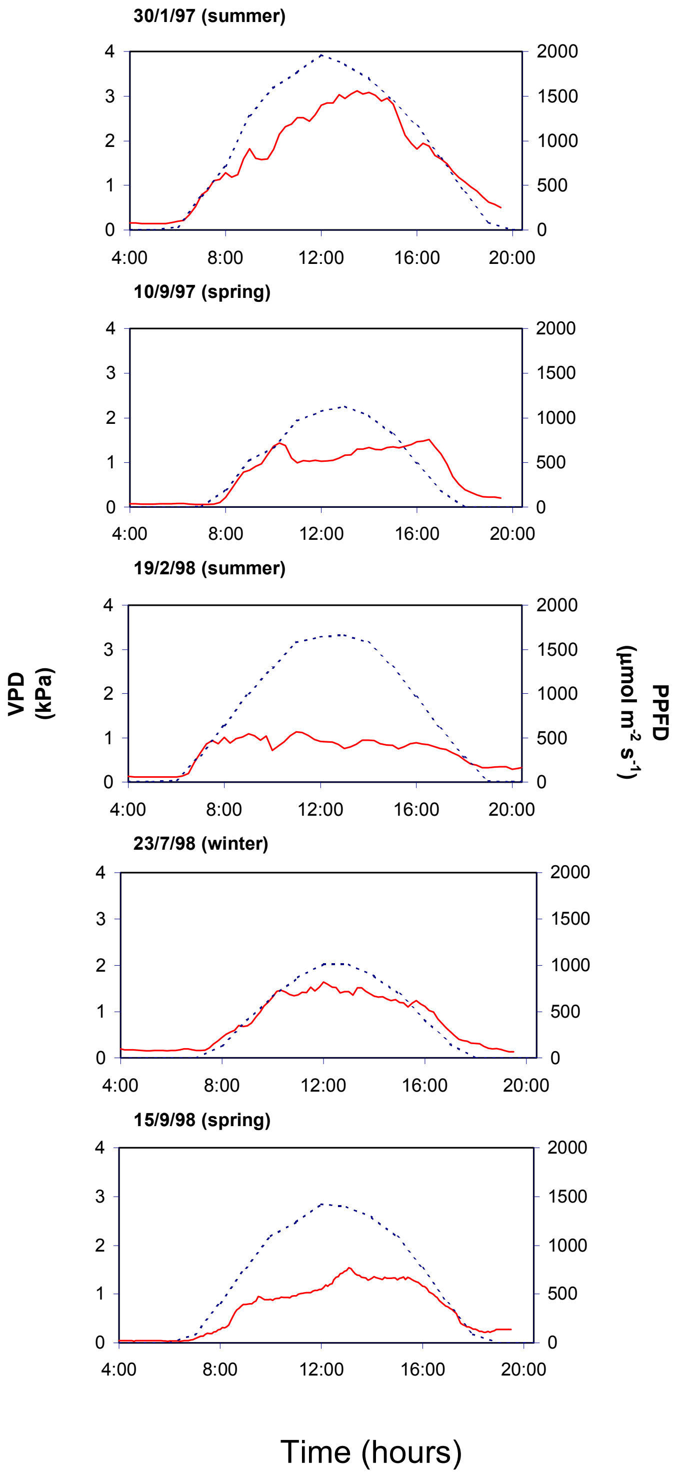


Figure 3.7: Diurnal variations in the atmospheric vapour pressure deficit (VPD; solid line) and photosynthetic photon flux density (PPFD; broken line) on the indicated experimental days.

Rainfall and maximum and minimum temperature data were taken from the 1960 to 1998 published records (SAWB, 1998). Average daily and monthly temperatures were calculated as the mean of daily minimum and maximum temperatures and the mean of the daily averages respectively.

Mean monthly temperature and total monthly rainfall for the period 1991 to 1998 are presented in Figure 3.5. The data for the period 1960 to 1998 are summarised as a climate diagram (Walter and Leith, 1967) as an inset in Figure 3.5. Monthly rainfall is highly variable with almost any month being excessively drier or wetter than the mean for that month. The spring and summer months are generally wetter than the winter months although large winter rainfall events occur as is evident from the large monthly rainfall measured in July 1998 (Figure 3.8). Lower humidity, rainfall and temperature characterise winter months. Table 3.1 summarises this average monthly data giving the average annual temperature and total rainfall figures. Average temperatures are not extreme fluctuating around 18°C (Table 3.1) but extremes and the range of temperature that may be measured on any one day can be considerable (Figure 3.6). The study period was one of lower rainfall than the 1960 to 1998 average (Table 3.1).

Table 3.1: Average annual temperature and total annual rainfall for the years 1991 to 1998 and for the 38 year period 1960 to 1998.

	1991	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1960 to 98**
Average annual temperature (°C)	18.4	18.5	18.4	18.1	17.6	17.6	17.1	17.9	17.9 ± 2.3
Total annual rainfall (mm)	528.4	465.2	550.0	587.2	583.4	639.1	488.0	538.6	654.3 ± 180.4

Wind direction along this section of coast is predominantly from the southwest and west during the winter months and from the northeast and east in the summer months (Avis, 1992). Wind results in sand movement downwind and although there is a considerable short term gross movement of sand, the alternately predominant wind in opposite directions results in a relatively small net sand movement in the longer term (Ketelaar, 1998).

Weather conditions on or prior to experimental days

Ambient temperature, relative humidity, vapour pressure deficit (VPD) and photosynthetic photon flux density (PPFD) incident on a horizontal surface measured from pre-awn to after sunset are given in Figures 3.6 & 3.7 (See: Chapter 4 for method details). All PPFD measurements made during the experimental days at Old Woman's River were associated with physiological measurements and were made at 90^o to the adaxial leaf surfaces and do not necessarily reflect incident light intensities. For this reason PPFD data presented in Figure 3.7 was obtained from the SAWB automatic weather station at Port Elizabeth. Here solar radiation data is measured with a horizontally placed Licor pyranometer (Licor Inc., USA) at standard instrument height and these measurements represent MJ m^{-2} integrated over an hour. These measurements were converted to Wm^{-2} using the conversion factor 277.8. Wm^{-2} were converted to PPFD using the equation: 1 Wm^{-2} of day light ≅ 1.895 μmol m^{-2} s^{-1} PPFD (Coombs *et al.*, 1985).

Minimum, maximum and average temperatures, average relative humidity and total daily PPFD values are given in Table 3.2. Distinct differences in average daily temperature between seasons were evident with summer temperatures being higher than either winter or spring temperatures. Similar trends in daily incident PPFD are also evident while trends in VPD and relative humidity were less obvious (Figures 3.6 & 3.7; Table 3.2).

Table 3.2: Average maximum and minimum temperatures, average relative humidity and average incident photosynthetic photon flux density (PPFD) measured on the indicated experimental days.

Date & season	**Average Temperature**	**Maximum Temperature**	**Minimum Temperature**	**Average RH**	**Total PPFD**
	(^oC)	(^oC)	(^oC)	(%)	mol m^{-2} day^{-1}
30/1/97 (summer)	23.6	31.2	17.1	70	54.7
10/9/98 (spring)	16.7	25.5	10.2	79	25.1
19/2/98 (summer)	19.11	23.4	13.9	79	44.2
23/7/98 (winter)	13.4	22.9	6.8	72	22.2
15/9/98 (spring)	16.4	25.9	6.7	81	35.1

Abbreviations: ambient relative humidity (RH), photosynthetic photon flux density (PPFD).

Rainfall prior to days on which experiments were conducted may have considerable effects on the physiology of the species investigated and hence the daily rainfall during the study period (1997 to 1998) is given in Figure 3.8. Experimental days are indicated on the figure. Cumulative amounts of rain for 7, 14, 21 and 30 days prior to each of the experimental dates is given in Table 3.3.

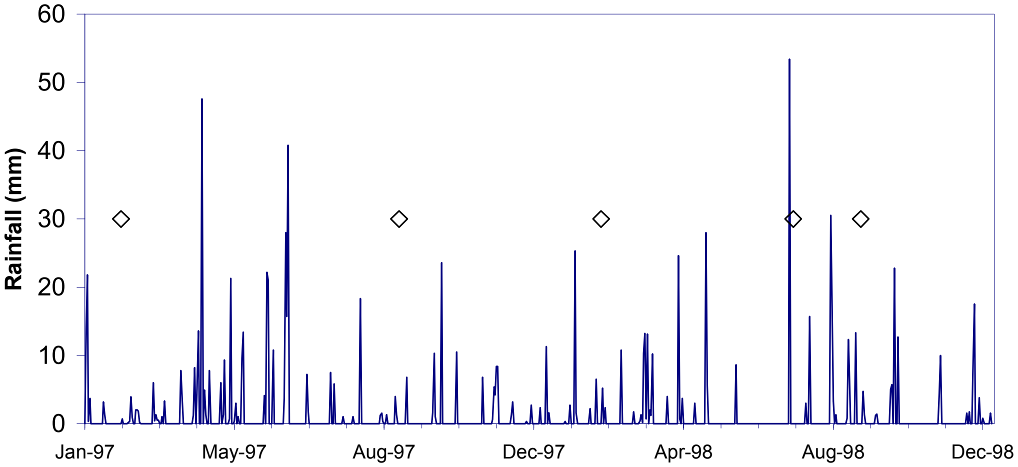


Figure 3.8: Daily rainfall at Great Fish Point weather station for the period 1 January 1997 to 31 December 1998. Experimental days are indicated by the open symbols.

For the three weeks prior to the 30/1/97 and 10/9/97 rainfalls of 2 and 9.6mm were recorded respectively. Whereas for the same number of days prior to 19/2/98, 23/7/98 and 15/9/98 rainfalls of 35.6, 53.4 and 35.2mm were recorded respectively.

Table 3. 3: Cumulative rainfall for 7, 14, 21 and 30 days prior to each of the indicated experimental days.

Date & season	Accumulative rainfall (mm)			
	7 days	14 days	21 days	30 days
30/1/97 (summer)	0.0	4.5	4.5	40.8
10/9/98 (spring)	1.0	1.0	2.0	15.3
19/2/98 (summer)	6.5	8.7	35.6	38.6
23/7/98 (winter)	53.4	53.4	53.4	53.4
15/9/98 (spring)	13.3	33.9	35.2	86.0

NOMENCLATURE AND DISTRIBUTIONS OF STUDIED SPECIES

The genus *Arctotheca*, a member of the Asteraceae, contains about eight species distributed in Africa and Australia. Whilst *Arctotheca populifolia* (Berg.) Norl. is this species recognised name the synonyms *Arctotheca nivea* (L.f.) Levin, *Arctotis verbasafolia* (Harv.) and *Arctotis populifolia* have been used. *A. populifolia* is found distributed around the entire southern African coast from southern Mozambique to the west coast of southern Africa and its distribution overlaps with that of temperate species in the western and southern Cape. *A. populifolia* also occurs in Australia as a dune pioneer species.

Ipomoea is a genus of some 500 species (Wilson, 1977) and *I. pes-caprae* (L.) R. Br. (Convolvulaceae) has some 21 synonyms. *I. pes-caprae* is found throughout the tropical and subtropical regions of the world (Devall and Thien, 1989), on five continents and most tropical islands (St. John, 1970). In southern Africa it is found from southern Mozambique to Algoa Bay (Port Elizabeth). On the west coast it is found in Angola near Benguela (Miur 1937; cited in Tinley, 1985) and continues up the west coast towards the tropics. Like *S. plumieri*, *I. pes-caprae* does not occur in the western and southern Cape.

The genus *Scaevola* (L.), in the family Goodeniaceae, is centred in Australia and includes over 400 species (Dyer, 1967). Although known by a number of synonyms (*Lobelia plumieri, Scaevola lobelia* and locally as *Scaevola thunbergii* Eckl. & Zeyh.) *Scaevola plumieri* (L.) Vahl. is recognised as being the accepted name (Jeffrey, 1979).

S. plumieri is found along many of the tropical sandy beaches in the Indo-Atlantic region. It is found along the east coast of the Americas, from north of Palm Bay in Florida south through the Caribbean Islands (Guppy, 1917), the Gulf of Mexico and central America (Espejel, 1987) to the coast of Brazil. In Brazil *S. plumieri* is found around Cape de Sao Roque and the city of Natal (N. W. Pammenter *pers. com.*) at least as far south as Rio de Janeiro (Guppy, 1917; Doing, 1985). *S. plumieri* is found on the west coast of Africa from Angola (Tinley, 1985) at least as far north as the Senegal coast of north-west Africa (Doing, 1985). Around the Indian Ocean coast *S. plumieri* is found as far east as India and Sri Lanka as well as the islands of Mauritius and Madagascar (Ridley, 1930). Along the east coast of Africa *S. plumieri* is found from Mombassa in Kenya (Doing, 1985; Tinley, 1985) on the African north-east coast (possibly further north) and south to Arniston near Cape Agulhas (Peter, 2000). *S. plumieri* does not occur in the western and southern Cape where the climate is temperate Mediterranean.

AN INTRODUCTION TO THE SPECIES

Arctotheca populifolia

The sea pumpkin *A. populifolia* is a succulent, thick stemmed pioneer species with leaves that are covered with a dense layer of grey hairs (Plate 3.2). The leaves are ovate and large (up to 70mm x 50mm) and leaves from different plants have leaf margins ranging from being serrulate to pinnatifid. *A. populifolia* is pauciennial and is not as long lived (*ca.* 2 yrs, Tinley, 1985) as either *I. pes-caprae* or *S. plumieri. A. populifolia* branches both on buried and unburied stems such that a single individual may rapidly spread forming a hummock dune under conditions of sand accretion**.** Buried stems produce adventitious roots at nodes. The underground stem connections may die so that several vegetatively produced ramets may occupy a hummock dune. *A. populifolia* flowers throughout the year and produces large quantities of seed that are dispersed locally and may rapidly establish in the immediate vicinity of the parent plant. As flowers mature they droop so that flower heads become buried releasing seeds locally (*pers. obs.*).

Ipomoea pes-caprae

The beach morning glory *I. pes-caprae* is a clonal, perennial vine with long trailing shoots (Plate 3.3) that grow linearly from the apical bud (Wilson, 1977). The leaves are large (up to 100 x 120 mm), non-succulent and like many of the Convolvulaceae exude a white sap if damaged. The plant reproduces vegetatively by producing axillary branches and adventitious roots at nodes (Bach, 1998). When stems become buried daughter ramets are produced from adventitious roots and axillary branches (Devall, 1992). As with *S. plumieri* this growth habit results in large individual plants or ramets forming large interconnected mats. I have encountered underground *I. pes-caprae* stems, following the erosion of a dune by a period of rough seas, which were approximately 80mm in diameter, suggesting that to attain such size individual plants are likely to be long-lived.

Plate 3.2: *A. populifolia* growing on the foredunes at Old Woman's River, Eastern Cape Province, Republic of South Africa.

I. pes-caprae produces large purple flowers in the summer resulting in a capsule containing four large hairy seeds. These appear to germinate readily (Knevel, *pers. com.*) although very few seedlings appear to establish (Balarin, 1996).

Plate 3.3: *I. pes-caprae* growing on foredunes at Old Woman's River, Eastern Cape Province, Republic of South Africa.

Scaevola plumieri

S. plumieri has large (up to 120 x 70 mm) broadly elliptic to obovate succulent leaves (Harte and Pammenter, 1983) borne on erect stems up to 80cm in length and randomly arranged (Plate 3.4).

Plate 3.4: *S. plumieri* growing on foredunes at Old Woman's River, Eastern Cape Province, Republic of South Africa.

Each shoot grows predominantly from its apical bud and the youngest leaves are vertically orientated. As leaves get older the angle made with the stem increases such that oldest leaves are inclined at angles greater than 90^o (Chapter 7). When stems become buried following the accumulation of wind borne sand, branching occurs from buried stems (Plate 3.5). The stems produce adventitious roots as they are buried. The end result is a large 'under-ground trunkless tree' with only the tips of the 'branches' emerging above the sand dune. Each individual plant with its network of interwoven below-ground stems can be quite massive as shown by invariable Inter-Simple Sequence Repeat (ISSR) sequence data for individual shoots collected from a large single foredune (Harman, 2000). Once established the major mode of clonal expansion is vegetative with very few new individual plants establishing from seed (Balarin, 1996). In the summer the plants flower and produce a large number of drupes which ripen to a deep purple and all fruit have fallen from the plants by March.

Plate 3.5: An example of *S. plumieri* stems that have been excavated by wind erosion revealing previously buried branching pattern.

LEAF, SHOOT AND CANOPY CHARACTERISTICS

Data gathered during experiments designed to test hypotheses about plant productivity, water and nutrient relations and physiology were used to tabulate some leaf, shoot and canopy characteristics of *A. populifolia*, *I. pes-caprae* and *S. plumieri* growing at Old Woman's River. The differences in these characteristics among the studied species are not discussed at length in this chapter but are highlighted in Chapters 4, 5, 6, 7 and 8.

Wet to dry weight conversions (Tables 3.4 A & B) were calculated from data gathered for the construction of pressure-volume curves (Chapter 5). This included measuring wet and dry weights of leaves, shoots and stems. For *A. populifolia* pressure-chamber measurements were made on individual leaves from different plants and not whole shoots as was the case for *S. plumieri* and *I. pes-caprae*. *A. populifolia* shoots cannot be inserted into a pressure-chamber as they are too thick, succulent and not robust enough.

Additional data on stem wet and dry weights were collected during primary productivity and biomass estimates (Chapter 4). The number of leaves or shoots analysed is given in Tables 3.4 A & B. In addition to these data further data for *S. plumieri* were available from Peter (2000) and Peter and Ripley (2001) and have been included in these tables.

Tables 3.4 A & B: Leaf (A) and stem (B) wet weight to dry weight conversions for *A. populifolia*, *I. pes-caprae* and *S. plumieri*. Fitted equations are linear. Values in A & B were calculated from individual *A. populifolia* plants and from individual *S. plumieri* and *I. pes-caprae* shoots. (C. Peter is acknowledged for some of the *S. plumieri* data.)

A

Species	**Leaf dry weight =**	r^2	**N (leaves)**	**N (shoots)**
A. populifolia	0.065 x wet weight	0.80	24	
I. pes-caprae	0.127 x wet weight	0.86	76	19
S. plumieri	0.131 x wet weight	0.93	1287	81

B

Species	**Stem dry weight =**	r^2	**N**
A. populifolia	0.160 x wet weight	0.96	20
I. pes-caprae	0.186 x wet weight	0.88	20
S. plumieri	0.322 x wet weight	0.94	20

Specific leaf area (SLA; Table 3.5) was calculated using data from plants collected for the measurement of nocturnal respiration rates (Chapter 4). The number of leaves and shoots measured is shown in Table 3.5. The aggregate leaf surface area of all the leaves on fresh shoots or plants were measured subsequent to which the leaves and stems were oven-dried and weighed. Leaf and stem dry weights were combined and used to calculate average shoot dry weights (Table 3.6). Data on average shoot dry weights and shoot densities (number of shoots per m^2 dune surface) for *S. plumieri* were also available from Peter (2000) and have been included.

Table 3.5: Average leaf weight and specific leaf area (SLA) of *A. populifolia*, *I. pes-caprae* and *S. plumieri*. Measurements were made on combined leaves from six individual shoots of each species.

Species	**Average leaf dry weight (g)**	**SLA (cm^2 g^{-1})**	**N (leaves)**	**N (shoots)**
A. populifolia	0.22 ± 0.1	60.6 ± 6.9	83	6
I. pes-caprae	0.42 ± 0.2	77.3 ± 5.2	76	6
S. plumieri	0.61 ± 0.4	35.8 ± 6.2	107	6

Data collected per unit ground surface area for above-ground biomass estimates of *A. populifolia* and *I. pes-caprae* (Chapter 4) included leaf dry weights. These were converted to leaf surface areas using the SLA values listed in Table 3.5 and were expressed as the total leaf surface area per unit ground surface area (Table 3.6) and termed leaf area index (LAI). As *A. populifolia* and *I. pes-caprae* have patchy distributions the values calculated are dependent on the soil surface area considered and caution is necessary particularly when comparing these values to LAI of species from other ecosystems. Included in Table 3.6 is the number of shoots per m^2 dune area. Peter (2000) calculated the LAI of *S. plumieri* by multiplying the average shoot surface area by the average number of shoots per m^2 and his data have been included in the table.

Table 3.6: *A. populifolia*, *I. pes-caprae* and *S. plumieri* canopy characteristics. The number of replicated measurements is given in the table. (C. Peter is acknowledged for some of the *S. plumieri* data.)

Species	**Average shoot dry weight (g)**	**Average number of shoots per unit dune surface area (m^2)**	**Leaf Area Index (LAI)**
A. populifolia	5.15 ± 1.67(N=6)	11.3 ± 2.5 (N=10)	0.23 ± 0.06
I. pes-caprae	12.84 ± 6.11(N=6)	21.7 ± 4.5 (N=10)	0.78 ± 0.26
S. plumieri	12.68 ± 2.98 (N=62)	21.43 ± 10.5 (N=20)	1.05 ± 0.51

CHAPTER 4
PLANT PRODUCTIVITY

INTRODUCTION

A consideration of the productivity of the dune pioneer species investigated in this study is essential as it defines the demands these plants have for resources. These demands include requirements for water, light and essential nutrients.

Dune environments as is outlined in Chapter 1 are low in nutrients, potentially water impoverished and are subjected to high levels of radiation (Barbour *et al*., 1985; Hesp, 1991; Davy and Figueroa, 1993). All of these abiotic factors have the potential of markedly limiting the productivity of plants growing in this environment. Additions of fertiliser (Willis, 1965; Garcia-Novo, 1976; Huiskes, 1980; Pavlik, 1983b; Fink and Zedler, 1990) and measurement of plant response to fluctuations in the water table (Martinez *et al.*, 1997) showed growth enhancement in response to nutrient supply and water availability. The effects of excess light on growth and productivity have not been researched in dune vegetation but have been shown to reduce productivity in field-grown *Zea mays* and *Brassica napus* (Farage and Long, 1987, 1991) during periods of low morning temperatures. In spite of these limitations dune pioneers need to continually grow in order to survive sand burial (Harris and Davy, 1988; Davey and Figueroa, 1993) and the physical damage (Grace and Russell, 1982) that is characteristic of this challenging environment. This raises the questions: How productive are the plants growing in this environment? How does productivity change with season? What limits productivity?

These questions have been approached at several levels: the photosynthetic and respiratory responses, response of leaf and stem non-structural carbohydrates, rates of leaf production and assessment of above-ground primary productivity. This multi-level approach was necessary as it not only gives answers to the above questions but also allows the differences or similarities between species to be ascertained. For example, suppose all three species achieve approximately the same production rates; is this because all three species have similar physiology or are different mechanisms involved in producing the same end result?

In addition to understanding the differences or similarities in production between the species, the construction of simple supply and demand budgets as has been carried out for water (Chapter 5) and nutrients (Chapter 6), requires the consideration of seasonal differences in supply and demand. Seasonal differences will determine the time scale for which budgets can be constructed. Norman (1993) recognises seasonal change as one of the complicating factors in modelling canopy responses. A lack of seasonal differences in demand may allow the construction of budgets for longer periods of time, uncomplicated by seasonal growth responses.

Photosynthetic and respiratory responses

Photosynthetic rates have been measured in a large variety of dune species both in the laboratory using pot-grown plants (De Jong, 1978; Pavlik, 1983a; Pammenter, 1983, 1985; Zhang, 1996) and in the field (Pavlik, 1983a; Elfman *et al.*, 1986; Ishikawa *et al.*, 1991; Yaun *et al.*, 1993). Rates generally range between 25 and 40 μmol CO_2 m^{-2} $s^{-1}$and show light saturation between half and full sunlight. Photosynthetic rates are very variable among species as well as with growth habit and the species living on coastal dunes include C_3 and C_4 grasses, annuals, herbaceous dicotyledonous plants and long-lived perennials. Consequently any generalisation concerning photosynthetic rates is meaningless. What is apparent is that temperate dune systems which are dominated by monocotyledonous grasses show marked seasonal changes in their light-saturated photosynthetic responses (Pavlik, 1983a) and also in responses at natural light intensities (Yuan *et al.,* 1993). Seasonal changes in photosynthetic rates in sub-tropical dune species have not been investigated.

Although photosynthetic rates are often affected by season the prevailing light conditions and more specifically the PPFD absorbed may be the most important factor determining productivity (Haxeltine and Prentice, 1996). Pammenter and Smith (unpublished manuscript) produced some strong evidence that *S. plumieri* is light limited due to self shading and leaf inclination angles despite living in the high light environment encountered on the beach (See Chapter 7).

Information about the respiration rates of dune species is sparse and confined mainly to potted plant experiments. Pavlik (1983a) calculated respiration rates for pot grown *Ammophila arenaria* and *Elymus mollis* and showed that rates increased when growth was stimulated by the addition of fertiliser. Seasonal responses and the proportion of photosynthetically assimilated carbon expended on respiration of field grown dune species is unknown but Lechowicz (1980) demonstrated increases in dark respiration with latitude for *Lathyrus japonicus.* He suggested increased respiratory costs at lower temperature and with shorter growing seasons. Data on seasonal changes in respiration rates for dune species are unavailable.

Non-structural carbohydrates

Little information is available on leaf, stem, or root non-structural carbohydrate concentrations in coastal dune species and these levels have not been measured for *A. populifolia*, *I. pes-caprae* or *S. plumieri* stems or leaves. *A. populifolia* and *I. pes-caprae* have succulent stems with large central cortices that could potentially serve as storage organs. The rhizomes of the temperate dune grass *Ammophila arenaria* do act as storage organs and sugar, but not starch, concentrations change on plant burial (Seliskar, 1994). Harris and Davey (1988) showed that *Elymus farctus* in response to plant burial reallocated carbon resources to maintain leaf mass at the expense of roots and stems. Buried plants had higher leaf concentrations of soluble carbohydrates both during the period of burial and during a subsequent non-buried period. The maintenance of leaf concentrations of soluble carbohydrates allowed the plants to quickly re-establish high photosynthetic rates when unburied.

The cycling and reallocation of carbohydrate reserves between roots and leaves has been shown in plants growing in other natural ecosystems. Carbohydrate reserves of small perennial alpine plants’ storage tissues are reported to be as high as 40-60% ($^{w}/_{w}$) (Archer and Tieszen, 1980; Mooney and Billings, 1961; Russell, 1940, cited in Bliss, 1985). Mooney and Billings (1960) showed that these plants which have highly seasonal growth (as do temperate dune species) relocate underground stored carbohydrates to the shoots in spring to support new growth. The carbohydrate reserves are gradually replenished during the growing season (Svoboda, 1977).

Plant growth

The growth and productivity of both adult plants and seedlings of dominant temperate dune species have been well researched. This is of particular interest because these species respond to sand-burial with increased growth (for a review see Hesp, 1991). Most of these species are monocotyledonous and hence the measures have generally included relative growth rates (Wallen, 1980), tiller production per plant (Willis, 1965), density of shoots per m^2 (Yaun *et al.*, 1993), leaf production per initial 100 leaves (Huiskes, 1980), tillering rates (Huiskes and Harper, 1979; Hertling, 1997) and rhizome extension (Watkinson *et al.*, 1979). There is little published data on biomass and more especially on above-ground productivity. Exceptions to this are the studies on *Ammophila arenaria* by Deshmukh (1977), Wallen (1980) and Pavlik (1983c). Deshmukh (1977) calculated primary production, biomass and loss by death of plants growing at various distances from the sea. Wallen (1980) included measurement of many growth parameters including green standing crop and productivity. These studies showed a decrease in productivity of *Ammophila arenaria* from pioneer dunes to older mature dunes. As this study considers plants colonising the pioneer dunes it is the *Ammophila arenaria* productivity data from the pioneer dunes that is of interest as this will make comparisons between temperate and sub-tropical systems possible.

Growth of non-temperate species has received less attention and most studies have concentrated on seedlings. Such studies include the calculation of relative growth rates of establishing seedlings of the dune species *Xanthium strumarium* growing in Maine, USA; the measurement of tiller length, mass and the number of leaves of *Triplasis purpurea* subject to salt-spray and burial in pot experiments (Cheplick and Demetri, 1999); above-ground and below-ground biomass production and relative growth rates of six pot grown species from the Gulf of Mexico (Martinez and Moreno-Casasola, 1996; 1998) and investigations on the effects of substrate salinity on the growth and photosynthetic response of pot grown *Ischaemum anthephoroides, Carex kobomugi* and *Calystegia soldanella* in Japan (Ishikawa *et al.,* 1991). The only non-temperate data available for South Africa is from monocotyledons. Hertling (1997) measured above- and below-ground biomass for the introduced grass *Ammophila arenaria* and the indigenous grasses

Elymus mollis and *Ehrhata villosa*. Comparable data for non-temperate dicotyledonous dune species are not available.

From the discussion above it is clear that growth is stimulated by sand-burial but also follows cyclic patterns related to climate (Davy and Figueroa, 1993). Seasonal growth and productivity has been shown in temperate (Huiskes, 1980) and sub-tropical dune systems (Pammenter, 1983; Steinke and Lambert, 1986).

Light stress

The beach environment has both high incident (Barbour *et al.*, 1985) and reflected light intensities (Chapman, 1976). This high incident light combined with potential water stress (Hesp, 1991) and low winter temperatures make beach plants potentially susceptible to light stress and ensuing photoinhibition. Despite this, photoinhibition has not been researched in dune species.

Photoinhibition and a reduction in photochemical capacity has been documented in both field grown crops (Groom *et al.*, 1990; Bolhar-Nordenkampf, 1991) and natural systems (Adams III, 1988; Adams III *et al.*, 1988; Fernandez-Baco *et al.*, 1998; Ogren, 1988; Demmig-Adams and Adams III, 1996; Niinemets *et al.*, 1998). The photoinhibition of natural terrestrial vegetation has been recently reviewed (Long and Humphries, 1994).

Photoinhibition is accompanied by a reduction in the photochemical capacity, an increase in the non-photochemical dissipation of energy, a decrease in the photochemical dissipation of energy and the de-epoxidation of the xanthophylls (Demmig-Adams and Adams III, 1996). The latter is one of the mechanisms whereby excess energy is channelled away from the photochemical processes and dissipated non-photochemically as heat (Gilmore *et al.*, 1996; Demmig-Adams *et al.*, 1988; Demmig-Adams, 1990). In addition there is evidence to suggest that there is a relationship between the size of the de-epoxidated xanthophyll pool (which varies between species) and the reduction in midday photochemical efficiency (Demmig-Adams and Adams III, 1996). Furthermore, one acclimation to the light environment in which a plant is grown can occur by the alteration of the size of the xanthophyll pool (Niinemets *et al.*, 1998). Sun-grown plants with high photosynthetic capacities have large xanthophyll pools but there appears to be considerable variation between species. Photoinhibition is related both to the light environment in which growth takes place and to absorbed light intensities. The light energy decreases exponentially as it is transmitted through the leaf and hence the photosynthetic apparatus near to the illuminated leaf surface is most susceptible to photoinhibition. Adams III *et al.* (1996) demonstrated that in succulent species there were larger pools of xanthophylls in the tissues of the upper leaf surfaces than the lower surfaces but that the proportion converted to the de-epoxidated states was the same throughout the tissue. The upper surface used as little as 25% of the absorbed light photochemically, the remainder being dissipated as heat. The lower surface used a considerably greater proportion of the light. This is of relevance to both *A. populifolia* and *S. plumieri* that possess succulent leaves.

Xanthophyll conversions are by no means the only mechanism whereby plants can cope with high light levels. Mechanisms such as paraheliotropisms have been demonstrated in soybean cultivars (Rosa and Forseth, 1995) with high light tolerant cultivars possessing steeper leaf angles at midday. Continued utilisation of reductant by the reductive (Baroli and Melis, 1998) and oxidative pentose phosphate pathways (Brestic *et al.*, 1995; Kozaki and Takeba, 1996; Heber *et al.,* 1996) and photosystem II (PSII) cyclic electron transport (Falkowski *et al.*, 1986) have been shown to decrease photoinhibition. Electron transport to oxygen (Park *et al.*, 1996) and the subsequent metabolism of resultant oxygen radicals has been shown to be an important dissipater of excess energy (Bielher and Fock, 1996; Noctor and Foyer, 1998).

METHODS

Climate measurements

Ambient temperature and relative humidity were recorded during the days when experiments were conducted using Weather Monitor II data loggers (Davis Instruments, Hayward, California, USA). These climatic data were downloaded using the Weather Monitor program Weatherlink 3.01 (Davis Instruments Corporation, 1994). Both of these parameters were used to calculate atmospheric vapour pressure deficit (VPD) where:

$$VPD = SVP_{Ta} - (SVP_{Ta} \times RH/100)$$

Here SVP_{Ta} is the saturation vapour pressure at ambient temperature and RH is the ambient relative humidity. Saturation vapour pressures were calculated from temperature according to the equations of Goff and Gatch (1946).

CO_2 assimilation

Photosynthetic measurements were made at approximately two-hour intervals on single leaves of three individual shoots or plants of each of the species beginning pre-dawn and continuing until after sunset. Fully expanded leaves (typically leaf 10 to 13) with similar orientations were selected for both the photosynthesis and chlorophyll fluorescence measurements. Leaves were numbered with the youngest visible leaf being designated leaf 1. Photosynthesis measurements were made using an LCA-II portable infrared gas analyser (IRGA), mass flow controller and Parkinson broad-leaf chamber (PLC; Analytical Development Corporation (ADC), Hoddeson, U.K.). The LCA-II IRGA was calibrated for CO_2 using certified calibration gas at a concentration of 406 μmol mol^{-1}. The LCA-II calibration was checked periodically throughout the course of this study and appropriate adjustments made where required. The data recorded in the field were logged on the LCA-II data logger (DL-2) and downloaded for subsequent analysis using the software Possim 1.0 (Sphynx Ink., 1996). This software was used to calculate gas exchange parameters according to the equations of von Caemmerer and Farquhar (1981) and leaf temperatures according to an energy budget equation (ADC, Hoddesdon, U.K.). Although the Parkinson leaf chamber measures the gas exchange from both leaf surfaces it allows illumination of only a single leaf surface and hence all calculations were based on the area of a single surface contained within the chamber.

Whole shoot respiration rates

Respiration rates were measured between 21:00 and 23:00 hrs on the 30/1/98 (summer) and 23/7/98 (winter) on three individual shoots or plants of each of the three species. Entire shoots were enclosed in thick polyethylene bags that were sealed with Prestik (© Bostik) and secured with insulation tape. Ambient air at a known flow rate set by a mass flow controller (ADC) was piped into the bag. Once the bag had inflated the air was allowed to exit via a second tube and was piped directly to an LCA-II infra-red gas analyser. Differential CO_2 measurements between incoming and exiting air were manually recorded during the two-hour period. At the end of the experiment the shoots were harvested their leaf areas calculated and shoots oven-dried to constant weight. Dry shoots were separated into leaves, stems and reproductive structures and dry weights of these components recorded separately. Respiration rates were expressed per unit leaf area although stem and fruit respiration would be included in the measured values.

Diurnal changes in chlorophyll fluorescence

Chlorophyll fluorescence measurements were made at approximately two-hour intervals on single leaves of three individual shoots or plants of each of the species beginning pre-dawn and continuing until after sunset. Leaves were selected for measurements as is outlined above but were not made on the same leaves as were used for the photosynthetic measurements. Measurements were made using a Plant Efficiency Analyser (PEA) (Hansatek, Kings Lynn, Norfolk, U.K.) and full 1-second induction curve data logged automatically. Measurements were made at either 100% or 80 % of PEA maximal light output (600 W m^{-2}) excitation intensity. Experimental trials were conducted to ensure that this was sufficient to produce maximal fluorescence from 2-hour dark adapted leaves. On the first experimental day (30/1/97) measurements were made on leaves that had been dark-adapted for both two hours and for 30 seconds prior to the construction of induction curves. For subsequent experiments measurements were made subsequent to only 30 second dark adaptation. Logged data were downloaded using the program Analyser (Hansatek, Kings Lynn, Norfolk, U.K.). PPFD incident on the leaves was measured just prior to each fluorescence measurement using a LiCor integrating radiometer (LiCor, Lincoln, Nebraska).

J,I & P fluorescence phase analyses

According to the methods of Strasser and Strasser (1995) fluorescence induction curves were used to calculate the maximum quantum yield of primary photochemistry (φ_{P0}), the photochemical rate constant (k_P) and the non-photochemical rate constant (k_N) using the software package Biolyser (©Laboratory of Bioenergetics, University of Geneva, Switzerland). A complete description of this method and the assumptions made are presented in Appendix A.

Recovery of chlorophyll fluorescence following a light treatment

Five similarly aged and orientated leaves on separate shoots or plants of each of the species were selected and enclosed in insulated, light proof foil bags during the evening prior to the experimental day. PEA leaf clips were pre-positioned on the leaf lamina care being taken to avoid major veins. The foil bags were removed early the next morning and a fluorescence induction curve constructed for each of the leaves using the instrument parameters outlined above. The leaves were then exposed to natural illumination and the PPFD incident on each leaf recorded at ten-minute intervals. After four hours an induction curve was constructed for each leaf following 30-second dark adaptation. The foil bags were replaced and at intervals over the subsequent few hours the bags were removed, leaves dark-adapted for a further 30 seconds and further induction curves constructed. See Figure 4.8 for the time intervals at which measurements were made. Data were logged, downloaded and analysed as outlined above. φ_{P0} was used to monitor the before and after illumination changes in photosynthesis.

Xanthophyll content

Leaves with similar orientation as those used for fluorescence and photosynthetic measurements, from three individual plants of each species were collected at two hourly intervals in the field and immediately frozen in liquid nitrogen. These were stored at -70^{o}C in the laboratory until analyses were performed. One gram of leaf tissue was ground in 1.5 ml of 100% acetone with an Ultra-Turrax tissue grinder (Janke and Kukel, Germany) and subsequently centrifuged at 7000 x *g* for five minutes. The pellet was re-suspended in 1.5 ml of acetone and re-centrifuged. Supernatants were pooled and filtered through a Cameo (Micron Separations Incorporated, USA) 0.45 µm nylon syringe filter. 20 µl samples of the filtrate were analysed on a Spherisorb ODS1 HPLC column (250 x 4.6mm) (Macherey-Nagel) with the following solvent protocol (modified method of Gilmore and Yamamoto, 1991): 10 minutes of acetonitrile: methanol: Tris-HCl buffer (85:25:4), 12 minutes of hexane: water (4:1) and 8 minutes of methanol. Peaks separated to baseline and were identified and quantified by running pure synthesised standards on the same system. Standards were kindly donated by Prof. Keith Cowan, University of Natal, Pietermaritzburg. Xanthophylls were expressed per unit chlorophyll (a + b).

Chlorophyll content

Acetone leaf extractions used for the xanthophyll analyses were diluted 1:1 with 100% analytical grade acetone and absorbances read at 662 and 645nm on a PU8670 Vis/NIR spectrophotometer (Phillips, U.K.). Pure acetone was used as the blank. The equations of Lichtenhaler (1987) were used to calculate chlorophyll a, chlorophyll b and chlorophyll (a+b) contents per gram fresh weight of tissue extracted. These values were also expressed per unit leaf area using leaf dry to wet weight and surface area to dry weight conversions (Table 3.4 & 3.5).

Non-structural carbohydrates

The same leaf samples as used for the quantification of xanthophyll intermediates were used for analyses of soluble sugars and starch. In addition on the experimental days of 19/2/98 (summer) and 23/7/98 (winter) sets of three leaves from individual plants of each species which had been pre-treated by shading were harvested at two-hour intervals throughout the day and frozen in liquid nitrogen. This pre-treatment consisted of erecting three days prior to the experiment 50% (measured) shade-cloth over an area of dune on which all three species were growing. The colorimetric method of Buysse and Merckx (1993) was modified as follows: 0.05g of frozen leaf was ground in 20 ml of 80% ethanol and centrifuged at approximately 27000 x *g* for 20 minutes. The pellet was re-suspended in a further 10 ml of 80% ethanol and re-centrifuged. Supernatants were pooled. Starch contained in the pellets was hydrolysed by suspending the pellet in boiling 3% aqueous hydrochloric acid solution for at least three hours, following which solutions were centrifuged and supernatants made up to 50 ml with 80% ethanol. Soluble starch standards were treated in the same fashion to ensure complete hydrolysis.

1 ml samples of ethanol extracted soluble sugars or hydrolysed starch were added to 1 ml of 28% phenol: water (w/w) following which 10ml of conc. H_2SO_4 was added and the tubes vortex-mixed. Colour reaction was allowed to develop for at least 15 minutes and

no longer than one hour before absorptions were measured at 490nm on a PU8670 Vis/NIR spectrophotometer (Phillips, U.K.). Appropriate standard curves were constructed using analytical grade sucrose and soluble starch. Wet to dry weight conversions (Table 3.4) were used so that soluble sugar and starch concentrations could be expressed per unit dry mass.

Growth analyses

Thin plastic coated wire was used to mark a central position on a stem and the number of leaves above and below this mark were counted. During subsequent (two month) periods the number of leaves above and below this mark were recounted and where necessary the wire was repositioned further up the plant stem. The level of the sand surface in relation to plant growth was recorded for *A. populifolia*. Initially twenty individual stems of each plant species were marked but due to sand-burial and plant death the number of stems monitored varied throughout the sampling period (see Table 4.5 for details). The number of leaves produced per plant was converted to leaf dry mass production using the average leaf dry weights (Table 3.5).

Annual shoot production and above-ground primary productivity

Once the average monthly number of leaves produced per shoot had been calculated it was possible to calculate the average monthly stem production. Sections of mature stems bearing the appropriate number (see footnote [4.1]) of leaf scars were excised from twenty individual plants of each species. Stem sections were oven dried to constant weight and dry weights recorded. Shoot primary production was calculated as the sum of the stem dry mass production and the leaf dry mass production and expressed as g dry mass yr^{-1}. Above-ground primary productivity (g dry mass m^{-2} yr^{-1}) was calculated by multiplying shoot primary production by the number of shoots per unit ground area of the dune (Table 3.6).

[4.1] The appropriate number of leaf scars was determined from the annual leaf production data.

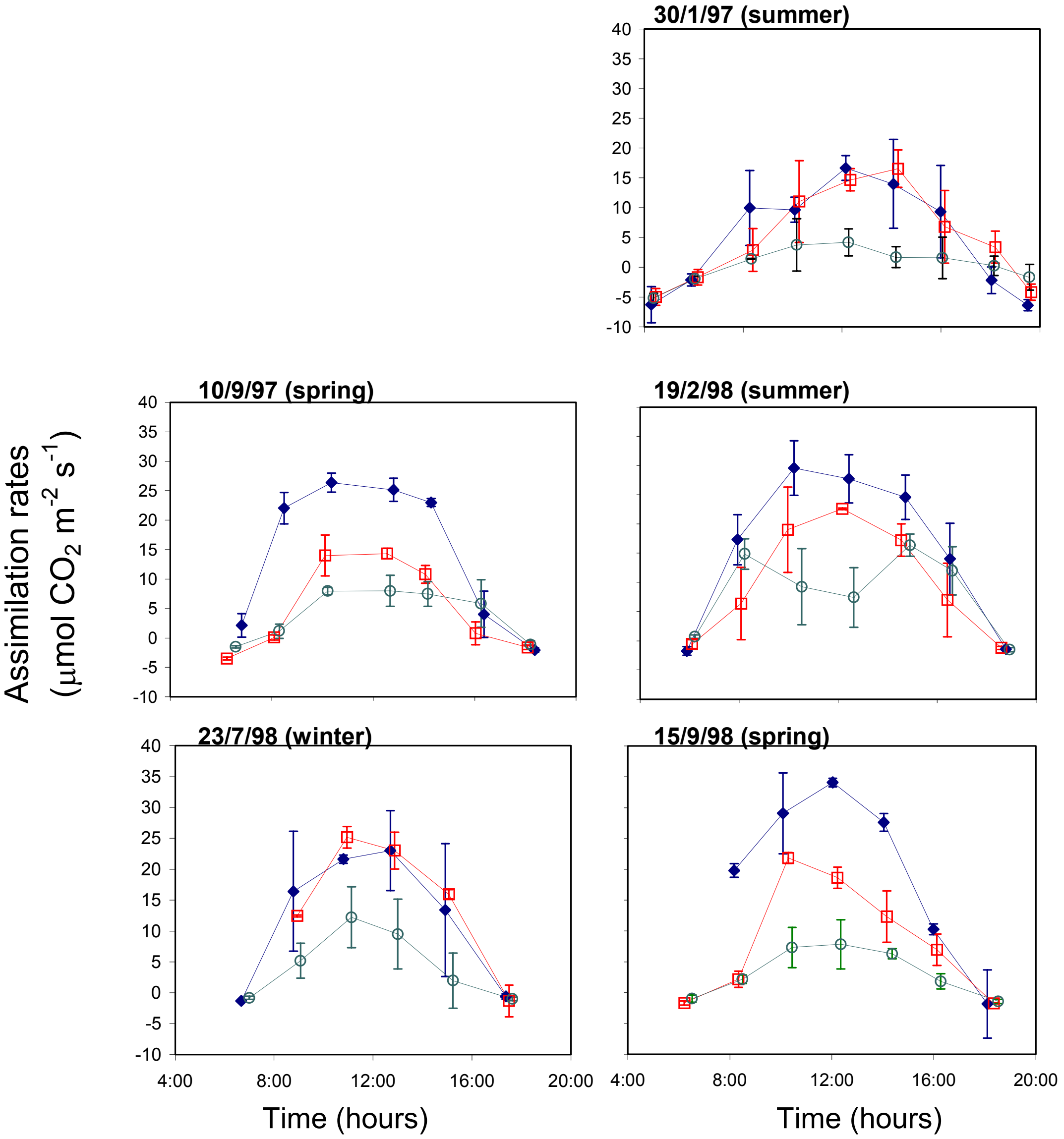


Figure 4.1: Diurnal changes in assimilation rates of *A. populifolia* (diamonds), *I. pes-caprae* (circles) and *S. plumieri* (squares) on the indicated experimental days. Standard deviations are indicated by the vertical bars. N=3.

Above ground biomass

Biomass data had been previously collected for *S. plumieri* (Peter, 2000 and Peter and Ripley, 2001). These data included average numbers of shoots per m^2 dune surface area, average shoot weights and average shoot surface areas (see chapter 3; Table 3.4 & 3.5). *S. plumieri* biomass was calculated by multiplying the average shoot density per m^2 by the average shoot dry mass (dead leaves not included). This type of data was not available for *A. populifolia* and *I. pes-caprae* and hence five randomly selected 1x1m plots in pure stands of each of these species were harvested and the harvests divided into stems and live leaves. These were oven dried to constant weight at 60^oC and combined dry weights expressed per m^2 of dune surface area.

Statistics

Where appropriate, data were compared using an analysis of variance (ANOVA) followed by Tukey tests if ANOVA showed significant differences at the 95% confidence level using the program Statistica '99 Edition, (© Statsoft, Inc., USA). Statistical model assumptions were tested for normality and square root transformations undertaken where necessary. The dependence of photosynthetic rate on various abiotic variables was assessed using a multiple stepwise regression (Statistica '99 Edition, © Statsoft, Inc., USA).

RESULTS

CO_2 assimilation

Diurnal changes in CO_2 assimilation rates measured on five separate days in different seasons throughout the course of the study are shown in Figure 4.1. Average peak assimilation rates (Figure 4.1 & Table 4.1) were significantly different between species when seasonal data were combined ($p<0.0001$). *A. populifolia* achieved the highest photosynthetic rates followed by *S. plumieri* and *I. pes-caprae*. Average peak CO_2 assimilation rates of 34.1 ± 1.4 $\mu mol\ m^{-2}\ s^{-1}$ for *A. populifolia* on the experimental day 15/9/98 (spring) are comparable to assimilation rates encountered in C_4 grasses which are considered to be amongst the most photosynthetically productive higher plants (Beale and Long, 1995).

The highest recorded average peak CO_2 assimilation rates of 25.1 ± 3.0 μmol m^{-2} s^{-1} and 16.3 ± 1.9 μmol m^{-2} s^{-1}, for *S. plumieri* and *I. pes-caprae* respectively are more typical of C_3 herbs.

Table 4.1: Average peak assimilation rates for the three species on days in the indicated seasons. If values obtained from the same species on different days were significantly different this is indicated by the bracketed numbers. The days are numbered as (1) - 30/1/97 (summer), (2) - 10/9/97 (spring), (3) -19/2/98 (summer), (4) -23/7/98 (winter) and (5) - 15/9/98 (spring). N=3. Peak assimilation rates are defined as the highest points on diurnal response curves of CO_2 assimilation rates (Figure 4.1).

	Average peak assimilation rates (μmol CO_2 m^{-2} s^{-1})		
Experimental day	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
30/1/97 (summer) (1)	16.7 ± 2.1 (3 & 5)	4.2 ± 2.3 (3 & 4)	16.6 ± 3.1
10/9/97 (spring) (2)	26.4 ± 1.7	8.0 ± 2.6	14.0 ± 0.9
19/2/98 (summer) (3)	29.6 ± 4.7 (1)	16.3 ± 1.9 (1 & 5)	22.6 ± 0.1
23/7/98 (winter) (4)	23.0 ± 6.5	12.2 ± 4.9 (1)	25.1 ± 3.0
15/9/98 (spring) (5)	34.1 ± 1.4 (1)	7.8 ± 4.0 (3)	21.8 ± 0.9
Average	**25.9**	**9.7**	**20.1**
Std. deviation	**6.9**	**5.2**	**4.5**

The results of a two-way ANOVA showed that the differences in peak assimilation rates among species ($p<0.0001$) and experimental days ($p<0.0001$) were significant. The interaction between species and experimental day was also significant ($p=0.0026$). This indicates that the various species responded differently on the various experimental days. Within a species there are significant differences between measuring days as is indicated in Table 4.1 but these differences do not relate to seasons as may have been expected. Some winter rates exceeded summer or spring rates. There appears to be no consistent pattern between species with the highest average rates being recorded on 15/998 (spring), 19/2/98 (summer) and 24/7/98 (winter) for the three species *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. However the peak rates were not significantly different from the next two highest rates in all three species and care must be taken in relating peak rates to a particular season. This is particularly so as each season is represented by only one day's data. The relationship of CO_2 assimilation to PPFD may explain the lack of a consistent pattern between seasons as each individual leaf has its own specific orientation and aspect on the dune and therefore receives its own particular light dose.

When assimilation rates were considered in chronological sequence they increased from values measured on 30/1/97 (summer) through to 19/2/98 (summer), decreased on 23/7/98 (winter) and subsequently increased on 15/9/98 (spring). This trend was apparent for all three species. There are exceptions to this in that the rates for *S. plumieri* increased from 19/2/98 (summer) to 23/7/98 (winter) and the rates for *I. pes-caprae* decreased from 23/7/98 (winter) to 15/9/98 (spring) but in both cases the differences are not significant. In addition to the effects of the prevailing climatic conditions it is possible that the plants pre-history may determine its subsequent performance.

Assimilation rate was strongly correlated with incident PPFD (Figure 4.2). Assimilation rates increased with increasing PPFD but the rate at which this increase occurred (efficiency of utilisation of incident PPFD) and the PPFD at which assimilation rate saturated were different for the three species. Differences in efficiency of utilisation of incident PPFD and light saturated photosynthetic rate are important in explaining the observed differences in productivity. This is explored at some depth in Chapter 7 where the measurements conducted under field conditions are compared with measurements conducted under far more controlled conditions. Some interesting differences in the photosynthetic characteristics of the three species are revealed.

On the different sampling days measured peak assimilation rates and the incident PPFD while these measurements were made can be superimposed on the data in Figure 4.2. This shows that the prevailing light intensity incident on the experimental day can account for the variability on different experimental days.

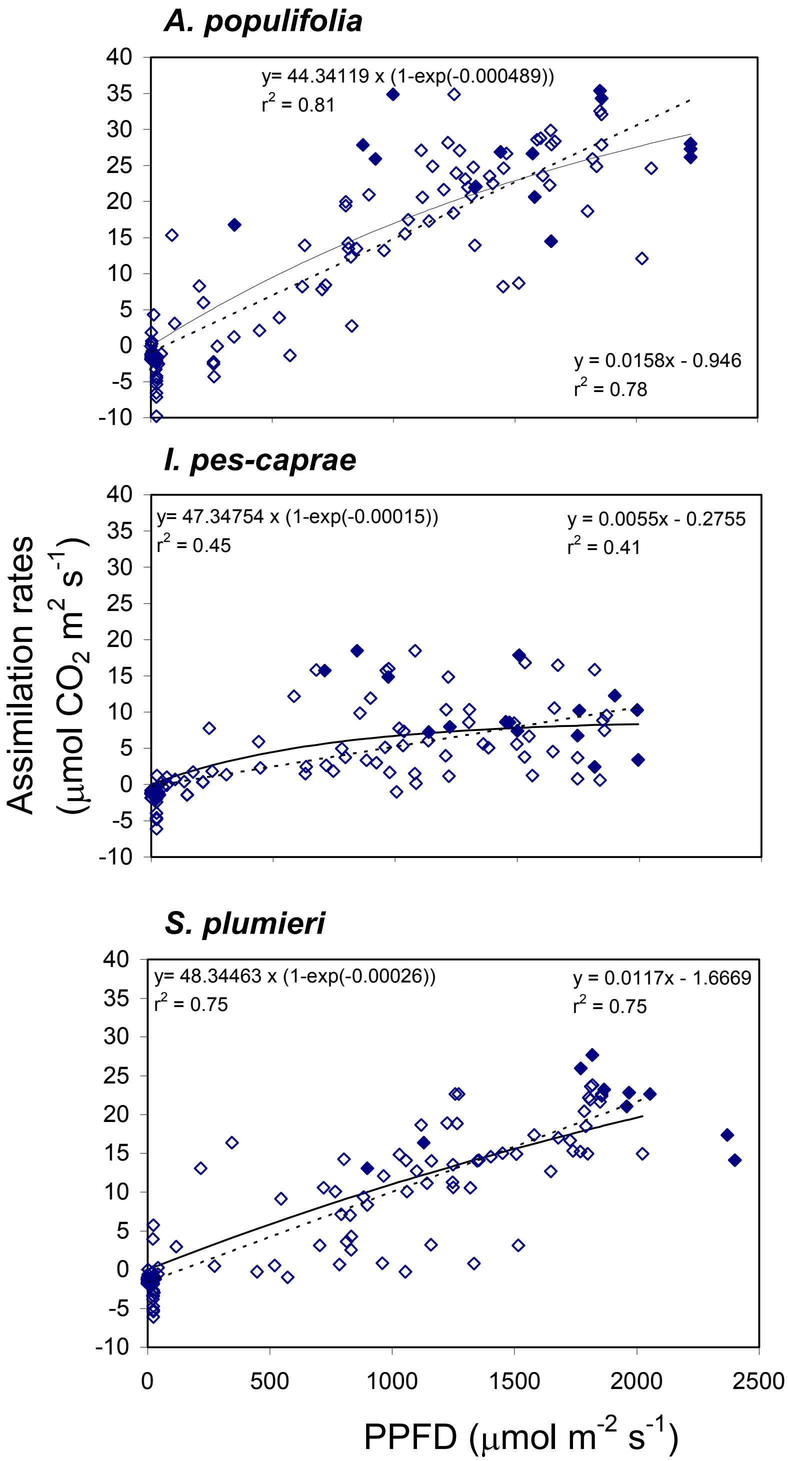


Figure 4.2: CO_2 assimilation rates (open symbols) of *A populifolia, I. pes-caprae* and *S. plumieri* related to photosynthetic photon flux density (PPFD). Solid lines are fitted saturation exponential equations and dotted lines are fitted linear equations. These equations are given on the figure. Included in the figure are the peak assimilation rates (solid symbols). Peak assimilation rates are defined as the highest points on the curves of diurnal CO_2 assimilation rates (Figure 4.1).

Data in Figure 4.2 was fitted with both saturated exponential and linear equations and these as well as the corresponding r^2 values for these fits are given on the figure. When data were fitted with linear equations, points where PPFD < 1 μmol m^{-2} s^{-1} were omitted. As is evident from the figure linear fits had r^2 values equal to or only slightly lower than saturated exponential equations. Because linear regressions provided adequate descriptions of the relationship between assimilation rate and PPFD, a multiple stepwise regression (which assumes a linear relationship between variables) was performed to determine which abiotic factor was the most important in controlling assimilation rate.

Abiotic factors measured included PPFD incident on the leaf, air temperature (T_a), leaf temperature (T_l), relative humidity (RH) and VPD. Table 4.2 lists the results of a stepwise multiple regression analysis of assimilation rates against abiotic variables. This statistical procedure identifies the abiotic variable that gives the highest adjusted r^2 value for a linear regression of assimilation rate against the abiotic variables. It then identifies two abiotic variables that in combination give the highest adjusted r^2 values and this is repeated until all the variables used in the analysis have been accounted for. It is evident from the results that PPFD accounts for the majority of the variability in assimilation rate for all three species where adjusted r^2 was 0.804, 0.447 and 0.828 for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. These r^2 values were increased by 0.9, 14.7 and 2.9 % with the inclusion of a second variable for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. The second variable that resulted in the maximal increase in the adjusted r^2 value was not the same for all three species and was T_l for *A. populifolia* and *I. pes-caprae* and RH for *S. plumieri*. The inclusion of a third variable further increased the adjusted r^2 values by 2.3, 8.9 and 1.5 % for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. The inclusion of the fourth variable had little effect on the adjusted r^2 values.

Table 4.2: Results of a stepwise multiple regression analysis of assimilation rates and the five abiotic variables measured or calculated at the same time that the rates were measured. For each species the variable(s) and the corresponding adjusted r^2 values are listed.

Species	**1 variable**	**2 variables**	**3 variables**	**All variables**
A. populifolia	PPFD (0.804)	PPFD, T_l (0.811)	PPFD, T_l & T_a (0.830)	All (0.831)
I. pes-caprae	PPFD (0.447)	PPFD, T_l (0.513)	PPFD, T_l & T_a (0.559)	All (0.555)
S. plumieri	PPFD (0.828)	PPFD, RH (0.852)	PPFD, RH & T_l (0.865)	All (0.867)

Abbreviations: Photosynthetic photon flux density (PPFD), Leaf temp (T_l), Ambient temperature (T_a), Relative humidity (RH)**.**

Whole shoot respiration

Results of a two-way ANOVA showed that respiration rates measured on the 19/2/98 (summer) and 23/7/98 (winter) were significantly different among species (combined seasons, p=0.0003) and between different experimental days (combined species, p< 0.0000). Rates for *A. populifolia* were higher than those of *I. pes-caprae* or *S. plumieri* and summer rates always exceeded winter rates (Table 4.3). Interspecific differences were not significant in summer but there were significant differences between winter rates of *A. populifolia* and the rates of the other two species. Average ambient temperatures during the two-hour measurement period were 17.9 ± 0.9 ^{o}C on the 19/2/98 (summer) and 9.1 ± 1.0 ^{o}C on the 23/7/98 (winter) and this may well account for some of the differences observed in CO_2 production.

Respiration has a Q_{10} of approximately two (Lambers, 1985) and this would predict that respiration rates would increase to 0.83, 0.26 and 0.39 μmol CO_2 m^{-2} s^{-1} with the observed 8.8 ^{o}C increase in ambient temperature from winter to summer for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. It is clear that this prediction only approximates values measured for *A. populifolia* and underestimates the values that were measured for *I. pes-caprae* and *S. plumieri*. Clearly these two species show seasonal changes in respiration rate that cannot be accounted for merely by the temperature differences.

The seasonal changes may be associated with changes in growth and maintenance. The three species investigated showed interspecific differences in daily amounts of CO_2

assimilated, leaf production and primary production (Figure 4.5 & Table 4.6). *A. populifolia* has the highest CO_2 assimilation and respiration rates (Table 4.1 & 4.3), produces the highest number of leaves on a monthly basis (Figure 4.5) and has the highest above-ground primary productivity (Table 4.6). This is followed by *S. plumieri* and then *I. pes-caprae* (Tables 4.1, 4.3, 4.5 and 4.6).

Table 4.3: Average whole shoot nocturnal respiration rates expressed per unit leaf area (see footnote [4.2]) measured between 21:00 and 23:00 hrs on the 30/1/98 (summer) and 23/7/98 (winter). If values obtained from different species and from the same species on different days were significantly different this is indicated by the bracketed numbers. Species measured on 23/7/98 are numbered as (1) – *A. populifolia*, (2) – *I. pes-caprae*, (3) – *S. plumieri* and species measured on 30/1/98 are numbered as (4) – *A. populifolia*, (5) – *I. pes-caprae*, (6) – *S. plumieri.* N=3.

	Average nocturnal whole shoot respiration rates (μmol CO_2 m^{-2} s^{-1})	
Species	**23/7/98 (winter)**	**30/1/98 (summer)**
A. populifolia	0.47 ± 0.15 (2, 3, 4 & 5)	0.92 ± 0.02 (1, 2 & 3)
I. pes-caprae	0.15 ± 0.03 (1, 4, 5 & 6)	0.78 ± 0.03 (1, 2 & 3)
S. plumieri	0.22 ± 0.01 (1, 4, 5 & 6)	0.68 ± 0.16 (2 & 3)

Integrated daily CO_2 assimilation

Peak assimilation rates are not good indicators of carbon assimilation for whole days as is evident when peak assimilation rates are compared with amounts of carbon assimilated over the whole day by individual leaves (Table 4.1 & 4.4). The latter was calculated from the integrated areas below the diurnal variations in assimilation rate (Figure 4.1). This integrated measure includes both the magnitude of the assimilation rates and the length of time over which the plant assimilates carbon. Positive CO_2 assimilation rates are extended over a longer period in summer than in either spring or winter, as is evident from diurnal variations in incident PPFD (Figure 3.7). A two-way ANOVA indicated that the differences in integrated carbon assimilation among species (p<0.0000), experimental days (p<0.0000) and for their interaction (p=0.0090) were significant. When species were combined experimental days 30/1/97 (summer), 23/7/98 (winter) and 15/9/98 (spring)

[4.2] A linear regression of whole shoot CO_2 exchange and shoot leaf surface area yielded a r^2 value of 0.88 while a regression of CO_2 exchange and shoot dry mass yielded a r^2 value of 0.16. This was possibly due to differences in the ratio of stem dry weight to leaf surface area between plants, with stems having lower respiration rates than leaves. Thus respiration rates were expressed per unit shoot leaf area.

were similar whilst 8/8/97 (spring) and 19/2/98 (summer) were distinct. Average integrated CO_2 assimilation rates for *A. populifolia*, *S. plumieri* and *I. pes-caprae* were 692.6 ± 211.6, 246.2 ± 147.4 and 453.7 ± 120.6 mmol m^{-2} day^{-1} respectively. Species differences between experimental days, like peak assimilation rates, do not follow patterns that might have been predicted by season with some winter amounts exceeding spring or summer amounts.

Table 4.4: Average daily amounts of CO_2 assimilated by the three species on days in the indicated seasons. Daily amounts of CO_2 assimilated were calculated by integrating the areas below diurnal assimilation rate curves (Figure 4.1). If values obtained from the same species on different days were significantly different this is indicated by the bracketed numbers. The days are numbered as (1) - 30/1/97 (summer), (2) - 10/9/97 (spring), (3) -19/2/98 (summer), (4) -23/7/98 (winter) and (5) - 15/9/98 (spring).

	Average Daily amounts of CO_2 assimilated (mmol m^{-2} day^{-1})		
Experimental day	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
30/1/97 (summer) (1)	566.5 ± 199.1	246.5 ± 77.1	568.0 ± 36.1
10/9/97 (spring) (2)	434.1 ± 150.6 (3 & 5)	95.8 ± 94.0 (3 & 5)	376.9 ± 117.6
19/2/98 (summer) (3)	859.3 ± 137.6 (2)	478.9 ± 101.3 (2)	566.3 ± 38.0
23/7/98 (winter) (4)	705.4 ± 19.0	223.5 ± 59.3	312.1 ± 65.7
15/9/98 (spring) (5)	861.6 ± 55.5 (2)	185.1 ± 50.0 (2)	445.4 ± 57.2
Average	**692.6**	**246.2**	**453.7**
Std. Deviation	**211.6**	**147.4**	**120.6**

Leaf and stem soluble sugar and starch

The basis of these experiments arose from the finding that the total amount of CO_2 assimilation on some of the experimental days was only marginally positive as in *I. pes-caprae* on the 10/9/97 (spring, Table 4.4). This value represents an average photosynthetic rate of 1.1 μmol CO_2 m^{-2} s^{-1} for those leaves. When compared to average nocturnal respiration rates on 30/1/98 of 0.78 μmol CO_2 m^{-2} s^{-1} (Table 4.3) indicates the potential for negligible net carbon assimilation. Caution must be taken in making the above comparison as photosynthetic rates were measured for selected leaves whilst respiration rates were measured for entire shoots. Total amounts of carbon assimilated by entire shoots may well be more or less than estimates from single leaves because of both leaf age effects and differences in leaf orientation (light interception). Entire shoot respiration rate includes not only the respiration of leaves but also reproductive structures and stems. Nevertheless such comparisons suggest that leaf and stem storage and reallocation of non-structural carbohydrates may be of importance. For this reason leaf soluble sugar and starch concentrations were analysed on all subsequent experimental days. In addition to the analysis of naturally illuminated plants some plants were pre-treated by shading in an attempt to assess the potential for carbohydrate relocation from stem to leaf.

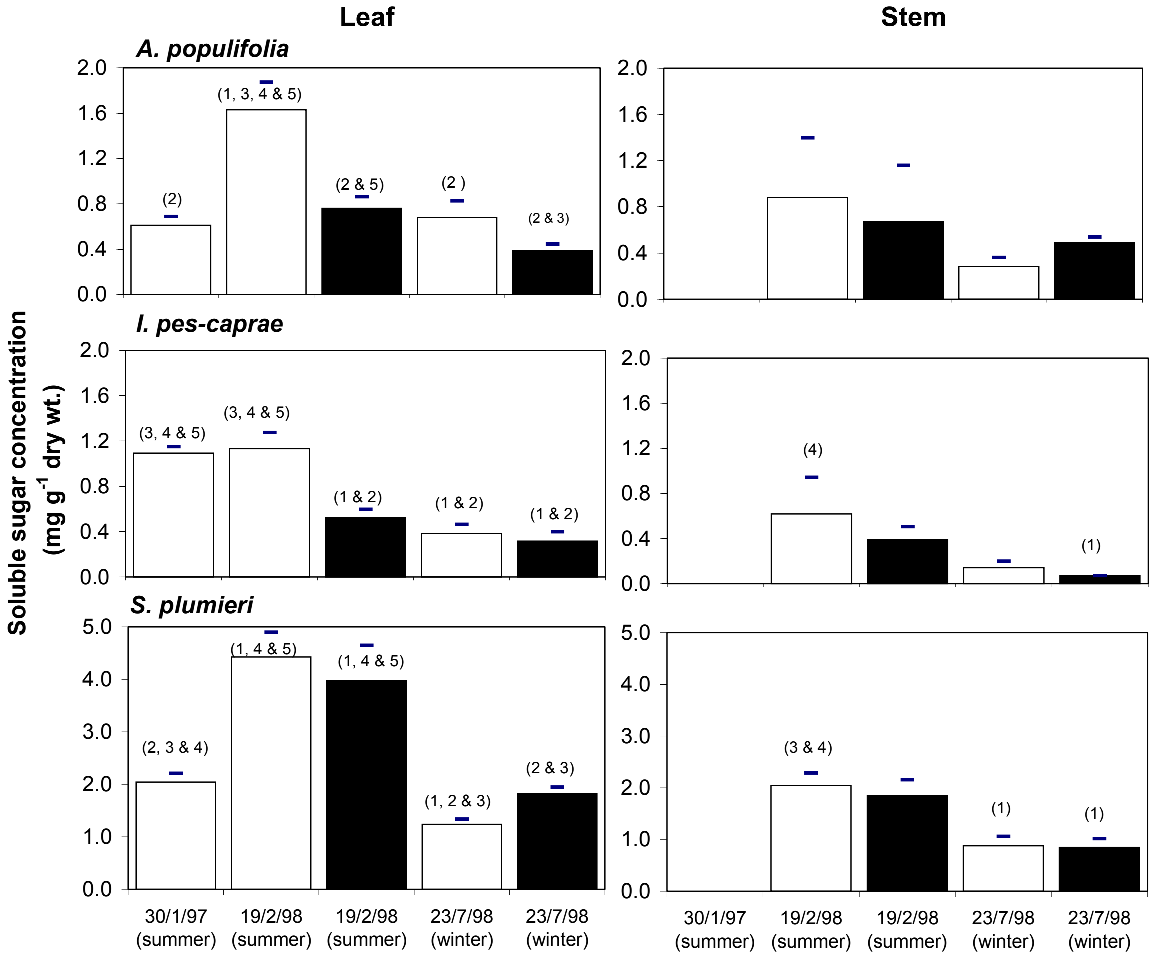


Figure 4.3: Average soluble sugar concentrations of leaves and stems harvested at midday on the indicated dates. In summer and winter 1998 in addition to samples harvested from plants incubated in natural light (open blocks) samples were collected from leaves that had been covered with 50% shade cloth for three days prior to sampling (patterned blocks). Standard deviations are shown by the horizontal bars. If values obtained from the same species on different days and on the same day from different shading treatments were significantly different this is indicated by the bracketed numbers. The days and shading treatments are numbered as (1) - 30/1/97 (summer), (2) - 19/2/98 (summer), (3) -19/2/98 (summer, shaded), (4) -23/7/98 (winter) and (5) - 23/7/98 (winter, shaded). Note different scale for *S. plumieri*. N=3.

Soluble sugars

The results of a two-way ANOVA indicated that the differences in soluble sugar concentrations of shaded and unshaded leaves among species ($p<0.0000$), experimental days ($p<0.0000$) and their interaction ($p<0.0000$) were significant. Unshaded leaves from *S. plumieri* contained on average greater concentrations of soluble sugars (2.57 ± 1.46 mg^{-1} g^{-1}dry wt.) than leaves from either *A. populifolia* (0.97 ± 0.52 mg^{-1} g^{-1}dry wt.) or *I. pes-caprae* (0.87 ± 0.38 mg^{-1} g^{-1}dry wt.). All three species showed seasonal variation in soluble sugar content (Figure 4.3) with summer leaves generally containing more sugar than winter leaves. Unshaded *A. populifolia* had leaf soluble sugar concentrations that were significantly higher on 19/2/98 (summer) than on 30/1/97 (summer) or 23/7/98 (winter). *S. plumieri* (30/1/98 and 19/2/98, summer) unshaded leaf sugar concentrations were significantly higher than the 23/7/98 (winter) values. *I. pes-caprae* unshaded leaves had significantly different sugar concentrations on three of the experimental days (30/1/97, 19/2/98 & 23/7/98) and summer values were higher than the winter values.

Shading significantly reduced the 19/2/98 (summer) leaf soluble sugar concentrations in *A. populifolia* and *I. pes-caprae* but not in those of *S. plumieri* and there were no significant differences in the 23/7/98 (winter) shaded or unshaded leaves.

The results of a two-way ANOVA of shaded and unshaded stem soluble sugars indicated that the differences among species ($p<0.0000$) and experimental days ($p<0.0000$) were significant. The interaction of species and experimental day was not significant ($p=0.4310$). *A. populifolia*, *I. pes-caprae* and *S. plumieri* unshaded stems contained on average 0.53 ± 0.46, 0.37 ± 0.23 and 1.55 ± 0.66 mg g^{-1} dry wt. soluble sugar respectively. Stems harvested from both unshaded and shaded plants of all three species in summer (19/2/98) contained higher sugar concentrations than those harvested in winter (23/7/98) but these concentrations were significant only for unshaded stems from *S. plumieri* (Figure 4.3). When data from unshaded and shaded plants was combined stem soluble sugar concentrations were on average significantly lower than leaf sugar concentrations for *A. populifolia* ($p=0.0280$), *I. pes-caprae* ($p=0.0001$) and *S. plumieri* ($p= 0.0012$).

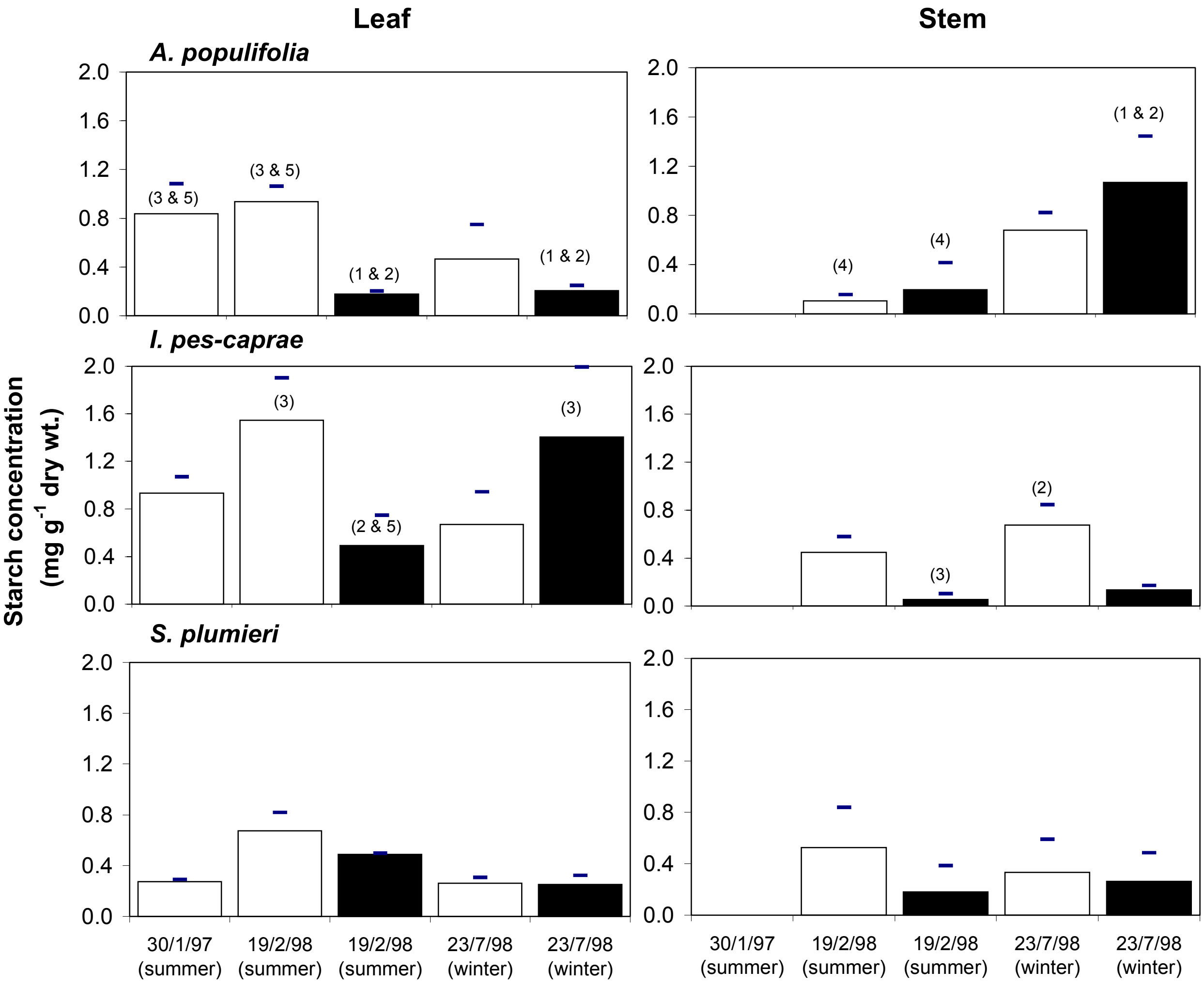


Figure 4.4: Average starch concentrations of leaves and stems harvested at midday on the dates indicated. In summer and winter 1998 in addition to samples harvested from plants incubated in natural light (open blocks) samples were collected from leaves that had been covered with 50% shade cloth for three days prior to sampling (patterned blocks). Standard deviations are shown by the horizontal bars. If values obtained from the same species on different days and on the same day from different shading treatments were significantly different this is indicated by the bracketed numbers. The days and shading treatments are numbered as (1) - 30/1/97 (summer), (2) - 19/2/98 (summer), (3) -19/2/98 (summer, shaded), (4) -23/7/98 (winter) and (5) - 23/7/98 (winter, shaded). Note different scale for *S. plumieri*. N=3.

Although the results were not statistically significant shading in summer for several days prior to harvesting resulted in the reduction of stem soluble sugar concentrations whereas in winter it did not. It is possible that the growth demands which were higher in summer than in winter (as was evident from measured assimilation rates, leaf production and respiration rates) reduced soluble sugar concentrations in leaves if photosynthetic rates were reduced by shading. The reduced leaf sugar concentrations were not compensated for by a reduction in stem sugar concentrations as there were no significant changes in these concentrations on shading. However this needs to be assessed in conjunction with leaf and stem starch concentrations which may be reallocated to maintain leaf carbohydrate concentrations.

Starch

The results of a two-way ANOVA indicated that the differences in shaded and unshaded leaf starch concentrations among species ($p<0.0000$), experimental days ($p<0.0000$) and between their interaction ($p=0.0004$) were significant. *A. populifolia*, *I. pes-caprae* and *S. plumieri* contained average unshaded leaf starch concentrations of 0.72 ± 0.30, 0.99 ± 0.42 and 0.42 ± 0.23 mg g^{-1} dry wt. respectively. Leaf starch concentrations were on average higher in summer (30/1/97 & 19/2/98) than in winter (23/7/98) however these differences were not significant (Figure 4.4). Shading significantly reduced the starch concentrations of *A. populifolia* and *I. pes-caprae* leaves on 19/2/98 (summer) but not on 23/7/98 (winter). Shading did not decrease *S. plumieri* leaf starch concentrations on either date.

A two-way ANOVA indicated that the differences in shaded and unshaded stem soluble starch concentrations among species ($p=0.2500$) were not significantly different. However differences between experimental days ($p=0.0015$) and for the interaction of experimental days and species ($p=0.0023$) were significant. Average unshaded stem starch concentrations were 0.34 ± 0.33, 0.49 ± 0.18 and 0.39 ± 0.28 mg g^{-1} dry wt. for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. *S. plumieri* showed no significant differences in stem starch concentrations measured on different experimental days nor between shaded and unshaded observations. Both *A. populifolia* and *I. pes-caprae* showed increases in stem starch concentration from the 19/2/98 (summer) to the 23/7/98 (winter) but these differences were only significant for shaded stem samples (Figure 4.4). When data from shaded and unshaded plants were combined differences in leaf and stem soluble starch concentrations were not significant for *A. populifolia* (p=0.2810) but were significant and higher in leaves than in stems of *I. pes-caprae* (p=0.0006) and *S. plumieri* (p=0.0360).

As the differences in unshaded leaf and stem soluble starch concentration were not significantly different on summer and winter experimental days and because the reduction in summer leaf starch concentrations due to shading were not accompanied by significant reductions in stem starch concentrations the reallocation of starch appears unimportant. Changes in leaf and stem starch concentration are more likely to be the result of changes in carbohydrate production by photosynthesis than by seasonal changes in the use of carbohydrates for storage or growth. However as stated above, there can be inter-conversion between soluble starch and sugar and the interaction between starch and sugar concentrations needs to be considered.

Total non-structural carbohydrates

In an attempt to investigate the possibility of the reallocation of carbohydrates between leaves and stems total non-structural carbohydrates were calculated as the sum of soluble sugars and starch (data not shown). Although a two-way ANOVA of shaded and unshaded leaf total soluble carbohydrate concentrations showed that differences among species, experimental days and between their interaction were significant (p<0.0001 for all), only the differences in values for *S. plumieri* on the experimental days of 19/2/98 (summer) and 23/7/98 (winter) were significant for the unshaded plants. *S. plumieri* differences in total soluble carbohydrates on these dates could be accounted for by the changes in soluble sugar concentration because only unshaded leaf soluble sugar and not starch concentrations were significantly different on these dates (Figures 4.3 & 4.4).

Similar to the findings for leaves, a two-way ANOVA indicated that the differences in shaded and unshaded stem total non-structural carbohydrate concentrations (sugars + starch) among species (p<0.0000), experimental days (p=0.0360) and between their interaction (p=0.0040) were significant. However there were no significant differences in the total amount of soluble carbohydrate on different experimental days for any one of the species.

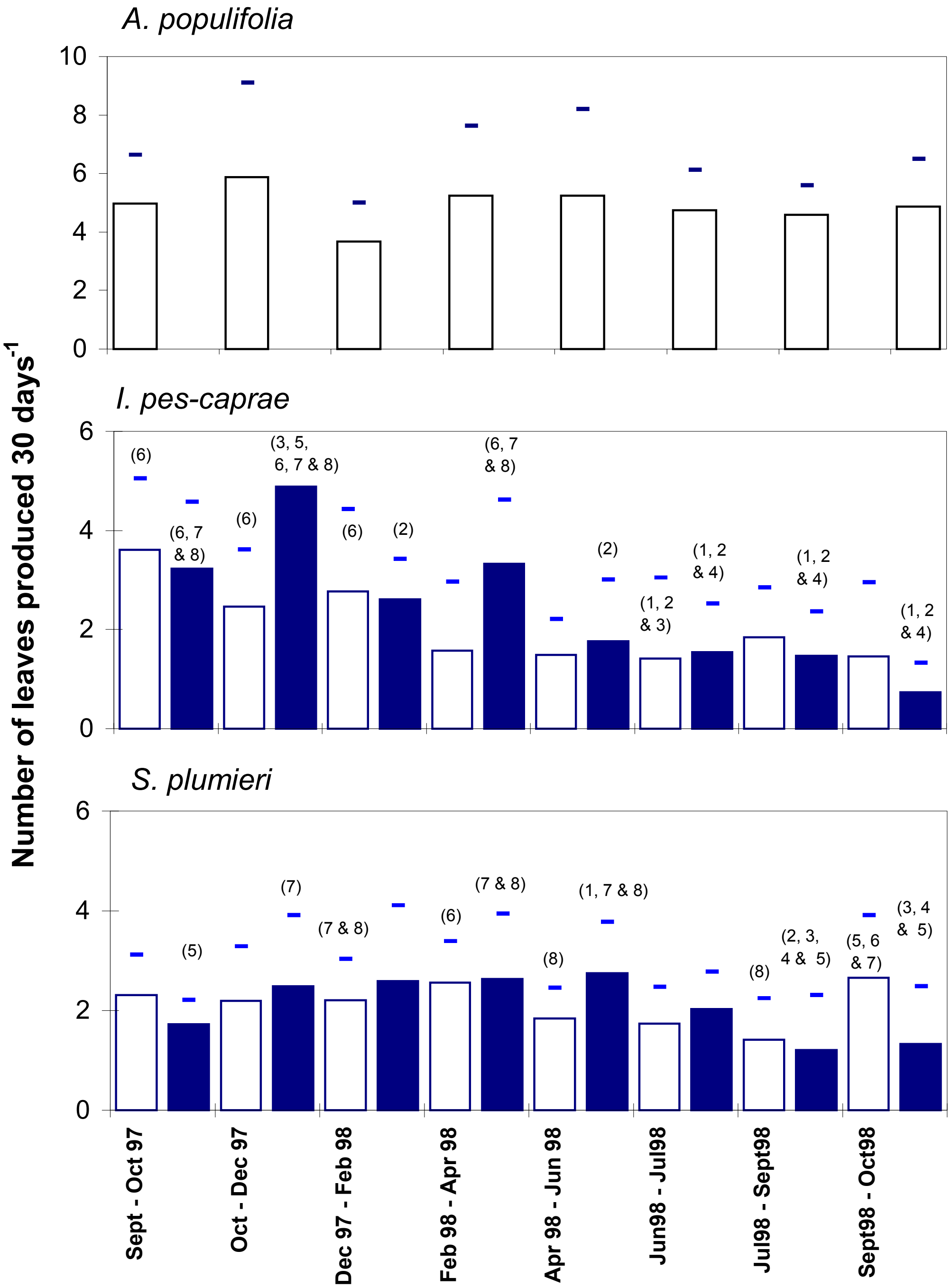


Figure 4.5: Monthly leaf production (open blocks) and loss (solid blocks) per shoot for the three indicated species. Standard deviations are indicated by the horizontal bars. If values obtained from the same species for different sampling periods were significantly different this is indicated by the bracketed numbers. The sampling periods are numbered as (1) - September to October 97, (2) - October to December 97, (3) - December 97 to February 98, (4) - February to April 98, (5) - April to June 98, (6) - June to July 98, (7) - July to September 98 and (8) - September to October 98. N for all species was variable (see Table 4.5 for details).

This observation together with the fact that some of the differences in stem starch and sugar concentrations on different experimental days were significant suggests a reciprocal response between stem soluble sugar and starch concentrations, albeit not a statistically significant one. When soluble sugar concentrations are higher in summer then starch concentrations are lower and *vice versa.* As sugar (Figure 4.3) and starch (Figure 4.4) concentrations in the succulent stems of *A. populifolia* and *S. plumieri* and the non-succulent stems of *I. pes-caprae* were either lower or not different from leaf concentrations the role of stems as carbohydrate stores is unlikely.

In conclusion none of the data shows the maintenance of leaf carbohydrate concentrations at the expense of stem reserves. This does not exclude the possibility of simultaneous reductions in leaf carbohydrate content on some experimental days and reallocation of reserves from the stems.

Leaf production and dry weight accumulation

Analysis of *A. populifolia* monthly leaf production proved to be difficult for a number of reasons: leaves are produced from more than one apical bud, individual shoots branch repeatedly on burial, some branches die back and individual plants are sporadically removed or buried by crisis events. As each shoot had its own adventitious roots monthly leaf production per shoot was calculated. Figure 4.5 shows the average leaf production per shoot per 30 calendar days for a variable number of plants (see Table 4.5 for details). Leaf production was not significantly different between months (p=0.4400). Due to the above mentioned difficulties leaf loss was not monitored for this species.

As *S. plumieri* and *I. pes-caprae* leaves are produced from single apical buds the analysis of results is straightforward. Both *I. pes*-caprae and *S. plumieri* produced more leaves in the summer months (October to April) than in winter months (June to August) although this trend was not significantly different between all summer and all winter sampling periods (Figure 4.5). *I. pes-caprae* leaf loss was higher in summer than in winter with losses in September to April being significantly higher than winter losses with the exception of the December 1997 to February 1998 sampling period where high variability obscured this effect. Similar trends were noted for *S. plumieri* with summer losses exceeding winter losses but higher variability reduced the significance of this effect.

Table 4.5: Average monthly leaf and stem production or loss per shoot monitored from September 97 to October 98 for *I. pes-caprae* and *S. plumieri* (See footnote [4.3]). When considering each of the above parameters, if ANOVA results indicated significant differences between species this is indicated by the bracketed numbers. Species were numbered as (1) - *A. populifolia*, (2) - *I. pes-caprae* and (3) - *S. plumieri*.

Species	**Monthly average**				
	Numbers of leaves:		**Dry mass (g) of**		
	Produced	**Lost**	**Leaves :**		**Stems:**
			Produced	**Lost**	**Produced**
A. populifolia	4.9 ± 2.2 (2 & 3)	—	1.08 ± 0.48 (3)	—	2.43 ± 1.47 (2 & 3)
I. pes-caprae	2.7 ± 2.3 (1)	3.3 ± 1.6 (3)	1.09 ± 0.76 (3)	1.39 ± 0.67 (3)	0.33 ± 0.10 (1 & 3)
S. plumieri	2.1 ± 0.9 (1)	2.1 ± 1.3 (2)	1.34 ± 0.61 (1 & 2)	1.27 ± 0.79 (2)	0.76 ± 0.29 (1 & 2)

Average monthly leaf production and losses are given in Table 4.5. *A. populifolia* had significantly higher average monthly leaf production than the other two species (p=0.000) possibly related to the high CO_2 assimilation rates recorded for this species. When leaf production was expressed as dry weight accumulation it was apparent that although *A. populifolia* produced the greatest number of leaves this did not translate into the greatest leaf dry weight accumulation due to the high water content of the succulent leaves of *A. populifolia*. When compared on a dry weight basis *S. plumieri* had significantly higher leaf dry weight production than either *A. populifolia* (p=0.028) or *I. pes-caprae* (p=0.0004).

Average monthly leaf loss was monitored only for *I. pes-caprae* and *S. plumieri* and there were significant differences between the two species when compared across all months (Figure 4.5). The relationship between the rates of leaf production and leaf loss determines leaf longevity. Leaf longevity was calculated from the average number of

---

[4.3] Initially 20 plants of each species were tagged but due to plant death and burial the number of plants sampled at each interval varied and is listed below:
A. populifolia: Sept – Oct 97 (N=15), Oct – Dec 97 (N=15), Dec 97 – Feb 98 (N=11), Feb – Apr 98 (N=7), Apr – Jun (N=7), Jun – Jul 98 (N=10), Jul --Sept (N=7), Sept – Oct (N=4).
I. pes-caprae: Sept – Oct 97 (N=17), Oct – Dec 97 (N=19), Dec 97 – Feb 98 (N=18), Feb – Apr 98 (N=16), Apr – Jun (N=12), Jun – Jul 98 (N=17), Jul – Sept (N=13), Sept – Oct (N=7).
S. plumieri : Sept – Oct 97 (N=17), Oct – Dec 97 (N=17), Dec 97 – Feb 98 (N=19), Feb – Apr 98 (N=16), Apr – Jun (N=18), Jun – Jul 98 (N=22), Jul --Sept (N=19), Sept – Oct (N=20).

leaves per shoot and average leaf production. This indicated that *S. plumieri* leaves were the longest lived (*ca.* 217 days) followed by *I. pes-caprae* (*ca.*176 days) and then *A. populifolia* (*ca.* 69 days).

The average monthly stem production was significantly different between species ($p<0.0000$) and *A. populifolia* invested 125% more dry mass in stems than in leaves (Table 4.5). *I. pes-caprae* and *S. plumieri* invested 70% and 43% more dry mass in leaves than in stems respectively.

Table 4.6: Above-ground biomass, average shoot primary production and above-ground primary productivity for *A. populifolia, I. pes-caprae* and *S. plumieri*. When considering each of the above parameters, if ANOVA results indicated significant differences between species this is indicated by the bracketed numbers. Species were numbered as (1) - *A. populifolia*, (2) - *I. pes-caprae* and (3) - *S. plumieri*.

Species	**Above-ground biomass (g dry mass m^{-2})**	**Shoot primary production (g dry mass shoot^{-1} yr^{-1})**	**Above-ground primary productivity (g dry mass m^{-2} yr^{-1})**
A. populifolia	63.1 ± 17.7 (3)	42.4 ± 18.7 (2 & 3)	479.5 ± 211.6 (2)
I. pes-caprae	134.6 ± 40.9	17.8 ± 15.0 (1 & 3)	386.5 ± 326.2 (1 & 3)
S. plumieri	271.4 ± 133.3 (1)	25.3 ± 10.3 (1 & 2)	541.4 ± 220.6 (2)

When stem and leaf dry weight production was summed and annual above-ground shoot primary production calculated differences between species were significant ($p<0.0000$) (Table 4.6). *A. populifolia* had the highest shoot primary production followed by *S. plumieri* and then *I. pes-caprae.* This is the same pattern as was noted for assimilation rates. Interestingly this pattern only emerges when stem dry mass production is included. This is due to the large investment of dry mass that *A. populifolia* puts into stems. When above-ground primary productivity was expressed per m^2 ground area the difference between *A. populifolia* and *S. plumieri* was not significant but both were significantly higher than values for *I. pes-caprae*.

Biomass estimates (Table 4.6) were significantly different between species ($p=0.015$). *S. plumieri* produced the highest values (271.4 ± 133.3 g m^{-2}) followed by *I. pes-caprae* (134.6 ± 40.9 g m^{-2}) and then *A. populifolia* (63.1 ± 17.7 g m^{-2}) although only the first and last mentioned species were significantly different.

Light stress

The beach environment has high incident and reflected light, low morning temperatures on occasion (see Chapter 2) and a potentially limited water supply, all of which have been found to be associated with photoinhibition in natural systems. Photoinhibition has been associated with a decrease in photochemical efficiency and the rate constant of photochemistry, increases in the rate constants of non-photochemical energy dissipation, and the de-epoxidation of the xanthophylls (Demmig-Adams and Adams III, 1996). Because of this these parameters were measured for *A. populifolia, I. pes-caprae* and *S. plumieri* leaves. Results were assessed for diurnal changes and for changes following exposure to natural illumination.

Diurnal changes in chlorophyll fluorescence

Leaves from all three species that were given a two-hour dark adaptation prior to chlorophyll fluorescence measurements showed no significant change in φ_{P0} values throughout the day. Average values measured on 30/1/97 (summer) for *A. populifolia*, *I. pes-caprae* and *S. plumieri* were 0.76 ± 0.05, 0.65 ± 0.12 and 0.76 ± 0.06 respectively. Presumably the two-hour dark adaptation was long enough to allow for the complete reversal of all the changes that allow the photosynthetic apparatus to adjust to a particular light environment.

More revealing measures are the chlorophyll fluorescence parameters of leaves incubated under natural conditions and given a 30-second dark adaptation prior to measurement. The dark interval is required for the re-opening of the reaction centres so that the subsequent OJIP transient will reflect their light induced closure (Kruger *et al.*, 1997). This 30-second dark adaptation does however drive the sample towards the dark-adapted state but Kruger *et al.* (1997) found that an adaptation of this duration was an optimal compromise re-opening all the reaction centres without causing a significant disturbance of the light-adapted state.

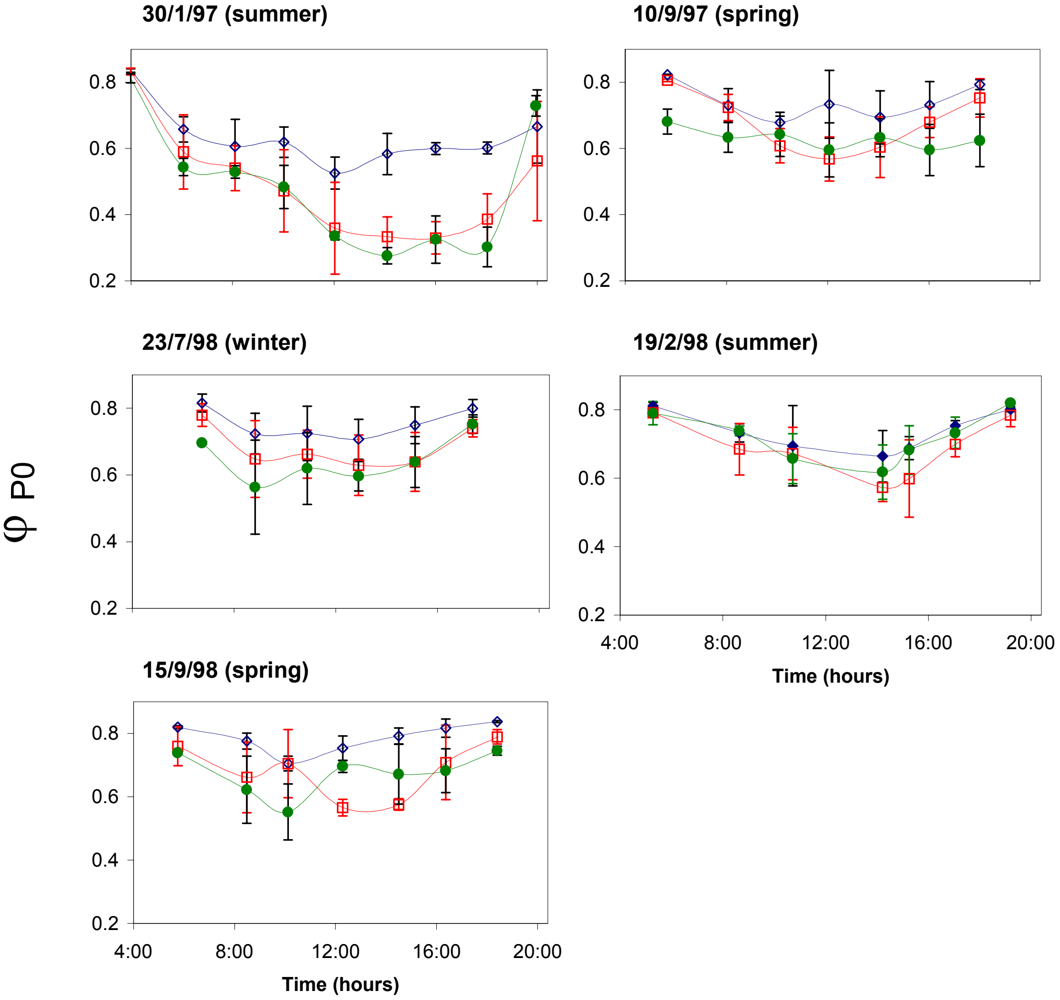


Figure 4.6: Diurnal changes in maximum quantum yield of primary photochemistry (φ_{P0}) of the three species *A. populifolia* (diamonds), *I. pes-caprae* (solid circles) and *S. plumieri* (squares) for indicated experimental days. Standard deviations are indicated by the vertical bars. N=3.

φ_{P0} in general was high in the morning decreased until midday and subsequently recovered in the afternoon (Figure 4.6) although there was considerable variation on different experimental days both in the extent of the reduction and in the subsequent recovery. The most obvious changes occurred in all three species on the experimental day 30/1/97 (summer) when φ_{P0} values were markedly reduced throughout the day but recovered in the afternoon and evening with the reduction in light intensity. Reductions in fluorescence yields have long been associated with both reversible and irreversible photoinhibition (Critchley and Smillie, 1981). Reductions in φ_{P0} between pre-dawn and midday were accompanied by changes in the rate constants for non-photochemical (k_N) and photochemical (k_P) energy dissipation (Figure 4.7). A two-way ANOVA showed that the differences in the percentage change in k_N between morning and noon among species ($p=0.0013$), experimental days ($p=0.0020$) and for their interaction ($p=0.0060$) were significant. The percentage changes in k_N between different experimental days were significant only in the case of *I. pes-caprae* and not for either *A. populifolia* or *S. plumieri* (Figure 4.7).

A similar two-way ANOVA of the percentage change in k_P showed that the differences measured on different experimental days ($p<0.0000$) and not among species ($p=0.3900$) nor between their interaction ($p=0.4400$) were significant. For all three species the percentage change in k_P value for experimental day 30/1/97 (summer) was significantly different to at least one of the values for the other experimental days and in the case of *S. plumieri* was significantly different to all the other values (Figure 4.7). k_N values for all species increased by between 16 and 216 % depending on species and experimental day and this was accompanied by either increases in k_P (7 to 26%) or by decreases in k_P (11 to 78%) (Figure 4.7).

The magnitude of the changes in k_N and k_P were related to the magnitude of the reduction in φ_{P0} for each individual replicate but when averages were calculated these trends became less obvious due to the between replicate variability. This variability may result from the different light environment that each individual leaf was exposed to in its natural orientation. In order to account for this variability when comparing chlorophyll fluorescence parameters measured on leaves with different orientations and on different experimental days fluorescence parameters were expressed in terms of light intensity measured at the leaf surface just prior to the chlorophyll fluorescence measurement. The slopes of observed trends were compared and differences between species and within species on different experimental days noted.

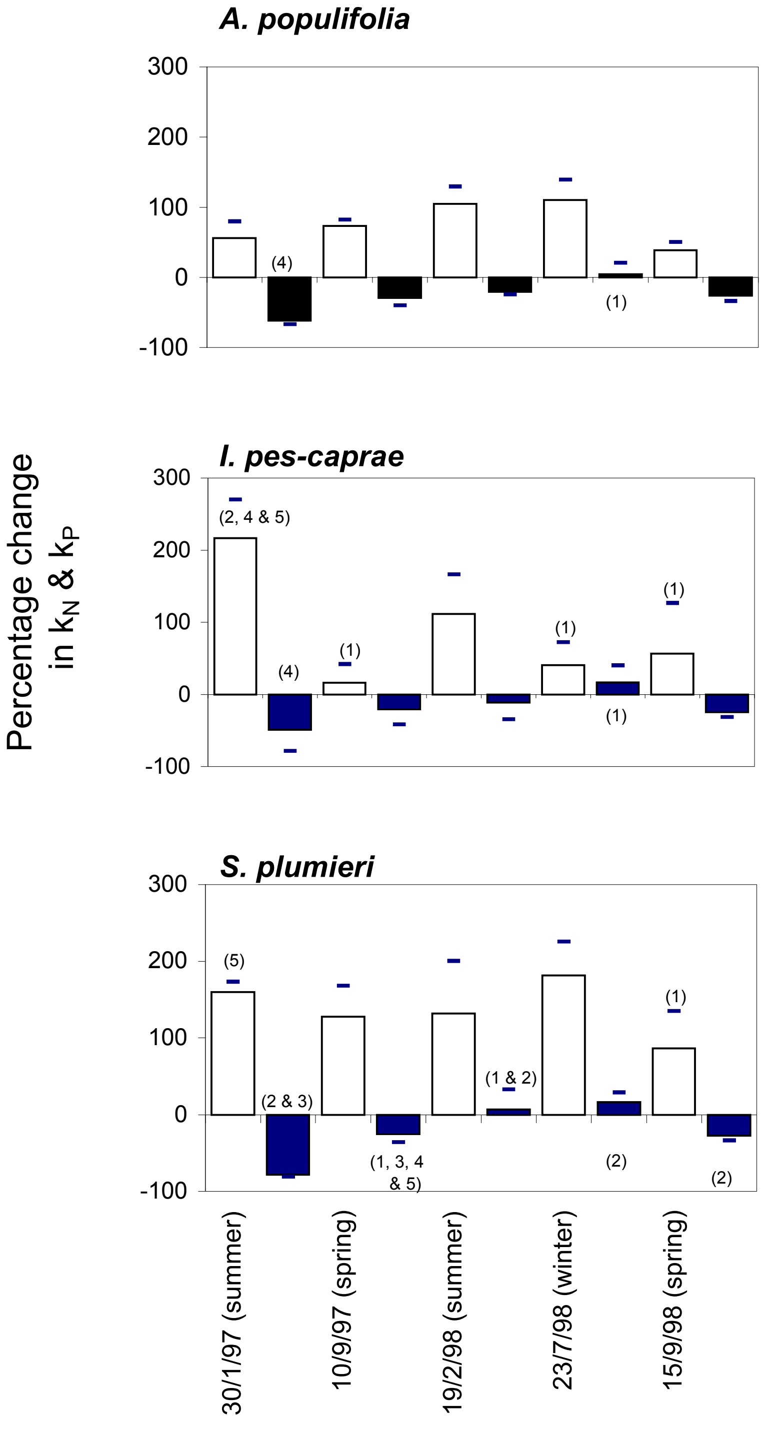


Figure 4.7: Percentage changes in photochemical rate constant (k_N; open blocks) and non-photochemical rate constant (k_P; solid blocks) from pre-dawn to midday for *A. populifolia*, *I. pes-caprae* and *S. plumieri* on the indicated experimental days. Standard deviations are indicated by the horizontal bars. If K_N or K_P values obtained from the same species for different experimental days were significantly different this is indicated by the bracketed numbers. The experimental days are numbered (1) - 30/1/97 (summer), (2) -10/9/97 (spring), (3) - 19/2/98 (summer), (4) - 23/7/98 (winter) and (5) - 15/9/98 (spring). N=9.

Plots of φ_{P0} and incident PPFD yielded straight-line relationships (data not shown). These relationships for each of the species consisted of fifteen curves (3 replicates on five experimental days) and yielded average r^2 values of 0.62 ± 0.2, 0.62 ± 0.3 and 0.52 ± 0.17 for *S. plumieri*, *A. populifolia* and *I. pes-caprae* respectively.

Results of a two-way ANOVA indicated that the differences in the slopes of these regressions among species (p=0.0002), experimental days (p=0.0110) and between their interaction (p=0.0060) were significant. However slopes for both *A. populifolia* and *S. plumieri* were not significantly different for the various experimental days. It was apparent that φ_{P0} responded in the same way to increasing light intensity on all experimental days for both these species. The slopes for *I. pes-caprae* on experimental days 30/1/97 (summer) and 10/9/97 (spring) were significantly different (p=0.0022) from one another. The slopes for *I. pes-caprae* on other experimental days were not significantly different. The photosynthetic apparatus of *I. pes-caprae* apparently responds differently to increasing PPFD in some seasons and this may relate to seasonal leaf vigour. Some older leaves of this species appeared chlorotic at the end and beginning of the growing seasons. Leaves with this appearance were avoided for physiological measurements and chlorophyll a:b ratios were not different between the experimental days 30/1/97 (summer) and 10/9/97 (spring) (Figure 4.9).

Recovery of chlorophyll fluorescence following a light treatment

Figure 4.8 indicates that there are differences in the φ_{P0} of dark adapted leaves and leaves that have been exposed to natural illumination and that with subsequent dark adaptation there is recovery to initial values. The reduction in φ_{P0} is due to the dissipation of energy both photochemically and non-photochemically. The relative proportions of these processes have been related to both the intensity of incident light and the pre-history of the leaf. The magnitude of the reduction in φ_{P0} and the length of time required for its recovery subsequent to a period of illumination differ both between species and with season.

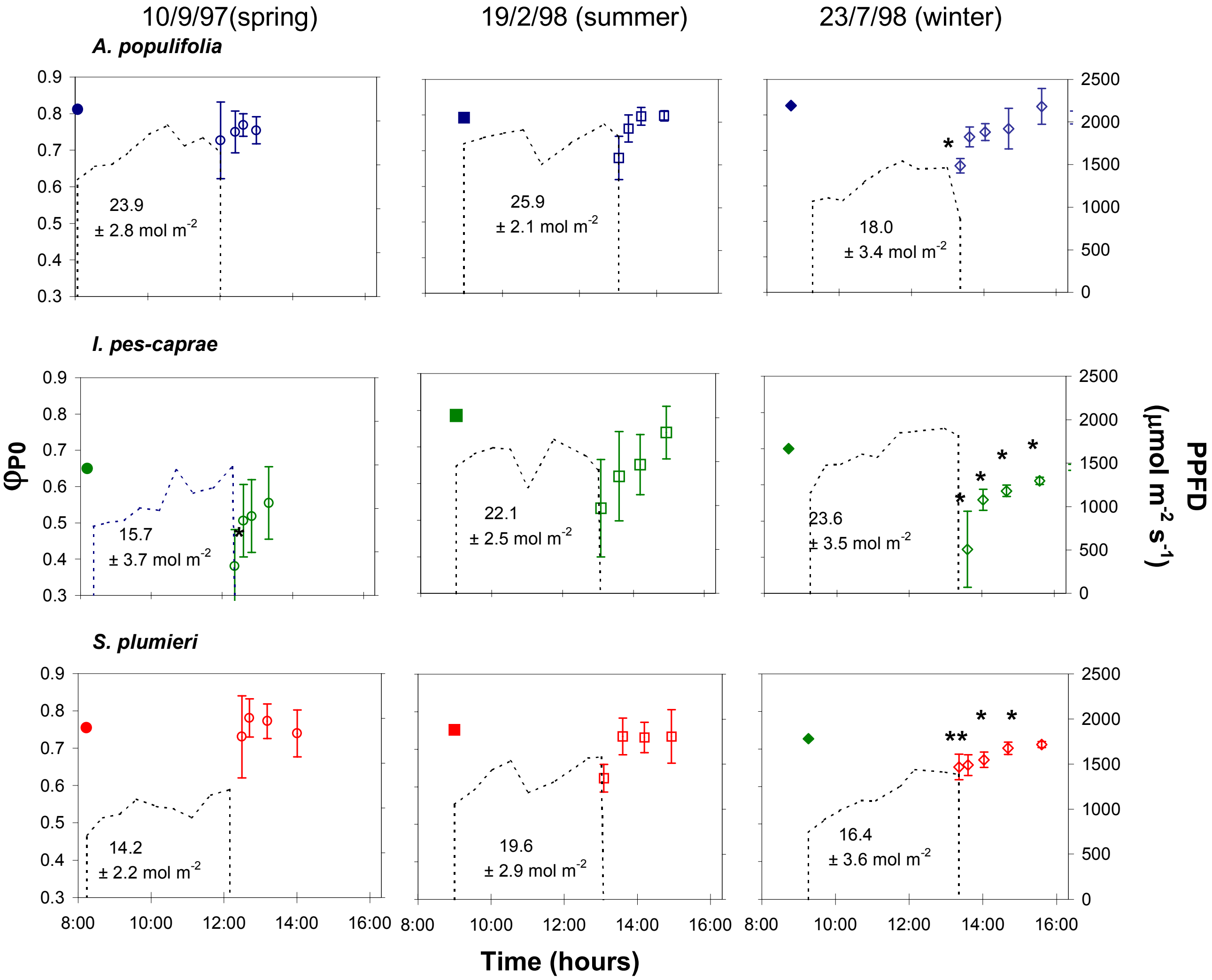


Figure 4.8: Average maximum quantum yield of primary photochemistry (φ_{P0}) prior to (solid symbols) and following a 4-hour incubation under sunlight (open symbols). The average photosynthetic flux density (PPFD) incident on the leaves during the four hours is indicated by the broken line. The total amount of photosynthetically active radiation incident during the 4 hour incubation is indicated on the graphs. φ_{P0} values that were significantly different from the initial value are indicated with a *. Standard deviations are indicated by the vertical bars. N=5.

On the 10/9/97 (spring) the only leaves to show significant reductions in φ_{P0} were those of *I. pes-caprae.* These rapidly recovered such that after 15 minutes values were not significantly different to the pre-illumination values. In addition to being the only species on the 10/9/97 to show a reduction in φ_{P0} the pre-illumination φ_{P0} values (0.6 ± 0.08) were significantly lower ($p = 0.0002$) than the pre-illumination values measured for all other species and seasons. They were also lower than the value that is considered typical for healthy tissue (± 0.8) (Bjorkman and Demmig, 1987).

On the 19/2/98 (summer) φ_{P0} values recorded prior to and immediately following the period of illumination were not significantly different for all three species. This was so despite the fact that the leaves of *A. populifolia* and *S. plumieri* had received the highest light dosage on this experimental day. φ_{P0} values of leaves for all species measured on the 23/7/98 (winter) showed both the greatest reduction and the slowest recovery following the four-hour incubation under natural light. On this day *A. populifolia*, *I. pes-caprae* and *S. plumieri* φ_{P0} values were reduced by 8, 40 and 9% respectively.

The leaves of *A. populifolia* recovered within 15 minutes but it took as long as 75 minutes for leaves from *I. pes-caprae* and *S. plumieri* to recover. The rate constants k_P and k_N were altered by the light treatment. Following incubation under natural light k_N increased by 48, 56 and 110% and k_P decreased by 12, 30 and 25% for *S. plumieri*, *A. populifolia* and *I. pes-caprae* respectively.

Succulent leaves from *A. populifolia* and *S. plumieri* showed smaller reductions in φ_{P0} than leaves from *I. pes-caprae* (Figure 4.6). In addition to which photosynthetic rate for *I. pes-caprae* and *S. plumieri* was unsaturated in full sunlight (Figure 4.2). Presumably as the incident light increases the amount of light transmitted through the leaf increases. This drives the photosynthesis of the opposite leaf surface and results in photosynthetic rates that do not saturate. As it is under conditions of excess reductant that photoinhibition has been shown to occur the continued use of reductant by unsaturated photosynthesis may reduce the requirement for the mechanisms associated with photoinhibition. In the succulent leaves the emitted fluorescence signal may originate from photosynthetic pigments both at the leaf surface and contained deeper within the tissues. It might thus be affected by the latter pigments not being light saturated.

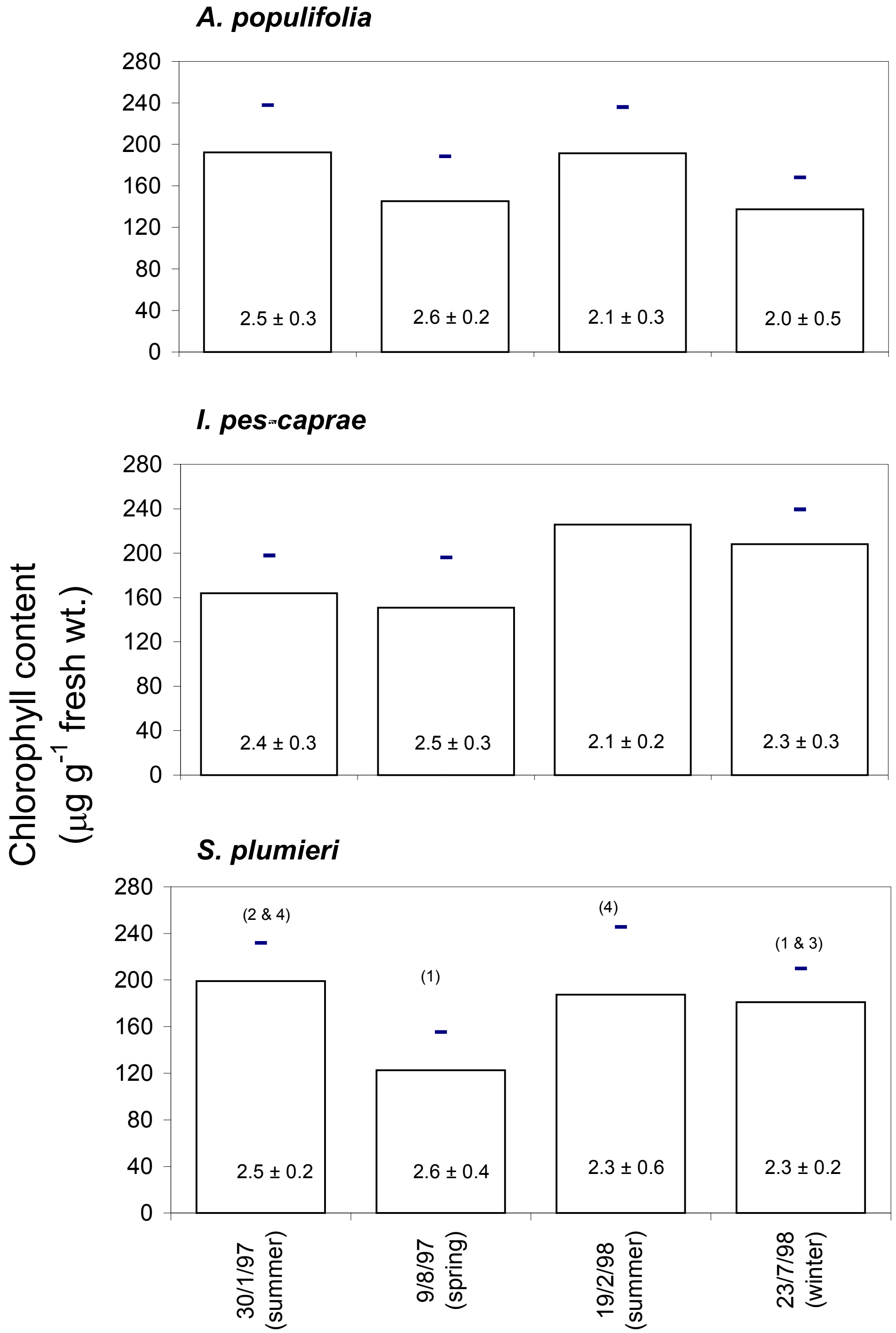


Figure 4.9: Average chlorophyll (a+b) content for *A. populifolia*, *I. pes-caprae* and *S. plumieri* leaves on the indicated experimental days. Standard deviations are indicated by the horizontal bars. If values obtained from the same species for different experimental days were significantly different this is indicated by the bracketed numbers. The experimental days are numbered (1) - 30/1/97 (summer), (2) -10/9/97 (spring), (3) - 19/2/98 (summer), (4) - 23/7/98 (winter) and (5) -15/9/98 (spring). N=9. The number within the blocks gives the average chlorophyll a:b ratios and their standard deviations.

Chlorophyll and xanthophylls

Chlorophyll and total xanthophyll (the sum of neoxanthin, violaxanthin, antherxanthin and zeaxanthin) contents were sampled at three time intervals on each experimental day (morning, noon and late afternoon). For all three species and on all experimental days there were no significant differences between total chlorophyll or total xanthophyll contents measured at these three different time intervals. For subsequent analyses data for each day were combined.

Chlorophyll concentration

The result of a two-way ANOVA indicated that the differences in leaf chlorophyll contents among species ($p<0.0000$), experimental days ($p<0.0000$) and between their interaction ($p<0.0000$) were significant. Chlorophyll (a + b) content of leaves from both *A. populifolia* and *I. pes-caprae* showed no significant differences between values from different days (Figure 4.9). *S. plumieri* summer leaf chlorophyll contents on 30/1/98 and 19/2/98 were significantly higher than winter values (23/7/98). When data for all seasons was combined *A. populifolia* had lower chlorophyll (a + b) contents (169.27 ± 46.56 µg g^{-1} fresh wt.) than *I. pes-caprae* (189.69 ± 59.66 µg g^{-1} fresh wt.) or *S. plumieri* (173.52 ± 49.11 µg g^{-1} fresh wt.). If the chlorophyll contents were expressed per unit leaf area using the wet weight to surface area conversion factors (Tables 3.4 and 3.5) then *S. plumieri* and *A. populifolia* had similar chlorophyll contents that were both significantly higher than those of *I. pes-caprae*. Chlorophyll contents per unit leaf area for *A. populifolia*, *I. pes-caprae* and *S. plumieri* were 13.87 ± 3.82, 6.15 ± 1.96 and 12.42 ± 3.51 µg cm^{-2} leaf area respectively. The average chlorophyll a:b ratios are similar between species and are shown in Figure 4.9.

Xanthophyll concentration

The results of a two-way ANOVA showed that the differences in total leaf xanthophylls on different experimental days ($p<0.0000$) were significant. This was not so for the differences among species ($p=0.1000$) nor between the interaction of species and experimental day ($p=0.1300$). Total leaf xanthophyll contents (Figure 4.10) did not show the same patterns of variation as was observed for chlorophyll (a + b). Values for *A.*

populifolia on experimental days 30/1/97 (summer) and 10/9/97 (spring) were significantly different from values on 19/2/98 (summer). Total xanthophyll content for *I. pes-caprae* and *S. plumieri* were not significantly different on any of the experimental days. Averages for *A. populifolia, I. pes-caprae* and *S. plumieri* were 760.8 ± 328.5, 787.8 ± 297.2 and 893.3 ± 387.3 mmol mol^{-1} chlorophyll (a+b), respectively.

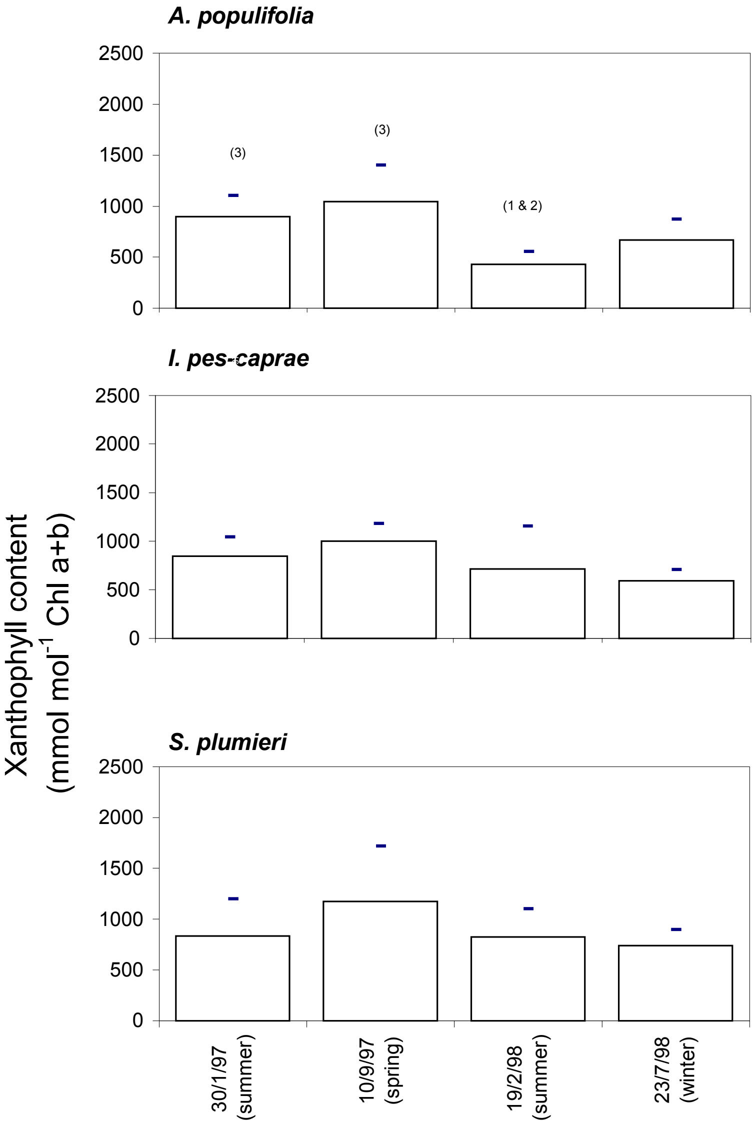


Figure 4.10: Average total xanthophyll content for *A. populifolia*, *I. pes-caprae* and *S. plumieri* on the indicated experimental days. Standard deviations are indicated by the horizontal bars. If values obtained from the same species for different experimental days were significantly different this is indicated by the bracketed numbers. The experimental days are numbered (1) - 30/1/97 (summer), (2) -10/9/97 (spring), (3) - 19/2/98 (summer) and (4) - 23/7/98 (winter). N=9.

The total xanthophyll pool size appeared unrelated to the average hourly incident PPFD data for Port Elizabeth. Linear plots of average total xanthophyll content against average hourly incident PPFD for each experimental day yielded r^2 values of 0.04, 0.09 and 0.01 for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively.

SAWB data which is integrated over one-hour periods was used in preference to the instantaneous data measured at the leaf surfaces prior to each fluorescence measurement as SAWB values are unaffected by leaf orientation. Furthermore the total xanthophyll pool size appeared unrelated to the average daily temperature measured at the study site and linear plots yielded r^2 values of 0.02, 0.05 and 0.04 for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively.

De-epoxidation states of xanthophylls

The de-epoxidation of the xanthophylls is strongly correlated to the non-photochemical dissipation of energy and hypotheses concerning the underlying mechanisms have been proposed (Gilmore *et al.*, 1996; Demmig-Adams *et al.*, 1988; Demmig-Adams, 1990). Reciprocal to the changes in φ_{P0} (Figure 4.6) DEPS increased from morning to midday and subsequently declined in the afternoon (Figure 4.11). This trend was generally not significant between measurement times on any particular experimental day with the exception of morning to noon changes in DEPS for *I. pes-caprae* on the 10/9/97 (spring). Hence data from all experimental days were combined. The lack of significant differences within an experimental day was probably a result of the variability in light intensity incident on the leaves in the field prior to sampling and due to the low number of replicates (N=3) measured at each time interval.

The results of a two-way ANOVA of combined data showed that there were significant differences both among species ($p=0.0020$) and between sampling times ($p<0.0000$). The interaction between sampling times and species was not significant ($p=0.8200$) showing that all three species responded in a similar way to changing time of day. In all three species the difference between morning and noon but not between noon and afternoon values for DEPS were significant (p=0.0110, p=0.0096 & p=0.0488 for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively).

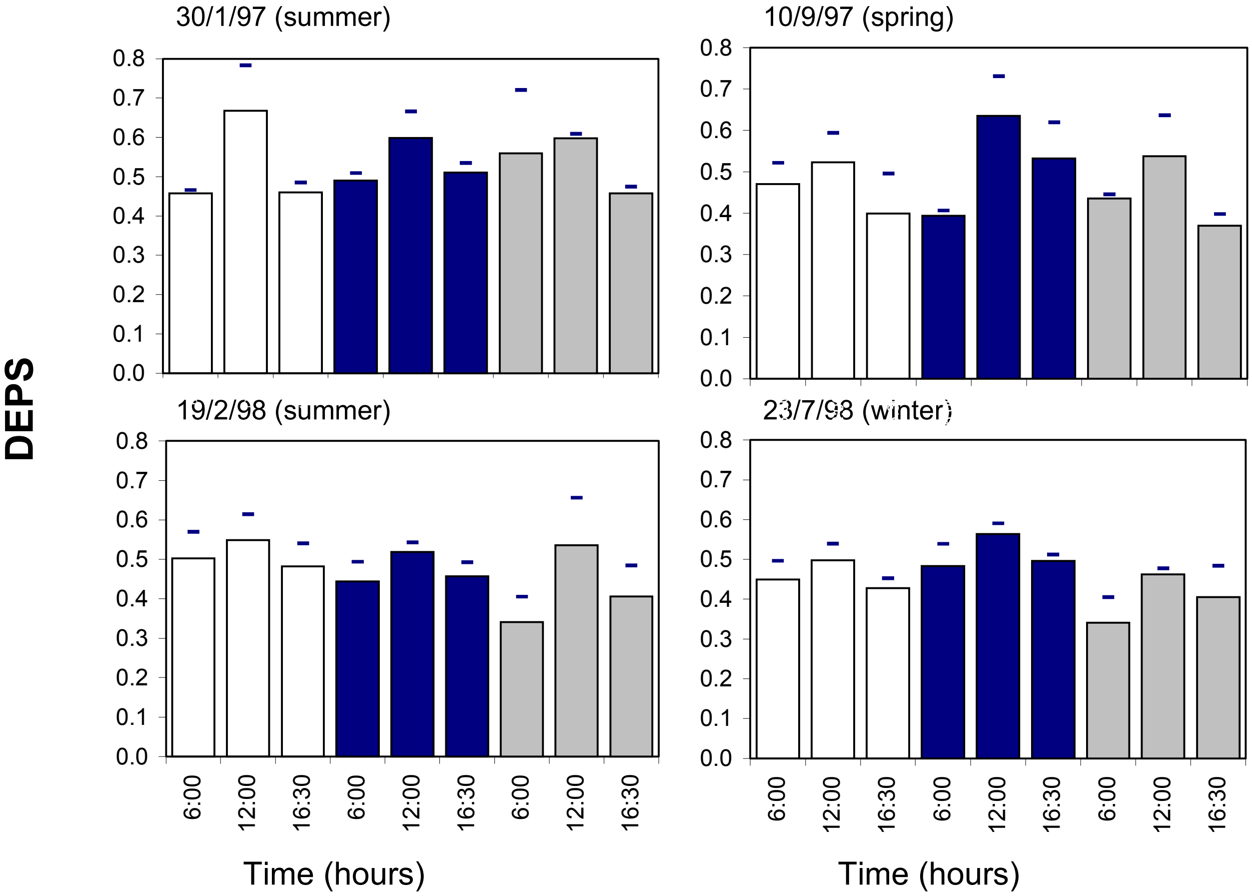


Figure 4.11: Diurnal changes in the de-epoxidation state of the xanthophylls (DEPS) of the three species *S. plumieri* (open blocks), *I. pes-caprae* (solid blocks) and *A. populifolia* (shaded blocks) for indicated experimental days. Standard deviations are indicated by the horizontal bars. N=3.

The lack of significant differences between noon and afternoon DEPS can be explained by different day lengths on the experimental days (Figure 3.7). For example differences in day length result in summer leaves sampled at 16:30 still receiving relatively high light intensities and hence maintain more of the xanthophylls in the de-epoxidated state. At the same time of day the leaves in winter would receive much less light and would hence respond accordingly. When combined data from mornings and afternoons was analysed the resultant variability in DEPS in the afternoons masked any significant trends.

When morning DEPS were subtracted from noon DEPS the differences were not significant between species (p=0.8200) nor between data from different experimental days (p=0.1386). As DEPS is a relative measure the above shows that of the total amount of contained xanthophylls the same proportion was converted into the de-epoxidated state from morning to noon by all three species. However there were not significant differences in the total amounts of xanthophyll that the three species contained.

DISCUSSION

Photosynthetic rates

The most important climatic variable for determining photosynthetic rate was incident PPFD (Figure 4.2 and Table 4.2). The causal limitation of productivity at the gas exchange level is difficult to determine due to the many abiotic variables that are covariate with PPFD. The dependence of both photosynthesis and net primary productivity on absorbed PPFD has been documented in both agricultural and forest ecosystems (Haxeltine and Prentice, 1996). An in-depth-look at the response of the studied species to light is conducted in Chapter 7. In addition to the effects of light on productivity biomass allocation patterns can affect productivity and growth (Abrahamson and Caswell, 1982). Allocation patterns were not directly investigated but differences in stem and leaf production suggest marked differences between species. In addition to which there are observable differences in the sizes of above-ground to below-ground biomass and of allocation to reproductive structures. This should be a subject of further study.

The maximum photosynthetic rates measured were quite different between species as was the daily amount of CO_2 assimilated per leaf (Table 4.1 & 4.4). *A. populifolia* achieved maximum rates (34.1 ± 1.4 μmol CO_2 m^{-2} s^{-1}; Table 4.1) that are comparable to C_4 species growing in an African savanna. For example the C_4 grasses *Digitaria eriantha, Panicum maximum* and *Eragrostis pallens* achieved maximum rates of 27.2, 27.9 and 33.4 μmol CO_2 m^{-2} s^{-1} respectively (Baines, 1989; Blackmore, 1992). The maximum assimilation rate for *A. populifolia* was higher than the maximum rate for *I. pes-caprae*

(16.3 ± 1.9 μmol CO_2 m^{-2} s^{-1}; Table 4.1) and *S. plumieri* (25.1 ± 3.0 μmol CO_2 m^{-2} s^{-1}; Table 4.1). The latter rates are comparable to the maximum rates measured for temperate C_3 and C_4 dune grasses. Maximum rates of 17 ± 3, 22.5 ± 3, 27.3, 30.7 μmol CO_2 m^{-2} s^{-1} have been measured for *Ammophila* arenaria (C_3), *Elymus mollis* (C_3) (Pavlik, 1983a)*, Ammophila breviligulata* (C_3) *and Calamovilfa longifolia* (C_4) (Yaun *et al.*,1993) respectively.

The differences in photosynthetic rates between the three species may be partly accounted for by the differences in the chlorophyll content of the leaves expressed on a leaf area basis. When expressed in such a way chlorophyll content is a functional measure as it defines the amount of pigment per unit of light interception. These values were highest for *A. populifolia* followed by *S. plumieri* and then *I. pes-caprae* and were 13.87 ± 3.82, 6.15 ± 1.96 and 12.42 ± 3.51 μg cm^{-2} leaf area respectively. Similarly average peak assimilation rates (Table 4.1) and average daily amounts of CO_2 assimilated (Table 4.4) were highest for *A. populifolia* followed by *S. plumieri* and then *I. pes-caprae*.

The three species investigated in this study represent early successional species (Tinley, 1985; Avis, 1992) and in this respect may be compared to early successional species in an Illinois (USA) succession where the early species include *Ambrosia trifida*, *Datura stramonium*, *Chenopodium album* and *Polygonum pennsylvanicum* (Bazzaz and Carlson, 1982). These species achieved light saturated photosynthetic rates of 33.8, 29.5, 26.7 and 23.4 μmol CO_2 m^{-2} s^{-1} respectively. These exceeded photosynthetic rates for later successional species. These rates are comparable to the rates measured for *A. populifolia*, *I. pes-caprae* and *S. plumieri* and suggest that high photosynthetic rates may be a characteristic of early successional species.

In order to emphasise the uniformity of the response of assimilation rate to PPFD between individual leaves and plants (Figure 4.2) these data were compared to data from Bazzaz (1993). This author compared the photosynthetic response to light of *Acer saccharum* measured (i) under controlled conditions in a greenhouse, (ii) under field conditions where measurements were made on a few individuals for a short duration and (iii) under field conditions over entire growing seasons. He points out that the last data give the most accurate assessment of light response in nature yet have the greatest variability. Data presented in Figure 4.2 were measured on many different individual leaves in different seasons and show much less variability than the data presented by Bazzaz (1993) for *Acer saccharum.*

Respiration rates

The higher and relatively little seasonally changed respiration rates for *A. populifolia* (Table 4.3) are possibly related to the higher rates of leaf production that were unchanged by season in this species (Figure 4.5). The difference between winter and summer respiration rates for *A. populifolia* could be accounted for by the seasonal differences in ambient temperature. In contrast the other two species had larger seasonal changes in dark respiration that could not be accounted for by the temperature differences (estimated from assumed value of Q_{10} =2) and are probably related to seasonal changes in growth rate. Respiration rates measured at night represented 5.9, 9.3 and 6.7% of the summer average photosynthetic rates determined within the same 24 hour period for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. In winter these values were reduced to 3.9, 3.3 and 1.7% respectively. These comparisons were made between whole shoot respiration and leaf assimilation rates. However a note of caution is required as whole shoot assimilation rates would include the effects of both leaf age and differential illumination. For the purpose of comparison data was expressed as a percentage of the maximum measured photosynthetic rates. These were 2.0, 1.2 and 0.9 % for the experimental day 23/7/98 (winter) and 3.1, 4.8 and 3.0 % for the experimental day 19/2/98 (summer) for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. These are considerably lower than similarly calculated values for the leaves of savanna species *Digitaria eriantha* and *Grewia bicolor* which were 15-18 % at 30^{o}C and 9-12% at 25^{o}C (Rey, 1982). Values measured for species in this study are similar to values measured for temperate beach grasses. *Ammophila arenaria* and *Elymus mollis* grown under controlled conditions (Pavlik, 1983a) had respiration rates expressed as percentages of maximal photosynthetic rates of 4.5% and 4.6% when supplied low nitrogen concentration and 6.4% and 10.3% when supplied high nitrogen concentration respectively.

Calculation of daily amounts of CO_2 assimilated includes the duration and rate of positive carbon assimilation (Table 4.4). This did not result in further clarification of the seasonal trends in productivity. Rather it emphasises that these species appear to respond to the prevailing conditions in an opportunistic fashion.

Soluble sugars and starch

Soluble sugar concentration was different on summer and winter experimental days (Figure 4.3) despite the fact that photosynthetic rates (Figure 4.1) showed little seasonal variation. Variation in stem soluble sugar concentrations for *I. pes-caprae* and *S. plumieri* were more seasonal than those for *A. populifolia* (Figure 4.3). *S. plumieri* had greater concentrations of sugar in both stem and leaves than the other two species indicating that this may be an important storage product in this species. Leaf starch concentrations in both stems and leaves were not significantly different on different experimental days (Figure 4.4). There was little evidence for the reallocation of stem reserves to the leaves. Had reallocation been important then it would be anticipated that on shading, the leaf concentrations of soluble sugars, starches, or total soluble carbohydrates would have been maintained at the expense of stem concentrations. This was not evident from the data collected. A decline in both leaf and stem concentration is more difficult to interpret as it could be the result of reduced carbohydrate production alone or a combination of both reduced carbohydrate production together with some resource reallocation. Reallocation has been reported before and Harris and Davy (1988) showed that *Elymus farctus* seedlings reallocated below-ground carbohydrates and increased foliar sugar concentration during and subsequent to a burial treatment. Seliskar (1994) measured both soluble sugar and starch concentrations in *Ammophila arenaria* rhizomes of control and buried plants and showed an increase in soluble sugar but not starch concentration on burial. The rhizomes contained at least three orders of magnitude more starch and 50 times more sugar than was measured in leaves or stems of the three species of plants investigated in this study. This data leads one to question the importance of these organs in carbohydrate storage in the three species studied.

Leaf production and longevity

Leaf production for temperate dune grasses has been demonstrated to be highly seasonal (Huiskes, 1980) and has been related to marked differences in abiotic factors between seasons. Similarly leaf production rates measured at both Mtunzini and Durban for *S. plumieri* have been shown to be seasonal with monthly averages for the two locations being 3.8 ± 1 (Pammenter, 1983) and 2.8 leaves shoot^{-1} month^{-1} (Steinke and Lambert, 1986) respectively. Not only is leaf production higher at these more northern locations but there was a more pronounced seasonal variation in the leaf production rates than those measured in this study (Figure 4.5). Steinke and Lambert (1986) showed that the winter months (June to September) had significantly lower rates of leaf production. Similarly Pammenter (1983) showed a 40 % reduction in leaf production in the winter months. In the present study only the June and July production rates were significantly different to some of the spring and summer months (Figure 4.5). Seasonal variation was much less pronounced. The lack of a seasonal response of both the photosynthetic rates and the growth rates may result from the latitudinal position of the study site. This location has both evenly distributed annual rainfall and moderate daily temperature (Chapter 3) resultant upon the influence of the Mediterranean climate from the west and the sub-tropical climate from the east (Schultze *et al.*, 1997). Differences between highest and lowest average monthly temperatures for the period 1990 to 1997 were 8.6^{o}C and 5.6^{o}C for Mtunzini and Old Woman's River respectively. Dune aquifers may also supply unlimited water reducing the importance of rainfall. This as well as other aspects of plant water acquisition and use are considered in Chapter 5.

Leaf longevity varied between the species with *A. populifolia* having the shortest lived leaves followed by *I. pes-caprae* and then *S. plumieri*. Specific leaf areas (SLA) for *S. plumieri* were considerably lower than those for *A. populifolia* and *I. pes-caprae* (Table 3.5). This may be related to high leaf longevity which Grime (1998) states may confer fitness in resource-poor environments where retention of captured resources is a high priority. The highest SLAs were measured for *A. populifolia* which had the shortest lived leaves, the highest growth rates and often grows closest to the swash zone where the continual input of debris may reduce resource limitations. SLA of *A. populifolia* and *I. pes-caprae* are similar to values calculated for savanna woody plants where values range from 53 cm^2 g^{-1} for *Strychnos pungens* to 78 cm^2 g^{-1} for *Ochna pulchra* (Scholes and Walker, 1993).

Shoot production

High photosynthetic rates (Table 4.1), respiration rates (Table 4.3) and leaf and stem production rates (Table 4.5) did translate into high shoot primary production (Table 4.6). This trend was apparent despite the fact that neither the dry matter contribution of flowers and fruits nor the daytime CO_2 exchange of these structures was assessed. Shoot primary production (Table 4.6) was significantly different between species and was much higher in *A. populifolia* than either *S. plumieri* or *I. pes-caprae.* This may in part explain this species success at living closest to the high water mark where sand-burial and stochastic events are highest. *I. pes-caprae* is found in areas where sand-burial appears to be less and is often associated with river mouths (*pers. obs.*). Possibly the lower primary productivity of this species limits its ability to withstand sand-burial. *S. plumieri* is found in an intermediate position and although it is often the dominant foredune species it is never found as close to the high water mark as is *A. populifolia*. Although sand-burial and, plant growth in relation to sand-burial, may be important determinants in limiting plant distributions on dunes there are other factors that also influence plant distribution. These include salt-spray tolerance, resistance to mechanical abrasion, soil pH, soil salinity and soil nutrient content (Boyce, 1954; Hesp, 1991; Davy and Figueroa, 1993).

Above-ground primary productivity

Above-ground primary productivity takes into account not only shoot primary productivity but also the density of shoots per unit of land area. Expressed on this basis the productivity of *S. plumieri* and *A. populifolia* were not significantly different although both values were higher than those measured for *I. pes-caprae* (Table 4.6).

The values measured for all three species are comparable both to values for African savannas (Scholes and Walker, 1993) which include woody, herbaceous and grass components and to values for *Spartina* marshes (Stroud and Cooper, 1968) (Table 4.7). The latter are considered very productive systems (Long and Woolhouse, 1979). Values for the dune species in this study are in excess of estimates for temperate dune systems (Deshmukh, 1977; Wallen, 1980), grasslands (Sims and Singh, 1978) and fynbos (Kruger, 1977) (Table 4.7). The comparison of productivity between the investigated species and species from other ecosystems must however be treated with some caution. *A. populifolia* and *I. pes-caprae* have patchy distribution and thus the number of stems or shoots per unit soil surface area, a parameter used in the calculation of primary productivity (see Methods), varies according to the soil surface area considered.

Biomass

Biomass estimates were similar to those measured for North American foredune species (Barbour and Robichaux, 1976) but were considerably lower than estimates for African savannas (Scholes and Walker, 1993) and fynbos (Stock and Allsop, 1992) (Table 4.7). The lack of a net increase in biomass and high rates of productivity implies high biomass turnover. The former was assumed based on equal rates of leaf production and loss for *I. pes-caprae* and *S. plumieri* although there is some stem accumulation particularly for *S. plumieri*. Biomass turnover is calculated from the proportion of above-ground living biomass replaced annually by above-ground primary production. Values were 760, 287 and 200% for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. The above assumption may be less valid for *A. populifolia* both because leaf loss was not monitored and because this species often forms small hummock dunes and during this process it is likely that the above-ground biomass increases.

Table 4.7: Annual above-ground primary productivity and biomass of species from various ecosystems.

Species or Community	Location and Habitat	Above-ground primary productivity (g dry mass m^{-2} yr^{-1})	Above-ground biomass (g dry mass m^{-2})
A. populifolia	Present study, Foredune	479.5 ± 211.6	63.1 ± 17.7
I. pes-caprae	Present study, Foredune	386.5 ± 326.2	134.6 ± 40.9
S. plumieri	Present study, Foredune	541.4 ± 220.6	271.4 ± 133.3
Ammophila arenaria [*2]	Eastern Scotland, Mobile dune	251.4 ± 22.1	
Ammophila arenaria [*2]	Eastern Scotland, Semi-fixed dune	76.5 ± 8.0	
Ammophila arenaria [*3]	Southern Sweden, 1 yr. old dune	63.6	
Ammophila arenaria [*3]	Southern Sweden, 10 yr. old dune	108.5	
Elymus mollis, Ammophila arenaria and *Ambrosia chamissonis* [*6]	North America, Sand dune		30 – 211
Ambrosia chamissonis, Ambronia maritima, Camissonia cheiranthifolia [*6]	California, Sand dune		20 – 174
Uniola paniculata, Panicum amarulum, Ipomoea stolonifera [*6]	Texas, Sand dune		103 – 211
Spartina alterniflora [*5]	North America, Salt marsh	279 – 500	
Average of ten different North American Grasslands [*4]	North America, Grassland	223	
Savanna woody plants [*1]	Southern Africa, Savanna	282.4	
Savanna herbs including grasses [*1]	Southern Africa, Savanna	157	
Total savanna (herbs + woody plants) [*1]	Southern Africa, Savanna	439.4	1627
Fynbos	Western Cape, SA,Fynbos	100 – 400[*9]	1496[*8]

[*1]Scholes and Walker, 1993; [*2]Deshmukh, 1977; [*3]Wallen, 1980; [*4]Sims and Singh, 1978; [*5]Stroud and Cooper, 1968; [*6]Barbour and Robichaux, 1976; [*7]Judd *et al*., 1977; [*8]Stock and Allsopp, 1998; [*9]Kruger, 1977. Values from Wallen (1980) were converted from calorific measures to above ground biomass in g dry mass m^{-2} using a factor of 18.8 kJ g^{-1} dry mass (Odum, 1971). Fynbos biomass was calculated as the average value from biomass data collected at 7 different areas in the Western Cape and these values included dead plant material (necromass).

Leaf area index

Low above-ground biomass with a high turnover resulted in low LAI. The values for the three species *A. populifolia*, *I. pes-caprae* and *S. plumieri* being 1.05 ± 0.51, 0.78 ± 0.26 & 0.23 ± 0.08 respectively. The LAI data from this study compare well with values of 1.0

and 0.95 measured for *S. plumieri* at Mtunzini (28^{o}E, 32^{o}S; Pammenter, 1985) and Old Woman's River (Peter, 2000). Values for *A. populifolia, I. pes-caprae* and *S. plumieri* are far below values measured for tropical forests and woodlands where LAI averaged across four different vegetation types was 5.2 (data extracted from Medina and Klinge, 1983), although the caution concerning the comparison of LAI for species with patchy distributions is reiterated (See Chapter 3). LAIs measured in this study are more comparable with those of dry sclerophyllous *Eucalyptus* forests of Australia where LAIs range between 1.0 and 3.0 (Carbon *et al.*, 1979; Anderson, 1981, cited in Jarvis and Leverenz, 1983) and values of 0.5 to 1.3 for deserts (data cited in Schultze, 1982). LAI has been related to both precipitation (Grier and Running, 1977) and potential evapotranspiration (Waring *et al.*, 1978). The low LAI of dune species may be essential in maintaining resource availability in an environment where the nutrient and water availability are potentially limiting (Pammenter, 1983; Barbour, 1990; Hesp, 1991). Nutrient and water acquisition, use and limitations are considered in detail in Chapters 5 & 6.

Light stress

A. populifolia and *S. plumieri* showed little evidence of light stress. Both recovering rapidly from light treatments (Figure 4.11) and showing little seasonal difference in response to incident light despite considerably different accumulative light dosages. Such recoveries following light treatments have been recorded for other species (Demmig-Adams and Adams, 1996) and the rates of recovery have been related to growth light intensities. The magnitude of the reductions in φ_{P0} may be related to the extent that the photosynthesis of these species is saturated by the midday light intensities.

A. populifolia showed the smallest midday reductions in φ_{P0} (Figure 4.6) in all seasons and the fastest recoveries following a light treatment. This is likely to be due to the hair layer of this species that not only reduces transmitted light but selectively reduces the amount of transmitted UV radiation (Ripley *et al.*, 1999) thereby reducing photoinhibition. Decreased reduction in photochemical efficiency has been demonstrated in *Senecio medley* leaves with hairs when compared to leaves with fewer hairs (Lang and Schindler, 1994).

Unlike *A. populifolia* and *S. plumieri*, *I. pes-caprae* is thin leafed, has much lower photosynthetic rates and is therefore potentially much more susceptible to light stress. There is evidence that this species is light stressed and its φ_{P0} was depressed the most by midday (Figure 4.6). It showed the slowest recovery following a light treatment and this was exacerbated in winter (Figure 4.11). Furthermore *I. pes-caprae* φ_{P0} values responded differently to increasing PPFD on various experimental days suggesting a changing sensitivity to photoinhibition in different seasons. This species shows signs of leaf senescence in the winter months although this was not evident as declining chlorophyll concentrations nor marked alterations to the chlorophyll a:b ratio (Figure 4.8). It must be pointed out that the leaves chosen for sampling and physiological measurements were visually healthy.

Only *S. plumieri* showed seasonal variation in chlorophyll (a+b) concentration (Figure 4.8) and chlorophyll a:b ratios for all species remained unchanged on different experimental days. Xanthophyll concentrations have been shown to change seasonally and Garcia-Plazaola *et al*. (1999) reported higher winter than summer xanthophyll concentrations in the Mediterranean Oak (*Quercus ilex*). These authors suggest that the higher winter xanthophyll concentrations offered added light stress protection when morning temperatures were relatively low and light intensities high. Despite similar conditions of low winter mornings temperatures (4 - 7^{o} C; Figure 3.6) on the beaches in the Eastern Cape, *A. populifolia*, *I. pes-caprae* and *S. plumieri* did not show differences in xanthophyll concentrations on different experimental days (Figure 4.9).

In all species total xanthophyll concentrations remained the same throughout the day suggesting that no *de novo* synthesis of these pigments occurred. This is in contrast to the findings of Schindler and Lichtenhaler (1996) for *Acer plantanoides* exposed to natural illumination. Furthermore the total xanthophyll pool size appeared unrelated to the incident PPFD. This is in contrast to the findings of Niinemets *et al.* (1998) who showed a strong correlation between integrated PPFD and total xanthophylls. The xanthophyll cycle showed significant conversion to the de-epoxidated states from the morning low irradiance to the midday high irradiance but this was only significant if all seasons' data were combined (Figure 4.10). Many researchers have noted similar diurnal conversions of the xanthophylls. For example Schindler and Lichtenhaler (1996) and Adams III & Barker (1998) and Haldimann (1997). There appeared to be little relationship between the DEPS and the extent of the reduction in φ_{P0} (Figure 4.6 & 4.10) although these analyses were conducted on different leaves from different shoots and the variation in the incident light between leaves and shoots may well have masked any relationship. Similarly Schindler and Lichtenhaler (1996) noted the lack of a clear correlation between the rise and fall of zeaxanthin concentration and changes in the chlorophyll fluorescence parameters. The average difference in the DEPS between samples harvested in the morning and those harvested at noon were 0.134 ± 0.074, 0.096 ± 0.079 and 0.118 ± 0.058 for *I. pes-caprae*, *A. populifolia* and *S. plumieri* respectively. Although these differences were not significant between species it is of interest that the species that had on average the lowest photosynthetic rates had the highest conversions to the de-epoxidated xanthophylls and visa versa. As the morning and midday xanthophyll analyses were made on leaves from different shoots the variability was high and could account for the lack of significance. This trend is similar to the findings of Adams and Demmig-Adams (1992) for pot-grown plants of several different species.

Reductions in φ_{P0} with increasing light intensity were evident for all species in all seasons and were accompanied by increases in k_N and by either small increases or decreases in k_P. φ_{P0} recovered in the afternoon in response to reducing light intensity (Figure 4.7). The extent of the midday reduction in φ_{P0} was not related to season as has been shown in the Mediterranean grassland species *Tuberaria guttata* and *Chamaemelum nobile.* These two species showed the greatest reduction in the high light intensities of midsummer (Fernandez-Baco *et al.*, 1998). The lack of a seasonal response again illustrates the opportunistic response of these species to the prevailing climatic conditions.

Table 4.8: Comparison of data presented for *A. populifolia*, *I. pes-caprae* and *S. plumieri* in Chapter 4. The number of symbols indicates the magnitude of the parameter relative to the smallest value measured for any one of the three species.

Measured parameter:	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
Primary carbon metabolism			
Peak photosynthetic rate	♦♦½	♦	♦♦
Daily amounts of CO_2 assimilated	♦♦♦	♦	♦♦
Dependence of A rate on PPFD	♦♦♦	♦	♦♦♦
Whole shoot respiration rates	♦♦♦	♦	♦♦
Soluble carbohydrates			
Leaf + stem non-structural CHOs	♦	♦	♦♦
Leaf starch	♦½	♦♦½	♦
Stem starch	♦	♦	♦
Leaf sugar	♦♦	♦	♦♦♦♦♦
Stem sugar	♦½	♦	♦♦♦♦
Production and productivity			
Leaf production	♦♦¼	♦¼	♦
Shoot primary productivity	♦♦½	♦	♦½
Above-ground primary productivity	♦	♦	♦½
Biomass	♦	♦♦	♦♦♦♦¼
Biomass turnover	♦♦♦♦	♦½	♦
Leaf longevity	♦	♦♦½	♦♦♦
Leaf Area Index (LAI)	♦	♦♦♦½	♦♦♦♦½
Specific Leaf Area (SLA)	♦¾	♦♦	♦
Physiological effects of light			
Susceptibility to light stress [4.3]	♦	♦♦	♦¼
Morning to noon change in k_N	♦	♦¼	♦¾
Morning to noon change in k_P	♦½	♦½	♦
Chlorophyll content	♦♦¼	♦	♦♦
Xanthophyll content	♦	♦	♦
Morning to noon change in DEPS	♦	♦	♦

Abbreviation: Photosynthetic photon flux density (PPFD), De-epoxidation states (DEPS). [4.3] Susceptibility to light stress was based on the extent of midday reductions in φ_{P0} (Figure 4.6) and recoveries from light treatments (Figure 4.8) averaged across all available data and was defined as: the percentage reduction in φ_{P0} from morning to midday x length of time required for φ_{P0} to recover following exposure to four hours of natural illumination.

Summary of inter-species comparison

Table 4.8 gives a summary of the Chapter 4 data that can be used to compare the three species, *A. populifolia*, *I. pes-caprae* and *S. plumieri*. Examination of this summary emphasises that the values of some the measured parameters were very different whilst others were similar. This allows some inferences to be made about the mechanisms or strategies that these three species use with respect to the inhospitable dune environment. The following discussion refers to Table 4.8 but references to the original figures and tables are also given.

A. populifolia was the most productive species with the highest assimilation rates (Figure 4.1 & Table 4.1), respiration rates (Table 4.3), daily amounts of CO_2 assimilated (Table 4.4), leaf and stem production per shoot (Figure 4.5 and Table 4.5) and shoot primary production (Table 4.6). Assimilation rates were closely correlated with incident PPFD (Figure 4.2). Leaf chlorophyll contents per unit leaf surface area (13.87 ± 3.82 µg cm^{-2}) and SLA (Table 3.5) were high maximising light interception with minimal carbon investment. High productivity may be related to the frequent availability of nutrients resulting from the decomposition of organic material in the swash zone and a requirement for fast growth following sand-burial. *A. populifolia* invested a large proportion of the above-ground production in stems (Table 4.5) and had shortest-lived leaves (50 ± 21 days) and an extremely high biomass turnover (760% yr^{-1}). Both above-ground stems and petioles were green and may have been photosynthetic (not measured). *A. populifolia* appeared to be insensitive to light stress having the smallest reductions in φ_{P0} (Figure 4.6) and the fastest recoveries in φ_{P0} (Figure 4.8) following exposure to full sunlight. This lack of sensitivity to light stress is possibly because of its leaf hairs (Ripley *et al*., 1999) and succulent leaves. *A. populifolia* is able to grow closest to the high-tide line (Lubke, 1983) where the conditions are the severest and this may be due to these characteristics.

S. plumieri performed in a similar way to *A. populifolia* but did not have as high assimilation rates (Figure 4.1 and Table 4.1), respiration rates (Table 4.3) nor shoot primary production (Table 4.6). Photosynthetic rates, like those of *A. populifolia,* were highly dependent on incident PPFD (Figure 4.2). Leaves were relatively long-lived (± 217 days) and this may be associated with lower SLA (Table 3.5) and higher primary productivity (Table 4.6). Higher primary productivity was a result of higher LAI (Table 3.6) rather than higher shoot productivity (Table 4.5). Resource availability may be lower for *S. plumieri* than for *A. populifolia* as the former species does not grow as close to the swash line (Lubke, 1983). Leaf and stem sugars were maintained at higher concentrations than in the other species (Figures 4.3 & 4.4) and like *A. populifolia, S. plumieri* appeared to be insensitive to light stress (Figures 4.6 & 4.8).

I. pes-caprae had the lowest photosynthetic rates (Figure 4.1 and Table 4.1), respiration rates (Table 4.3), daily amounts of CO_2 assimilated (Table 4.4), leaf and stem production per shoot (Figure 4.5 and Table 4.5) and shoot primary production (Table 4.6). Leaves contained the lowest amounts of chlorophyll per unit leaf area (6.15 ± 1.96 $\mu g\ cm^{-2}$) and appeared to be sensitive to light stress (Figures 4.6 & 4.8). The latter may be associated with low and light saturated photosynthetic rates (Figure 4.2) and thin non-succulent leaves. *I. pes-caprae* leaves were nearly as long lived (±176 days) as *S. plumieri* leaves and had high SLA (Table 3.5).

Despite these differences *A. populifolia, I. pes-caprae* and *S. plumieri* had above-ground primary productivity values that were similar (Table 4.6) and because of differences in biomass turnover had very different above-ground biomass values (Table 4.6).

CONCLUSIONS

Despite the limitations of viewing five intensive snap-shots of the physiology of these species on days in different seasons and the limitation that the instrumentation used required the selection of fine weather days, certain conclusions can be reached.

- All three species appear to respond to prevailing climatic conditions with respect to carbon acquisition and this seems to be largely determined by the light environment.

- Both *S. plumieri* and *I. pes-caprae* showed seasonal differences in biomass production. *A. populifolia* appears to maintain its productivity throughout the year.

- Differences measured in both photosynthetic rates and respiration rates translated into both leaf production rates and shoot primary productivity.

- Given the marked differences in rates of photosynthesis, respiration and leaf production the final summation of all of these measures (above-ground primary productivity) was not considerably different among species. This suggests different physiological solutions to solving the same challenges presented by this environment.

- Primary productivity was relatively high in comparison to other natural ecosystems despite the beach environment being low in nutrients and having potentially low water availability.

- LAI in mono-specific stands of all three species was low and may relate to resource availability. To be highly productive in what is considered a resource limited environment requires that a large soil surface and volume be available to individual plants or growing shoots.

- Between species differences in above-ground biomass (in which there is little net increase) and productivity are the result of differences in biomass turnover and leaf longevity. These may be related to SLA and resource availability. If resources are limited then longer-lived leaves with greater carbon investment and higher SLA might be expected.

- The high light intensity environment on the beach and the resultant potential light stress appear to have been dealt with differently by the species under investigation. *A. populifolia* and *S. plumieri*, both succulents, have photosynthetic rates that remain unsaturated even at the highest light intensities encountered on the beach. The continued utilisation of reductant at these high light intensities may well prevent photoinhibition (Osmond, 1981; Baroli and Melis, 1998) thus reducing the reduction of φ_{P0} and the requirement for non-photochemical energy dissipation. In contrast *I. pes-caprae* has a relatively lower photosynthetic rate that saturates in full sunlight. This species shows a greater reduction in φ_{P0} and a larger midday conversion to the de-epoxidated xanthophylls. *I. pes-caprae* respond differently to increasing light intensity in different seasons suggesting a seasonal change in the mechanisms associated with photoinhibition.

CHAPTER 5
PLANT WATER RELATIONS

INTRODUCTION

The beach environment has traditionally been thought of as arid and the lack or limited availability of water to be a primary stress (Rozema *et al*., 1985; Barbour *et al*., 1985; Barbour, 1990; Hesp, 1991; Davy and Figueroa, 1993). Limited water availability may not be due to low rainfall but may arise as a result of the coarse substrate with low water retention, high evaporation, low boundary layer resistance, high solar radiation, high soil salinity and high temperature (Barbour *et al*., 1985). In addition beach plants possess xeromorphic attributes often associated with desert plants, which contribute to the perception that this is a xeric environment.

Not all researchers view coastal dunes as xeric environments and De Jong (1979) concluded the sand water content is invariably sufficient to meet plant demands. This was based on the high leaf water potentials developed by dune plants in comparison to xerophytes. Other workers have also measured dune plant water potentials and shown that values are rarely more negative than –2.0 MPa (Dubois, 1977, cited in Barbour *et al*., 1985; Pavlik, 1984, 1985).

Despite the lack of consensus about the aridity of beach environments the maintenance of plant water status is essential for plant growth and productivity (Parker, 1968; Boyer, 1995). Plant water status is dependent both on the loss of water by transpiration and the acquisition of water from the substrate. In turn the water content of the beach substrate is dependent on water inputs by rainfall, fog, dew and groundwater. Losses are through plant water uptake, direct evaporation from the sand and deep drainage.

Answering questions about dune plants’ water requirements, water use and related physiology, remains one of the key issues in understanding plant function in the beach environment. Clearly these key issues need to be understood thus the following questions were asked: What is the source of plant water? What volumes of water are required? Is water supply limiting? How efficiently is water used? How are water use and acquisition controlled physiologically?

Water relations of dune soils

Dunes sands are coarse in nature and have low organic content and this is particularly so for newly-formed pioneer dunes. Young foredune sands are relatively unstructured and the small loss in water-holding capacity subsequent to incineration suggests that organic material within the sand adds little to its water-holding capacity (Salisbury, 1952). Dune sands have low water retention properties and sand water-holding capacities measured by various authors range from 1.5 to 7% ($^{w}/_{w}$) (Salisbury, 1952; Oosting, 1954; Ranwell, 1972; van der Valk, 1974; Huikes, 1979; Peter, 2000). The water content of the sand up to about one meter above the water table is increased by capillary rise (Ranwell, 1959; Packham and Willis, 1997). In addition the water table fluctuates with the tide. Fresh water floats on seawater and is driven vertically by the rising tide but has a lag of about forty-eight hours in the rise and fall within the dune compared with the tidal rise and fall (Hill and Handley, 1914, cited in Salisbury, 1952).

The water potential of sand drops dramatically only after the water content has declined to below 1% (Buckman and Brady, 1969). Salisbury (1952) observed that annuals on the Braunton dunes in the United Kingdom (U.K.) wilted once water content declined to 0.5%($^{w}/_{w}$). He thus concluded that most of the soil water between the field capacity and 0.5% is available to dune plants.

Water inputs

Four sources of water may be available to beach plants: rainfall, fog and dew, a water table within the root zone and internal dew formation (Barbour *et al.*, 1985).

Rainfall

The distribution and absolute amount of rainfall on beaches of the eastern coast of South Africa are highly variable and are related to latitude (Stone *et al.*, 1998). The East Coast of South Africa is subject to a transition in climate from Mediterranean in the southeast to sub-tropical in the northeast. This results in changes in seasonal variation, temporal distribution and the magnitude of annual rainfall at different locations. The substrate because of its course texture and low organic content has poor water-holding capacity and hence the distribution of rainfall may be more important than the total annual rainfall (Fitter and Hay, 1987).

Fog and Dew

Fog which has been considered an important water source in some dune systems (Barbour *et al*., 1985) is infrequent on the beaches of the Eastern Cape (Tinley, 1985). Data for the period 1990 to 1999 from the SAWB recorded at 8:00hrs in East London showed that on average 1.2 ± 0.8 days month^{-1} had fog. Furthermore by 14:00hrs 75% of these fogs had dissipated.

Any fog or dew that wets the plants and surface layers of the dune sands is rapidly lost during the first few hours of sunshine (*pers. obs.*). Although this may be of limited importance as a source of root water the evaporation of dew from the plant surfaces rather than that of plant water may represent an important water saving. Dew drips from leaves and run-off down the stem directly into the soil would need to penetrate through the sand to depths greater than 10cm before it would be available to plant roots. As the surface layers of sand are devoid of roots and are highly mobile (and thus represent conditions that are not conducive for root formation) it is unlikely that water acquisition in this manner is important.

Internal dew formation

Olsen-Seffer (1909) introduced the term ‘internal dew formation’ to describe the process whereby water vapour diffuses from the underlying water table vertically and condenses within the dune below a cold surface sand layer. Salisbury (1952) presented evidence that this process is a significant source of water for dune plants. Subsequent experiments conducted during a period of no rain showed that the upward movement of water maintained sand water potentials of –0.2 to –0.5 MPa in comparison to –1 to –1.5 MPa measured in sand when the upward movement was prevented (De Jong, 1979). The water-holding capacity of the sand is low and hence relatively small fluxes of water could account for the maintenance of the soil water potentials. This small flux in water vapour implies a limited volume of supplied water for use by the plants. This small volume was estimated to be sufficient to maintain small shallow rooted annuals and perennials on the Braunton dunes (Salisbury, 1952) but similar estimates have not been made for large tropical and sub-tropical dune species.

Ground-water

Ground-water or dune aquifers are a feature of many dune systems (De Jong, 1979; Packham and Willis, 1997; Grootjans *et al*., 1998) and ground-water is certainly available in coastal dunes on the south eastern Cape coast of South Africa. A large Cenozoic deposition indicative of coastal aquifers is centred in Algoa Bay and extends at least 100 km up and down the coast (Campbell *et al*., 1992). This aquifer is used to supply water to several of the small coastal towns including Boknes, Cannon Rocks, Alexandria and Port Alfred. The depth of this aquifer below the surface appears to be variable and depends on the permeability of the sand or gravel, the thickness of the deposit, the width of the dune field and the height of the dunes (Campbell *et al*., 1992). Whether indigenous dune plants utilise aquifer water is unknown in southern Africa.

Water losses

High temperatures, high light intensities, strong wind and open canopies all contribute to high evaporative demand (Barbour *et al*., 1985). Evaporative demand results in water loss both directly from the sand and as transpiration from the vegetation.

Direct evaporation

According to Hesp (1991) direct evaporation from the sand surface may be important only until the surface layers have dried and produce a thermal over-blanket which can markedly reduce subsequent water loss (Hyde and Wasson, 1983).

Transpiration rates

The leaf to atmosphere vapour pressure deficit and leaf conductance determine water loss via plants (transpiration). The leaf to atmosphere vapour pressure deficit defines the water concentration gradient from the leaf to the atmosphere. Leaf temperature and the water potential of the evaporative surfaces determines the concentration of water in the intercellular spaces. If it is assumed that the water potential of the evaporative surface is zero and that the cells are turgid then the vapour pressure of the intercellular space is the saturation vapour pressure at leaf temperature (Beadle *et al*., 1993). However cells of transpiring leaves often range between –1.0 to –6.0 MPa but this introduces only a small error (Kramer, 1983).

Leaf conductance is largely determined by stomatal conductance which is regulated by the plant in response to several parameters. Stomatal response and the resultant effect on gas exchange are considered in some detail in Chapter 7. When leaves possess thick waxy cuticles as is the case with dune plants cuticular transpiration is low (Willis and Jefferies, 1963; Schonherr, 1982; Fitter and Hay, 1987). In addition to the controlled conductance of water efflux offered by the stomata the boundary layer of moisture-laden air surrounding the leaf causes a reduction in conductance. The magnitude of the boundary layer conductance is determined by wind-speed and leaf morphology (Nobel, 1981).

Canopies consisting of many leaves have additional factors affecting transpiration. These include increased boundary layer resistance which is determined by canopy structure (Baldocchi, 1993). Dune pioneer species have open canopies which are less affected by canopy boundary layers (Peter and Ripley, 2001) and this in combination with the frequently windy conditions encountered at the beach simplifies the canopy response.

Leaf transpiration and whole-plant water loss have been measured for a range of dune plant species. Salisbury (1952) by measuring the weight loss of eight small potted dune species (annuals and perennials) estimated the average transpiration rate as 2.0 mmol H_2O m^{-2} s^{-1}. More recent studies using porometers and infra-red gas analysers have provided data on both peak and average transpiration rates. Examples include De Jong (1978), Pavlik (1983a, 1985), Pammenter (1985) and Peter and Ripley (2001). The species researched include a range of C_3 and C_4 grasses, annuals and long-lived perennials. These species were subjected to various conditions and hence a wide range of transpiration rates have been recorded which reveal no obvious characteristics unique to dune species.

Water Use Efficiency

In order to obtain CO_2 plants require large wet internal surface areas and these surfaces are susceptible to evaporative water loss (Cowan, 1982; Kramer, 1983). The amount of carbon gained per unit of water lost equates to water use efficiency (WUE). Water use efficiency may be determined instantaneously by comparing photosynthetic and transpiration rates (A/E) or can be determined over a longer time scale by comparing total plant dry mass gain to water loss. Szarek and Ting (1975) list typical water use efficiency ranges for C_3, C_4 and CAM plants as 1.67 to 2.2, 2.9 to 4, and 6.7 to 40 mmol CO_2 mol^{-1} H_2O respectively. In addition to differences between plants with different photosynthetic types plants from arid environments have higher water use efficiency than plants from mesic environments (Caldwell, 1985; Ehleringer and Cooper, 1988). However not all xeric species have high water use efficiency. Several studies have shown that the more xeric species keep their stomata open for longer during drought resulting in decreased water use efficiencies (Bunce *et al.*, 1977; Hinckley *et al.*, 1979).

Water use efficiencies of a limited number of dune species have been reported. De Jong (1978) reported laboratory measured values of 7.3, 7.7, 7.1 and 10.7 mmol CO_2 $mol^{-1}H_2O$ for *Atriplex leucophylla* , *Cakile maritima, Ambrosia chamissonis* and *Abronia maritima* respectively. Dubois (1977, cited in Barbour, 1985) compared WUE of C_3 and C_4 beach grasses in Georgia over a summer season and measured average values of 7.7 and 10.7 mmol CO_2 $mol^{-1}H_2O$ respectively. Zhang (1996) investigated the effects of sand-burial and nutrient supply on the normalised WUE of greenhouse grown *Cakile edentula*. Values are also available for greenhouse grown *Cakile maritima* (Holton, 1980) and *Ammophila arenaria* and *Elymus mollis* (Pavlik, 1983a). The only reported values for a sub-tropical dune species are for potted *S. plumieri* (4.5 mmol CO_2 $mol^{-1}H_2O$ measured at a VPD of 1 kPa; Pammenter, 1985) and for *A. populifolia* where leaf hairs were shown to increase water use efficiencies (Ripley *et al*., 1999). Hence a comparison of values measured for dune species in this study with reported values for other dune species and xeric species is of interest.

Plant Water Potential

Water potential is defined as the difference in free energy of the water in a system. It is determined by osmotic, matrix and pressure constraints and the free energy of pure water (Slayter and Taylor, 1960). Both plant and leaf water potentials must be lower than soil water potentials if water is to be continually taken up from the soil. Leaf water potentials are the result of soil water potentials (water availability), hydraulic conductance and transpiration rates. Because of this, leaf water potentials need to be considered in combination with rates of transpiration. Low leaf water potentials during periods of reduced transpiration and the lack of a nocturnal or pre-dawn reduction in leaf water potential are indicative of low soil water availability or low hydraulic conductance.

A number of studies have recorded leaf water potentials of dune plants (De Jong, 1979; Dubois, 1977, cited in Barbour, 1985; Pammenter, 1983; Pavlik, 1985; Yaun *et al*., 1993). Recorded values range from –0.3 to –2 MPa and none reach the very negative potentials measured for plants from xeric environments.

Components of plant water potential

Techniques for measuring, the theoretical consideration of and the potential limitations in assessing the components of plant water potential have been extensively discussed (See Slayter and Taylor, 1960; Tyree and Hamel, 1971; Sinclair and Venables, 1983; Koide *et al*., 1989; Boyer 1995; Tyree, 1999). The components of plant water potential include osmotic and turgor potential. Turgor potential is of importance when considering plant function as growth and cell extension is possible only when turgor is positive (Bradford and Hsiao, 1982; Boyer, 1995). In addition to which short-term productivity is reliant upon the maintenance of stomatal conductance to CO_2. High stomatal conductance relies on the maintenance of plant water status a condition accompanied by positive turgor (Passioura, 1982). The elasticity of plant cells determines the relative amount of water that leaves can lose before turgor is lost (Boyer, 1995).

Osmotic potential is determined by the concentration of osmotically active substances in the cells and is affected by the uptake of solutes, the production of osmotically active substances through metabolism and the reduction of cell water content by transpiration (Bewley and Krochko, 1982). Some plants have been shown to actively osmoregulate accumulating osmotically active substances such as proline, sorbitol and glycinebetaine in order to maintain leaf water potentials that are more negative than soil water potentials (Hsiao *et al*., 1976; Tyree and Jarvis, 1982; Morgan, 1984). The maintenance of stomatal conductance following osmotic adjustment results in increased net carbon gain as has been demonstrated for *Amaranthus palmeri* (Ehleringer, 1983).

Osmotic adjustment has been demonstrated for a range of coastal plants (Stewart *et al.,* 1979; Gorham *et al*., 1981) by directly assessing accumulated solutes. In addition osmotic adjustment of the dune plants *Ammophila arenaria* and *Elymus mollis* was demonstrated using pressure-volume analysis (Pavlik, 1985). Pavlik (1985) also showed that the diurnal variations in these species' leaf water potentials was mainly as the result of altered turgor potential and not osmotic potential and that the osmotic adjustment was related to longer term seasonal changes.

No information is available for osmotic adjustment and changes in the cell elasticity related to turgor maintenance for subtropical and tropical dune species. The determination of leaf water potential ($\Psi_{(leaf)}$), turgor potential (Ψp) and osmotic potential ($\Psi\pi$) are therefore crucial to understanding the performance of a plant under potentially water limited conditions (Passioura, 1982).

Source of plant water

Plant roots generally have access to two types of water (i) ground-water which includes water in the saturated soil zone and (ii) seasonal soil water derived from rain. Because there is no isotopic fractionation of water during its uptake at plant roots (Dawson and Ehleringer, 1991) and provided that rain-water and ground-water are isotopically distinct the relative amounts of these two types of water taken up by plants can be quantified (White, 1988). Many such studies have considered the differences in deuterium to hydrogen ratios between rain-water and ground-water and the water contained in plants. Examples of such studies include Schiegl and Vogel (1969), White *et al*. (1985), Dawson and Ehleringer (1991) and Flanagan *et al*. (1992). As with deuterium to hydrogen ratios the ratios of ^{18}O to ^{16}O of rain-water and ground-water are often distinct (Gat, 1980) and can be used to differentiate between these sources of plant water (Ehleringer and Dawson, 1992). Ground-water typically shows a stable isotopic ratio whilst rain-water is both affected by season and magnitude of rainfall (Gat, 1980). The seasonal influence is due to a differential effect that temperature has on the relative evaporation rates of the two isotopes (^{16}O and ^{18}O). This results in summer rain-water having more heavy isotopes than does winter rain-water. Ground-water is not subjected to evaporation and hence reflects the weighted average of annual precipitation inputs. In addition to enrichment by evaporation ocean waters are enriched with heavy oxygen and hydrogen isotopes and any mixing of fresh aquifer-water with sea-water is likely to result in water with ratios that are distinct from fresh-water or rain-water (Ehleringer and Dawson, 1992). This should allow the identification of this water as a source for dune plants.

No direct information on the source of plant water nor of the relative utilisation of rain-water and ground-water is available for dune plants. Indirect evidence such as observations of rooting depths (Salisbury, 1952; Wilson, 1977; De Jong, 1978; Martinez and Moreno-Casasola, 1998) and the effects on species compositions following hydrological perturbations (Grootjans *et al*., 1998) suggests that dune species are able to utilise ground-water.

Estimates of volume of water available to plants

Two authors have previously made estimates of the amounts of water in dune sands that are available to plants. By determining weight loss from potted plants Salisbury (1952) measured the transpiration rates of eight species of dune plants (mainly annuals) and compared these values with the amount of water available in the volume of dune sand exploited by the plants. Using a sand-water content of 4% he estimated that there was sufficient water for only about 3 to 5 days of transpiration at the measured rates. However the plants were observed growing on dunes where there had been no rain for up to 6 weeks. Based on measurements of the increases in sand-water content from day to night Salisbury (1952) calculated that the process of internal dew formation supplied sufficient water to sustain the measured transpiration rates.

Pammenter (1983) constructed water budgets using measured transpiration rates for *S. plumieri* growing at Mtunzini on the north coast of KwaZulu Natal. Using average transpiration rates, a sand-water content of 5% and assuming that plants utilised sand to a depth of only 2 m he estimated that the plants growing on a square meter of dune surface could be sustained for 54 days by the volume of water contained in the sand. In addition to this the annual rainfall at Mtunzini exceeded estimates of annual volumes of water transpired and led Pammenter (1983) to conclude that it was not necessary to invoke internal dew formation as a water source for the plants.

To investigate the sufficiency of water supply in relation to transpiration losses both annual and monthly water budgets were calculated.

Adaptive features affecting plant water relations

Dune plants have many adaptive features that have been attributed to water and other correlated stresses. Water stress in dune systems is often accompanied by high light intensity, high temperature and wind exposure (Barbour, 1990; Hesp, 1991). Adaptive features include: small leaves, leaf rolling, paraheliotropism, slow growth, high root to shoot ratios, leaf pubescence, epicuticular wax layers, succulence, accumulation of solutes, efficient water use, various root adaptations and aerodynamically appropriate growth forms (Rozema *et al.*, 1985; Barbour, 1990; Hesp, 1991). Barbour (1990) cautions that some of these may be in response to limited nitrogen availability and not to water stress.

Little research has been conducted to establish what physiological function many of these adaptations confer. Many supposed functions have been extrapolated from studies of plants in other ecosystems. Examples being succulence and the presence of leaf hairs. A particular adaptation may in fact be in response to a number of stresses. Ripley *et al.* (1999) showed that the leaf hair layer of *A. populifolia* not only increased water use efficiency but also increased productivity by reducing photoinhibition. The measurements made in this investigation may reveal the physiological function of some of these adaptations.

Root Systems

Dune species generally have high root to shoot ratios (Packham and Willis, 1997) and have evolved various rooting patterns for utilising water resources. A C_4 species such as *Atriplex leucophylla* with a short taproot and extensive fine shallow roots develops a relatively negative midday leaf water potential that relaxes slowly during the latter part of the day (De Jong, 1979). In contrast *Abronia chamissionis* has a large taproot in excess of a meter in length and exhibits little seasonal fluctuation in leaf water potential. It does not develop very negative midday leaf water potentials. Leaf loss may also help to contain seasonal variations in the leaf water potential of this species. A third and intermediate strategy with regard to water relations is the possession of intermediate root lengths utilised by species such as *Abronia maritima* and *Cakile maritima.* Salisbury (1952) recognised three different rooting depths with annuals rooting between 5 and 20cm, small herbaceous perennials with roots extending deeper than 1m and large perennials rooting to even greater depths. *Chamaecrista chamaecristoides* growing *in situ* in the Gulf of Mexico has long roots with few ramifications reaching great depths (Martinez and Moreno-Casasola, 1998). Other southern Californian perennials such as *Ambronia maritima* and *Ambrosia chamissonis* also have long taproots (Fink and Zedler, 1990).

The rooting patterns of the species investigated in this study were not quantified but several observations are of relevance. *I. pes-caprae* and *S. plumieri* are deep rooted and excavations showed that their roots could be found down to depths of 3m at which point the permanent water table is encountered. As sand accretes and the dune height is increased *S. plumieri* produces adventitious roots from buried stems. *I. pes-caprae* has been reported to produce some roots that penetrate into the dune vertically to great depths (Wilson, 1977). This plant also has long surface runners which produce roots at nodes. *A. populifolia* is more shallowly rooted and roots extend down to only about one metre although there is considerable variation. The central taproot of *A. populifolia* often appears to die back at depths greater than half a metre and it is the adventitious roots that appear viable at greater depths. On burial the stem produces adventitious roots. These species unlike annuals of temperate dunes (Salisbury, 1952) have no roots in the top 10 cm of sand. The more stable supply of water in the sand at depths greater than 10 cm is what is of significance to the deeper-rooted perennials investigated in this study.

METHODS

Climate measurements

Ambient temperature and relative humidity were recorded and used to calculate atmospheric vapour pressure deficit (VDP) as explained in the previous chapter (Climate measurements, Chapter 4).

Transpiration rates

Beginning pre-dawn and continuing until after sunset gas exchange measurements were made at approximately two-hour intervals on single leaves of three individual shoots or plants of each of the species. For details of leaf selection, measurements and calculations see the previous chapter (CO_2 assimilation, Chapter 4). The LCA-II IRGA was calibrated for RH using a dew-point apparatus. Under most field circumstances the LCA-II (ADC, Hoddesdon, U.K.) over-estimates transpiration rates because it pumps dry air (desiccated with magnesium perchlorate) over the leaf, substantially increasing the leaf to atmosphere water concentration gradient. This dry air is equivalent to an atmospheric relative humidity of 0%. However measured values for atmospheric relative humidity were never below 35%. To correct for this over-estimation 20 measurements were made on individual leaves of different plants of each of the three species using both an LCA-II and a LiCOR 6400 (LiCor, Lincoln, Nebraska). The LiCOR 6400 passes un-dried atmospheric air over the leaf. Measurements were made at different times of the day on the 27/6/2000 so that a wide range of transpiration rates were recorded. Linear fits were used to compare the two instruments and to correct all LCA-II transpiration data. In addition to the data collected in this study data from Peter (2000) for *S. plumieri* collected at Old Woman's River, Port Alfred and Port Edward (1997 to 1999) has been included (for which C. Peter is gratefully acknowledged). Peter's data was collected from a range of leaves chosen from various points along the stem but excluded the youngest and oldest leaves. Measurements were made with an LCA-II (ADC) and were corrected as is outlined above. Transpiration rates were expressed on a leaf area basis as mmol H_2O m^{-2} s^{-1}.

Average volumes of water transpired m^{-2} leaf area day^{-1} were calculated by integrating the areas below the diurnal responses of transpiration rate. Where appropriate these were converted to litres of water lost m^{-2} dune surface area day^{-1}, using the equation:

$$\text{Volume transpired } (\text{l m}^{-2} \text{ dune area day}^{-1}) = \frac{\text{volume of water transpired } (\text{mol m}^{-2} \text{ leaf area day}^{-1}) \text{ x LAI}}{18 \ (\text{g mol}^{-1}) \text{ x } 1000 \ (\text{g l}^{-1})}$$

LAI (Leaf Area Index) values are given in Table 3.6.

Water use efficiency was calculated according to the formula:

$$\text{Water use efficiency (WUE)} = \frac{\text{Assimilation rate (A, } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1})}{\text{Transpiration rate (E, mmol H}_2\text{O m}^{-2} \text{ s}^{-1})}$$

This was calculated both for instantaneous data and data integrated over the entire day. When the latter was calculated by integrating the area below diurnal assimilation and transpiration curves the amounts of CO_2 assimilated and water transpired were only calculated for that period of the day when CO_2 assimilation rates were positive.

Leaf water potentials

Leaf water potentials were measured at the same time intervals on leaves or shoots. Shoots with orientations similar to those shoots used for gas exchange and chlorophyll fluorescence were measured. A Schönlander pressure-chamber (own design, constructed by Grahamstown Engineering, Grahamstown, South Africa) was used to measure leaf water potentials of *A. populifolia* and shoot water potentials of *I. pes-caprae* and *S. plumieri*. *A. populifolia* has stems that are succulent, soft and unsuitable for sealing in a pressure chamber and so individual leaves were used for this species. Both leaf (*A. populifolia*) and shoot (*I. pes-caprae* and *S. plumieri*) water potentials are referred to as $\Psi_{(leaf)}$ throughout.

One of the basic assumptions of this technique is that the osmotic potential of xylem-water is zero. Measurements of the osmotic potential of the xylem-water exuded under pressure were made with a HR-33T psychrometer and a L-51A leaf-chamber (Wescor Instruments). Filter paper was wetted with the exudate and inserted into the chamber. Following a period of equilibration at constant temperature psychrometric measurements were made. Values of –0.137, 0.004 and –0.087 MPa were measured for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. These values are acceptably small relative to the leaf water potentials measured with the pressure-chamber.

Pressure-volume curves

At the end of each experimental day at least three plants from each species were harvested, had their stems cut under water and were left to rehydrate overnight. The following day these plants were used to construct pressure-volume curves according to the methods described by Boyer (1995). Leaf or shoot water potentials of the rehydrated plants were recorded and then leaves or shoots were left to lose water by transpiration before subsequent balance pressures were re-measured. This was repeated until water potentials were linearly related to water loss with at least five points on the straight line. Pressure-volume curves were fitted with the non-linear regressions of Sinclair and Venables (1983), as follows:

$$\Psi_{leaf} = a \text{ x } [(1/(1-(1- RWC +d)/b)) – \exp (-c \text{ x } (1- RWC +d))]$$

Where: a = osmotic potential at full turgor ($\Psi\pi$,0); b = ratio of symplastic water to total leaf water ; c = curvature factor exponentially relating Ψp and RWC; d = correction factor for leaf water potential at full turgor and RWC = relative water content.

$$\text{RWC} = \frac{\text{(leaf or shoot rehydrated wt.} - \text{dry wt.)} - \text{accumulative wt. of water lost}}{\text{(leaf or shoot rehydrated wt.} - \text{dry wt.)}}$$

Parameters a, b, c and d were estimated for each individual pressure-volume curve by non-linear curve fitting using Statistica '99 Edition, (© Statsoft, Inc., USA).

Each individual replicate (see footnote [5.1] for replication details) was fitted by the procedure described above and the fitted functions used to derive $1/\Psi$ values at particular RWCs. The same RWCs were chosen for all the replicates of a particular species such that average values and standard deviations could be calculated from the derived values for each species and experimental day (Figure 5.5).

Significant points were extrapolated or calculated for each individual pressure-volume curve. Points included values at full turgor ($\Psi\pi$,0 and c) and values at the turgor loss point (RWC, $\Psi\pi$).

$\Psi\pi$ was calculated according to the equation $\Psi\pi = a / \text{RWC}$ (modified from Sinclair and Venables, 1983. See results section for explanation). The RWC at turgor loss point was estimated visually from the plotted curves and the $\Psi\pi$ at this RWC calculated according to the equation: $\Psi\pi = a / \text{RWC}$.

Diurnal changes in the components of leaf water potential

Diurnal changes in the components of leaf water potential ($\Psi_{\text{leaf}} = \Psi\pi + \Psi p$) were calculated by interpolating the leaf RWC corresponding to each Ψ_{leaf} from a combined pressure-volume curve constructed for each species. Justification for combining data from different experimental days for each of the species is given in the results section below. Combined curves were fitted according to the methods of Sinclear and Venables (1983). Fitted parameters and interpolated RWC were used to calculate osmotic

---

[5.1]

	Number of replicate pressure-volume curves constructed on indicated day				
Species	30/1/97	10/9/97	19/2/98	23/7/98	15/9/98
A. populifolia	3	3	5	4	3
I. pes-caprae	4	3	4	3	3
S. plumieri	2	3	3	3	3

potentials according to the modified equation: $\Psi\pi = a / RWC$. Turgor potentials were calculated according to the following formula: $\Psi p = \Psi_{leaf} - \Psi\pi$.

Leaf water potentials of plants growing on a small isolated dune

Excavation of *A. populifolia*, *I. pes-caprae* and *S. plumieri* showed that these three species have extensive underground connections to other shoots and that this was particularly so for the latter two species. This raised the question as to whether these underground stem connections were functional or merely the remnants of these plants growth habits. To investigate this a small dune containing both *I. pes-caprae* and *S. plumieri* was isolated from a larger adjacent foredune by digging a trench around it. All below-ground stems connecting the two dunes were identified. Leaf water potentials for *I. pes-caprae* and shoot water potentials for *S. plumieri* were measured for plants growing on the smaller and larger dunes and then the underground connecting stems were cut. For the subsequent 3 to 4 days (19 to 22 February 1998) leaf or shoot water potentials were measured on both dunes at 14:00 and 19:00 hrs. Measurements were not replicated for *S. plumieri* due to the small number of shoots occupying the small isolated dune. Whole shoots are required for measuring the water potentials of *S. plumieri* as the leaf petioles are unsuitable for pressure-chamber measurements. Triplicate measurements were made on *I. pes-caprae* as individual leaves could be inserted into the pressure-chamber.

$\delta\,^{18}O$ values of rain, ground and stem water

In order to investigate whether plants were using rain-water or ground-water the $^{18}O/^{16}O$ ratios of rainwater and groundwater were compared to the ratios of water that was vacuum distilled from below-ground stems. All $^{18}O/^{16}O$ ratio determinations were performed by Ematek (Council for Scientific & Industrial Research, Pretoria, South Africa). $^{18}O/^{16}O$ ratios are reported as δ values, that is, deviations from the international standard Vienna SMOW (Standard Mean Ocean Water). This sample is set to 0.0 $^{0}/_{00}$.

Deviations are reported as:

$$\delta(^{0}/_{00}) = \frac{(R_{standard} - R_{sample})}{R_{standard}} \times 10^3$$

Where R is the isotopic ratio of $^{18}O/^{16}O$.

Ground-water was collected via a PVC plastic pipe (50mm I.D.) which was buried vertically 3m deep at the base of a sand dune central to the study site. The buried end of the tube was closed with fine nylon mesh and this was protected with a layer of coarser plastic mesh. The end of the tube was permanently below the level of ground-water. Samples were collected on 9/9/97, 21/2/98, 8/6/98, 19/7/98, 23/7/98 and 14/9/98, by lowering a weighted boiling-tube down the plastic pipe. Collected samples (150 ml) were sealed in boiling-tubes with rubber bungs and tightly wrapped with insulation tape.

Rain-water was collected on numerous occasions (see Figure 5.8 for dates) at Kleinemonde approximately 5 km from the study site and sealed in glass bottles. P. Cowley is gratefully acknowledged for performing this task. *S. plumieri* stem water was vacuum distilled directly from five underground stem sections collected from individual shoots on each of the days 9/9/97, 21/2/98 and 22/7/98. Stem sections approximately 150mm in length were excised and immediately sealed in boiling-tubes stoppered with rubber bungs and wrapped with insulation tape. Stem sections were sent to Ematek where the vacuum distillations were performed.

The isotopic signal of rainfall is affected by the magnitude of the rainfall event (Gat, 1980) and thus a weighted average value for rainfall was calculated. The $\delta\ ^{18}O$ value of each rain sample was plotted against the magnitude of the rainfall event and yielded a straight line ($r^2 = 0.66$). This relationship was used to predict $\delta\ ^{18}O$ values for all the rainfall events during the period when the isotopic study was conducted (August 1996 to October 1998) and an average value was calculated.

Water budgets

Investigation of the sufficiency of water supply in relation to transpirational losses required that both annual and monthly budgets be calculated for the three species investigated in this study in high and low rainfall years. Annual water budgets and estimates of sand-water content and the lengths of time that such water content can sustain transpiration rates are useful. However they do not take into consideration the dynamic changes that occur on a day to day basis as the result of rainfall inputs and transpirational losses. To investigate these dynamic changes the daily changes in sand-water content (Capacitance Budgets) were estimated for the three species assuming various starting conditions.

Annual and monthly water budgets

A statistically significant correlation between transpiration rate and VPD (Figure 5.2) allowed daily, monthly and annual water losses to be predicted from climatic data. VPD is calculated from relative humidity and ambient temperature (see Chapter 4). Average relative humidity and temperature were used to calculate average daily VPD. This was then used to predict average daily transpiration rate using the regressions of E against VPD (Figure 5.2). The average daily transpiration rate was integrated over 24 hours to predict average daily volume of water transpired. Integrating transpirational water loss over 24 hours makes the assumption that nocturnal stomatal closure does not occur in these plants. This assumption is supported by the fact that all three species have positive pre-dawn and early evening leaf conductances and transpiration rates (Figure 5.1). Furthermore the pre-dawn and evening data was included in the analyses of the relationship between E and VPD (Figure 5.2). Average daily ambient temperature and relative humidity measurements are available for Port Alfred from the South African Weather Bureau (SAWB). Port Alfred is a coastal town situated approximately 15 km to the south west of the Old Woman's River study site which was assumed to have similar climatic conditions.

Annual and monthly water budgets were calculated by comparing rainfall inputs to transpirational water losses. Water losses predicted for single leaves were scaled from leaf to one m^2 of dune surface using the leaf area indices (Table 3.6) listed in Chapter 3. Annual budgets were calculated for an average (1978 to 1998, 618 mm), a wet (1994, 693 mm) and a dry (1997, 546 mm) year whilst monthly budgets were calculated for 1994 and 1997. The following assumptions were made when calculating the budgets (i) the plants occupying one m^2 of dune were assumed be part of a greater continuous homogenous canopy so that the water available to the plants is primarily from the column of sand below that one m^2 surface area and (ii) leaf and canopy transpiration rates were assumed to respond in the same way to changing VPD. This second assumption has been tested for *S. plumieri* by Peter (2000) and Peter and Ripley (2001) who showed that whole shoot water loss when measured with the shoot *in situ* in the canopy was the same as leaf water loss when both were expressed per unit leaf surface area. This was attributed to the open nature of the *S. plumieri* canopy and the frequent windy conditions on the beach which reduces boundary layer effects. Like *S. plumieri*, *A. populifolia* and *I. pes-caprae* have open canopies and it is likely that canopy boundary effects are unimportant.

Capacitance budgets

Rainfall rapidly percolates into the dune sand and this water is available for plant use.
As mentioned in the introduction the water-holding capacity of beach sand ranges between 1.5 and 7% ($^{w}/_{w}$) (Salisbury, 1952; Oosting, 1954; Ranwell, 1972; van der Valk, 1974; Huiskes, 1979) and water potentials are only dramatically decreased when the sand-water content is reduced to around 0.5%($^{w}/_{w}$). At this point beach plants have been observed to wilt (Salisbury, 1952). The water between these limits is likely to be available to plant roots. This necessitates consideration of the role of the sand as a water store (capacitor) which may buffer periods when the plants transpire greater volumes of water than is available via rainfall. In order to investigate this, daily compounded sand-water content was calculated for a particular volume of sand (see below) by assuming a starting content to which daily rainfall was added and from which daily transpirational losses were subtracted. The compounded sand water content was not allowed to exceed the sand-water holding capacity (field capacity).

The starting sand-water content and the water-holding capacity used were equal and the budget was repeated using three different water content values 1.5, 4 and 7% ($^{w}/_{w}$). This was based on the range of 1.5 to 7% quoted in the literature (Salisbury, 1952; Oosting, 1954; Ranwell, 1972; van der Valk, 1974; Huiskes, 1979). In addition sand-water content was established empirically by digging holes between 1 and 3.5 metres deep in non-vegetated dunes at various beaches (Port Alfred, Kenton–on-Sea and Old Woman's River) and collecting sand samples at progressive depths of 250mm. The sand samples were enclosed in double plastic bags and returned to the laboratory. On two of the five occasions when holes were dug it had rained on the one or two days prior to the sampling and thus sand-water contents are likely to be good estimates of the sand water-holding capacity. Wet weights were established and then samples were dried to constant weight at $60^{o}C$ and percentage soil water content calculated on a mass to mass ($^{w}/_{w}$) basis. Below the initial dry layer (100mm) sand-water content was 4.28 ± 0.96 % ($^{w}/_{w}$) and remained at this content until depths approached the water table.

In addition to the above assumptions when constructing the capacitance budgets the following assumptions were made:

i) A sand bulk density of 1.3 kg l^{-1} was assumed (Pammenter, 1983).

ii) *A. populifolia* which is relatively shallow rooted was assumed to root to a depth of one metre and hence the plants occupying one m^2 of dune surface area would use one m^3 of sand volume.

iii) *I. pes-caprae* and *S. plumieri* are much deeper rooted and were assumed to extend their roots 3m down to the water table and hence the plants occupying one m^2 of dune surface would use 3 m^3 of sand volume. Roots of all three species have been encountered at these depths during various excavations on the beach.

iv) The loss of water directly from the sand surface was assumed to be minimal due to resistive and insulating properties created by the dry surface layer of sand, a phenomenon discussed by Hesp (1991).

Statistics

Where appropriate data were compared using an analysis of variance (ANOVA) followed by Tukey tests if ANOVA showed significant differences at the 95% confidence level using the program Statistica '99 Edition, (© Statsoft, Inc., USA). Statistical model assumptions were tested for normality and square root transformations undertaken where necessary. The dependence of transpiration rates and instantaneous water use efficiencies on various abiotic variables was assessed using a multiple stepwise regression (Statistica '99 Edition, © Statsoft, Inc., USA). Non-parametric data from pressure-volume curves was compared using a Kruskal-Wallis ANOVA.

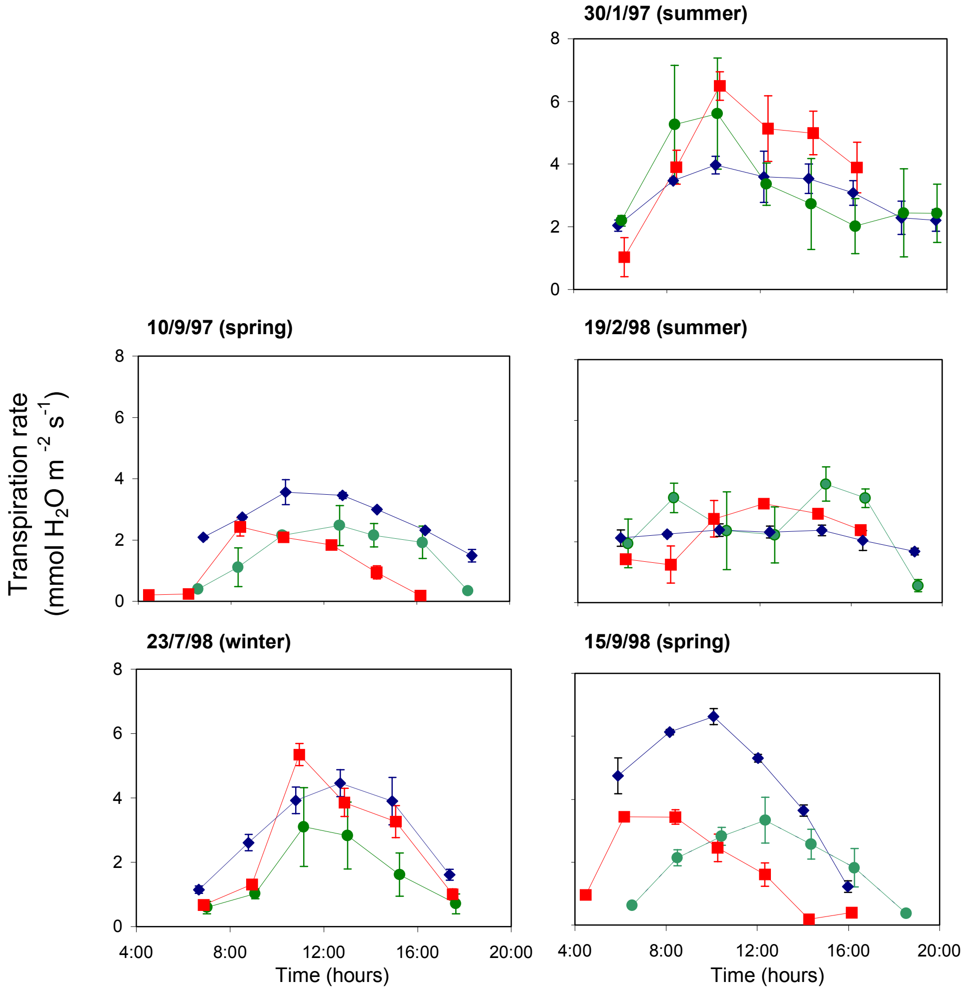


Figure 5.1: Diurnal changes in transpiration rates of *A. populifolia* (diamonds), *I. pes-caprae* (circles) and *S. plumieri* (squares) for the indicated experimental days. Standard deviations are indicated by the vertical bars. N=3.

RESULTS

Transpiration

Transpiration rates are a function of the leaf to atmosphere vapour pressure deficit and the leaf conductance. Vapour pressure deficit is dependent on leaf temperature, ambient temperature and the relative humidity. As the day proceeds, the co-variants incident light intensity, leaf temperature, ambient temperature, VPD and relative humidity alter. The former four increasing to maxima around midday before declining to minima after dark. Relative humidity produces a reciprocal response (Figure 3.4 & 3.5). As a result of this, driven by the leaf to atmosphere vapour pressure gradient, transpiration rate increased from dawn to peak around midday and then decline until darkness. This typical pattern was evident on all experimental days in all species (Fig 5.1) with the exception of the response by *I. pes-caprae* on the 30/1/97 (summer) and 19/2/98 (summer) where midday reductions of stomatal conductance produced reductions in transpiration rates. This was far more pronounced on the 19/2/98 (summer) resulting in a diurnal response with two peaks.

Table 5.1: Average peak transpiration rates for the three species on days in the indicated seasons. If values obtained from the same species on different days were significantly different this is indicated by the bracketed numbers. The days are numbered as (1) - 30/1/97 (summer), (2) - 10/9/97 (spring), (3) -19/2/98 (summer), (4) -23/7/98 (winter) and (5) - 15/9/98 (spring). N=3. Peak transpiration rates are defined as the highest points on diurnal responses of transpiration rates (Figure 5.1)

	Average peak transpiration rates per unit leaf surface area (mmol H_2O m^{-2} s^{-1})		
Experimental day	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
30/1/97 (summer) (1)	3.9 ± 0.8 (5)	5.9 ± 1.2 (2, 3, 4 & 5)	6.1 ± 1.2 (2, 3 & 5)
10/9/97 (spring) (2)	3.6 ± 0.4 (5)	2.6 ± 0.5 (1)	2.4 ± 0.3 (1 & 4)
19/2/98 (summer) (3)	2.4 ± 0.2 (4 & 5)	3.5 ± 0.5 (1)	3.3 ± 0.1 (1 & 4)
23/7/98 (winter) (4)	4.3 ± 0.3 (3 & 5)	3.1 ± 1.2 (1)	5.4 ± 0.3 (2 & 3)
15/9/98 (spring) (5)	6.6 ± 0.3 (1, 2, 3 & 4)	3.3 ± 0.7 (1)	3.4 ± 0.1 (1)
Average	**4.2**	**3.7**	**4.1**
Std. deviation	**1.5**	**1.4**	**1.5**

The results of a two-way ANOVA showed that the differences in peak transpiration rates among species (p=0.0775) were not significant. However differences among experimental days ($p<0.0001$) and for the interaction of species and experimental day ($p<0.0001$) were significant. Average peak transpiration rates of the three species ranged between 2.39 and 6.63 mmol H_2O m^{-2} s^{-1} (Table 5.1). These rates are comparable to those measured for temperate dune grasses (Pavlik, 1985) despite lower average daily temperatures in temperate regions. Within a species there were significant differences between rates measured on the various experimental days (Table 5.1).

For all three species the 30/1/97 (summer) peak rates were significantly different to at least the 15/9/98 (spring) rates. In the case of *A. populifolia* this difference was an increase and in both *I. pes-caprae* and *S. plumieri* the difference was a decrease in rate. Differences do not relate to seasons as may have been expected. Some winter rates exceeded summer or spring rates. There appears to be no consistent pattern in rate between species. The highest average rates were recorded on 15/9/98 (spring), 30/1/97 (summer) and 30/1/97 (summer) for the three species *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively.

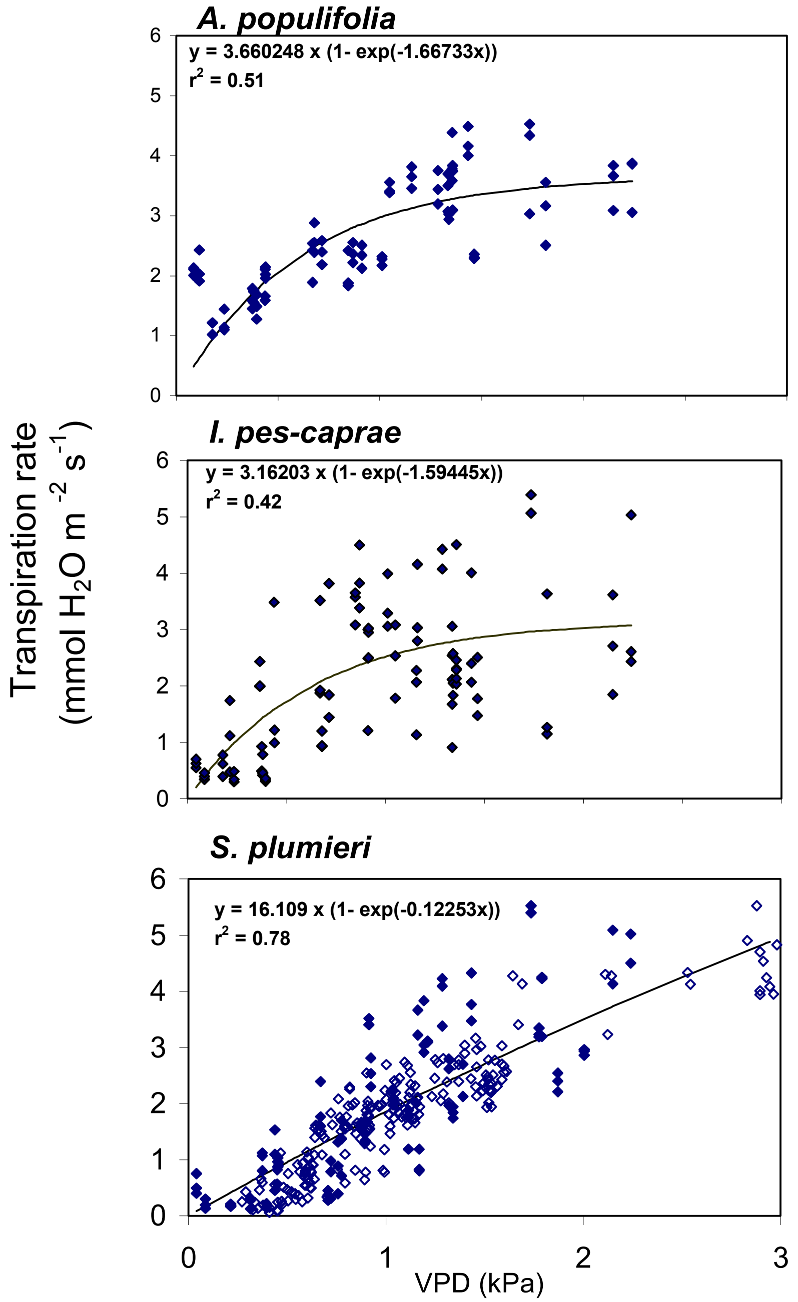


Figure 5.2: Transpiration rates of *A. populifolia, I. pes-caprae* and *S. plumieri* related to atmospheric vapour pressure deficit (VPD; solid symbols). Additional *S. plumieri* data (open symbols) are from Peter (2000). Solid lines are fitted saturation exponential equations. N = 89, 114 & 327 *for A. populifolia*, *I. pes-caprae* and *S. plumieri*, respectively.

The relationship of transpiration rate to VPD (Figure 5.2) may explain the lack of a consistent pattern between seasons. VPD is determined by ambient temperature and relative humidity parameters that did not vary with season as may have been predicted (Figure 3.7). Some winter experimental days maximum ambient temperatures exceeded maxima measured on either summer or spring experimental days and relative humidity showed reciprocal responses (Figure 3.6).

Transpiration rate is the result of the leaf to atmosphere vapour pressure deficit and leaf conductance. If leaf conductance is below maximal and unlimited by stomatal conductance then the response of E to VPD is linear. Above this point when either stomata reduce leaf conductance or maximal leaf conductance is attained then the response of E to VPD saturates. These typical saturated exponential responses were observed for all three species (Figure 5.2). *S. plumieri* for which the most data has been accumulated had the highest r^2 value of 0.78 and the relationship for *A. populifolia* and *I. pes-caprae* yielded lower r^2 values of 0.51 and 0.42 respectively. Linear plots improved the r^2 values to 0.61 and 0.86 for *A. populifolia* and *S. plumieri* respectively but decreased the r^2 for *I. pes-caprae* to 0.33. The lower r^2 value and the greater degree of variability in the data for the response of E to VPD for *I. pes-caprae* was in part the result of stomatal regulation. Midday reductions in stomatal conductance on some experimental days reduced midday transpiration rates (Figure 5.1 & Figure 7.5).

A. populifolia and *I. pes-caprae* showed similar responses to increasing VPD as was evident when the parameters from the fitted equations were compared (Figure 5.2). *S. plumieri* showed less saturation in response to increasing VPD and the parameters of the fitted equation for this species were quite different to those of *A. populifolia* and *I. pes-caprae*. Some physiological explanations for these differences are discussed in Chapter 7. The saturated exponential relationships were used to predict water losses by transpiration from climatic data obtained from the SAWB. These relationships were chosen rather than the linear relationships which yielded higher r^2 values for *A. populifolia* and *S. plumieri.* The linear relationships were abandoned because they had positive y-intercepts implying positive transpiration rates at 0 VPD. This situation is not physically possible. If linear plots of E to VPD were forced through the zero intercept then r^2 values were reduced to – 0.11, 0.2 and 0.66 for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively.

Results from a stepwise multiple regression of E against ambient temperature, leaf temperature, ambient humidity, PPFD and VPD showed that the abiotic variable that accounts for the majority of the variation in E was different for all three species (Table 5.2). These variables are however not independent. For example as light intensity increased both ambient and leaf temperature also increased with a concomitant decrease in relative humidity (Figure 3.6 and 3.7). Data used in this analysis for *S. plumieri* did not include data from Peter (2000). Variables that accounted for the highest proportion of the variation in E were RH, PPFD and VPD for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. The addition of a second abiotic variable (VPD for *A. populifolia*, T_a for *I. pes-caprae* and PPFD for *S. plumieri*) increased the adjusted r^2 values by 5, 23 and 9% for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. The addition of third, fourth and fifth variables resulted in small increases or decreases in the adjusted r^2 value.

Integrated areas below diurnal transpiration rate curves were used to calculate average daily amounts of water transpired. A two-way ANOVA indicated that the differences in the average daily amounts of water transpired among species (p=0.0433), experimental days (p<0.0001) and between their interaction (p<0.0001) were significant.

Table 5.2: Results of a stepwise multiple regression analysis of transpiration rates and the five abiotic variables measured or calculated at the same time that transpiration rates were measured. For each species the variable(s) and the corresponding adjusted r^2 values are listed.

Species	**1 variable**	**2 variables**	**3 variables**	**All variables**
A. populifolia	RH (0.655)	PPFD, VPD (0.691)	PPFD, VPD & RH (0.706)	All (0.699)
I. pes-caprae	PPFD (0.405)	PPFD, T_a (0.498)	PPFD, T_a & VPD (0.502)	All (0.507)
S. plumieri	VPD (0.652)	VPD, PPFD (0.713)	VPD, PPFD & T_l (0.717)	All (0.725)

Abbreviations: Photosynthetically active radiation (PPFD), Vapour pressure deficit (VPD), Relative humidity (RH), Ambient temperature (T_a), Leaf temperature (T_l).

Amounts of water lost per day, averaged for all experimental days, were 124.3 ± 20.6, 107.0 ± 47.7 and 109.9 ± 50.7 mol m^2 day^{-1} for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively (Table 5.3).

Table 5.3: Average daily amounts of water transpired by the three species on days in the indicated seasons. If values obtained from the same species on different days were significantly different this is indicated by the bracketed numbers. The days are numbered as (1) - 30/1/97 (summer), (2) - 10/9/97 (spring), (3) -19/2/98 (summer), (4) -23/7/98 (winter) and (5) - 15/9/98 (spring). Daily amounts of water transpired were calculated by integrating the areas below diurnal transpiration rate curves (Figure 5.1).

	Average daily amounts of water transpired per unit leaf surface area (mol m^{-2} day^{-1})		
Experimental day	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
30/1/97 (summer) (1)	128.6 ± 15.8	161.4 ± 80.5 (2 & 4)	196.9 ± 37.8 (2, 3, 4 & 5)
10/9/97 (spring) (2)	146.5 ± 7.6	73.0 ± 13.3 (1)	57.7 ± 7.8 (1)
19/2/98 (summer) (3)	105.0 ± 7.7	126.6 ± 22.2	93.9 ± 8.1 (1)
23/7/98 (winter) (4)	118.5 ± 3.6	76.7 ± 19.5 (1)	111.0 ± 5.8 (1)
15/9/98 (spring) (5)	123.0 ± 34.8	97.3 ± 10.2	89.9 ± 2.0 (1)
Average	**124.3**	**107.0**	**109.9**
Std. Deviation	**20.6**	**47.7**	**50.7**

A. populifolia showed no significant differences in average daily amounts of water transpired between experimental days while both *I. pes-caprae* and *S. plumieri* showed significant differences between experimental days (Table 5.3). *I. pes-caprae* average daily water loss for the 30/1/97 (summer) was significantly higher than values for 10/9/97 (spring) and 23/7/98 (winter). S. *plumieri* value for 30/1/97 was significantly higher than values measured on all other experimental days. Of all experimental days VPD reached highest values on the 30/1/97 (Figure 3.7) and this could explain the greater water losses measured on this day for *I. pes-caprae* and *S. plumieri*. As with CO_2 assimilation, production and productivity data, *A. populifolia* appears to be least affected by seasonal changes.

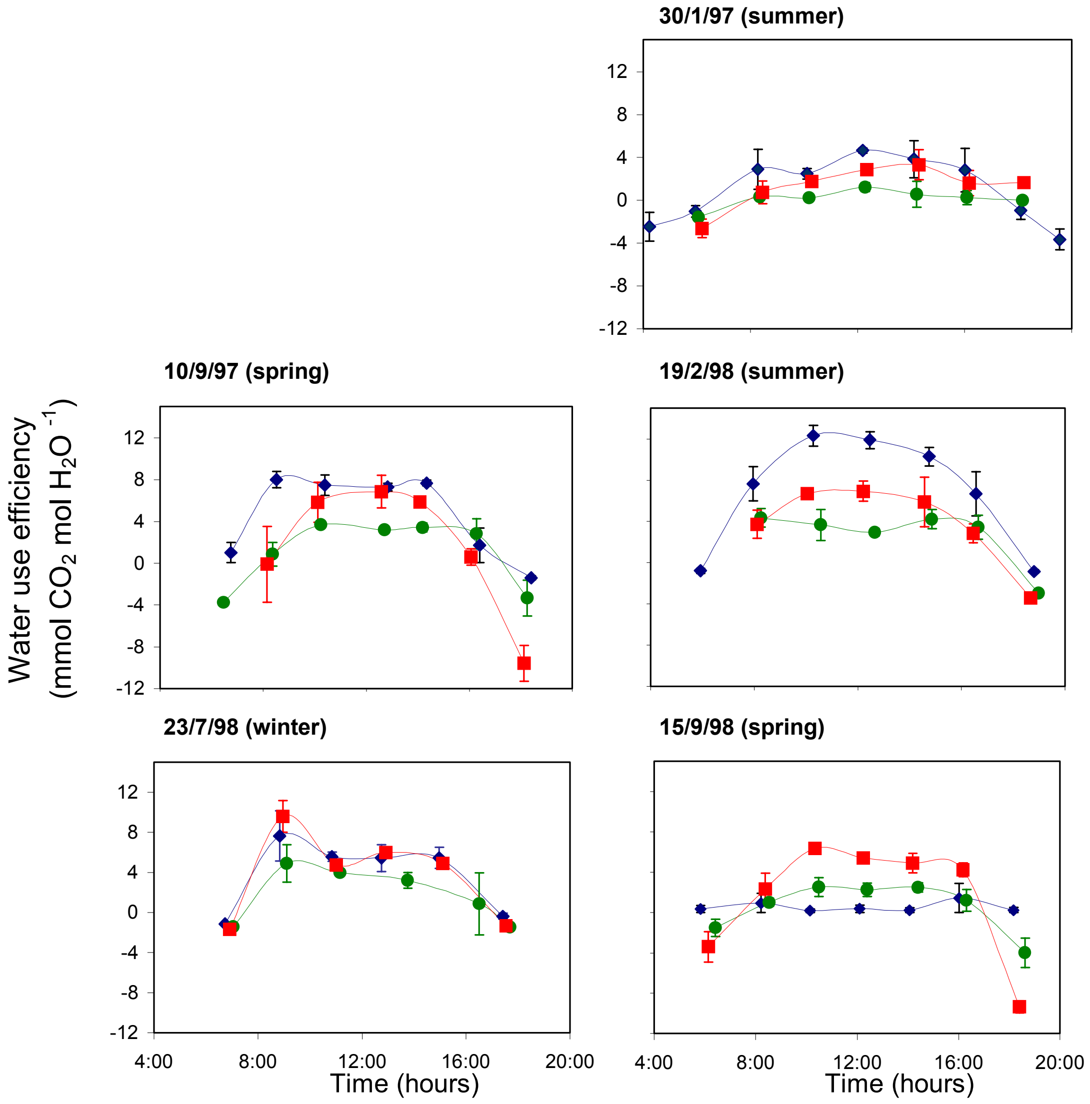


Figure 5.3: Diurnal changes in instantaneous water use efficiency of *A. populifolia* (diamonds), *I. pes-caprae* (circles) and *S. plumieri* (squares) for the indicated experimental days. Standard deviations are indicated by the vertical bars. N=3.

Water use efficiency

As the day proceeds the abiotic factors driving both photosynthesis (PPFD) and transpiration (VPD) increase to peak at or just after midday and then decrease during the afternoon. In response to this photosynthesis and transpiration show similar diurnal patterns. However the increase in photosynthesis was proportionately greater than the increase in transpiration. For example on the 19/2/98 (summer) transpiration and assimilation rates for *A. populifolia* increased 0.9 and 17.9 fold from dawn to midday respectively. In response to this, instantaneous WUE increased throughout the morning to peak at midday before declining in the afternoon (Figure 5.3). Differences existed between experimental days as a result of variation in abiotic factors. Results of a stepwise multiple regression analysis (Table 5.4) showed that for all three species as was the case for assimilation rate, PPFD was the most important abiotic factor determining the variability in instantaneous WUE. The addition of a second variable (T_l for *A. populifolia* and *I. pes-caprae* and T_a for *S. plumieri*) increased the adjusted r^2 values by 2.3, 11.3 and 14.0 % respectively. For all three species the third variable identified was RH and when all variables were included the adjusted r^2 values were 0.64, 0.73 and 0.60 for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively.

Table 5.4: Results of a stepwise multiple regression analysis of instantaneous water use efficiency and the five abiotic variables measured or calculated at the same time that former rates were measured. For each species the variable(s) and the corresponding adjusted r^2 values are listed.

Species	**1 variable**	**2 variables**	**3 variables**	**All variables**
A. populifolia	PPFD (0.621)	PPFD, T_l (0.635)	PPFD, T_l & RH (0.648)	All (0.644)
I. pes-caprae	PPFD (0.513)	PPFD, T_l (0.571)	PPFD, T_l & RH (0.710)	All (0.730)
S. plumieri	PPFD (0.479)	PPFD, T_a (0.546)	PPFD, T_l & RH (0.598)	All (0.600)

Abbreviations: Photosynthetically active radiation (PPFD), Relative humidity (RH), Ambient temperature (T_a), Leaf temperature (T_l).

Results of a two-way ANOVA of daily water use efficiencies calculated from the areas below diurnal transpiration and CO_2 assimilation curves showed significant differences among species, experimental days and for the interaction of both. *A. populifolia* had the highest daily WUE when averaged across all seasons (5.89 ± 2.42 mmol mol^{-1}) followed by *S. plumieri* (4.67 ± 1.84 mmol mol^{-1}) and *I. pes-caprae* (2.33 ± 1.14 mmol mol^{-1}) (Table 5.5).

Only *A. populifolia* showed significant differences in daily WUE. Daily WUE on the 10/9/97 (spring) was significantly lower than values obtained on the 19/2/98 (summer) and on 15/9/98 (spring) (Table 5.5). The low WUEs measured on this day (10/9/97, spring) were the result of both high transpiration rates (Table 5.3) and low CO_2 assimilation rates (Table 4.4).

Table 5.5: Average daily water use efficiencies for the three species on days in the indicated seasons. If values obtained from the same species on different days were significantly different this is indicated by the bracketed numbers. The days are numbered as (1) - 30/1/97 (summer), (2) - 10/9/97 (spring), (3) -19/2/98 (summer), (4) -23/7/98 (winter) and (5) - 15/9/98 (spring). Daily water use efficiencies were calculated by dividing the integrated areas below diurnal CO_2 assimilation curves (Figure 4.1) by the integrated areas below diurnal transpiration rate curves (Figure 5.1). Only when the CO_2 assimilation rates were positive were the areas below the curves used.

	Average daily water use efficiency (mmol CO_2 mol^{-1} H_2O)		
Experimental day	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
30/1/97 (summer) (1)	4.58 ± 2.14	1.63 ± 0.49	2.95 ± 0.55
10/9/97 (spring) (2)	3.00 ± 1.20 (3 & 5)	1.38 ± 1.36	6.56 ± 2.11
19/2/98 (summer) (3)	8.49 ± 0.70 (2)	3.77 ± 0.30	6.04 ± 0.24
23/7/98 (winter) (4)	5.95 ± 0.05	2.97 ± 0.83	2.38 ± 0.68
15/9/98 (spring) (5)	7.44 ± 2.35 (2)	1.89 ± 0.38	4.96 ± 0.74
Average	**5.89**	**2.33**	**4.67**
Std. deviation	**2.42**	**1.14**	**1.84**

Leaf water potential

Leaf water potentials are mainly determined by the rate at which water is lost in comparison to the rate at which water is supplied to the leaves from the soil via the conduction system (Hanson and Hitz, 1982). High transpiration rates in combination with low soil water content and low conduction rates results in low leaf water potentials. Leaf water potentials typically show reciprocal responses to diurnal changes in transpiration rate, declining to minima at or just after midday and recovering during the afternoon (Hanson and Hitz, 1982). All three species showed these typical responses (Figure 5.4) although there were differences between species and on different experimental days.

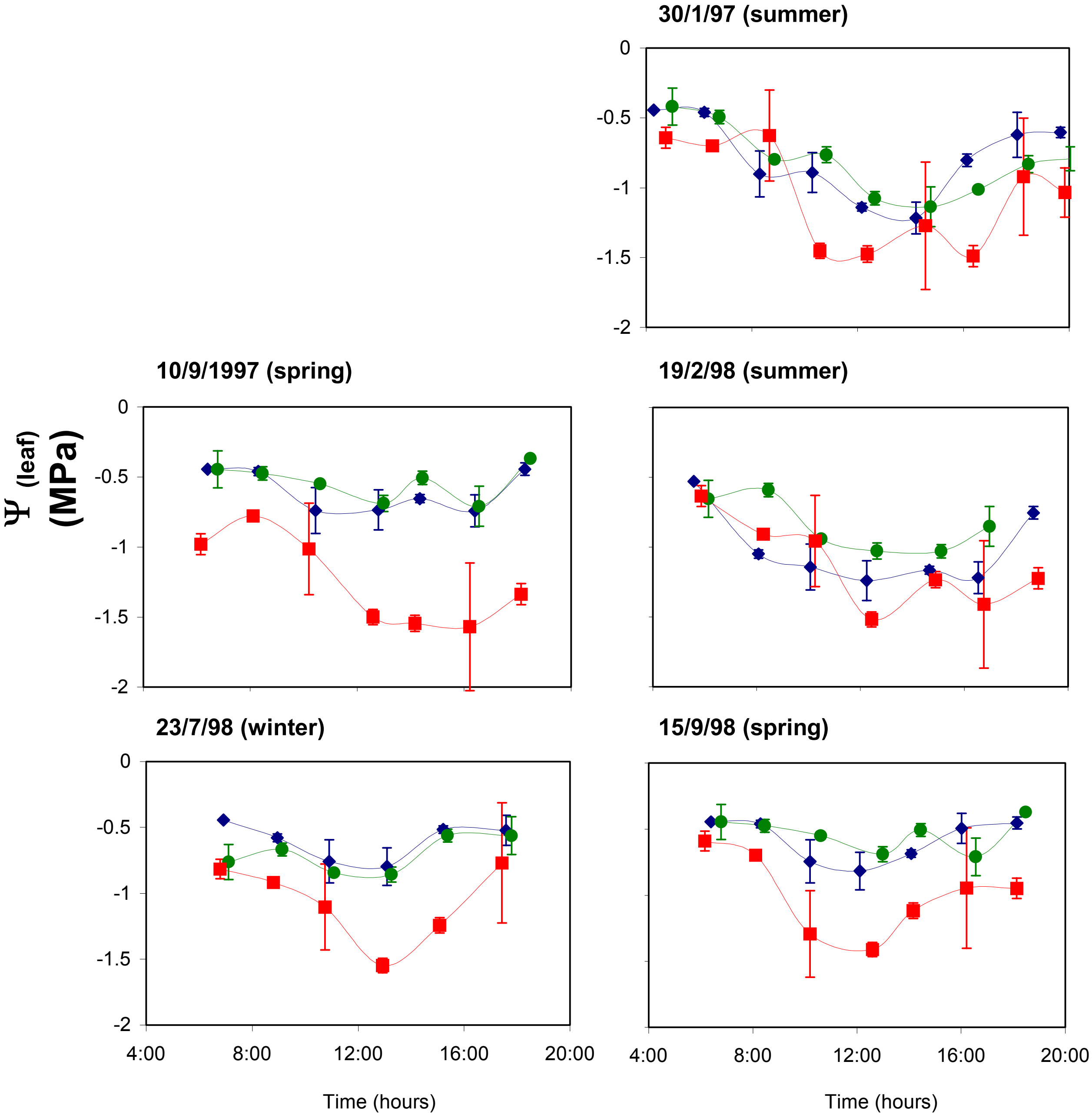


Figure 5.4: Diurnal changes in leaf water potential ($\Psi_{(leaf)}$) of *A. populifolia* (diamonds), *I. pes-caprae* (circles) and *S. plumieri* (squares) for the indicated experimental days. Standard deviations are indicated by the vertical bars. N=3.

Results of a two-way ANOVA showed that the differences in minimum leaf water potentials among species ($p<0.0001$), experimental days ($p<0.0001$) and for their interaction ($p<0.0001$) were significant. When averaged over all the experimental days *S. plumieri* minimum water potentials (-1.56 ± 0.08 MPa) were lower than the average values for either *A. populifolia* (-1.01 ± 0.04 MPa) or *I. pes-caprae* (-0.93 ± 0.05 MPa) (Table 5.6). Both *A. populifolia* and *I. pes-caprae* showed significant differences between experimental days with the minimum values measured on 10/9/97 (spring) and 19/2/98 (summer) being significantly different from values for the other experimental days. Both these experimental days had received low rainfall in the 14 day period prior to the experimental day (Table 3.3). However cumulative rainfall prior to the experimental day 30/1/98 was also low and yet the minimum leaf water potentials were not, making conclusions about such correlation difficult. *S. plumieri* minimum water potentials were not significantly different on different experimental days.

Table 5.6: Minimum daily average leaf or shoot water potentials for the three species on days in the indicated seasons. If values obtained from the same species on different days were significantly different this is indicated by the bracketed numbers. The days are numbered as (1) - 30/1/97 (summer), (2) - 10/9/97 (spring), (3) -19/2/98 (summer), (4) - 23/7/98 (winter) and (5) - 15/9/98 (spring). Minimum leaf or shoot water potentials are defined as the lowest points on the diurnal responses of leaf or shoot water potentials (Figure 5.4).

	Minimum daily average leaf or shoot water potential (MPa)		
Experimental day	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
30/1/97 (summer) (1)	-0.81 ± 0.04 (2 & 3)	-0.88 ± 0.03 (2 & 3)	-1.57 ± 0.16
10/9/97 (spring) (2)	-1.28 ± 0.06 (1, 4 & 5)	-1.17 ± 0.11 (1, 4 & 5)	-1.60 ± 0.09
19/2/98 (summer) (3)	-1.31 ± 0.04 (1, 4 & 5)	-1.10 ± 0.00 (1, 4 & 5)	-1.60 ± 0.10
23/7/98 (winter) (4)	-0.81 ± 0.02 (2 & 3)	-0.75 ± 0.05 (2 & 3)	-1.59 ± 0.01
15/9/98 (spring) (5)	-0.83 ± 0.04 (2 & 3)	-0.75 ± 0.05 (2 & 3)	-1.47 ± 0.04
Average	**-1.01**	**-0.93**	**-1.56**
Std. deviation	**0.04**	**0.05**	**0.08**

Pressure-volume curves

Curve-fitting to individual pressure-volume curve replicates produced r^2 values that ranged from 0.94 to 0.99 with the majority of r^2 values being greater than 0.98. Figure 5.5 shows average derived pressure-volume curves constructed for the three species on the various experimental days.

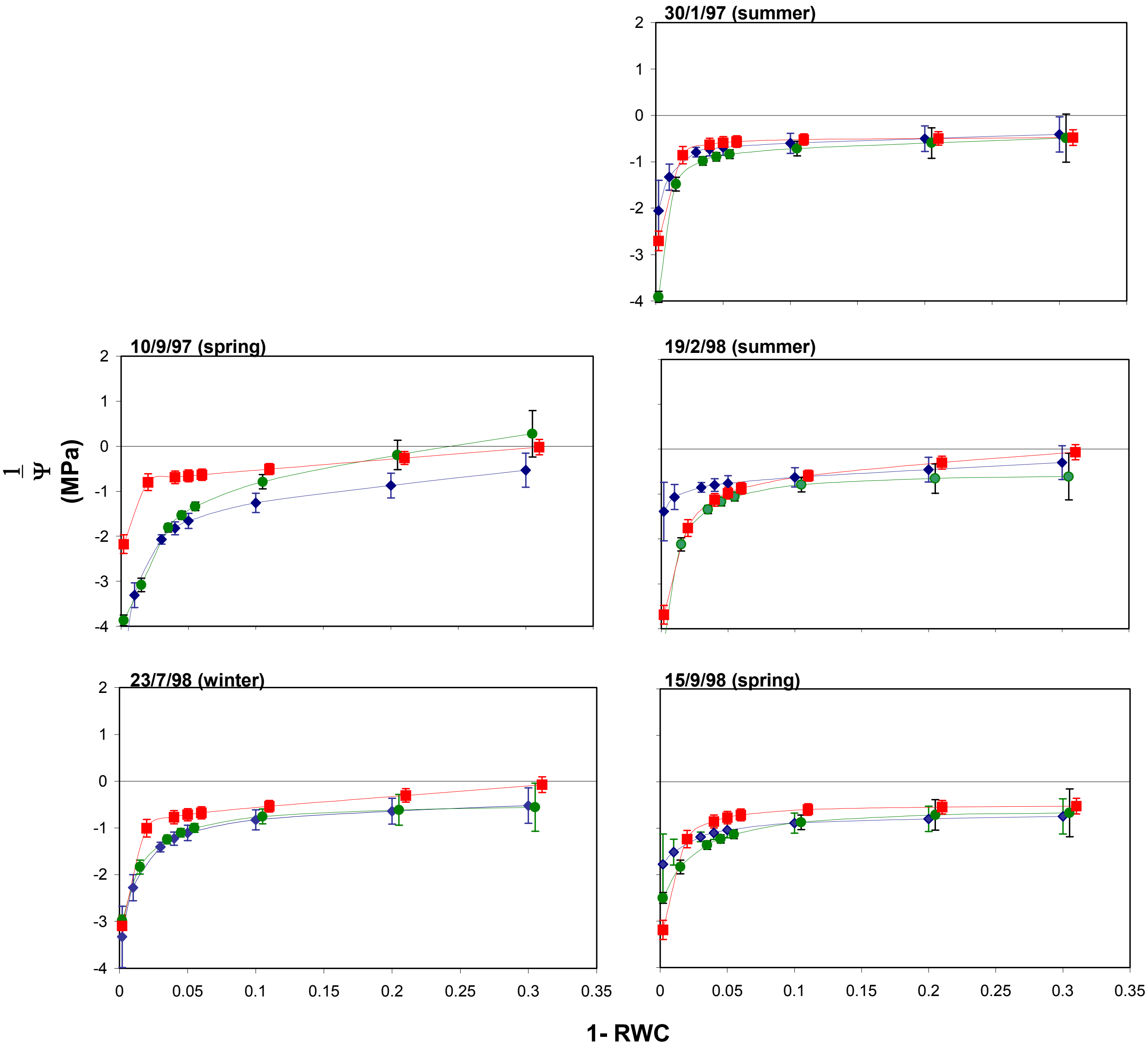


Figure 5.5: Average derived pressure-volume curves for *A. populifolia* (diamonds), *I. pes-caprae* (circles) and *S. plumieri* (squares) for the indicated experimental days. Standard deviations are indicated by the horizontal symbols. The number of replicates for *A. populifolia, I. pes-caprae* and *S. plumieri* are tabulated in the footnote 5.1.

In order to compare these curves significant points were obtained from each individual replicate pressure-volume curve and included values at full turgor (Ψπ,0 and the curvature factor) and values at the turgor loss point (RWC, Ψπ=Ψp). The intercept on the 1–RWC axis gives the symplastic to total leaf water ratio (= b, from curve fitting).

Theoretically this intercept can not be greater than 1 but due to low slopes of the relationship between $\Psi\pi$ and 1-RWC it was often found to be greater than 1 (Figure 5.5). This may have been due to tension in the protoplast-compartment, caused by resistance of the cell walls to collapse (Boyer, 1995), or the dilution of cell sap by appoplastic water (Tyree, 1976). This value (b) is used to calculate the bulk modulus of elasticity ($\varepsilon = (b-(1-RWC)) \times c \times \Psi p$; Sinclair and Venables, 1983) but the calculated value of ε is nonsensical if $b>1$. Thus in order to compare the relationship between Ψp and RWC, c (the curvature factor exponentially relating Ψp and RWC) was compared between species and on different experimental days. In addition to affecting the calculation of ε, b is used to correct $\Psi\pi$ to the symplastic volume according to the equation $\Psi\pi = a/(1-(1-RWC)/b)$ (Sinclair and Venables, 1983). Because b increased to values greater than 1 the above equation was modified to $\Psi\pi = a/(1-(1-RWC)) = a/RWC$. This represents the bulk averaged osmotic potential of the symplastic and appoplastic water.

The four significant points from individual replicate pressure-volume curves were compared between different experimental days and between individual species using a Kruskal-Wallis non-parametric ANOVA. The RWC where $\Psi p= 0$ for *A. populifolia* and *I. pes-caprae* were significantly different between different experimental days at the 95% confidence level but not at the 98% level (Table 5.7). Similarly the curvature factor (c) for *S. plumieri* was significantly different for different seasons at the 95% but not at the 96% level. The value of $\Psi\pi$ at RWC=1 was not significantly different on different days for any of the species. This indicates an absence of osmotic adjustment between experimental days.

Because differences were not highly significant and replication was not high (N ranged from 2 to 5, see footnote $^{5.1}$ for details) the significant points for each individual species from individual replicates on different experimental days were combined. Combined species specific data was compared and only the RWC where $\Psi p=0$ ($p=0.0233$) was significantly different at the 95 but not the 98% level.

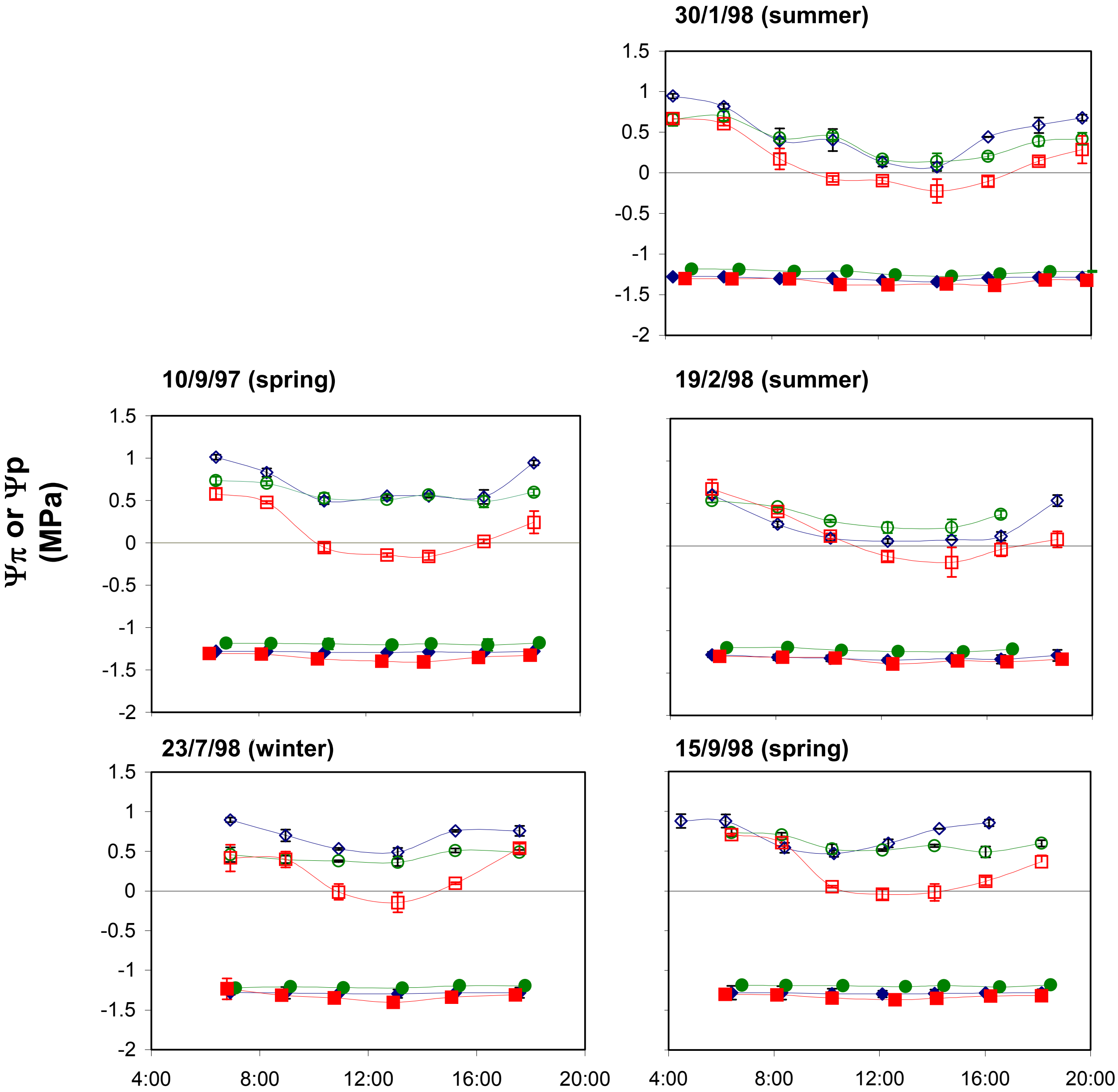


Figure 5.6: Diurnal changes in osmotic potential (Ψπ; closed symbols) and turgor potential (Ψp; open symbols) of *A. populifolia* (diamonds), *I. pes-caprae* (circles) and *S. plumieri* (squares) for the indicated experimental days. Standard deviations are indicated by the vertical bars. N=3.

Components of leaf water potential

Leaf water potential is the sum of osmotic and turgor potential and the diurnal changes in these on the various experimental days are given in Figure 5.6. For all three species the changes in $\Psi_{(leaf)}$ result from changes in Ψp and not in $\Psi\pi$. *S. plumieri* showed the largest variation in Ψp (Figure 5.7) and this corresponded to the largest variation in $\Psi_{(leaf)}$ (Figure 5.4). *S. plumieri* turgor potentials were reduced to zero on all five experimental days. These zero turgors were synchronised with the midday minima in $\Psi_{(leaf)}$ and with the peaks in transpiration rate (Figure 5.1). *A. populifolia* and *I. pes-caprae* turgor potentials never decrease to zero although both approached zero on the 30/1/97 (summer) and on the 19/2/98 (summer) (Figure 5.6).

Table 5.7: Significant points averaged for all experimental days extracted from pressure-volume curves for *A. populifolia*, *I. pes-caprae* and *S. plumieri*. The p-values indicate differences between values obtained on different experimental days for each individual species. For the number of replicate pressure-volume curves constructed on each experimental day for each species see footnote [5.1].

Average significant points extracted from pressure-volume curves						
SPECIES	C (curvature factor)		$\Psi_{(leaf)}$ where: RWC=1 (MPa)		$\Psi\pi$ where: RWC=1 (MPa)	
	Average	P	Average	p	Average	p
A. populifolia	47.23 ± 52.90	0.566	-0.38 ± 0.20	0.023	-1.24 ± 0.53	0.178
I. pes-caprae	23.51 ± 19.57	0.106	-0.23 ± 0.12	0.041	-1.48 ± 0.47	0.229
S. plumieri	58.34 ± 50.68	0.040	-0.21 ± 0.04	0.053	-1.46 ± 0.35	0.053

Average significant points extracted from pressure-volume curves						
SPECIES	Ψp where: RWC=1 (MPa)		RWC where: Ψp=0		$\Psi_{(leaf)}$ where: Ψp=0 (MPa)	
	Average	P	Average	p	Average	p
A. populifolia	0.86 ± 0.59	0.178	0.94 ± 0.03	0.023	-1.31 ± 0.54	0.163
I. pes-caprae	1.25 ± 0.49	0.566	0.89 ± 0.04	0.025	-1.65 ± 0.55	0.229
S. plumieri	1.26 ± 0.34	0.255	0.91 ± 0.03	0.199	-1.61 ± 0.41	0.070

Abbreviations: leaf water potential ($\Psi_{(leaf)}$), turgor potential (Ψp), osmotic potential ($\Psi\pi$), relative water content (RWC).

Leaf water potentials of plants growing on a small isolated dune

After severing the underground inter-dunal stem connections between *S. plumieri* plants and between *I. pes-caprae* plants the leaf or shoot water potentials of the plants on the smaller isolated dune decreased with time in comparison to controls (Figure 5.7).

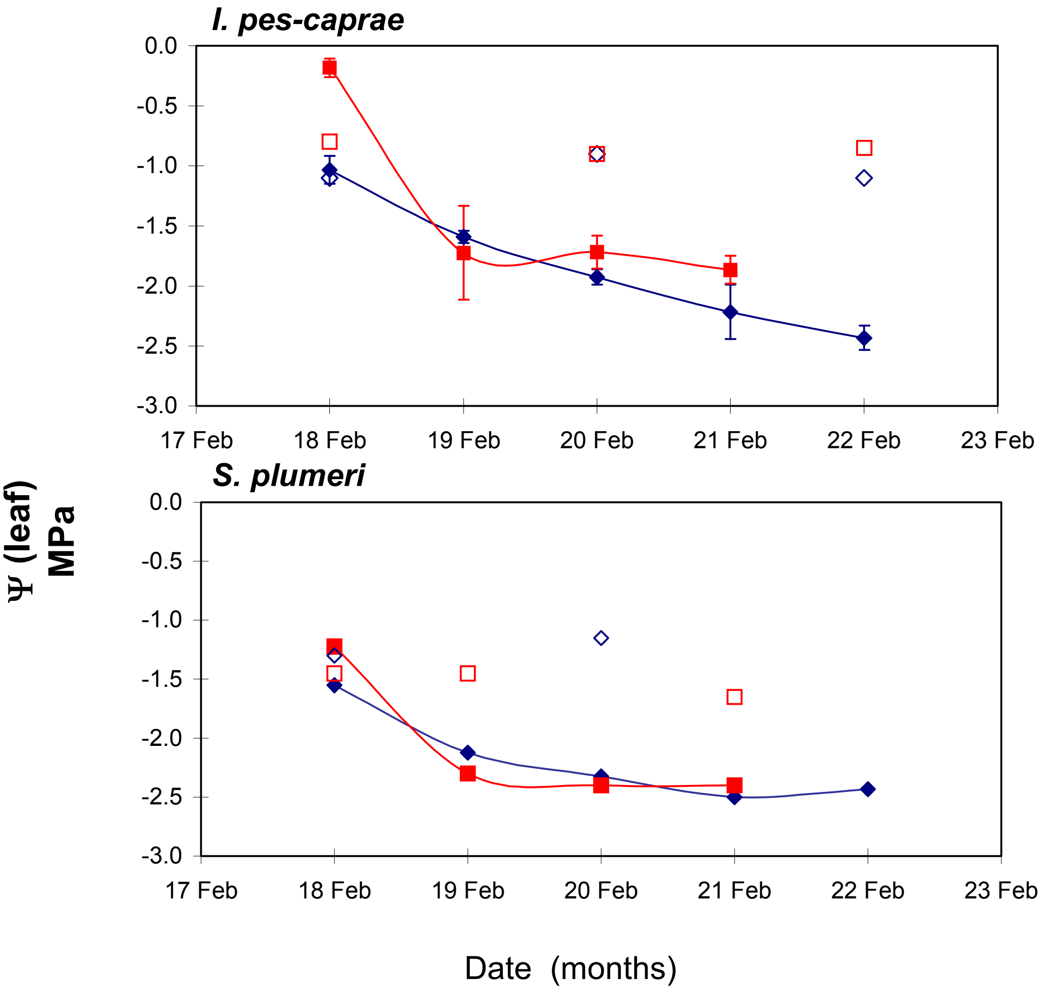


Figure 5.7: Changes in leaf or shoot water potential ($\Psi_{(leaf)}$) of *I. pes-caprae* and *S. plumieri* measured at 14:00 (solid diamonds) or 19:00 (solid squares) for a number of days following the excision of underground connections between a small foredune on which experimental plants were growing and a larger inland dune. Leaf or shoot water potentials of plants growing on the larger inland dune are shown as open symbols. Measurements for *I. pes-caprae* were replicated and vertical bars indicate standard deviations. N=3. *S. plumieri* measurements were not replicated.

There was little or no difference between water potentials measured at 14:00 and 19:00 indicating that the water potentials of plants on the smaller isolated dune did not recover in the evening once transpiration rates declined. This evening recovery was apparent for control plants (Figure 5.7) and was apparent when diurnal leaf or shoot water potentials were monitored (Figure 5.4). Despite the fact that the plants growing on the isolated dune had adventitious root systems (Plate 5.1) they were dependent on underground connections to the larger dune to maintain their water supply.

Plate 5.1: Excavated *I. pes-caprae* and *S. plumieri* shoots displaying adventitious roots.

$\delta\,^{18}$O values of rain, ground and stem water

S. plumieri plant stems sampled on the 9/9/97 had isotopic δ^{18}O values (-4.3 ± 0.28 $^{0}/_{00}$) that were the same as values measured for ground-water (-4.5 $^{0}/_{00}$) and both of these values were clearly distinct from the weighted average rain-water δ^{18}O values(-2.5 $^{0}/_{00}$) (Figure 5.8). On the 20/2/98 the values for stem-water, weighted average rain-water and ground-water were indistinct at –2.38 ± 0.35, -2.5, and –2.9 $^{0}/_{00}$ respectively. Clearly on this sampling date the lack of differences in the signals from rain- and ground-water did not allow the source of plant-water to be determined.

On the third stem sampling date (22/7/98) stem values had declined to –4.22 ± 0.26 $^{0}/_{00,}$ values comparable to those obtained for stems on 9/9/97. The ground-water samples collected prior (8/6/98 & 19/7/98) and subsequent (23/7/98 & 14/9/98) to this stem sampling date (22/7/98), had δ^{18}O values that exceeded the weighted average values for rain-water.

Port Alfred Weather Station (20 km south west of the study site) recorded rain on 7/6/98 (33.2mm), 19/7/98 (14.8mm), 20/7/98 (106mm) and 21/7/98 (5.8mm). Analyses showed that heaviest rainfall produced the largest δ^{18}O values. Percolation of rain-water to the ground-water table following heaviest rainfall may account for the increased ground-water δ^{18}O values obtained prior to and subsequent to the stem sampling date of 22/7/98.

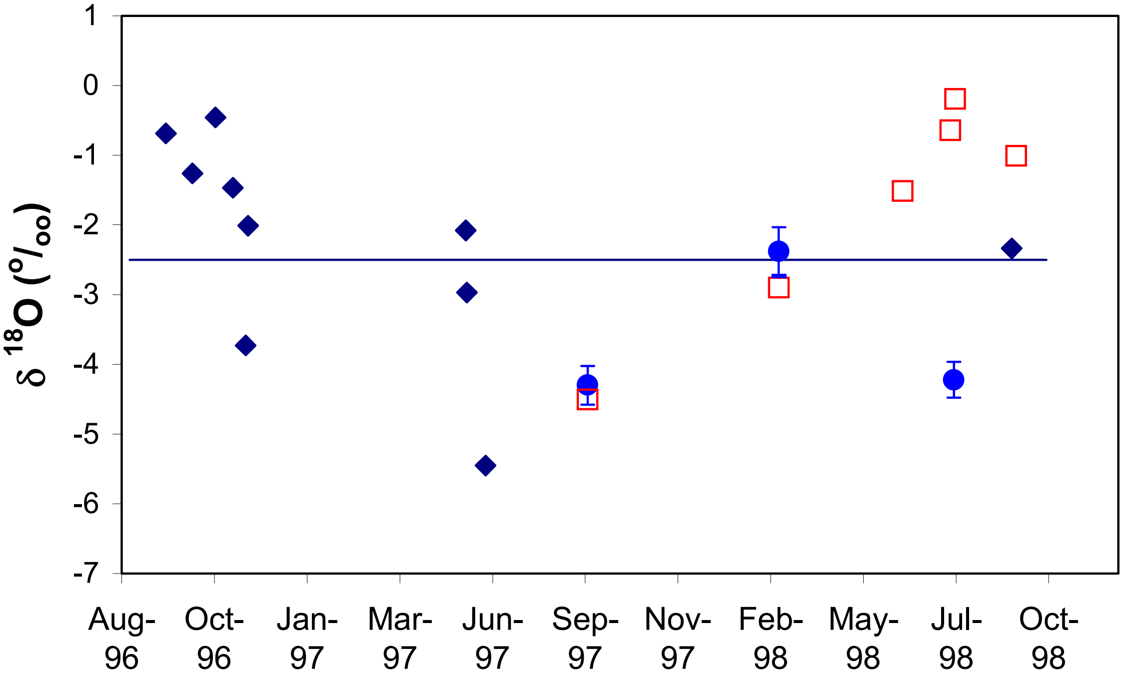


Figure 5.8: Relative deviations in the ^{18}O/^{16}O ratio (δ^{18}O) of rain (diamonds), ground-water (squares) and water vacuum-distilled from *S. plumieri* stems (circles) collected on the indicated dates. The solid line indicates the average weighted δ^{18}O value for rainfall. N=5 for stem samples and standard deviations are given by the vertical bars.

Water Budgets

Annual and monthly budgets

Table 5.8 lists annual budgets for *A. populifolia, I. pes-caprae* and *S. plumieri* on an average, a high and a low rainfall year. For all three species the difference between rainfall and transpiration is positive for the average and the high rainfall year. However in the low rainfall year the budget for *I. pes-caprae* was negative. The differences in the predicted volumes of water transpired per unit dune surface area for the three species arises from differences in the relationship between E and VDP (Figure 5.2) and in LAIs (Table 3.6). The majority of average daily VPDs were less than 1 kPa and at these VPDs the relationships between E and VPD would predict the highest transpiration rates per unit leaf area for *A. populifolia* followed by *I. pes-caprae* and then *S. plumieri*. However because the LAI measured for *A. populifolia* was the lowest (0.23) the predicted volume of water transpired per unit dune surface area was also the lowest. *I. pes-caprae* had the second highest predicted transpiration rates (at VPDs less than 1 kPa) and this in combination with LAI = 0.78 predicted the highest transpirational water loss per unit dune surface area. *S. plumieri* had the highest LAI (1.05) and the lowest predicted transpiration rates (at VPDs less than 1 kPa). This resulted in predicted volumes of water transpired per unit dune surface area that were intermediate between those of *A. populifolia* and *I. pes-caprae*.

The variation in E was better related to changes in VPD for *S. plumieri* than for either *A. populifolia* or *I. pes-caprae* as was evident from the r^2 values of 0.78, 0.51 and 0.42 respectively (Figure 5.2). This better relationship for *S. plumieri* was also evident from mean loss function values, larger values indicating a greater scatter of data around the fitted function. The mean loss function is defined as: ($\sum\{\text{observed} - \text{predicted}\}^2$/number of observations). Mean loss values were 0.40, 0.98, 0.37 for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. Low r^2 values and high mean loss function values particularly for *I. pes-caprae* indicate that the predictions from the relationship of E to VPD must be treated with a certain amount of caution.

Predicted volumes of water transpired per m^2 of dune surface for an average year (Table 5.8) were converted to predict average daily water losses by dividing by 365.25 days. Predicted volumes were 0.57, 1.6 and 1.2 l m^{-2} dune surface day^{-1} for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. The average daily volumes of water transpired by *A. populifolia, I. pes-caprae* and *S. plumieri* for the five experimental days were 0.51, 1.5 and 2.07 l m^{-2} of dune surface respectively. Estimates for *A. populifolia* and *I. pes-caprae* are similar to the measured average water losses (Table 5.8). This was not surprising as predicted water losses are based on the data from the five experimental days. Predicted and measured average daily water losses for *S. plumieri* are considerably different. This results from the relationship of E to VPD being based not only on the data from the five experimental days (101 data points) but also on an additional 226 data points from Peter (2000) (Figure 5.2). The high r^2 value (0.76) and low mean loss function (0.37) indicates that the relationship of E to VPD was highly predictive for this species.

Table 5.8: Annual water budgets for *A. populifolia, I. pes-caprae* and *S. plumieri* determined in average, high and low rainfall years. Volumes are calculated per unit dune surface area.

Volumes of water ($l\ m^{-2}\ yr^{-1}$)	**Average rainfall (1978-1998)**		
	A. populifolia	***I. pes-caprae***	***S. plumieri***
Rainfall	618 ± 168	618 ± 168	618 ± 168
Transpiration	208 ± 12	593 ± 34	430 ± 30
Difference	**410**	**25**	**188**

Volumes of water ($l\ m^{-2}\ yr^{-1}$)	**High rainfall (1994)**		
	A. populifolia	***I. pes-caprae***	***S. plumieri***
Rainfall	745	745	745
Transpiration	225	639	464
Difference	**520**	**106**	**281**

Volumes of water ($l\ m^{-2}\ yr^{-1}$)	**Low rainfall (1997)**		
	A. populifolia	***I. pes-caprae***	***S. plumieri***
Rainfall	546	546	546
Transpiration	197	559	392
Difference	**349**	**-13**	**154**

In addition to annual rainfall the temporal distribution of rainfall is important for plant water relations (Fitter and Hay, 1987). Monthly rainfall and predicted monthly transpirational losses were compared per unit dune surface area (Figure 5.9). For all three species during both high and low rainfall years some monthly budgets were negative. This result suggests that when considering short time periods the water supply may in fact be limiting. *A. populifolia* had the smallest and least frequent negative budgets. *I. pes-caprae* had both the largest and most frequent negative budgets as a result of the predicted high volumes of water transpired per unit dune surface area. *S. plumieri* had frequency and size of negative budgets that were intermediate between those of *A. populifolia* and *I. pes-caprae*.

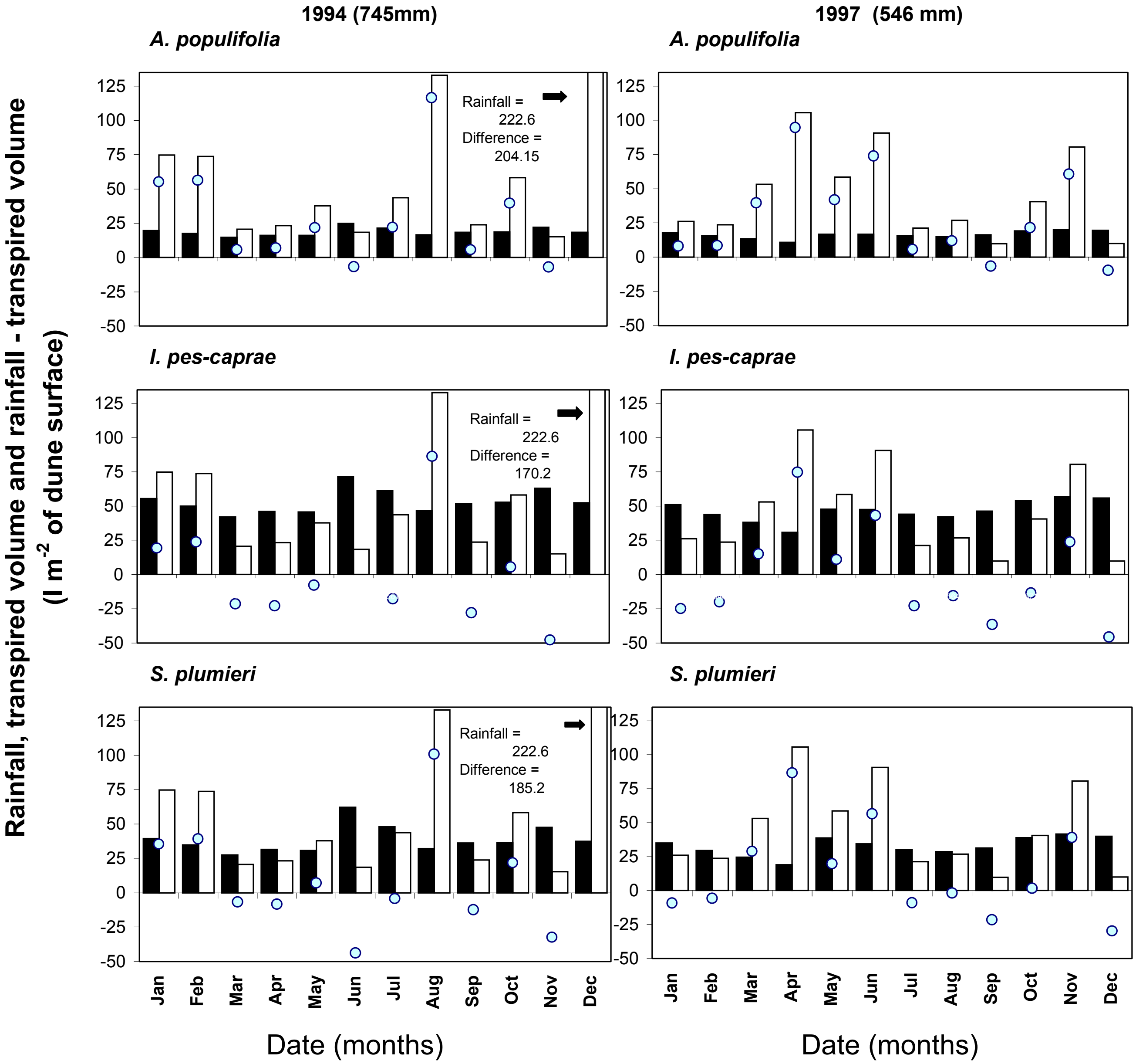


Figure 5.9: Monthly rainfall (open blocks), predicted transpiration (solid blocks) and rainfall minus transpiration (circles) for *A. populifolia, I. pes-caprae* and *S. plumieri* in low (1997, 546 mm) and high (1994, 745 mm) annual rainfall years. Note that monthly rainfall and rainfall minus transpiration for December 1994 are shown on the figure.

Some of the water held in the sand by capillarity is available to plants and it is necessary to account for this when budgeting water supply. The water in the sand between 7 and 0.5% is likely to be available to plants (Salisbury, 1952). The upper limit of sand-water is determined by the sand water-holding capacity. Plants cannot extract the water below 0.5% because the water potentials required to do this are more negative than the water potentials generated by these plants. The model was repeated using three water-holding capacities, 1.5, 4 and 7%. The starting water content was arbitrarily set at the water-holding capacity. In 1994 at water-holding capacities of 4 or 7% only *I. pes-caprae* dune soils had water contents depleted to levels approaching 0.5% (Figure 5.10). This occurred in the June to August period when rainfall was low and again in the November to December period due to a combination of low rainfall and high transpiration (Figure 5.3).

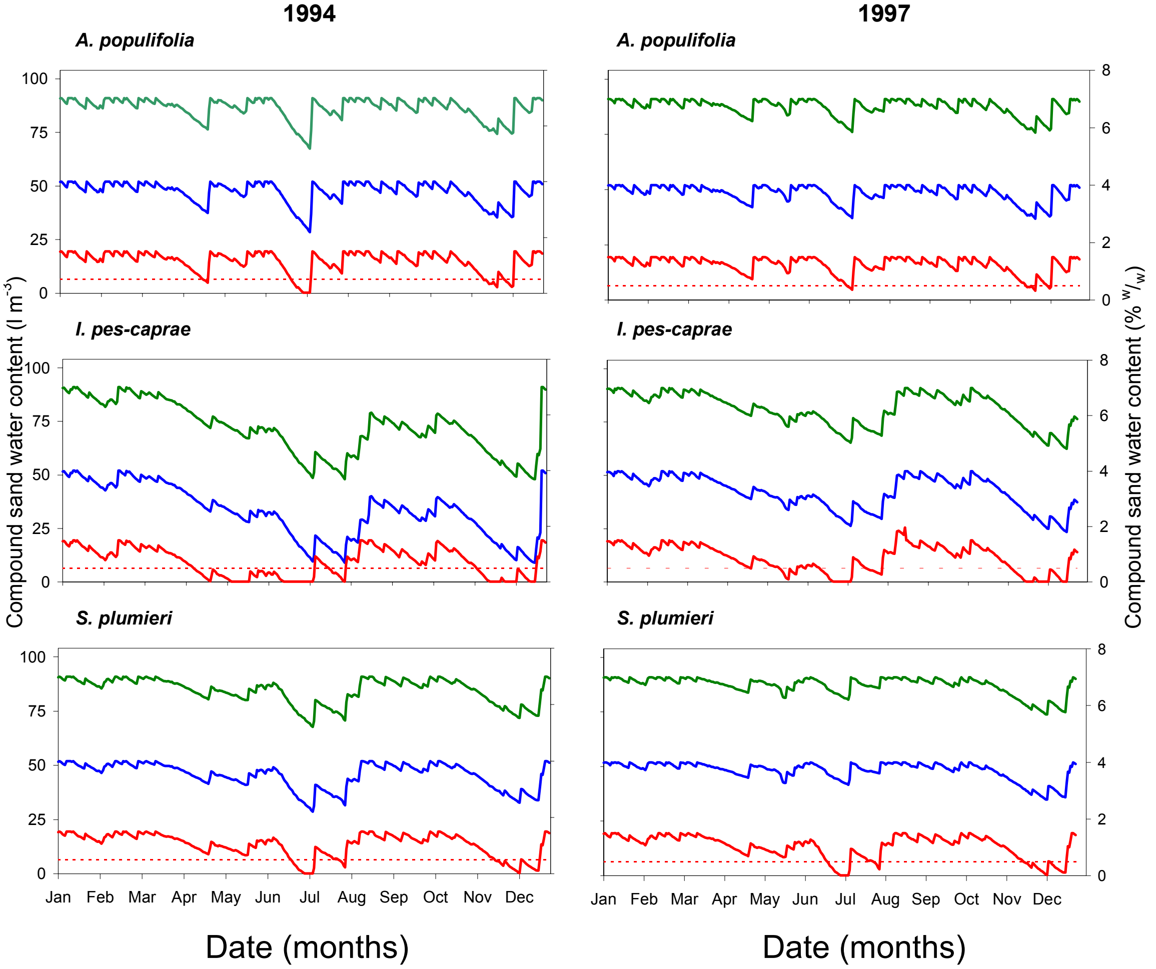


Figure 5.10: Compounded sand water content for one square metre of *A. populifolia, I. pes-caprae* and *S. plumieri* vegetated dunes in low (1997, 546 mm) and high (1994, 745 mm) rainfall years. The volume of water in the sand at the start of the budget and the water holding capacity were assumed to be equal. The model was repeated with three different values 1.5 % (red line), 4 % (blue line) and 7% (green line). The minimum sand water content at which water is available to plants (0.5%) is indicated by the red broken line.

At water-holding capacity of 1.5% all three species reduced sand water content below 0.5. In 1997 (the low annual rainfall year) the temporally even distribution of rain maintained a higher sand-water content than did the more sporadic yet higher rainfall of 1994. Because of its low water use per unit dune surface area *A. populifolia* was less affected by differences in rainfall distribution than the other two species.

DISCUSSION

Transpiration rate

Comparative transpiration data are listed in Table 5.9. Ranges in transpiration rates measured for *A. populifolia, I. pes-caprae* and *S. plumieri* are comparable to rates measured for *S. plumieri* growing at Mtunzini in KwaZulu Natal and also to rates for the monocotyledonous dune grasses *Elymus mollis* and *Ammophila arenaria* (Pavlik, 1984). These similarities in transpiration rates are apparent despite the wide differences in plants and probable differences in environmental factors. Comparative diurnal data from other dicotyledonous dune species is scarce although Packham (cited in Packham and Willis, 1997) presents diurnal data for two temperate dicotyledonous dune species *Cynoglossum officinale* and *Hydrocotyle vulgaris.* Rates ranged from 9 to 16.5 mg g fresh wt.$^{-1}$ hr^{-1}for *Cynoglossum officinale* and from 9.5 to 17.5 mg g fresh wt.$^{-1}$ hr^{-1} for *Hydrocotyle vulgaris.*

Using surface area to dry weight and dry weight to wet weight ratios (Tables 3.4 & 3.5) the daily ranges in transpiration rates of A. *populifolia*, *I. pes-caprae* and *S. plumieri* were calculated per unit wet weight. Ranges were 0.9 to 2.8, 1.1 to 6.3, and 0.6 to 3.1 mg g wet wt.$^{-1}$ hr^{-1}for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. These values are far lower than the values measured by Packham (cited in Packham and Willis, 1997). However comparisons between species on a wet weight basis are of limited value due to the highly variable wet weight per unit surface area measured between species. Because of this succulent leaves will always have low values.

Transpiration rates measured for the three species in this investigation are neither as high as average rates measured for savanna broad-leafed woody species following periods of rainfall (Table 5.9), nor are they as low as values for temperate montane conifers (Wyoming and Colorado), dry mountain fynbos (Cape Province) and cold desert species (California). Pammenter (1983) considered that transpiration rates of *S. plumieri* were fairly high and usually fell within the range 0.8 to 3.9 mmol H_2O m^{-2} s^{-1} characteristic of a wide variety of species growing under diverse conditions (Leopald and Kriedeman, 1975).

Relatively high single-peaked diurnal responses in transpiration are characteristic of well-watered plants (Schulze and Hall, 1982). Some plants can exhibit single-peaked responses following long-term drought but in these the transpiration rates are low (Stocker, 1956). The combination of relatively high transpiration rates and single-peaked diurnal responses (Figure 5.1) suggest that plants in this study were well supplied with water. However this was not always the case as in the summer (30/1/97 and 19/2/98) *I. pes-caprae* had two-peaked diurnal responses. The response on the 19/2/98 was the more obvious (Figure 5.1) and was the result of reduced stomatal conductance during the midday period (Figure 7.5). The reduced stomatal conductances reduced transpiration rates and $\Psi_{(leaf)}$ during this period (Figure 5.4). Different interspecific stomatal behaviour and its effect on transpiration rates has been noted for other dune (Willis and Jefferies, 1963) and xeric C_3 species (Schultze *et al*., 1974, 1975, 1980). The role of stomata in controlling water loss is discussed in Chapter 7.

When calculated from average leaf water content and average daily transpiration rates (Table 5.3) daily amounts of water transpired represent 94.3, 216.6 and 106.7% of leaf water content for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. The more succulent leaves of *A. populifolia* and *S. plumieri* with their greater water content have lower rates of water replacement. The replacement rates measured for *A. populifolia*, *I. pes-caprae* and *S. plumieri* are far lower than estimates for the dune species *Senecio jacobaea* which replaced leaf water every 45 minutes (Willis and Jefferies, 1963). These differences could result from differences in transpiration rates, differences in leaf water content or a combination of both. Although Willis and Jefferies (1963) quote transpiration rates for *Senecio jacobaea* (15 to 20 mg g fresh $wt.^{-1}min^{-1}$) the absence of surface area to weight ratios in their report precludes direct comparison with the data gathered in this study.

Table 5.9: Transpiration rates of various species growing in the indicated habitats at various geographical locations. Transpiration rates are expressed per unit leaf surface area.

Species	**Location and Habitat**		**Transpiration rate mmol H_2O m^{-2} s^{-1}**
A. populifolia ^{*1}	Eastern Cape, RSA, Coastal dunes		1.1 – 6.6
I. pes-caprae ^{*1}			0.4 – 5.9
S. plumieri ^{*1}			0.2 – 6.5
S. plumieri ^{*2}	KwaZulu Natal, RSA, Coastal dunes		1.2 – 6.13
Elymus mollis ^{*3}	Oregon, North America, Coastal dunes		3.8 – 5.8
Ammophila arenaria ^{*3}			3.9 – 6.6
Burkea africana ^{*4}	Nylsvlei, RSA, Savanna		18.3
Ochna pulchra ^{*4}			14.9
Grewia flavescens ^{*4}			13.0
Abies lascocarpa ^{*5}	South East Wyoming, USA	Western Coniferous Forest	0.1 - 4.5
Picea engelmannii ^{*6}	Central Colorado, USA		0.1 – 0.8
Pinus contorta ^{*7}	South East Wyoming, USA		0.8 – 1.7
Ceratoides lanata ^{*8}	Great Basin, California, USA, Cold Desert		1.1
Protea laurifolia ^{*9}	Western Cape, RSA, Dry mountain fynbos		0.2 – 4.7
Rhus dissecta ^{*9}			0.3 – 2.5
Rafnia capensis ^{*9}			0.1 – 2.7
Salvia sp. ^{*9}			0.4 – 2.6

^{*1} Present study; ^{*2} Pammenter, 1983; ^{*3} Pavlik, 1984; ^{*4} Scholes and Walker, 1993; ^{*5} Knapp and Smith, 1981 cited in Smith, 1985; ^{*6} Kaufmann, 1975 cited in Smith, 1985; ^{*7} Fletcher, 1976 cited in Smith, 1985; ^{*8} Caldwell, 1985; ^{*9} von Willert et al., 1989.

Transpiration rates were largely determined by the prevalent ambient conditions with PPFD, RH, T_a and VPD being important in determinants (Table 5.2). As conditions did not vary appreciably on experimental days (Figure 4.6 and 3.7, Chapter 3) total amounts of water transpired on a daily basis were generally not significantly different (Table 5.3). Exceptions include the 30/1/97 (summer) where the amount of water lost by *I. pes-caprae* and *S. plumieri* were significantly higher than the amounts lost on other experimental days. These higher transpiration rates could be accounted for by the higher VPDs measured on this day (Figure 3.7). The reason for *A. populifolia* not having higher transpiration rates on this day was not apparent. The diurnal response of stomatal conductance was not different from the responses measured on the other experimental days (Figure 7.5).

Water use efficiency

Variation in the instantaneous water use efficiencies are largely determined by the photosynthetic rate (Figure 4.1) which as the day proceeds increase by several orders of magnitude more than does the transpiration rate (Figure 5.1). As a result the most important determinant in the variation in WUE is the abiotic factor that has the largest effect on photosynthetic rate namely PPFD (Table 5.4 and Figure 4.2). On four of the five experimental days *I. pes-caprae* showed the lowest water use efficiencies at all measurement intervals (Figure 5.3). Daily water use efficiencies were significantly different between species with *A. populifolia* being the most efficient (Table 5.5). This species has leaf hairs which have been shown to decrease transpiration rates by a greater relative amount than assimilation rates (Ripley *et al*., 1999) and this results in high WUE. In addition the leaf hairs appear to trap dew so that the water lost by the leaves for the first few hours in the morning is not internal water but is water from the hair layer. This phenomenon is discussed in Chapter 7.

Pammenter (1983) calculated average WUE values of 3.1 ± 0.19 mmol mol^{-1} for *S. plumieri* under field conditions at Mtunzini. He excluded gas exchange data at light intensities below 100 μmol m^{-2} s^{-1}. His values are similar to the average value (4.67 ± 1.84) calculated for *S. plumieri* in this investigation. Average WUE of 5.89 ± 2.42 and 4.67 ± 1.84 mmol mol^{-1} for *A. populifolia* and *S. plumieri* respectively are typical of values (2.9 to 4 mmol mol^{-1}) listed for C_4 species (Szarek and Ting, 1975). However the average values of 2.33 ± 1.14 mmol mol^{-1} for *I. pes-caprae* are more typical of values (1.67 to 2.2 mmol mol^{-1}) listed for C_3 plants (Szarek and Ting, 1975). The water use efficiencies particularly for *A. populifolia* and *S. plumieri* are as high as values of 3.9 mmol mol^{-1} measured for *Atriplex confertifolia* a C_4 desert species (Caldwell *et al*., 1977). These high water use efficiencies are a result of high photosynthetic rates (Table 4.1) rather than low transpiration rates (Table 5.1). Hence in this case high water use efficiencies do not necessarily imply adaptation to a xeric environment. Plants adapted to xeric conditions may in fact have reduced water use efficiencies and Bunce *et al*. (1977) and Hinckley *et al*. (1979) showed that xeric species were capable of keeping their stomata open during periods of drought and under these conditions had reduced WUE. The unsaturated response of assimilation rate with increasing PPFD measured for *A. populifolia* and *S. plumieri* (Figure 4.2) ensures that the A/E ratio is maintained even at high light intensity.

Leaf water potentials

Diurnal responses in leaf water potential are typical of mesic plants (Hanson and Hitz, 1982) with minimal $\Psi_{(leaf)}$ never being decreased below –1.55 MPa. These values are similar to those measured for other dune species and deep-rooted fynbos species. Table 5.10 lists the minima and the ranges of water potentials measured for various species including dune species from North America and South Africa. Of interest are the very negative water potentials developed by the chaparral species of North America and the shallow rooted fynbos species (*Diosma hirsuta*). Both live in environments that have annual rainfall not dissimilar to the amounts measured for Old Woman's River study site. This emphasises the apparent water availability in these dunes.

Garcia-Novo (1979) showed a linear relationship between the annual range of leaf water potentials and the range in height of the soil-water table for coastal shrub vegetation in Spain. Species on dry elevated ground developed leaf water potentials as low as –7 MPa. In contrast species in low-lying areas of high water availability developed water potentials of –1 to –1.5 MPa. These values are comparable to water potential measured in this study.

Barbour *et al*. (1985) differentiated between deep rooted *Ambronia maritima* and *Ambrosia chamissonis* which developed minimal water potentials of –1.25 MPa and *Artiplex leucophylla* and *Cakile maritima* which are shallow rooted and develop minimal water potential of –1.8 MPa.

Table 5.10: Water potentials developed by various dune and other plant species growing at the indicated locations with listed annual rainfalls.

Species	Vegetation type and/or root system	Location	Dawn water potential range	Minimum midday $\Psi_{(leaf)}$	Rainfall at location
			MPa	MPa	mm yr^{-1}
A. populifolia [*1]	Rooted to *ca.*1m	Old Woman's River, coastal dunes	-0.44 to –0.53	-1.2	618
I. pes-caprae [*1]	Rooted to *ca.*3m		-0.42 to –0.76	-1.1	618
S. plumieri [*1]	Rooted to *ca.* 3m		-0.63 to –0.98	-1.55	618
S. plumieri [*2]	Deep rooted succulent	Mtunzini, South Africa	-0.16	-1.65	1279
Abronia maritima [*3]	Tap root to 3m water table	Northern & Southern California	-0.75 to -1.25	-1.8	820 & 390 respectively
Ambrosia chamissonis [*3]	Tap root to 3m water table		-0.3 to –1.1	-1.8	
Atriplex leucophylla [*3]	C_4, Rooted in swash zone		-0.3 to –1.8		
Cakile maritima [*3]	Shallow rooted		-0.3 to –1.8		
Heterotheca subaxillaris [*4]	C_3 herb	Georgian Coast		-1.2	
Croton punctuatus [*4]	C_3 herb			-1.0	
Iva imbricata [*4]	C_3 shrub			-1.8	
Atriplex arenaria [*4]	C_4			-2.0 to –2.2	
Uniola paniculata [*4]	C_4			-2.0 to –2.2	
Spartina patens [*4]	C_4 grass			-2.0 to –2.2	
Ellymus mollis [*5]	C_3 grass	Oregon Coast		-1.66	
Ammophila arenaria [*5]	C_3 grass			-1.5	
Plantago maritima [*6]	Salt marsh halophyte	Norfolk		- 2.75	
Arctostaphylos glauca, Adenostoma fasciculatum, Ceanothus greggii [*7]	Chapparral evergreen sclerophylls	North America		< -6.0	400 - 650
Protea laurifolia, Protea neriifolia [*8]	Deep rooted fynbos	Algeria, South Africa	-0.5 to –0.8	-1.0 to –1.8	675
Diosma hirsuta [*8]	Shallow rooted fynbos	Algeria and South Africa	-0.9 to –2.7	-1.8 to –3.9	675
Leucadendron pubescencens, Protea laurifolia [*9]	Deep rooted fynbos	Cedarberg, South Africa	-0.3 to –0.5	-1.1 to –1.4	675
Elytropappus gnaphaloides [*9]	Ericoid fynbos		-0.6 to –0.9	-1.9 to –2.0	675

Abbreviation: Leaf water potential ($Y_{(leaf)}$) [*1] Present study; [*2] Pammenter, 1983; [*3] De Jong, 1979; [*4] Dubois, 1977, cited in Barbour *et al.*, 1985; [*5] Pavlik, 1985; [*6] Jefferies *et al.* , 1977; [*7] Mooney et al., 1977; [*8] Miller et al., 1983; [*9] von Willert *et al.*, 1989.

A. populifolia and *I. pes-caprae* rarely developed water potentials below –1.2 MPa whilst *S. plumieri* developed potentials of –1.5 MPa on all experimental days. However unlike the species studied by Barbour *et al*. (1985) there was no clear correlation between the minimal water potentials developed and rooting pattern of the plants.

Both *I. pes-caprae* and *S. plumieri* are deep rooted whilst *A. populifolia* is not as deep rooted. Despite this *S. plumieri* developed the most negative water potentials. This could be the result of differences in hydraulic conductance rather than the root water availability (Koide et al., 1989).

Pressure-volume curves and the components of water potential

Differences in physiologically important parameters on different experimental days (extracted from the pressure-volume curves) were not highly significant (Table 5.7) and thus allowed for the combining of data for individual species. Subsequent calculation of the components of $\Psi_{(leaf)}$ from average pressure-volume curves constructed for each species showed that daily changes in water potential were the result of changes in turgor rather than osmotic potential, a phenomenon which has been shown for the dune grasses *Ammophila arenaria* and *Elymus mollis* (Pavlik, 1984).

In contrast to the findings of Pavlik (1984) for *Ammophila arenaria* and *Elymus mollis,* seasonal adjustment in osmotic potential was not evident (Table 5.7 and Figure 5.5). The lack of osmotic adjustment further suggests that water availability is not limiting. The accumulation of various stress metabolites has been shown in a range of coastal plants (Smirnoff and Stewart, 1985). These were attributed to drought stress but also conferred heat stability to some enzymes. Greenhouse experiments have been used to show osmotic adjustment in response to limited water supply in *Ziziphus mauritiana* (Clifford *et al*., 1998) and *Quercus wislizenii* (Momen *et al*., 1992). Osmotic potentials remained depressed even after re-watering in the former but not latter species. Saline soil-water and the resultant reduction in soil-water potential has also been shown to result in osmotic adjustment in coastal and salt marsh halophytes (Stewart *et al*., 1979). It appears that in the species studied in this investigation osmotic adjustment is unnecessary as they have a plentiful supply of non-saline water possibly consequent upon their deep root-systems and low water demands.

Leaf expansion rates are affected by transpiration rate presumably via the effects on plant water potential (Clifton-Brown and Jones, 1999). The turgor potentials of *S. plumieri* were reduced to zero on all experimental days during the midday period (Figure 5.6). This may affect leaf expansion. However turgor potentials recovered in the afternoon as transpiration rates declined and thus are unlikely to have adversely affected growth in the long-term. The RWC at which Ψp was reduced to zero was highest for *A. populifolia* followed by *S. plumieri* and then *I. pes-caprae* (Table 5.7). These differences are not highly significant and fall within the ranges of 0.94 to 0.9, and 0.9 to 0.875 measured for the dune grasses *Elymus mollis* and *Ammophila arenaria* respectively (Pavlik, 1984).

Difficulties encountered in the measurement of the ratio of symplastic to total plant water are not unique to this study. Richter (1997) considers that this ratio and the calculated bulk modulus of elasticity are unreliable. He comments that this data should be interpreted with extreme caution if used at all. As a result of this the appoplastic to total plant water ratios have not been reported and differences in cell elasticity between species and on different experimental days have not been emphasised. Differences in cell elasticity as indicated by the curvature factor c (Figure 5.7), could have resulted from differences in growth with young expanding tissue being less rigid than older or dormant tissue (Tyree and Jarvis, 1982).

δ ^{18}O values of rain-, ground- and stem-water

Unfortunately the lack of distinction between δ ^{18}O values of rain-water and ground-water does not allow the source of plant water to be identified (Figure 5.8). Nor, as has been shown for other plants (Ehleringer and Dawson, 1992; Flanagan *et al*., 1992), could the water use patterns between these two sources be ascertained in the present study. On one occasion the plant water appeared to have δ ^{18}O values that were similar to ground-water and clearly different from the weighted average value for rain. On certain dates all three types of water had similar values. On the last four sampling occasions (8/6/98, 19/7/98, 23/7/98 & 14/9/98) the ground water δ ^{18}O values were similar for high rain and were much higher than the ground-water values measured previously (21/2/98 & 9/9/97; Figure 5.8). What is apparent was that both ground-water and plant-water change and that the changes in ground-water are related to large rainfall events. It is tempting to attribute the changes in plant water δ ^{18}O values to the utilisation of different proportions of ground-water or rain-water on different occasions but the data do not allow for this conclusion to be reached. However the data does show that rain-water rapidly percolated into the coarse, organically-poor beach sand and contributed to the ground-water table. This ground-water or the water that rises above the water table both through capillarity and as water vapour is likely to be available to the deep-rooted species.

Frequent sampling of ground-water, rain-water and plant-water prior to and following heavy rainfall may be more revealing about the source of plant water and how it changes with rain-water availability.

Water Budgets

Pammenter (1983) estimated that the water in the dune sand was sufficient to supply *S. plumieri* for long periods of time (52 days). Similar estimates based on water-holding capacity of 4% and rooting depth of 1 m for *A. populifolia* and 3 m for *I. pes-caprae* and *S. plumieri* suggests that sufficient water is available to supply typical transpiration rates for periods of 33, 34 and 24 days respectively. This is in strong contrast to the estimates of 3 to 5 days calculated by Salisbury (1952) for dune annuals and small perennials. Those species were shallow rooted in comparison to the species investigated in this study. The rapid depletion of sand-water led Salisbury to conclude that internal dew formation was necessary to supply water as plants had been observed to survive periods of up to six weeks without rain. On only one occasion between 1/1/90 and 31/12/99 did a period without rain exceed 24 days. As Pammenter (1983) concluded it appears unnecessary to invoke the process of internal dew formation as a source of water for dune plants. That is not to say that this process does not occur. Salisbury (1952) suggests that internal dew formation could supply 9 litres of water per m^3 of beach sand based on an average nocturnal increase in the sand-water content of ± 0.7%. Using this value and the assumed volumes of sand utilised by *A. populifolia*, *I. pes-caprae* and *S. plumieri* the volume of water supplied by this process was estimated. Estimates were that this process could supply *A. populifolia* with 9 litres day^{-1} and *I. pes-caprae* and *S. plumieri* with 27 litres day^{-1}. These values are far in excess of estimates of the average daily volumes of water transpired by *A. populifolia* (0.51 l day^{-1}), *I. pes-caprae* (1.5 l day^{-1}) and *S. plumieri* (2.1 l day^{-1}) per m^2 of dune. In fact the excesses are so large that they could maintain plants even if they utilised far less sand volume than assumed above. Further research is required to establish to the number of days a year that climatic conditions are favourable for internal dew formation and how significant this process is in supplying water to the dune species in southern Africa.

Comparisons of annual rainfall and transpired volumes suggested that even in low rainfall years the rainfall was not limiting (Table 5.8). However this is an over-simplification as it ignores both the seasonal differences in rainfall distribution, the water storage capacity of the sand and any percolation processes. When comparisons were made on a monthly basis it was apparent that all three species were subjected to periods when transpiration exceeded rainfall (Figure 5.9). This was particularly so for *I. pes-caprae.* With its relatively high transpiration rate (Table 5.3) and high LAI (Table 3.6) it developed both the most frequent and the largest negative monthly budgets. Water budgets suggest that of the three species *I. pes-caprae* was on occasion the most susceptible to water limitations (Table 5.8 and Figures 5.9 & 5.10). Interestingly physiological data obtained on two of the experimental days showed that *I. pes-caprae* regulated water loss with midday reduction in transpiration rate (Figure 5.1) and stomatal conductance (Figure 7.5).

When the water-holding capacity of the beach sand was included at values of 4% ($^{v}/_{v}$) or higher, none of the species reduced sand-water content down to 0.5% at which point it is unlikely that the plants could continue to extract soil-water (Figure 5.10). The water-holding capacity of 4% appears to be a realistic value as on all occasions when excavations were made in the dunes, water content of approximately this magnitude was measured. It is possible that the process of internal dew formation maintained sand water contents at around 4% and the process should not be discounted as a possible source of water. If the water-holding capacity of sand is in fact as low as 1.5% then upon occasion all three species reduced sand-water content below 0.5 % (Figure 5.10).

Capacitance budgets assume that all the water in the soil (down to 0.5%) is available to the plants. This may not be true. Once soil-water content is reduced below field capacity the hydraulic conductivity is such that water diffuses relatively slowly through the soil. Hence the amount of water available to the plants depends on the rooting density a parameter that was not quantified. In view of this the calculated volumes of available soil water may be over-estimates.

Leaf water potentials of plants growing on a small isolated dune

Much of the data presented in this chapter suggests that on the experimental days water availability was not limiting. This was apparent from the relatively high transpiration rates with single-peaked diurnal responses, the leaf water potentials that were not very negative and which recovered by dawn and the positive water budgets. Only *I. pes-caprae* on the 30/1/97 and 19/2/98 showed any evidence of possible water limitation and had a two-peaked diurnal response in transpiration rate as the result of midday stomatal closure.

The maintenance of the plant's water status particularly in the cases of *S. plumieri* and *I. pes-caprae* appears to be dependent on extensive below-ground connections between shoots and extensive root systems. If the between-shoot connections were cut the remaining adventitious roots were insufficient for the maintenance of plant water status (Figure 5.8).

In addition to the extensive root systems the low LAI (Table 3.6) results in a relatively low transpiration demand per unit ground surface area. The development of a low LAI may ensure that resources such as water and nutrients are not limiting. It is interesting that *A. populifolia* in comparison with the other two species is neither rooted as deeply nor has as extensive under-ground stem connections but has the lowest LAI. This low

LAI also makes this species less susceptible to seasonal differences in the distribution of rainfall (Figure 5.10) and may be one of the reasons why it is able to maintain a high growth rate throughout the year (Figure 4.5). The density of plant cover has been related to plant water potentials of coastal shrubs in Spain. Initially as plant cover increased the water potentials declined until a threshold was reached following which plant cover declined with further decreases in water potential (Garcia-Novo, 1979). This could be viewed as evidence that limited water resources limit the leaf area that can be supported.

Summary of inter-species comparison

Table 5.11 gives a summary of the data from Chapter 5 that can be used to compare the three species *A. populifolia*, *I. pes-caprae* and *S. plumieri*. The three species are not very different with respect to the quantities of water used per unit leaf area but show major differences when water acquisition and use per unit dune area are considered.

Despite high transpiration rates and because of low LAI *A. populifolia* used the least amount of water per unit dune surface area. The demand for water appears to be more than adequately met if the species exploits dune sands to a depth of about one metre (Table 5.8). Due to the low requirements for water this species was least affected by seasonal rainfall distribution (Figures 5.9 & 5.10). This may offer an explanation as to why growth and productivity are not seasonal (Figure 4.5; Tables 4.5 & 4.6, Chapter 4). In addition this species had the highest WUE (Table 5.5), maintained the highest leaf water potentials (Figure 5.4) and was never recorded as having zero turgor potential (Figure 5.6). All these features are likely to be advantageous to a species living closest to the high water mark where sporadic yet frequent crisis events determine that growth and reproduction must be completed rapidly. Tinley (1985) reports that *A. populifolia* lives for only about two years and the monitoring of leaf production (Chapter 4) showed that many plants died within one year.

Due to a combination of high transpiration rate (Figure 5.1 & Table 5.1) and relatively high LAI (Table 3.6, Chapter 3) *I. pes-caprae* used the largest amount of water per unit dune surface area although the often unpredictable response of E to VPD decreases the confidence of such estimates. Water budgets suggest that this species is the most likely to be limited by water availability (Table 5.8; Figures 5.9 & 5.10). Limitations in water availability may result in the physiological regulation of water loss for which there is some evidence. This includes: two-peaked diurnal responses in transpiration rate (Figures 5.1); midday recovery of $\Psi_{(leaf)}$ (Figure 5.6); and midday reductions in stomatal conductance (Figure 7.5). Despite this *I. pes-caprae* showed no evidence of osmotic regulation a feature that has been shown for other dune species (Pavlik, 1983; Smirnoff and Stewart, 1985).

Table 5.11: Comparison of data presented for *A. populifolia*, *I. pes-caprae* and *S. plumieri* in Chapter 5. The number of symbols indicates the magnitude of the listed parameter relative to the smallest value measured for any one of the three species.

Measured parameter:	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
Transpiration and water use efficiencies			
Average peak E	♦	♦	♦
Average daily amount of water lost	♦¼	♦	♦
Dependence of E rate on VPD $^{5.2}$	♦	♦	♦♦
Average peak WUE	♦♦	♦	♦♦
Average daily WUE	♦♦½	♦	♦♦
Dependence of WUE on PPFD $^{5.2}$	♦¼	♦	♦
Leaf or shoot water potentials			
Average minimum $\Psi_{(leaf)}$	♦	♦	♦¾
Average $\Psi\pi$, where RWC=1	♦	♦	♦
Average RWC, where Ψp=0	♦	♦	♦
Average $\Psi_{(leaf)}$, where Ψp =0	♦	♦¼	♦¼
Water budgets			
Excess rainfall in annual budgets $^{5.3}$	♦♦♦♦♦♦♦♦ ♦♦♦♦♦♦♦♦	♦	♦♦♦♦♦♦ ♦½
Number of negative budget months $^{5.3}$	♦	♦♦♦¼	♦♦♦
Number of days sand-water is reduced to below 0.5% $^{5.4}$	♦	♦♦♦♦♦	♦½

Abbreviations: transpiration rate (E), atmospheric vapour pressure deficit (VPD), water use efficiency (WUE), photosynthetic photon flux density (PPFD), leaf water potential ($\Psi_{(leaf)}$), turgor potential (Ψp), osmotic potential ($\Psi\pi$), relative water content (RWC). $^{5.2}$The dependence of E on VPD, and WUE on PPFD, was based on a comparison of r^2 values for these relationships. $^{5.3}$Excess rainfall for annual budgets was based on average yearly values and monthly budgets on the average of 1994 and 1997 data. $^{5.4}$The number of days on which sand-water was reduced to 0.5% was calculated as an average of 1994 and 1997 data using a water-holding capacity of 1.5%.

These osmoregulating species inhabit the coasts of Oregon and the United Kingdom where seasonal differences are marked and predictable. It is possible that in the unpredictable conditions encountered on the Eastern Cape coast the investment of resources in osmoregulatory compounds is disadvantageous.

When calculated per unit of dune surface area the volumes of water transpired by *S. plumieri* were intermediate between the volumes transpired by the other two species (Table 5.8). The response of E to VPD was more predictable and did not saturate even at the highest VPDs recorded (Figure 5.2). This species had the lowest minimum leaf water potentials (Figure 5.4) despite having apparently greater water availability than *I. pes-caprae* (Table 5.8; Figures 5.9 & 5.10). This is possibly due to lower hydraulic conductivity.

CONCLUSIONS

As with the measurement of productivity (Chapter 4) the measurement of plant water relations was limited to five intensive snap-shots on days in different seasons. Despite this certain conclusions can be reached:

- The beach environment in the Eastern Cape and the studied species can be considered mesic as was evident from the plant physiological parameters measured and the predicted water availability.

- The water supplied by rainfall and the water stored in the dune sands is sufficient to meet the requirements of the plants although water budgets calculated for *I. pes-caprae* suggest that this species may on occasion be water limited.

- The source of plant water cannot be determined using isotopic ratios but water budgets suggest that it is not necessary to invoke either the utilisation of ground water nor the process of internal dew formation to supply sufficient water to meet the demands.

- Low LAI in combination with deep rooting and below ground-stem connections are necessary for maintenance of favourable water relations.

- The plants do not develop leaf water potentials that are more negative than –1.55 MPa (values that are typical of mesophytes and not xerophytes).

- The plants do not show changes in osmotic potential, an indication that they do not need to osmoregulate.

- Turgor potential for the most part remains positive throughout the day, a condition suitable for the maintenance of growth that may be essential to cope with sand accretion.

- All three species show relatively high transpiration rates and only *I. pes-caprae* showed any evidence of strong limitations of transpiration rate through reductions in midday stomatal conductance.

- All three species have relatively high water use efficiencies as a result of high assimilation rates rather than low transpiration rates.

CHAPTER 6
PLANT NUTRIENT RELATIONS

INTRODUCTION

Macronutrients are required in relatively large quantities as they are components of organic compounds such as proteins and nucleic acids, or they act as osmotica. Foredune sands are low in nutrient concentrations and nutrient availability has been proposed as one of the factors limiting effective plant productivity on coastal foredunes (Morris *et al*., 1974; Pavlik, 1983a; Lammerts and Grootjans, 1997).

Researchers have answered many of the questions concerning the nutrient relations of coastal dune vegetation (see reviews by Barbour *et al*., 1985; Hesp, 1991). Studies have focused on issues such as nutrient inputs, nutrient contents of plant tissues, mineralization rates, plant response to nutrient addition and nutrient allocation or reallocation patterns. These studies have generally focused on temperate systems and have drawn conclusions about nutrient limitations by comparisons with nutrient rich systems. Few studies have related nutrient availability to the demand for nutrients created through plant growth. An exception to the latter is a study by Fay and Jeffery (1992) whose preliminary findings were that mineralization of organic nitrogen supplied by wind-blown sand was sufficient to meet the demands created by root and shoot growth.

In sub-tropical dune systems the relationship between nutrient supply and demand is a central factor in the understanding of dune systems. Hence the following questions were asked. Which nutrients are in short supply? How much of these nutrients are required to support plant growth? Does the supply of nutrients merely meet the demand or are nutrients supplied in excess? Do the plants recycle limiting nutrients internally? Are some nutrients overabundant creating the potential for stress?

Nutrient availability

Beach sands are low in nutrients generally but particularly in nitrogen (Willis, 1965; Barbour *et al*., 1985; Ehrenfeld, 1990; Cain *et al*., 1999) and phosphorus (Willis, 1965; Salisbury, 1952; Harte and Pammenter, 1983). Sand consists largely of chemically inert quartz (Buckman and Brady, 1969) and in combination with low organic content, leaching and low nutrient input, results in low nutrient availability (Barbour *et al*., 1985).

Of all the macronutrients, nitrogen nutrition has received the most attention. This is because nitrogen concentrations in dune sands are extremely low (Harte and Pammenter, 1983; Gerlach *et al*., 1994) and yet the plant requirements for nitrogen are the highest.

The major nitrogen input is via detritus deposition in the swash. However nitrogen is also available in seawater where concentrations are highly variable (0.036 to 1000 ppm) and from fog (1 to 7 ppm) (Barbour *et al*. 1985, and authors cited therein).

Following nitrogen, leaf potassium concentrations of dune plants were found to be the highest of four cations measured (Na^+, Mg^{2+}, Ca^{2+}, and K^+). This despite the low concentrations of potassium in meteorological inputs (van der Valk, 1974b) and in dune soils (Art *et al*., 1974; Pammenter, 1983). This has led to the conclusion that potassium availability is potentially limiting. Barbour *et al.* (1985) comment on the lack of data from research on the potassium nutrition of beach plants in America and that such information would be of particular interest due to the interaction between sodium and potassium uptake in high sodium environments.

Little is known about the phosphorus nutrition of dune plants (Barbour *et al*., 1985) although the concentrations found in dune sand and in dune plants have been recorded by Johnson (1963, cited in Barbour *et al*., 1985), Ernst (1983) and Harte and Pammenter (1983).

It is important to consider not only the absolute concentrations of nutrients in dune soils but also their flux, mineralization and turnover. The rapid transformation of ammonium to nitrate was measured in immature soils from foredunes (Skiba and Wainwright, 1984). Gerlach (1993) reported very low organic nitrogen mineralization to ammonium and nitrate in primary dune sands. The values increased along the coastal dune succession and were highest in soils below dune forests. Unlike nitrogen mineralization rates, the proportion of the total amount of nitrogen that is annually turned over was highest in young dune sands (Gerlach *et al*., 1994).

Skiba and Wainwright (1984) showed that sulphur oxidation rates in dune sands were similar to rates measured in garden soils. The rates of oxidation were related to soil carbon and nitrogen content, soil pH and vegetation cover.

Nutrient inputs

Meteorological inputs from sea-spray and fog are the major suppliers of sodium, magnesium, calcium, potassium and phosphorus and sand concentrations reflect their relative abundance in seawater (van der Valk, 1974; Art *et al.,* 1974, Clayton, 1972; Holton, 1980). These nutrients are indirectly available to plants by the interception of fog that collects and flows down the stems rather than by direct foliar absorption (van der Valk, 1977).

Soil nutrient concentrations are kept low through leaching as the substrate is course and contains little organic matter (Barbour *et al*., 1985). van der Valk (1974) in a nutrient study on the Welsh coast showed that nutrient (K^+, Na^+, Ca^{2+} and Mg^{2+}) input from salt spray exceeded the amounts lost through leaching. The surplus accumulated in coastal vegetation.

Some dune species have access to ground-water that contains nutrients and are also periodically inundated with seawater. Seawater contains both macronutrients and micronutrients (Goldberg, 1963; Hesp, 1991). Little or no research has been conducted to investigate the role of ground-water as a potential source of nutrients. In contrast to coastal dunes the importance of ground-water in dune slacks has been researched (van Dijk and Grootjans, 1993; Sival and Grootjans, 1996; Grootjans *et al*., 1998). The eutrophication of ground-water has been shown to affect vegetation composition, cover and biomass (van Dijk and Grootjans, 1993).

Organic matter is deposited on the beach by the sea (Hesp, 1991; Davy and Figueroa, 1993) and is distributed by sand-movement (Hesp, 1991 and references cited therein). This deposited matter serves as a source of nutrients and is the major source of nitrogen. Fay and Jeffrey (1992) measured higher concentrations of nitrogen in foreshore and freshly blown sand than in older dune-sands and view the foreshore as a reservoir of available nitrogen. They showed that the mineralization of organic nitrogen contained within these sands allowed for the growth of A*mmophila arenaria* subsequent to its burial. Furthermore the availability of nitrate in the foreshore sands has been shown to differentially induce nitrate reductase activity in various dune species (Garcia-Novo, 1976; Holton, 1980).

Nitrogen fixation by rhizosphere bacteria has been demonstrated in some dune grasses (Ahmad and Neckelman, 1978; Day *et al*., 1975) but the importance of this source of nitrogen in the nutrition of dicotyledonous species in tropical and sub-tropical regions is unknown. In addition to rhizosphere nitrogen fixation, nodulated plant species have access to atmospheric nitrogen via their symbiotic relationship with bacteria. Nitrogen fixation by nodulated dune species has been researched in the USA (Holton and Johnson, 1979; Holton, 1980) but not in the coastal flora of the Eastern Cape Province. Unlike some temperate dune systems there are relatively few legumes found growing on the Eastern Cape coast (Hertling, 1997). The introduced *Acacia cyclops,* which is nodulated, has become successfully invasive along this coast.

Not only are nutrient contents, rates of turnover and mineralization important, but so are the spatial and temporal distributions of these nutrients within the foredunes. In foredune-sands nitrogen is heterogeneously distributed both spatially and temporally down to the smallest scales that have been measured (Cain *et al*., 1999). Small-scale heterogeneity can have large impacts on the performance of individual plants (Antonovics *et al*., 1987; Miller *et al*., 1995). For example the distribution of organic litter has been shown to markedly influence the size of *Salsola kali* individuals (Lee *et al*., 1983). Furthermore local areas of high nitrogen availability were found not to be persistent (Cain *et al*., 1999). The other nutrients are distributed more homogeneously as they originate mainly from fog and sea-spray (van der Valk, 1974).

Nutrient addition

Addition of nutrients to naturally and laboratory-grown dune species have been shown to increase plant biomass (Willis, 1965; Pavlik, 1983b; Dougherty *et al*., 1990; Zhang, 1996), change nutrient and carbon allocation patterns (Pavlik, 1983b; Zhang, 1996) and photosynthetic rates (Pavlik, 1983a; Zhang, 1996). This evidence has been used to support the hypothesis that nutrients limit dune species productivity. However Barbour *et al.* (1985) caution that many plants regardless of habitat respond to the addition of fertilizer and one can infer little about their nutritional ecology from such studies. They also suggest that a comparative study of the response of coastal dune and non-dune species to the addition of nitrogen would be more relevant.

Studies on dune plants have been conducted under conditions of reduced nitrogen availability (Pavlik, 1983 a, b, c; Holton, 1980). Plants showed a decrease in leaf area, plant dry weight, leaf area ratio, dark respiration rates, tissue nitrogen contents and an increase in root to shoot ratios. There were some differential interspecific responses. For example *Cakile maritima* increased leaf conductance which maintained photosynthetic rates but decreased WUE. *Ammophila arenaria* and *Elymus mollis* decreased leaf conductances and this resulted in increased WUE.

Nutrient remobilization

Limitations in nutrient availability are likely to result in conservative nutrient use by foredune plants. This includes the remobilization of nutrients from older to younger leaves and reproductive structures (Pemadasa and Lovell, 1974; Harte and Pammenter, 1983). Remobilization is defined as the net export of nutrients from ageing leaves to younger leaves (Marshner, 1995). This has been shown to occur within clones (Nobel and Marshall, 1983) and from vegetative to reproductive structures (Nobel and Marshall, 1983; Ernst, 1983; Harte and Pammenter, 1983). Dune slack pioneer species have interspecific differences in their adaptations to a low supply of the major nutrients. The pioneer sedge *Schoenus nigricans* has a low demand for phosphorus and nitrogen in comparison with herbaceous pioneer species or later-successional grasses (Grootjans *et al*., 1998; and references cited therein). This low demand for phosphorus was attributed to high internal cycling of these nutrients.

Of the macronutrients (K^+, N, P, Mg^{2+}, Na^+, Cl^-) chloride and phosphorus are remobilizable whilst calcium and magnesium are not. Remobilization occurs via the phloem and the latter two nutrients have low phloem mobility. The remobilization of nutrients in the phloem is a normal feature throughout the life of an individual leaf (Marshner, 1995). For example in nitrate fed wheat plants, of the nitrate transported to the leaves in the xylem, 79% was cycled back to the roots via the phloem. In the roots 21% was incorporated into new tissue while the remainder was cycled back to the leaves in the xylem (Simpson *et al*., 1982). Similar cycling has been shown for other macronutrients (Jeschke and Pate, 1991).

Nutrient budgets

Fay and Jeffrey (1992) showed that estimates of nitrogen requirements for shoot and root growth were less than the amounts made available through soil organic nitrogen mineralization. They concluded that wind-blown sand (which contained organic matter) was the most important source of nitrogen and that the subsequent mineralization of this organic nitrogen was sufficient to supply the luxuriant growth of dune vegetation of the North Bull Island, Dublin.

Similar nutrient budgets can be constructed by considering the supply of nutrients from the soil-solution in comparison with the demand for nutrients that is created by growth. The supply can be estimated from the volume and nutrient content of the water that is taken up by plants. This supply would represent the minimum available to the plant as it assumes that nutrients are acquired in the same concentrations as are found in the soil-solution. Such budgets may be complicated because plant roots may selectively take up nutrients and because nutrients are recycled within plants. The amounts of nutrients contained within plant organs are not always directly related to the transpiration rates or volume of water transpired by that organ but this varies among nutrients (Marschner, 1995). This is dependent on nutrient mobility. The amounts of the less mobile nutrients (Ca^{2+}, Na^{+}, and Si^{+}) are more closely related to transpiration than to the amount of nutrients re-cycled within the plant (N, P and K^{+}).

Salt accumulation

Salt concentrations of dune sands below the surface layers are low, as a result of leaching (Boyce, 1954; Rozema *et al*., 1985). However the continual leaching of the superficially deposited salts makes these ions available to dune plants and may increase the salinity of the soil-solution. However the salinity of the soil-solution appears not to limit the particular species that can thrive in the foredunes. Oosting and Billings (1942), Boyce (1954) and Barbour *et al*. (1973) could show little correlation between soil salinity and species distribution as one moves inland within the dune system.

Salt-spray and the foliar absorption of salt may be more important considerations than soil salinity. A correlation between salt-spray tolerance and species distribution along a successional gradient within dunes has been demonstrated by various authors (Oosting and Billings, 1942); Avis 1992). Furthermore salt-spray loads and intensities are correlated with the distance inland from the beach (Avis and Lubke, 1985). Foliar depositions as a result of salt-spray are thought to enter leaves through lesions (Barbour *et al*., 1985). The foliar accumulation of salts results in the development of leaf succulence in dicotyledonous plants and this has been related to salt tolerance. Succulent leaves contain 4 to 6 times the chloride concentration of non-succulent leaves from the same species (Barbour *et al.*, 1985).

Another potential source of salt is dune aquifer-water which may contain significant quantities of both sodium and chloride. The utilization of this water and the direct foliar absorption of salt from spray may lead to the foliar accumulation of these ions. This may be one of the factors that determine leaf longevity. A linear foliar accumulation of sodium with no remobilization has been demonstrated throughout the life of *S. plumieri* leaves (Harte and Pammenter, 1983). Maximum levels accumulated surpassed levels measured for the halophyte *Avicennia marina* (Drennan and Pammenter, 1982) and Harte and Pammenter (1983) concluded that it was unlikely that *S. plumieri* possessed a sodium exclusion mechanism. It is possible that the accumulation of these ions determines leaf senescence.

METHODS

Dune sand nutrient contents

Holes were excavated at the seaward side of the base of the foredune adjacent to location where all the physiological measurements of 21/2/98 and 25/7/98 were taken. 500 g soil samples at depths of 50, 150 and 250 cm were enclosed in double plastic bags and stored at –40°C. Samples were analysed for potassium, nitrogen and phosphorus by Matrolab, Capetown. The amount of nitrogen was measured by the Kjeldahl method in which a weighed mass of soil was digested with hydrogen sulphate and selenium as a catalyst. The digest was then distilled. The distillate was collected in a saturated boric acid indicator solution and then titrated with standard acid. Potassium was determined as follows: 0.05g of soil were added to a 50 ml solution of 1M ammonium acetate cooled to 20 ± 0.2 °C and shaken at 180 oscillation min^{-1} for 30 minutes. The solution was rapidly filtered and potassium in the clear filtrate determined by atomic absorption spectroscopy using appropriate standards.

Phosphorus was determined by Bray extraction (Bray and Kurtz, 1945). 50ml of Bray extraction solution (3.756 mM NH_4F + 2.126 mM HCl in distilled water) was added to 6.67 g of soil and the mixture shaken at 180 rpm in a reciprocating shaker. Immediately after shaking 2 drops of a flocculent (Superfloc N-100,Cyanamid, 1g l^{-1}) was added and the mixture filtered (Whatman No. 30). The first fraction of the filtrate was discarded. Phosphorus was determined using the molybdenum blue colorimetric method which uses 1,2,4-amino naphthol sulphonic acid as a reducing agent.

Ground-water and dune-water nutrient content

Ground-water samples were collected on the 21/2/98, 29/10/98, 12/2/99 and 26/3/99 and sealed in boiling-tubes. For details of ground-water sampling refer to Chapter 5. Water was extracted from beach-sand by centrifugation and is referred to as sand-water throughout. Sand was removed to a depth of 20 cm and then 10 kg of sand was collected and sealed in a container to prevent evaporation. This was repeated on the same dates that ground-water samples were collected. Sub-samples of this were centrifuged in 500 ml bottles at 1000 x *g* for 15 minutes. The dry upper portion of the centrifuged sand was discarded and the wet sand from the bottom of the centrifuge tubes was pooled. This wet sand was placed into 12 ml centrifuge tubes which were then inserted into larger 100 ml centrifuge tubes. The inner tube had a small perforation in its base so that on centrifugation water would exit the inner tube and accumulate in the larger outer tube. This system of double tubes was centrifuged at 4000 x *g* for 15 minutes. The extracted water was pooled and sealed in a boiling-tube.

Water samples were analysed for potassium, nitrogen, phosphorus and sodium by Matrolab, Capetown. Total available nitrogen was calculated as the sum of ammonium nitrogen, nitrate and nitrite. Ammonium nitrogen was determined as follows: 15ml of 45% ($^{m}/_{v}$) sodium hydroxide was added to a 50ml water sample and distilled. The distillate was collected in a saturated boric acid indicator solution and then titrated with standard acid. Nitrate and nitrite were then determined by adding 0.2g Devarda alloy to the remaining sodium hydroxide and sample solution, followed by further distillation. The distillate was collected in a second saturated boric acid indicator solution and then titrated with standard acid.

Phosphorus concentrations were determined as follows: 10 ml of water sample were added to 10 ml of a solution of ammonium-molybdate and ammonium-vanadate. 30ml of distilled water was added and the solution allowed to stand for 30 minutes. Absorbance was then read at 470 ηm. A standard curve was constructed using 10, 25 and 50 μmol mol^{-1} calibration standards treated in the same way as the samples.

Following appropriate dilution potassium and sodium concentrations in the water samples were determined by atomic absorption spectroscopy and were compared to calibration standards.

Above-ground shoot nutrient contents

On two dates (21/2/98 and 25/7/98) three plants or shoots from each species were collected, the living leaves and stem removed and washed twice with distilled water before drying to constant weight at 60^{o}C. Leaves and stems from each plant or shoot were pooled and milled to a fine powder. Pooled milled samples were sent to Matrolab for potassium, nitrogen, phosphorus and sodium analysis. Following the digestion of 1 g of ground oven dried leaf sample in 15 ml of 45% sodium hydroxide, ammonium nitrogen, nitrate and nitrite were determined by the Kjeldhal method, as described above. Total nitrogen was calculated as the sum of ammonium, nitrate and nitrite.

Potassium and sodium concentrations were determined by atomic absorption spectroscopy following appropriate dilution of acid digests of ashed leaf samples.

Nitrogen use efficiency (NUE) was calculated as follows (Chapin and Van Cleve, 1989):

$$\text{NUE (g g}^{-1}\text{)} = \frac{\text{above-ground plant dry mass (g)}}{\text{above-ground plant N content (g)}}$$

Nutrient budgets

Nutrient supply and demand were calculated per unit dune surface area according to the following formula:

Nutrient supply (g m^{-2} yr^{-1}) = water nutrient concentration (g l^{-1}) x annual volume of water lost (l m^{-2} yr^{-1})

Nutrient demand (g yr^{-1} m^{-2}) = Shoot or plant nutrient concentration (g g^{-1}) x shoot primary productivity (g dry wt. yr^{-1}) x number of shoots per unit dune surface area (shoot m^{-2})

Values for the variables can be found in the following figures and tables: Average water nutrient concentration (Figure 6.2), average annual volume of water lost (Table 5.8), average shoot or plant nutrient concentration (Table 6.1), average shoot primary productivity (Table 4.6) and average number of shoots per m^2 (Table 3.6).

A range and the mean nutrient demand and supply were calculated. The higher value in the range was calculated by adding the standard deviation to the mean for each variable in the equations above and the lower value was calculated by subtracting the standard deviation. Mean values were calculated from the mean of each of the variables.

Effect of leaf age on nutrient content and remobilization

Three shoots or plants of each species were harvested on the 21/2/98 and the 25/7/98 and individual leaves labelled and washed twice in distilled water. Leaves were numbered sequentially with the smallest visible leaf being assigned the number 1. This sequence is hereafter referred to as the assigned leaf numbers. The buds and smallest leaves that had insufficient dry mass to be analysed independently were pooled (up to five small leaves). Because of this when leaf weight to assigned leaf number relationships were plotted the first five or six leaves were omitted. Leaves were oven dried to constant weight at 60^{o}C and individual weights recorded. Individual leaves were then ashed at 300^{o}C in a muffle furnace and the ash added to 10 ml of distilled water in polycap vials. Chloride concentrations were measured with a chloride ion-specific electrode (Eutek Inc., USA) that was calibrated hourly with analytical grade sodium chloride standards. Chloride concentrations were expressed as g 100g^{-1} dry weight.

Leaf ash suspensions were sent to Matrolab (Capetown) for analysis of macronutrients. 5 ml of concentrated hydrochloric acid and a few drops of nitric acid were added to each ashed suspension and after a few minutes made up to 50 ml with distilled water. A 10 ml sub-sample of this was used to determine phosphorus concentrations as for water samples above.

Potassium, calcium, magnesium and sodium concentrations were determined by atomic absorption spectroscopy following the appropriate dilution of the acid digest (as above). These concentrations were expressed as g 100g^{-1} dry mass.

Statistics

Where appropriate the data were compared using an analysis of variance (ANOVA) followed by Tukey tests if ANOVA showed significant differences at the 95% confidence level using the program Statistica '99 Edition (© Statsoft, Inc., USA). Statistical model assumptions were tested for normality and square root transformations undertaken where necessary.

RESULTS

Dune-sand nutrient content

The nutrient contents of beach sand are considered to be low (Salisbury, 1952; Willis, 1965; Barbour *et al*., 1985; Pammenter, 1983; Ehrenfeld, 1990; Cain *et al*., 1999). Nutrient concentrations for a variety of sands from different localities are listed in Table 6.1.

Table 6.1: Nutrient contents of a wide range of sands and soil. N=6 for analyses performed in this study.

	Nutrient contents (mg 100g^{-1} dry sand)					
	Na^{+}	**Ca^{2+}**	**Mg^{2+}**	**P**	**K^{+}**	**Total N**
Diverse range of soils [*1]	2.3 – 23.0	8 - 200	4.9 – 48.6	0.31 – 30.1	0.39 – 39.1	98 – 490
Dune sands [*1]	2.3 – 13.7	40 – 200	2.4 – 9.7	0.3 – 0.6	1.56 – 3.91	1.4 – 14
Dune sands [*2]	–	–	–	0.32 ± 0.02	2.57 ± 0.41	1.6 ± 1.4[*5]
Beach plane and foredunes [*3]						1.3 – 12.8
Dune sand, Noordwijkerhout [*4]	10.8 ± 2.1	276 ± 28.0	1.9 ± 0.2	0.74 ± 0.2	1.5 ± 0.1	17.2 ± 1.0
Dune sand, Castricum [*4]	11.7 ± 2.3	340 ± 24.0	4.1 ± 0.2	0.49 ± 0.1	1.6 ± 0.4	29.9 ± 2.1

[*1] Harte and Pammenter (1983), Mtunzini, Kwa-Zulu Natal; [*2] Present investigation, Old Woman's River; [*3] Gerlach *et al.* (1994), Spiekeroog, East Fristan Islands, German Bight; *[4] Ernst (1983), The Netherlands. In the present study total nitrogen = NH_3 + NO_2^{-} + NO_3^{2}. In other studies it includes organic nitrogen. Data from Gerlach *et al.* (1994) was converted from kg ha^{-1} 30 cm surface layer to mg 100 g^{-1} using the bulk density of sand as 1.3 kg l^{-1}.

The concentrations of nitrogen, phosphorus and potassium are comparable between the two South African sites, Mtunzini and Old Woman's River. Sodium, calcium, magnesium and phosphorus concentrations fall within the range measured for a wide variety of sands. However nitrogen concentrations are far lower and were similar to values recorded for sand from Spiekerroog, Germany (Gerlach *et al.* (1994). Values for soil total nitrogen, calcium, sodium and phosphorus from the two Netherlands sites (Noordwijkerhout and Castricum) were higher than those from the South African or German sites. Potassium in South African dune sands remained much lower than the values for sand from the Netherlands sites.

The nitrogen, phosphorus and potassium concentrations at different sampling depths were not significantly different (p= 0.4641, 0.1036 and 0.8180 respectively) when data from the two sampling dates were combined (Figure 6.1).

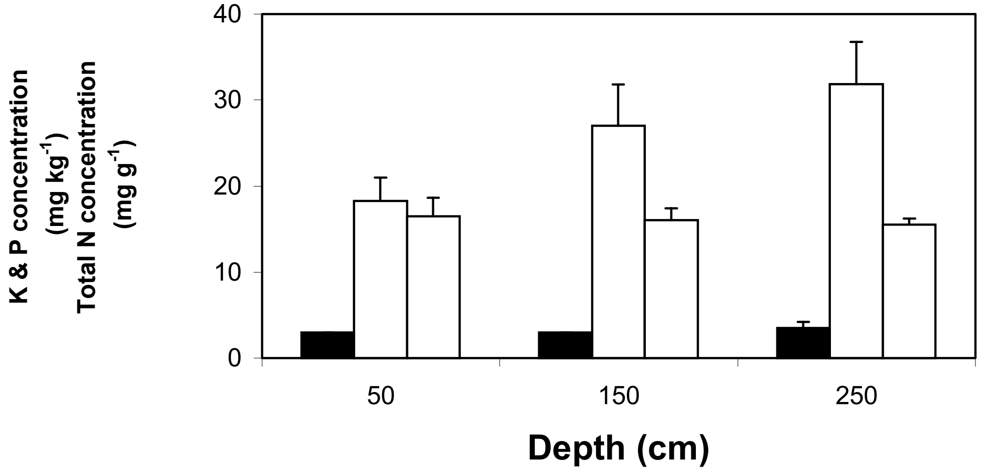


Figure 6.1: Average sand concentrations of phosphorus (P; solid block), potassium (K^+; open block) and total nitrogen (N; patterned block) at three depths sampled on two dates (18/2/98 and 25/7/98). Concentrations were not significantly different at the different soil depths. Standard deviations are indicated by the vertical bars. N=4.

The lack of variation suggests that nutrient availability in the sand above the capillarity saturated zone associated with the ground-water table is relatively uniform. As sand samples were not collected at a number of different locations within the foredune, spatial variation was not assessed. Other authors have shown this to be quite considerable (Cain *et al.*, 1999). Nutrient availability showed considerable temporal variation (Figure 6.2) which was different for the individual nutrients. This variability was assessed for potassium, nitrogen, phosphorus and sodium in both ground-water and water extracted from the beach sand by centrifugation.

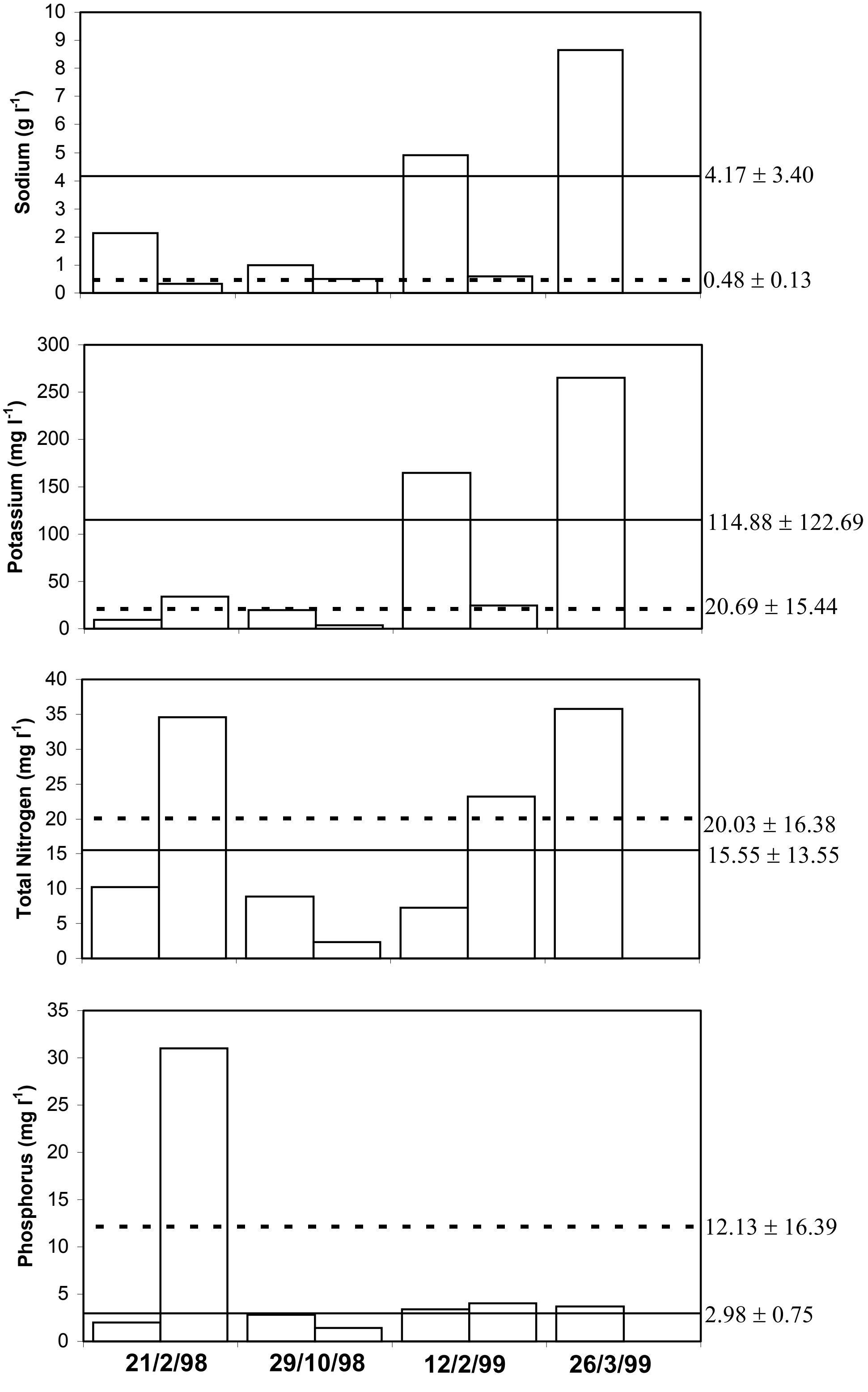


Figure 6.2: Ground-water (open blocks) and sand-water (patterned blocks) concentrations of sodium, potassium, total nitrogen and phosphorus sampled on the indicated dates. Average concentrations and standard deviations for ground-water (solid line) and sand-water (broken line) are indicated on the right of the figure.

Sodium and potassium in the ground-water showed similar temporal patterns of variation with the higher concentrations being recorded on the 12/2/99 and the 26/3/99. The highest values recorded approach the concentrations of these ions found in seawater i.e. 15.5 g l^{-1} and 379 mg l^{-1} for sodium and potassium respectively (Taiz and Zeiger, 1991). Furthermore the higher values recorded on the afternoons of the 12/2/99 and 26/3/99 were when the tide was high. This suggests inundation of the ground-water with seawater. Total nitrogen concentrations were similar on the first three sampling dates but were more than doubled on the third sampling date. Phosphorus concentrations remained relatively constant on all sampling dates.

Water centrifuged from beach sand had low sodium and phosphorus concentrations on all sampling dates. Total nitrogen and phosphorus concentrations varied markedly on different sampling dates (Figure 6.2).

Above-ground shoot or plant nutrient content

The potassium, nitrogen and phosphorus concentrations of shoots or plants of the three species were assessed on two occasions (Figure 6.3). Total nitrogen was significantly different between species (p=0.0045) with *I. pes-caprae* concentration from shoots sampled on the 21/2/98 (summer) being significantly higher than concentrations measured in *S. plumieri* shoots on the 25/7/98 (winter). Neither the differences between sampling days (p=0.6649) nor the interaction between sampling day and species (p=0.7671) were significant. For a single species there were no significant differences in nutrient concentrations from samples collected on different dates.

Nitrogen use efficiencies (NUE) were calculated for the above-ground plant parts and were 72.5 ± 11.9, 65.7 ± 5.24 and 103.0 ± 25.7 g g^{-1} for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. The higher values indicate a higher above-ground biomass production per unit of nitrogen use (Chaplin and van Cleve, 1989).

Shoot or plant phosphorus concentrations were significantly different among species ($p=0.0137$) and on different sampling dates ($p=0.0228$) but the interaction of these was not significant ($p=0.1563$). *A. populifolia* phosphorus concentrations sampled on the 21/2/98 (summer) and on the 25/7/98 (winter) were significantly lower than in *I. pes-caprae* shoots sampled on the 21/2/98 (summer). In addition the *A. populifolia* concentrations sampled on the latter date were significantly different from both *I. pes-caprae* and *S. plumieri* shoots sampled on the 25/7/98 (winter).

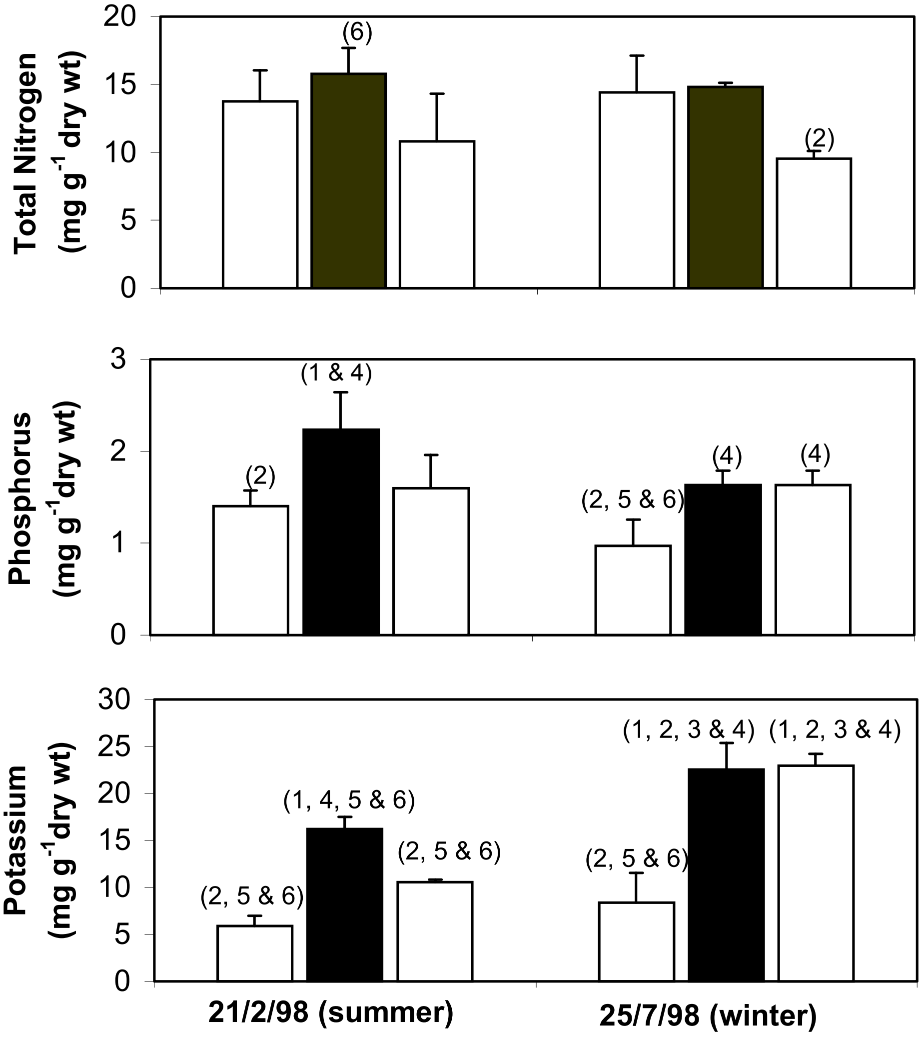


Figure 6.3: Above-ground shoot or plant concentrations of total nitrogen, potassium and phosphorus for *A. populifolia* (open blocks), *I. pes-caprae* (solid blocks) and *S. plumieri* (patterned blocks) on the two indicated sampling dates. If nutrient concentration sampled for the different species and on different dates were significantly different this is indicated by the bracket numbers. Samples collected on the 21/2/98 being designated (1) - *A. populifolia,* (2) - *I. pes-caprae* and (3) - *S. plumieri* 25/7/98. Samples collected on the 25/7/98 being designated (4) - *A. populifolia,* (5) - *I. pes-caprae* and (6) - *S. plumieri*. Standard deviations are indicated by the vertical bars. N=3.

The differences in shoot or plant potassium concentrations were significant among species ($p<0.0001$), between sampling dates ($p<0.0001$) and for their interaction ($p=0.0029$). *A. populifolia* plants contained significantly lower concentrations of potassium than did *I. pes-caprae* or *S. plumieri* shoots sampled on the 25/7/98 (winter) and *I. pes-caprae* shoots sampled on the 21/2/98 (summer) (Figure 6.3). Both *I. pes-caprae* and *S. plumieri* shoots sampled on the 25/7/98 (winter) contained greater concentrations of potassium than did shoots sampled on the 21/2/98 (summer).

Although the shoot or plant concentrations of nitrogen and phosphorus were significantly different among species and between sampling dates, there were not significant differences between sampling dates for a particular species (Figure 6.3). There were intraspecific differences in shoot potassium concentrations for *I. pes-caprae* and *S. plumieri* on the two sampling dates. From the presented data it is not possible to determine whether this is due to differences in potassium availability or demand. However leaf production rates of *I. pes-caprae* and *S. plumieri* were significantly higher in summer than in the winter of 1998 (Figure 4.5). *A. populifolia* did not have significant differences in plant potassium concentrations sampled on the 21/2/98 and 25/7/98 (Figure 6.3) nor did this species display differences in leaf production rates throughout the year (Figure 4.5).

Nutrient budgets

The analysis of water rather than soil allowed for the estimation of nutrient supply. Nutrient supply by ground-water or sand-water was calculated as the product of the estimated volumes of water taken up by the plants (Chapter 5) and the concentration of nutrients in that water. It was assumed that nutrients were taken up in the concentrations in which they are found in these water sources. Thus these calculations represent the minimum supply of these nutrients. These estimates were compared with the nutrient demand created by shoot or plant production. Calculating nutrient demand is complicated because nutrients may be remobilized from older to younger leaves. This complication was accounted for, as the average nutrient content from all the leaves on whole shoots or plants were used in these calculations. Because the sampling of ground-water and sand-water for nutrient content spanned a period of about a year, this time frame was chosen to construct nutrient budgets. The ranges in and the mean nitrogen, phosphorus and potassium supply and demand are given in Figure 6.4 and 6.5.

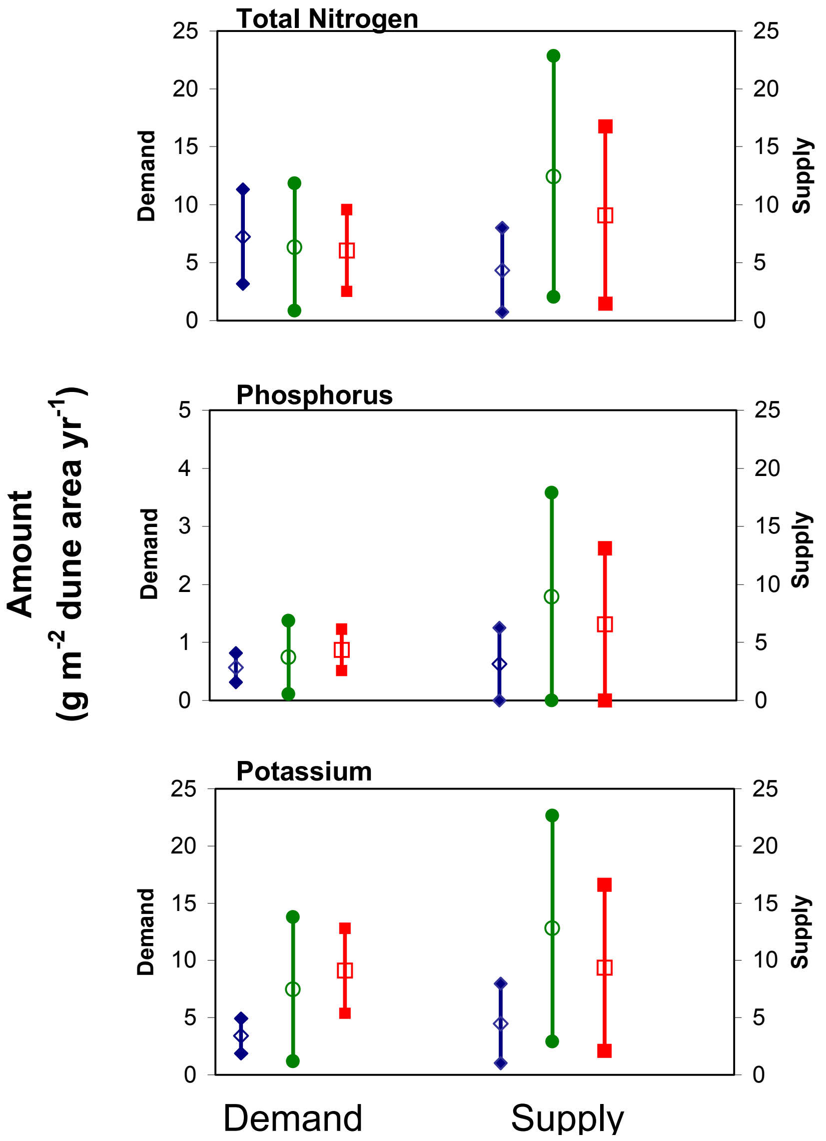


Figure 6.4: The average annual demand (amount in open symbols, range in closed symbols) and the sand-water supply (amount in open symbols, range in closed symbols) of the indicated nutrient per m^2 of dune for *A. populifolia* (diamonds), *I. pes-caprae* (circles) and *S. plumieri* (squares). Note the differences in scale for phosphorus supply and demand.

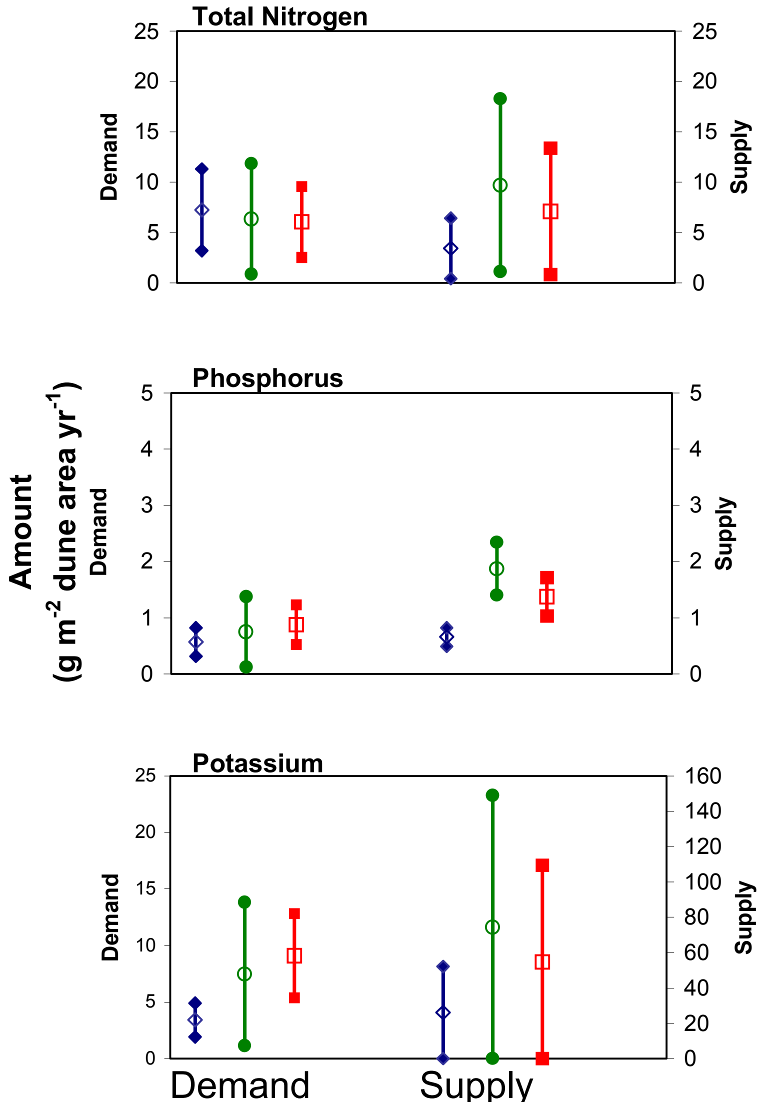


Figure 6.5: The average annual demand (amount in open symbols, range in closed symbols) and the ground-water supply (amount in open symbols, range in closed symbols) of the indicated nutrient per m^2 of dune for *A. populifolia* (diamonds), *I. pes-caprae* (circles) and *S. plumieri* (squares). Note the differences in scale for potassium supply and demand.

From the data presented it is evident that the ranges in supply for the annual budgets were large. This resulted mainly from the large variation of measured ground-water and sand-water nutrient concentrations. Phosphorus supply by ground-water was an exception and had a much smaller range in values due to the low and less variable phosphorus concentrations measured in ground-water.

The average sand-water and ground-water supply of nitrogen, phosphorus and potassium were estimated to be at least equal to or greater than the average demand for these nutrients by *I. pes-caprae* and *S. plumieri* (Figure 6.4 and 6.5). Similarly phosphorus and potassium demands by *A. populifolia* were equal to or less than the supply by ground-water or sand-water. However average nitrogen demand by *A. populifolia* was 66% and 113% higher than the average supplies by sand-water and ground-water respectively.

When considering the ranges in supply and demand the highest demand values were always higher than the lowest supply values and visa versa (Figures 6.4 and 6.5). Higher resolution in sampling particularly of ground-water and sand-water nutrient concentrations would be required in order to ascertain if nutrient supply is limiting in the short-term.

Leaf nutrient concentrations

The assigned leaf number is related to leaf age but this relationship is not necessarily linear as is evident from the changing rates of leaf production during the year (Figure 4.5). On average, leaf production rates were 4.9 ± 2.2, 2.7 ± 2.3 and 2.1 ± 0.9 leaves month^{-1} for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. Thus on average each leaf produced approximates a 6.1, 11.1 and 13.0 day period for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. In order to simplify subsequent language "assigned leaf number" has been termed "leaf age" hereafter.

The average weight of *A. populifolia* and *S. plumieri* leaves sampled on 21/2/98 increased linearly with increasing leaf age. However the average weight of *A. populifolia* and *S. plumieri* leaves sampled on the 25/7/98 initially increased with age and then decreased (Figure 6.6). *I. pes-caprae* leaves sampled on both dates showed a similar trend. Linear regressions were fitted to the initial increasing linear portions from combined replicates of each species on particular sampling dates (Table 6.2). When individual replicates were examined there was considerable variation within species and on different sampling dates. This is evident when the linear regressions for individual replicates (Table 6.4 and 6.5) are compared to values from combined responses (Table 6.2).

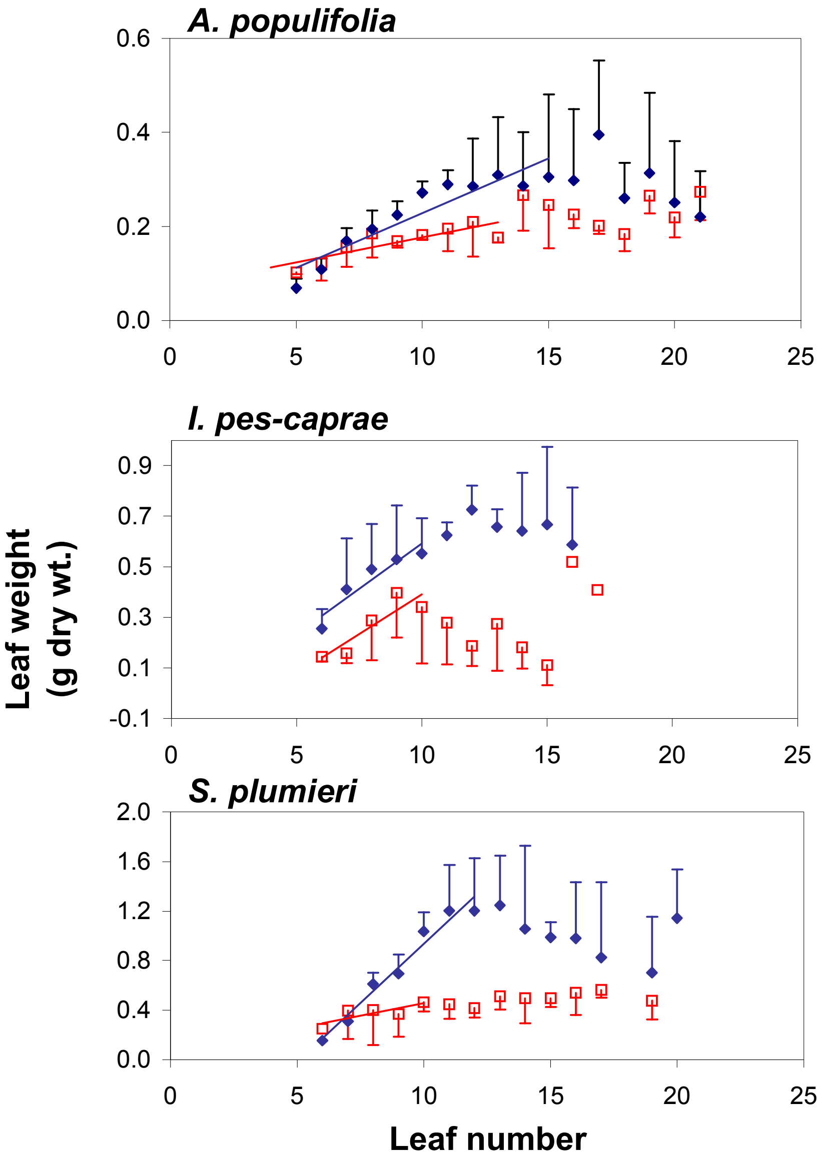


Figure 6.6: Average leaf dry weight sampled on the 21/2/98 (summer; open symbols) and on the 25/7/98 (winter; closed symbols) in response to increasing assigned leaf number. Standard deviations are given by the vertical bars. N=3.

An ANOVA showed that the initial linear response of leaf weight to age (slopes of regressions for individual replicates) were significantly different between sampling dates for *A. populifolia* ($p=0.0064$), *I. pes-caprae* ($p<0.0001$) and *S. plumieri* ($p<0.0001$). This was so despite the shoots having been sampled from similar locations on the dunes. Because leaf weights increased sequentially (at least initially) and because some plants showed declining leaf weight in the older leaves, the leaf nutrients were expressed as concentrations rather than contents. Had leaf weights after an initial increase, remained relatively constant, then the expression of this data as leaf contents would have been preferred. Under these conditions simple increases or decreases in leaf nutrient content are indicative of nutrient import and export respectively. When leaf weight continues to change with increasing leaf age then the consideration of the simultaneous changes in leaf weight and macronutrient concentration with increasing leaf age (assigned leaf number) allows conclusions to be reached concerning the net import or export of nutrients.

Table 6.2: Results of linear regressions of leaf weight related to leaf age (assigned leaf number) for *A. populifolia*, *I. pes-caprae* and *S. plumieri.* Linear regressions were fitted to initial increasing linear portions of data from combined replicates of each species on a particular sampling date. The leaves numbered 1 to 5 were omitted from the analyses (see methods for an explanation).

		Linear regression		
Species	**Date**	r^2	Slope (g leaf $^{-1}$)	Intercept (g)
A. populifolia	21/2/98	0.41	0.0117	0.0615
	25/7/98	0.61	0.026	0.041
I. pes-caprae	21/2/98	0.51	0.091	-0.068
	25/7/98	0.41	0.072	0.162
S. plumieri	21/2/98	0.75	0.082	-0.068
	25/7/98	0.68	0.168	-0.114

If leaf weight increases linearly with increasing leaf age then the corresponding changes in leaf nutrient concentrations can be interpreted as follows. Constant leaf nutrient concentrations imply a net import of nutrients and suggest that this process may be regulated. Increasing nutrient concentration implies the net import of nutrients. Decreasing nutrient concentrations implies either the net export of nutrients or the dilution of existing or imported nutrient concentrations due to increasing leaf weight. It is possible to distinguish between the latter two processes by considering the rate of decreasing leaf nutrient concentration in conjunction with the rate of change in leaf weight (Figure 6.7). In order to make such a comparison it is necessary to ensure that leaf weight and nutrient concentrations have the same units. Hence individual replicate data was normalised to the maximum leaf weight and maximum nutrient concentration respectively (see Appendix B).

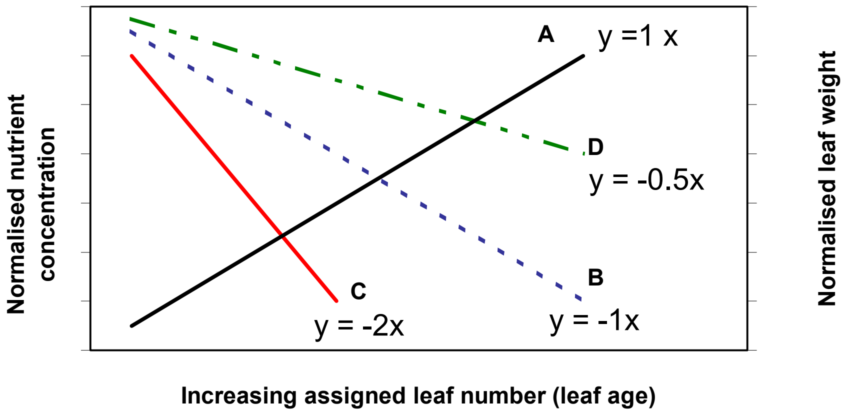


Figure 6.7: Theoretical changes in normalised leaf weight (A) and decreasing normalised nutrient concentrations (B, C and D) related to increasing leaf age (assigned leaf number). The slopes of linear regressions are given on the figure. As leaf weights and nutrient concentrations have different units, data was normalised to the maximum leaf weights and nutrient concentrations respectively and are hence unitless. Slope A + slope B = 0 therefore import = export and both may =0. Slope A + slope C < 0 therefore export > import. Slope A + slope D > 0 therefore import > export.

If leaf weight continues to increase linearly at a constant rate with leaf age and the nutrient concentration declines at the same rate then there is no net export or import of nutrients (i.e. import = reallocation and both import and export may = 0). However if nutrient concentrations decline at higher rates than the rates of leaf weight increase, then nutrients are being exported (i.e. export > import). If nutrient concentrations decline at rates lower than those of leaf weight increase, then there is a net import of nutrients (i.e. import > export).

However if leaf weights initially increase and remain relatively constant thereafter, it is necessary to analyse the initial linear portion of the relationship independently of the rest of the curve. The interpretation of such linear data is explained above. The constant leaf weight portion of the curves can be interpreted as follows. A constant leaf nutrient concentration implies neither net import nor export (import = export, both may = 0); an increasing leaf nutrient concentration implies a net import of nutrients (import > export); and a decreasing leaf nutrient concentration implies a net export of nutrients (export > import).

Constant or increasing leaf nutrient concentration with increasing leaf age implies a net import of nutrients and consideration of the simultaneous rates of changes in leaf weight is not necessary. Conclusions about nutrients that show such changes can be obtained from average data (Figure 6.6 and Table 6.3).

Conclusions about the net export of nutrients under conditions of declining leaf nutrient concentration can be reached only by simultaneous consideration of both the rates of the changes in leaf weight and nutrient concentration. As there was variation between replicates in the response of changing leaf weight with assigned leaf number it was necessary to perform these analyses on individual normalised replicate data (See Appendix B, Figure 6.10, 6.11 and 6.12). Average or combined data obscured otherwise obvious trends. This was apparent if the r^2 values for the combined data (Table 6.2) were compared to the values for individual replicates (Table 6.4 and 6.5). These detailed analyses were performed only on nutrients that had declining concentrations with increasing leaf weight and the results are presented following a general description of the changes in leaf macronutrient concentrations as related to increasing leaf age.

Macronutrients

The average responses of leaf nutrient concentration to leaf age are given in Figure 6.8. The young expanding leaves (leaves numbered 1 to 6) often show rapid declines in the concentration of leaf nutrients as the result of changes in dry mass, rather than changes as the result of the import or export of nutrients. Because of this the nutrient data from these leaves was omitted from the data analysis. Table 6.3 gives the results from linear regressions of combined replicate data for each sampling date and species.

The change in macronutrient concentration with leaf age differs for nutrient, species and for sampling date. This was evident from the r^2 values, slopes of the regressions and the results of the two-way ANOVA of sampling date, leaf age and nutrient concentration (Table 6.3).

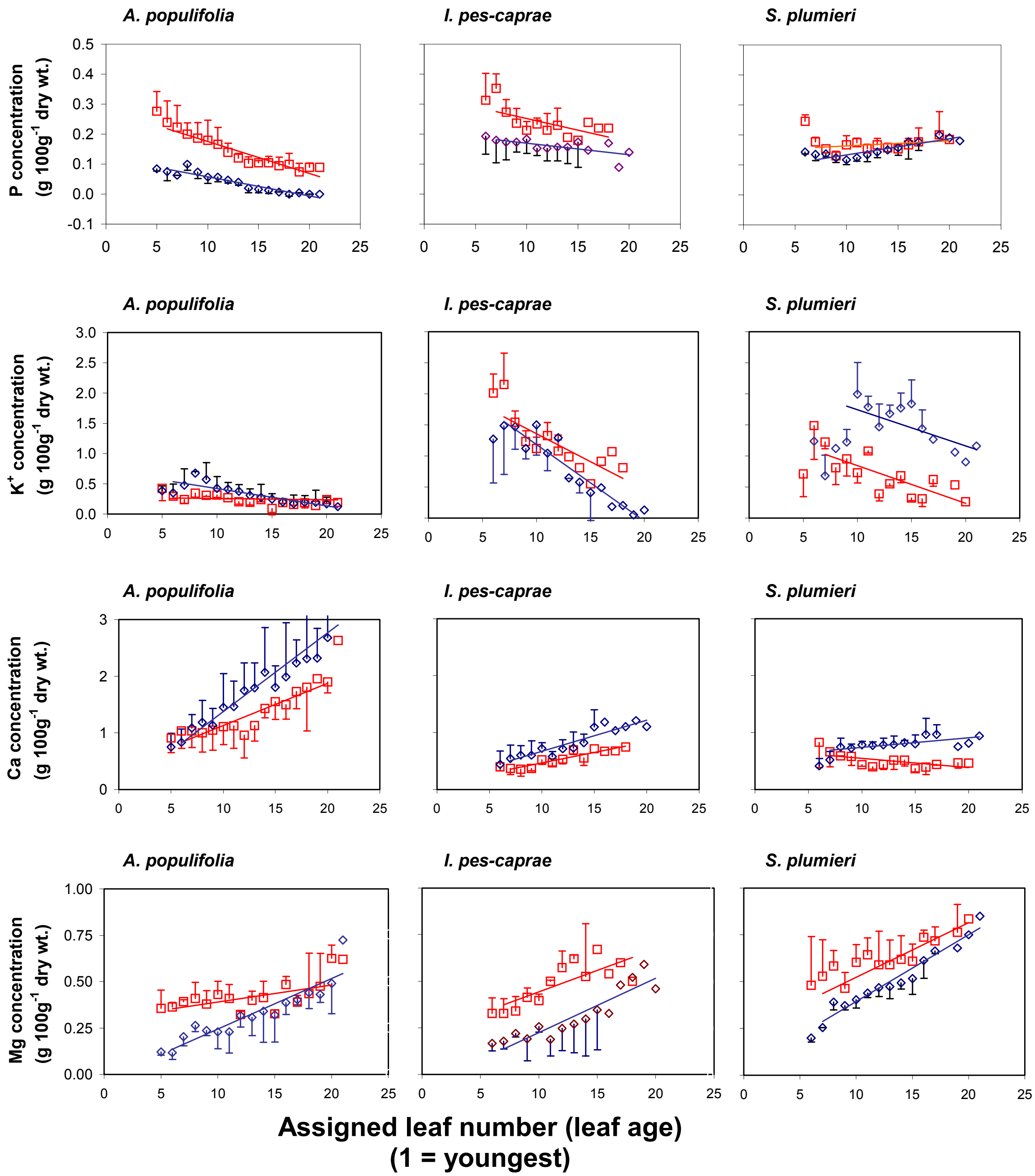


Figure 6.8: Average phosphorus, potassium, calcium and magnesium concentrations in leaves sampled on the 21/2/98 (summer; open symbols) and on the 25/7/98 (winter; closed symbols) in response to changing leaf age. Standard deviations are given by the vertical bars. Linear regressions were fitted to the data from combined replicates. The data from the first six or seven leaves was omitted. N=3.

Table 6.3: Results from a two-way ANOVA and from a linear regression analysis of macronutrient concentrations related to assigned leaf number (leaf age). The leaves of the three species were harvested on the indicated dates. Linear regressions were fitted to combined data for each species from each sampling date (See Figure 6.8 for leaf numbers used in regressions). N=3.

	Species	**Date**	Summary statistics (p values)			Linear regression		
			Date	Assigned leaf #	Interaction	r^2	Slope	Intercept
							g 100g^{-1}leaf^{-1}	g 100g^{-1}
P	*A. populifolia*	21/2/98	<0.0001	<0.0001	0.7656	0.59	-0.011	0.253
		25/7/98				0.75	-0.006	0.102
	I. pes-caprae	21/2/98	<0.0001	0.1285	0.3517	0.26	-0.010	0.314
		25/7/98				0.66	-0.003	0.190
	S. plumieri	21/2/98	<0.0001	<0.0001	0.0001	0.02	0.001	0.161
		25/7/98				0.48	0.006	0.096
K^+	*A. populifolia*	21/2/98	0.0012	0.0002	0.5103	0.30	-0.011	0.338
		25/7/98				0.42	-0.024	0.628
	I. pes-caprae	21/2/98	0.2674	0.0011	0.5382	0.52	-0.167	2.020
		25/7/98				0.58	-0.124	1.948
	S. plumieri	21/2/98	<0.0001	0.0766	0.0003	0.61	-0.066	1.248
		25/7/98				0.18	-0.050	2.044
Ca^{2+}	*A. populifolia*	21/2/98	<0.0001	<0.0001	0.8023	0.57	0.087	0.515
		25/7/98				0.64	0.137	0.421
	I. pes-caprae	21/2/98	<0.0001	0.0030	0.8556	0.67	0.041	0.216
		25/7/98				0.54	0.055	0.331
	S. plumieri	21/2/98	<0.0001	0.9887	0.0147	0.23	-0.021	0.685
		25/7/98				0.24	0.021	0.617
Mg^{2+}	*A. populifolia*	21/2/98	<0.0001	<0.0001	0.1280	0.23	0.011	0.314
		25/7/98				0.63	0.026	0.061
	I. pes-caprae	21/2/98	<0.0001	0.0032	0.3838	0.48	0.030	0.266
		25/7/98				0.42	0.025	0.072
	S. plumieri	21/2/98	<0.0001	<0.0001	0.8281	0.49	0.031	0.341
		25/7/98				0.85	0.035	0.186
Na^+	*A. populifolia*	21/2/98	<0.0001	0.0042	0.6785	0.15	0.088	1.941
		25/7/98				0.42	0.135	0.644
	I. pes-caprae	21/2/98	<0.0001	0.2323	0.8649	0.07	0.110	2.360
		25/7/98				0.10	0.042	1.125
	S. plumieri	21/2/98	<0.0001	<0.0001	0.3135	0.37	0.271	1.821
		25/7/98				0.82	0.333	-0.851
Cl^-	*A. populifolia*	21/2/98	<0.0001	0.9853	0.9877	0.01	0.052	12.653
		25/7/98				0.14	0.201	7.729
	I. pes-caprae	21/2/98	<0.0001	0.9947	0.9716	0.03	0.243	11.529
		25/7/98				0.55	-0.235	9.072
	S. plumieri	21/2/98	<0.0001	0.6858	0.2670	0.05	0.133	14.505
		25/7/98				0.07	0.147	7.219

All leaf macronutrient concentrations were significantly different between leaves of different age and between the two sampling dates. Leaf potassium concentrations in *I. pes-caprae* leaves were the exception (Table 6.3). The interaction of sampling date and leaf age was significant only for *S. plumieri* phosphorus, potassium and calcium concentrations. This indicated that in the majority of cases the change in nutrient concentration with leaf age responded in the same way in plants sampled on both dates.

A. populifolia and *I. pes-caprae* leaf phosphorus concentrations declined with increasing leaf age. *S. plumieri* leaf phosphorus concentrations initially declined and then remained constant or increased with increasing leaf age (Figure 6.8). The increase in phosphorus concentrations in *S. plumieri* leaves sampled on the 25/7/98 indicated the net import of this nutrient. Likewise the constant phosphorus concentration in leaves from *S. plumieri* sampled on the 21/2/98 (Figure 6.8) indicated a net import of phosphorus as leaf weight increased linearly with increasing leaf age (Figure 6.6 and Table 6.2).

Potassium concentrations decreased with increasing leaf age in all three species although the magnitude of this response was different both interspecifically and on different sampling dates (Figure 6.8 and Table 6.3).

Leaf calcium concentrations increased with leaf age in *A. populifolia* and *I. pes-caprae,* indicating the net import of this macronutrient. The calcium concentrations in *S. plumieri* leaves were not significantly different between leaves of different age (p=0.9887), indicating that concentrations remained relatively constant. This occurred despite increases in leaf mass, albeit for only the initial 11 or 14 leaves, indicating the net import of this nutrient. Magnesium concentrations increased with increasing leaf age in all three species (Figure 6.8 and Table 6.3), indicating the net import of this nutrient.

In the case where nutrient concentration decreased with increasing leaf age a detailed comparison of these rates of decrease were made with rates of change in leaf weight. This was undertaken both for leaf phosphorus and potassium concentration.

Table 6.4: Results from linear regressions of changes in normalised leaf weights and phosphorus concentrations as related to increasing leaf age (assigned leaf number) for leaves from *A. populifolia*, *I. pes-caprae* and *S. plumieri* sampled on the indicated dates. Linear regressions were fitted to individual replicates using the range of numbered leaves as indicated. The highest leaf number is indicated in brackets. Plots of individual replicate nutrient concentration, leaf weight and leaf age are given in Appendix B.

		Range of numbers used in each analysis				Linear regressions								If: 1) Wt. slope + nut. slope <0 then net export 2) Wt. slope + nut. slope > 0 then net import 3) Wt. slope = nut. slope then import = export	
						r^2				Slopes					
		Portion one		Portion two		Portion one		Portion two		Portion one		Portion two		Wt. slope + nut. slope Portion 1	Wt. slope + nut. slope Portion 2
	Replicate	[P]	Wt.	[P]	Wt.	[P]	Wt.	[P]	Wt.	[P]	Wt.	[P]	Wt.		
A. populifolia (21/2/98)	1	5-14(21)	5-21	15-21	—	0.94	0.69	0.44	—	-0.065	0.028	-0.007	—	-0.037; export	0.021; import
	2	5-14(21)	5-21	15-21	—	0.89	0.79	0.35	—	-0.055	0.032	-0.017	—	-0.023; export	0.015; import
	3	5-16(21)	5-16	—	—	0.90	0.90	—	—	-0.076	0.069	—	—	-0.007; export	—
A. populifolia (25/7/98)	1	5-14(21)	5-12	*	13-21	0.86	0.91	—	0.41	-0.076	0.145	—	-0.019	0.069; import	—
	2	5-16(16)	5-13	—	14-16	0.97	0.88	—	0.95	-0.11	0.101	—	-0.057	0.007; import	-0.115; export
	3	5-21(21)	5-17	—	18-21	0.84	0.94	—	0.11	-0.063	0.072	—	-0.032	0.009; import	-0.095; export
I. pes-caprae (21/2/98)	1	6-18(18)	6-10	—	11-18	0.61	0.86	—	0.01	-0.028	0.221	—	0.003	0.193; import	-0.025; export
	2	6-13(13)	6-13	—	—	0.29	0.46	—	—	-0.035	0.083	—	—	0.048; import	—
	3	6-15(15)	6-15	—	—	0.80	0.56	—	—	-0.044	0.055	—	—	0.011; import	—
I. pes-caprae (25/7/98)	1	6-20(20)	6-12	—	13-20	0.76	0.98	—	0.18	-0.034	0.125	—	-0.012	0.091; import	-0.046; export
	2	6-16(16)	6-16	—	—	0.53	0.87	—	—	-0.024	0.062	—	—	0.038; import	—
	3	6-16(16)	6-12	—	13-16	0.41	0.98	—	0.96	-0.041	0.075	—	-0.124	0.034; import	-0.165; export
S. plumieri (21/2/98)	1	6-10(18)	6-11	11-18	12-18	0.17	0.92	0.87	0.52	-0.013	0.129	0.054	0.04	0.116; import	0.094; reduced import
	2	6-15(19)	6-14	16-19	15-19	0.74	0.76	0.94	0.51	-0.045	0.075	0.096	0.038	0.030; import	0.134; import
	3	6-14(19)	6-19	15-19	—	0.73	0.80	0.33	—	-0.044	0.04	-0.014	—	-0.004; low export	0.026; import
S. plumieri (25/7/98)	1	6-17(17)	6-12	—	13-17	0.85	0.82	—	0.85	0.034	0.125	—	-0.075	0.159; import	-0.041; export
	2	6-10(18)	6-14	11-18	15-18	0.69	0.85	0.92	0.82	-0.033	0.092	0.052	-0.092	0.059; import	-0.04; export
	3	6-11(20)	6-13	12-20	13-20	0.57	0.98	0.55	0.35	-0.033	0.075	0.0404	-0.038	0.042; import	0.002; reduced import

* Nutrient data for leaves 14-21 is missing

The individual replicate responses to increasing leaf age were either monophasic or biphasic (See Appendix B). When the responses were monophasic linear regressions were fitted to all the leaf data (excluding leaves numbered 1-5 or 6, see above). For the biphasic responses linear regressions were fitted to both portions of the curves (termed portions one and two hereafter). The criterion used for deciding to which portion of the curve a particular data point should be assigned was based on maximising the r^2 values for both portions of the curve. The results are listed in Table 6.4 and 6.5 and include the results of the linear regressions, the range of assigned leaf numbers included in the analysis and the conclusion that can be drawn from a comparison of the rates of change in nutrient concentration and leaf weight. When comparing the slopes of the changes in leaf weight and leaf nutrient content with increasing leaf age one is considering the average response for the range of leaves to which the line was fitted. Furthermore the assumption has been made that all the leaves will proceed through a sequential change in nutrient content and weight as was noted for the leaves that are on the plants or shoots at the time of sampling. However this may not be so and the oldest leaves may have been produced under limiting conditions or when the plants were small. Leaves produced subsequently may well attain much larger sizes and may not lose weight over time.

Phosphorus

For all replicates of *A. populifolia* sampled on the 21/2/98 rates of decrease in phosphorus concentration with increasing leaf age exceeded the rates of leaf weight increase. Hence the average response over the range of leaves was the net export of nutrients (Table 6.4). Recall that the smallest leaves were not large enough to analyse individually and thus the change in nutrient status of these (up to leaf numbered five) is not included in these analyses. It is possible that nutrients are imported into these young leaves. Two of the replicates had reduced decreases in phosphorus concentration from leaf 15 to 21 while leaf weight continued to increase with leaf age indicating that phosphorus was imported into these leaves (Table 6.4). For all replicates of *A. populifolia* sampled on the 25/7/98 rates of decrease in phosphorus concentration with increasing leaf age were less than the rates of leaf weight increase. This indicates that phosphorus was imported (Table 6.4).

Two of these replicates had biphasic responses and phosphorus was exported from the older leaves.

The response of *I. pes-caprae* leaf phosphorus concentration to leaf age was more uniform than that of *A. populifolia.* Phosphorus was imported during portion one of all the responses of leaves sampled on both dates (Table 6.4). In three replicates, biphasic responses occurred and phosphorus was exported during the latter portion of these responses.

For the majority of *S. plumieri* replicates (portion one), as leaf age increased the decline in leaf phosphorus concentrations was less than the increase in leaf weight (Table 6.4). This showed that for the range of leaves used for portion one analyses, phosphorus was imported. An exception was replicate 3 sampled on the 21/1/98 (summer) which showed a low export of phosphorus. All the replicates had biphasic responses and two showed an import of phosphorus, two a reduced import and two an export of phosphorus from the older leaves. The apparent export of phosphorus from the older leaves was the result of declining leaf weight with increasing age rather than a decrease in the rate of phosphorus accumulation (see Figure 6.11, Appendix B). This interpretation may result from the erroneous assumption that all leaves will follow the sequential changes in leaf weight represented by the leaves on the shoot at the time of sampling. If the assumption was made that in the latter portion of the response of phosphorus concentration to leaf age, leaf weight remained constant (slope = 0), then all replicates would appear to be importing phosphorus into leaves of all ages.

Potassium

The majority of replicates of *A. populifolia* sampled on both dates showed rates of decrease in potassium concentration with increasing leaf age that were less than the rates of leaf weight increase. Hence the average response for the range of leaves used for portion one analyses, was the net import of potassium (Table 6.5). All the replicates that showed bi-phasic responses exported potassium from the older leaves (Table 6.5).

Table 6.5: Results from linear regressions of changes in normalised leaf weights and potassium concentrations as related to increasing leaf age (assigned leaf number) for leaves from *A. populifolia*, *I. pes-caprae* and *S. plumieri* sampled on the indicated dates. Linear regressions were fitted to individual replicates using the range of numbered leaves as indicated. The highest leaf number is indicated in brackets. Plots of individual replicate nutrient concentration, leaf weight and leaf age are given in Appendix B.

		Range of numbers				Linear regressions								If: 1) Wt. slope + nut. slope <0 then net export 2) Wt. slope + nut. slope > 0 then net import 3) Wt. slope = nut. Slope then import = export	
						r^2				Slopes					
		Portion one		Portion two		Portion one		Portion two		Portion one		Portion two		Wt. slope + nut. Slope Portion 1	Wt. slope + nut. slope Portion 2
Replicate		$[K^+]$	Wt.	$[K^+]$	Wt.	$[K^+]$	Wt.	$[K^+]$	Wt.	$[K^+]$	Wt.	$[K^+]$	Wt.		
A. populifolia (21/2/98)	1	5-14 (21)	5-21	15-21	—	0.79	0.69	0.65	—	-0.065	0.028	0.034	—	-0.037; export	-0.031; export
	2	5-14(21)	5-21	15-21	—	0.09	0.79	0.35	—	-0.014	0.032	—	—	0.018; import, low r2	—
	3	5-16 (16)	5-16	—	—	0.63	0.90	—	—	-0.058	0.069	—	—	0.011; import	—
A. populifolia (25/7/98)	1	5-14(21)	5-12	15-21	13-21	0.24	0.91	N/A	0.41	-0.020	0.145	—	-0.019	0.125; import	-0.039; export
	2	5-16(16)	5-13	—	14-16	0.84	0.88	—	0.95	-0.106	0.101	—	-0.057	-0.005; low export	-0.163; export
	3	5-21(21)	5-17	—	18-21	0.70	0.94	—	0.11	-0.045	0.072	—	-0.032	0.027; import	-0.077; export
I. pes-caprae (21/2/98)	1	6-18(18)	6-10	—	11-18	0.43	0.86	—	0.01	-0.038	0.221	—	0.003	0.183; import	-0.041; export
	2	6-13(13)	6-13	—	—	0.83	0.46	—	—	-0.073	0.083	—	—	0.010; import	—
	3	6-15(15)	6-15	—	—	0.90	0.56	—	—	-0.074	0.055	—	—	-0.019; export	—
I. pes-caprae (25/7/98)	1	6-20(20)	6-12	—	13-20	0.89	0.98	—	0.18	-0.074	0.125	—	-0.012	0.051; import	-0.086; export
	2	6-16(16)	6-16	—	—	0.93	0.85	—	—	-0.078	0.062	—	—	-0.016; export	—
	3	6-16(16)	6-12	—	13-16	0.71	0.98	—	0.96	-0.054	0.075	—	-0.124	0.021; import	-0.178; export
S. plumieri (21/2/98)	1	6-12(18)	6-11	13-18	12-18	0.71	0.92	<0.01	0.52	-0.126	0.129	-0.001	0.040	0.003; low import	0.039; import
	2	6-19(19)	6-13	—	14-19	0.74	0.85	—	0.71	-0.050	0.095	—	0.0485	0.045; import	-0.002; export
	3	6-19(19)	6-19	—	—	0.68	0.78	—	—	-0.049	0.040	—	—	-0.009; low export	—
S. plumieri (25/7/98)	1	6-14(17)	6-13	15-17	14-17	0.94	0.76	0.68	0.75	0.077	0.104	-0.072	-0.076	0.181; import	-0.148; export
	2	6-14(18)	6-14	15-18	15-18	0.89	0.85	0.60	0.82	0.080	0.092	-0.078	-0.092	0.172; import	-0.170; export
	3	6-14(20)	6-13	15-20	15-20	0.57	0.98	0.34	0.35	0.046	0.075	-0.070	-0.038	0.121; import	-0.108; export

For the majority of *I. pes-caprae* replicates (portion one), as leaf age increased the decline in leaf potassium concentrations was less than the increase in leaf weight. This showed that for the range of leaves used for portion one analyses, potassium was imported. Replicates 3 (21/1/98) and 2 (27/5/98) were exceptions with apparent export from the range of leaves measured. However both of these replicates have low r^2 values for either the regression of leaf weight or potassium concentration to increasing leaf age (Table 6.5). Three replicates showed bi-phasic responses with potassium being exported from the older leaves.

For five of the six *S. plumieri* replicates (portion one), as leaf age increased the decline in leaf potassium concentrations was less than the increase in leaf weight. This showed that for the range of leaves used for portion one analyses, potassium was imported. The one exception showed a weak export of potassium from portion one (replicate 3, 21/1/98) and had slopes of decreasing potassium and increasing leaf weight with increasing leaf age that were almost equal (Table 6.5). Five of the *S. plumieri* replicates had bi-phasic responses with three displaying export, one reduced import and one the import of potassium to the older leaves (Table 6.5).

Sodium and chloride

The utilisation of saline ground-water or the foliar uptake of salt spray could result in the foliar accumulation of sodium and chloride. The accumulation of such ions may be one of the determinants of leaf longevity. In order to investigate this possibility the accumulation of sodium and chloride was related to leaf age (assigned leaf number).

Leaf sodium concentrations increased in both *A. populifolia* and *S. plumieri* with increasing leaf age (Figure 6.9) although the strength of this linear relationship varied among species and on different sampling dates. The results of a two-way ANOVA indicated significant differences between leaves of different age (Table 6.3). Unlike in *A. populifolia* and *S. plumieri,* sodium concentrations of *I. pes-caprae* leaves did not vary with leaf age (Figure 6.9 and Table 6.3). This was despite the fact that leaves would have continually transpired and taken up water. This suggests that *I. pes-caprae* may exclude salt-uptake at its roots or may use only non-saline sand-water.

Chloride concentrations in the leaves of all three species either remained relatively unchanged or declined with increasing leaf age (Figure 6.9). Chloride concentrations were not significantly different between leaves of different age (Table 6.3). Constant or declining concentrations suggest that plants use either water that is low in chloride content or that they possess mechanisms for the preclusion of its uptake.

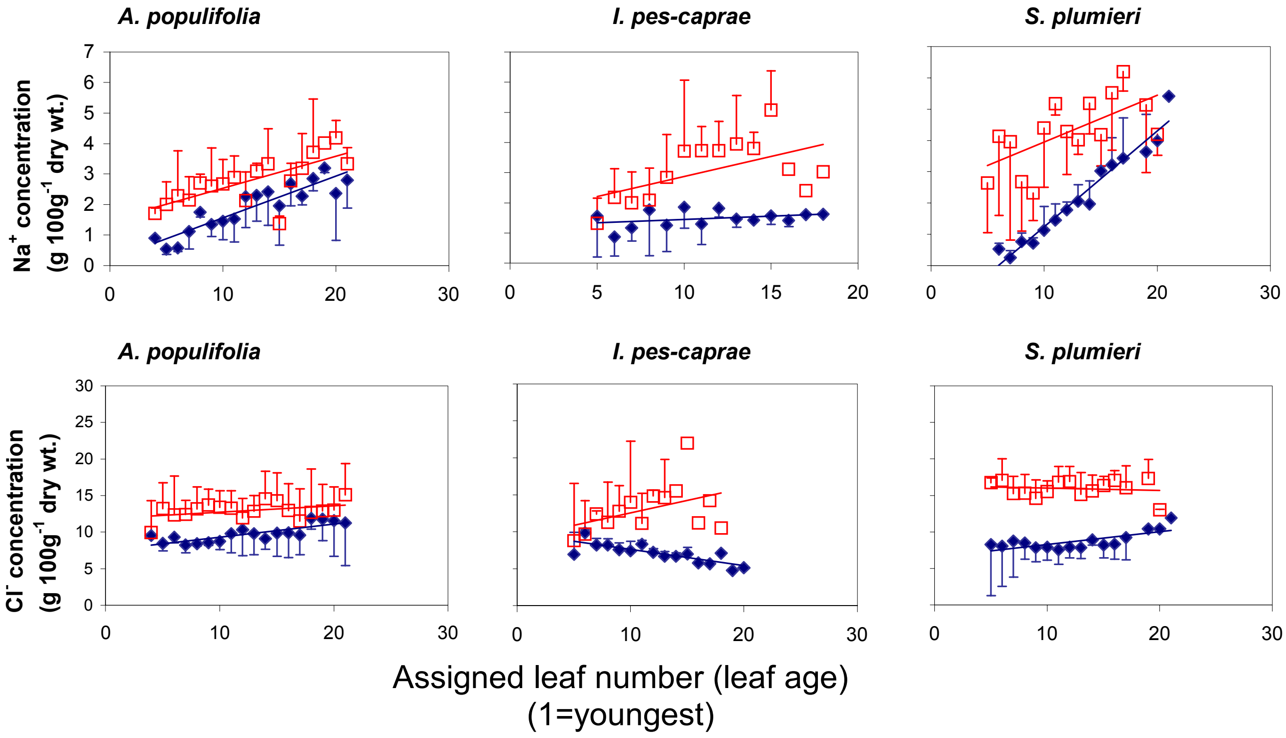


Figure 6.9: Average leaf sodium and chloride concentrations sampled on the 21/2/98 (summer; open symbols) and the 25/7/98 (winter; closed symbols). Standard deviations are given by the vertical bars. Linear regressions were fitted to the data from combined replicates and the data from the first five leaves was omitted. N=3.

DISCUSSION

Dune sand and water nutrient contents

As shown by other researchers in temperate (Salisbury, 1952, Willis, 1965; Barbour *et al*., 1985; Ehrenfeld, 1990; Cain *et al*., 1999) and subtropical climates (Harte and Pammenter, 1983), the nutrient content and particularly the potassium, nitrogen and phosphorus concentrations of dune soils are low (Table 6.1).

These nutrients did not vary in concentration with depth but varied temporally as has been shown for nitrogen (Cain *et al.*, 1999). The spatial variation was not measured but is likely to be considerable, based on the spatial patterns of debris deposition that were observed on the foreshore (*pers. obs.*). Possibly the growth-form of large dicotyledonous species such as *I. pes-caprae* and *S. plumieri*, with their highly connected below-ground stems that can produce adventitious roots, allow them to take advantage of spatially heterogeneous nutrient availability. Below-ground architecture may allow these spatially heterogeneously acquired nutrients to be reallocated amongst shoots and below-ground plant parts. Information available on nutrient remobilization in clonal dune plants shows the nutrients are supplied to growing and reproductive tissue at the expense of older leaves (Nobel and Marshall, 1983). Individual shoots that are supplied with wind-blown sand, a source of nitrogen (Gerlach, 1993), certainly respond with increased vigour. Adjacent clonal stems remain non-vigorous in appearance (*pers. obs.*) questioning the translocation of nutrients between shoots. Further research should investigate such phenomena.

Above-ground plant nutrient content

Average above-ground nitrogen concentrations for *A. populifolia*, *I. pes-caprae* and *S. plumieri* plants were 14.1 ± 2.3, 15.3 ± 1.3 and 10.2 ± 2.4 mg g^{-1} dry wt. respectively. The former two values are very similar to the value of 14 mg g^{-1} dry wt. for plant that is considered sufficient for adequate growth (Marshner, 1995) whilst the latter value for *S. plumieri* was 27% lower.

Average plant potassium concentrations for *A. populifolia*, *I. pes-caprae* and *S. plumieri* were 7.1 ± 2.5, 19.4 ± 4.0 and 16.8 ± 6.8 mg g^{-1} respectively and range from 0.7 to 1.8 times the value of 9.8 mg g^{-1} that is considered sufficient for adequate growth (Marshner, 1995).

Above-ground plant phosphorus concentrations of 1.93 ± 0.43 mg g^{-1} dry wt. for *I. pes-caprae* exceeds the average phosphorus concentration of 1.85 mg g^{-1} dry wt. that is considered sufficient for adequate growth. The values of 1.18 ± 0.32 and 1.62 ± 0.25 for the two succulent species *A. populifolia* and *S. plumieri*, were less than this value.

Nutrient budgets

Above-ground nutrient concentrations of *A. populifolia*, *I. pes-caprae* and *S. plumieri* were generally not atypical for higher plants. The product of these concentrations and above-ground productivity gives the nutrient demand for above-ground production. The average nitrogen and phosphorus demands per unit dune surface area for the three species were not very different (Figure 6.4). However due to the low leaf potassium concentrations of *A. populifolia* (Figure 6.3), the demand for potassium was less than the demands either by *I. pes-caprae* and *S. plumieri.*

Mean nutrient supply for growth exceeded nutrient demand of *S. plumieri* and *I. pes-caprae* for the three nutrients potassium, nitrogen and phosphorus. This estimate was based only on the demand created by above-ground growth. Had below-ground growth been included then the increased demand may have resulted in demand exceeding supply as was evident for the nitrogen demand of *A. populifolia* (Figure 6.4 and 6.5). However nutrient concentration of water extracted from sand just above the swash zone may contain higher nitrogen concentrations than measured for sand sampled at the base of the foredunes. As *A. populifolia* frequently thrives in this zone, the mean negative nitrogen budget for this species may be misleading. Extensive spatial and temporal sampling of nutrient input and the nutrient demand created by below-ground growth is necessary to resolve such issues.

With the exception of nitrogen demand by *A. populifolia* the average demand for potassium, nitrogen and phosphorus created by above-ground productivity are adequately met by nutrient supply from either ground-water or sand-water (Figures 6.4 and 6.5). Similar preliminary findings showed that the demand for nutrients, particularly nitrogen, by *Ammophila arenaria* growth is adequately met by the supply through the mineralization of organic nitrogen (Gerlach *et al*., 1994).

As supply was calculated using the annual estimates of volumes of water transpired caution about the accuracy of these estimates particularly for *I. pes-caprae* are reiterated (see Chapter 5).

Effect of leaf age on nutrient concentration and remobilization

The demand calculation used in these budgets assumed similar demand throughout the entire plant and does not take into consideration the remobilization of nutrients from older to younger leaves. Not only is there considerable nutrient cycling within plants but ageing of leaves is associated with the net export of nutrients from older leaves. This process involves the utilisation of mineral nutrients stored in vacuoles, the breakdown of storage proteins and finally the breakdown of cell structures and enzyme proteins (Marschner, 1995). Harte and Pammenter (1983) have demonstrated the export of nitrogen from older to younger leaves in *S. plumieri*. Pemensa and Lovell (1974) and Ernst (1983) have noted similar translocations from vegetative to generative plant parts for dune annuals and for *Phleum arenarium* respectively. Although nitrogen concentration of individual leaves was not measured in this study there was evidence for the remobilization (net export) of potassium in all three of the species studied (Table 6.5). The net export of potassium from *S. plumieri* leaves confirms the findings of Harte and Pammenter (1983) who measured the net export of potassium initiated prior to the leaves being fully expanded in *S. plumieri*.

There was evidence for the remobilization of phosphorus in *I. pes-caprae* and *S. plumieri* leaves sampled on two dates following initial periods of net import (Table 6.4). However this was so only for the *A. populifolia* leaves sampled on the 25/7/98 and not the 21/1/98

(Table 6.4). The lack of consistent intraspecific patterns on different sampling dates may result from the heterogeneous availability of nutrients. If nutrients are available in sufficient concentrations the redistribution of nutrients internally is reduced (Marschner, 1995).

The mobility of calcium and magnesium in phloem is low (Marschner, 1995) and therefore these nutrients are not cycled internally nor is there a remobilization associated with leaf ageing. Rather these nutrients accumulate in vegetative tissue (Figure 6.8 and Table 6.3), a phenomenon noted for many plant species including the dune species *S. plumieri* (Harte and Pammenter, 1983). The accumulation of calcium has been related to transpiration rates with rapidly-transpiring organs accumulating more calcium than reproductive structures with lower rates (Lange and Thorpe, 1989).

Both sodium and chloride are phloem-mobile (Marschner, 1995) and the net accumulation of sodium, noted particularly in *S. plumieri* leaves (Figure 6.9), may result from the translocation of sodium from younger to older leaves. This has been recognised as an important mechanism in natrophobic species (Marschner, 1995). The oldest *S. plumieri* leaves are routinely dropped and this may be a mechanism for ridding the plant of excess sodium. The preferential remobilization of certain nutrients from the senescing leaves of plants grown on saline substrates has been demonstrated (Greenway, 1962, cited in Marschner, 1995). This author showed that whilst nutrients such as potassium were preferentially remobilized, sodium and chloride were not and older leaves bore the symptoms of the toxicity caused by these nutrients.

Harte and Pammenter (1983) comment that the concentrations of sodium found in dry *S. plumieri* leaves is considerably higher than that measured in dry *Avicennia marina* leaves (a mangrove halophyte). However when measured in fresh leaves the concentration was lower in the leaves from *S. plumieri*. These authors concluded that it is unlikely that *S. plumieri* has a sodium exclusion mechanism at the root level but that the development of leaf succulence maintains sodium at lower concentrations. *S. plumieri* leaves are much longer lived (217 ± 31 days) than leaves from either *A. populifolia* (51 ± 20 days) or *I.*

pes-caprae(176 ± 86 days) and hence higher sodium concentrations in *S. plumieri* leaves may be the result of a longer duration of accumulation. Alternatively the other species *A. populifolia* and *I. pes-caprae* may possess root exclusion mechanisms.

Chloride concentrations did not accumulate in the leaves of *A. populifolia*, *I. pes-caprae* nor *S. plumieri* (Figure 7.9). Values averaged across both sampling dates show leaf concentrations of 2.9, 2.6 and 2.3 g $100g^{-1}$ dry wt. for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. These are similar to the range of values (2.4 to 5.2 g $100g^{-1}$ dry wt.) measured for the seaward-facing leaves of *Ilex vomitoria*, *Quercus virginiana* and *Myrica cerifera* (Boyce, 1954). Leaves of these three species sampled from the leeward side of the plants had much lower salt concentrations (0.8 g $100g^{-1}$ dry wt.) than those from the seaward side. Boyce (1954) concluded that chloride was not relocated from the seaward to the landward side of these species.

Summary of inter-species comparison

Table 6.6 gives a summary of the data in Chapter 6 that can be used to compare the three species *A. populifolia*, *I. pes-caprae* and *S. plumieri*. From the table it is evident that plant potassium and nitrogen concentrations were not very interspecifically different. However the phosphorus concentrations of *A. populifolia* plants were less than the concentrations in the other two species (Figure 6.3). The plant nitrogen and phosphorus concentrations and above-ground shoot productivity (Table 4.6) results in above-ground nitrogen and phosphorus demands. These demands were not very different in the three species. However the average demand for potassium by *I. pes-caprae* and *S. plumieri* was at least twice as high as that of *A. populifolia* (Table 6.6).

Differential demands for nutrients have been noted for various dune-slack pioneers (Grootjans *et al*., 1998) and were attributed to interspecific differences in the internal cycling of nutrients. Although *A. populifolia*'s demand for potassium was low the internal cycling or remobilization of potassium in this species did not exceed values measured for either *I. pes-caprae* or *S. plumieri* (Table 6.5 and 6.6).

Table 6.6: Comparison of data presented for *A. populifolia*, *I. pes-caprae* and *S. plumieri* in Chapter 6. The number of symbols indicates the magnitude of the indicated parameter relative to the smallest value measured for any one of the three species. See Appendix B for the calculation of nutrient budget, accumulation, and import or export values. Included in the appendix is information on which parameters can be compared.

Measured parameter:	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
Average above-ground plant nutrients			
Average [K^+]	♦	♦½	♦½
Average [N]	♦½	♦½	♦
Average [P]	♦	♦♦¾	♦¼
Average nitrogen use efficiency	♦	♦	♦½
Nutrient budgets (negative values indicate that demand>supply)			
K^+ demand	♦	♦♦¼	♦♦¾
K^+ supply from sand-water minus demand	♦	♦♦♦♦♦	¼
K^+ supply from ground-water minus demand	♦	♦♦♦	♦♦
N demand	♦¼	♦	♦
N supply from sand-water minus demand	–♦	♦♦	♦
N supply from ground-water minus demand	–♦♦♦♦	♦♦♦	♦
P demand	♦	♦¼	♦♦½
P supply from sand-water - demand	♦	♦♦♦	♦♦¼
P supply from ground-water - demand	♦	♦♦♦♦♦♦♦♦♦♦ ♦ ¾	♦♦
Nutrient accumulation			
Ca^{2+}	♦♦♦♦♦♦♦♦	♦♦♦½	No accumulation
Mg^{2+}	♦	♦♦	♦♦½
Na^+	♦♦♦♦♦♦♦♦½	♦♦♦♦♦¾	♦♦♦♦♦♦♦♦♦ ♦♦♦♦♦♦♦♦♦ ♦
Cl^-	No accumulation		
Nutrient import or export (export indicated by negative values)			
K^+ (portion 1)	♦	♦¾	♦♦♦¾
K^+(portion 2)	–♦♦¼	–♦♦♦¼	–♦♦♦¼
P (portion 1)	♦	♦♦♦♦♦♦♦♦♦♦ ♦♦♦♦♦♦♦♦♦♦ ♦♦♦	♦♦♦♦♦♦♦♦♦ ♦♦♦♦♦♦♦♦♦ ♦♦♦♦
P (portion 2)	– ♦♦♦♦♦♦♦♦♦♦ ♦♦♦♦½	– ♦♦♦♦♦♦♦♦♦♦ ♦♦♦♦♦♦♦♦♦♦ ♦♦♦♦♦	♦♦♦♦♦♦♦♦♦

Average nitrogen use efficiency for above-ground plant parts was highest for *S. plumieri* and lowest for *I. pes-caprae*. This indicates that *S. plumieri* accumulates more above-ground biomass per unit nitrogen use (Table 6.6). This measure includes the stems which contain less nitrogen than leaves (Chapin and van Cleve, 1989) and is hence influenced by the proportion of leaf to stem mass.

The supply of nutrients from water contained within the dune sands or from ground-water, meets and in many instances exceeds, the demands created by above-ground growth of *I. pes-caprae* and *S. plumieri* (Figure 6.4 and 6.5). The inclusion of the nutrient demand created by below-ground production remains of great interest and practical methods for assessing the below-ground production of such large dicotyledonous plants remains a challenge for the future.

The high above-ground primary productivity (Table 4.6) and relatively smaller volumes of water transpired (Table 5.8) by *A. populifolia* resulted in nutrient budgets that were less positive for potassium and phosphorus than were those measured for the other two species (Table 6.6). *A. populifolia* nitrogen budgets were negative. The remobilization of nitrogen within plants and particularly *A. populifolia* plants may be of considerable importance for future research.

The main difference between the nutrient supply by ground-water or sand-water was that supply of potassium from the ground-water was six times higher than the supply from sand-water (Figures 6.4 and 6.5). However the supply of phosphorus and nitrogen from ground-water was 4.8 and 1.3 times lower than the supply from sand-water (Figure 6.4 and 6.5).

Calcium accumulation was higher in *A. populifolia* than in *I. pes-caprae* leaves and both were higher than that for *S. plumieri* leaves which did not accumulate calcium (Table 6.6). *S. plumieri* accumulated more magnesium and sodium than either *A. populifolia* or *I. pes-caprae* (Table 6.6). *S. plumieri* leaves are the longest lived and hence each individual leaf transpired larger quantities of water during its life-span than those of either of the other two species. This may result in this greater accumulation of these ions. Furthermore there may also be remobilization of sodium to older senescing *S. plumieri* leaves. All three species accumulated more sodium than either calcium or magnesium.

All three species initially (portion 1) showed a net import of phosphorus and potassium (Table 6.6). With the exception of *S. plumieri*, all subsequently exported phosphorus and potassium from the older leaves (portion 2, Table 6.6). The response of the initial import of potassium was highest in *S. plumieri.* The subsequent export was similar in all three species. The initial import of phosphorus was much higher in *I. pes-caprae* and *S. plumieri* than in *A. populifolia*. Furthermore *S. plumieri* continued to import phosphorus into the older leaves while both *A. populifolia* and *I. pes-caprae* exported phosphorus from the older leaves (Table 6.6).

CONCLUSIONS

- Nutrient content of foredune sands from the Eastern Cape are low particularly in potassium, nitrogen and phosphorus.

- In addition to the supply of nutrients via the soil solution (sand-water), the ground-water also contains nutrients which may be utilised by dune species.

- Dune species nutrient content is typical of higher plants and not particularly interspecifically different. The differences in nutrient demand are the result of differences in plant productivity per unit dune surface area.

- The supply of nutrients from either ground-water or sand-water is adequate to meet the demands of above-ground production with the exception of nitrogen supply to *A. populifolia*.

- There is evidence for the reallocation of potassium and phosphorus although considerable variation was encountered both intraspecifically and interspecifically.

- Nutrients that are less mobile in phloem (Ca^{2+}, Mg^{2+} and Na^{+}) accumulated in the leaves of all three species. Chloride concentrations are interspecifically constant.

CHAPTER 7
PHYSIOLOGY

INTRODUCTION

The three previous chapters addressed questions concerning productivity, water and nutrient relations. The results presented in these chapters yielded information about the plants' requirements and responses as well as about some underlying physiological mechanisms. These mechanisms give further insight into and understanding of the observed responses. These mechanisms are presented here together with data showing the response of entire *S. plumieri* shoots to light and the role of the leaf hair layer on leaves of *A. populifolia*.

Collectively the data presented contributes to the understanding of how these species function in the foredune environment. The principal questions asked were: Are there physiological explanations for the observed differences in productivity and water and nutrient relations among the three species investigated? Do selected species have unique adaptations to the dune environment and if so how do these adaptations function?

Leaf light responses

For over a century botanists have recognised that light dramatically influences plant growth through its effects on photosynthesis. Because of this the response of photosynthesis and of primary productivity to light have been the subject of much investigation. These responses have been extensively reviewed (Rabinowitch, 1951; Bjorkman, 1973; Osmond *et al*., 1980).

At low PPFD, photosynthesis is linearly related to PPFD and the efficiency of light utilisation, or the quantum yield, is given by the slope of the response. At higher PPFD the increase in CO_2 assimilation is less than proportional to the increase in PPFD and ultimately assimilation ceases to increase with increasing PPFD (light saturation) (Bjorkman, 1981). Calculation of the efficiency of light utilisation requires the amount of light absorbed by the leaf to be quantified and this requires sophisticated instrumentation. Hence light response curves are often constructed using incident light. Under such conditions the initial linear response defines the efficiency of utilisation of incident PPFD (Long and Hallgren, 1985). The point at which the photosynthetic response to PPFD intercepts the x-axis is termed the light compensation point and is a measure of dark respiration (Bjorkman, 1981). At the compensation point, dark respiration generates CO_2 within the leaf tissue at the same rate as it is used by photosynthesis and hence the response does not pass through the zero intercept.

The light response of plants is markedly influenced by the illumination conditions to which the plants are subjected during growth. This affects pigment composition, pigment content, PSI:PSII ratio and the thylakoid morphology (Bjorkman, 1981). Gabrielsen (1948) found that even in plants having widely different chlorophyll contents there was a correlation between efficiency of utilisation of incident light and chlorophyll content. However there are not marked differences in the utilisation of incident light of related species nor of the same species grown under different light intensities. The differences in the utilisation of incident light are determined largely by light absorption properties and not the subsequent utilisation of absorbed light (Bjorkman, 1981). The light-saturated CO_2 assimilation rate is limited by CO_2 availability and its effects on the carboxylation activity of ribulose-1,6-bisphosphate carboxylase oxygenase (Rubisco). Light-saturated assimilation rate may also at times, be limited by the capacity of triose-phosphate metabolism and the recycling of inorganic phosphate to the chloroplast (Bjorkman, 1981).

De Jong (1978) working on the North American dune plants measured the response of CO_2 assimilation rate to increasing PPFD and showed typical C_3 high light intensity responses for *Abronia maritima*, *Ambrosia chamissonis* and *Cakile maritima.* The response saturated at around 800 μmol m^{-2} s^{-1}. The C_4 species *Atriplex leucophylla* saturated at higher light intensity. A response that is typical of C_4 species and results from the lack of a CO_2 limitation of carboxylation, consequent upon the efficiency of phosphoenolpyruvate carboxylase and the CO_2 pumping mechanism.

Pammenter (1985) investigated greenhouse grown *S. plumieri* and found that photosynthetic rates saturated at approximately 1000 μmol m^{-2} s^{-1} but cautioned that greenhouse grown *S. plumieri* may show atypical responses. Subsequent work by Pammenter and Smith (unpublished) showed that *S. plumieri* did not have completely saturated photosynthetic rates even at an intensity as high as 2000 μmol m^{-2} s^{-1}. They attributed this to the transmission of light from the illuminated side to the opposite side of the leaf which continued photosynthesising. Similar responses were found for *A. populifolia* (Ripley *et al*., 1999) and *I. pes-caprae* was found not to be saturated when absorbed PPFD was 1000 μmol m^{-2} s^{-1} (Adams *et al*., 1988).

In this investigation light response curves were constructed for the three species because light was shown to be the most important abiotic variable in determining CO_2 assimilation and productivity (Chapter 4, Productivity). Furthermore interspecific differences in assimilation rates may be explained by differences in the ϕ_i, dark respiration rates and A_{max}.

Leaf CO_2 responses

CO_2 response curves are a means by which the capacity of a plant to assimilate CO_2, unaffected by the influence of stomata, can be assessed (Sêstak, Câtsky and Jarvis, 1971; Farquhar *et al.,* 1980; Farquhar and von Caemmerer, 1982). By measuring the rates of CO_2 and H_2O exchange and associated water vapour concentration gradients, the leaf conductance to CO_2 can be calculated. Hence intercellular CO_2 concentrations (Ci) can be estimated. The direct response of CO_2 assimilation rates to Ci is initially linear but ultimately assimilation ceases to increase with increasing Ci (Figure 8.2). The slope of the initial increase gives the carboxylation efficiency of CO_2 assimilation. The rate of CO_2-saturated assimilation gives the rate of ribulose-1,6-bisphosphate (RuBP) regeneration which at these assimilation rates is limited by the supply of ATP and NADPH (Farquhar and Sharkey, 1982). The point at which the photosynthetic response to Ci intercepts the x-axis is termed the CO_2 compensation point and is a measure of photorespiration (Farquhar and von Caemmerer, 1982). At the compensation point photorespiration generates CO_2 within the leaf tissue at the same rate as it is used by photosynthesis and hence the response does not pass through the zero intercept. Additionally as the leaf conductance is calculated and because leaf conductance approximates stomatal conductance, the limiting effect of stomatal conductance on assimilation can be assessed (Farquhar and Sharkey, 1982). These techniques have become routinely used in laboratory and field ecophysiological studies.

Laboratory studies on *Ammophila arenaria* and *Elyms mollis* showed that the addition of nutrients affected both the carboxylation efficiency and the rate of RuBP regeneration (Pavlik, 1983). De Jong (1978) working on the North American dune plants *Abronia maritima*, *Ambrosia chamissonis*, *Cakile maritima* and *Atriplex leucophylla* measured differences in the carboxylation efficiency and in the rates of RuBP regeneration between the C_3 and C_4 species and among the C_3 species. CO_2 responses of sub-tropical dune species have not been reported although Pammenter (1985) measured a CO_2 compensation point of 60 μmol mol^{-1} for *S. plumieri*, a value that is typical for C_3 plants (Coombs, 1985). In this investigation CO_2 response curves were constructed for the three species studied because interspecific differences in the significant points that can be interpolated from these curves, may offer explanations of why the CO_2 assimilation rates and productivity of these three species were different (see Chapter 4, Productivity)

Stomatal control

Central to leaf gas exchange physiology, is the controlling role of the stomata. These multi-sensory hydraulically-operated valves respond to light (intensity and wavelength), CO_2 concentration, relative humidity and plant water status (Mansfield and Davies, 1981). Diurnal stomatal behaviour as well as the responses of CO_2 assimilation, transpiration and intercellular CO_2 concentrations to changing stomatal conductance is informative. Stomata have been shown to have one-peaked and two-peaked diurnal responses (Schulze and Hall, 1982). The latter is caused by midday stomatal closure which has been considered a water conserving mechanism. However generalisations concerning diurnal stomatal responses and their ecological significance must be treated cautiously. Under conditions of low water availability, the desert species *Atriplex hymenelytra* maintained one-peaked diurnal responses albeit with very low rates of CO_2

and H_2O exchange (Pearcy *et al.*, 1974). Diurnal stomatal response needs to be considered in conjunction with the rates of gas exchange.

The consideration of the simultaneous changes in photosynthesis (A) and transpiration (E) in relation to leaf conductance has been used to evaluate the role of stomata (Hall and Schultze, 1980; Farquhar *et al.*, 1980; von Willert *et al.*, 1989). Uniform response of A and E to changing leaf conductance are indicative of the optimisation of the amount of CO_2 gained for the amount of water lost (Cowan and Farquhar, 1977; Cowan, 1982). Constant ratios of $\delta A/\delta E$ ($\delta A/G_{Leaf} \div \delta E/G_{Leaf} = \delta A/\delta E$) during periods of changing ambient conditions have been cited as evidence for this optimisation (Schulze and Hall, 1982). However not all species display this optimal stomatal behaviour and Thomas *et al.* (1999) showed that in two tropical rainforest tree species $\delta A/\delta E$ increased with increasing leaf to air vapour pressure deficit. The current investigation included the consideration of the effects of changing leaf conductance on assimilation, transpiration and intercellular CO_2 concentration for *A. populifolia*, *I. pes-caprae* and *S. plumieri*.

Whole-plant or canopy light responses

Studies on crop plants and forests have shown that productivity is more closely related to intercepted PPFD (Hesketh, 1980; Jarvis and Leverenz, 1983) and the efficiency with which this intercepted light is used than to the light-saturated rates of photosynthesis. This has been attributed to mutual shading within the canopy which results in many of the leaves photosynthesising at non-light-saturated rates (Woledge, 1977). This is intuitive but has been investigated only for canopies with high LAI. Dune vegetation and particularly sub-tropical foredune vegetation have low LAI (Chapter 3, The study site and plants investigated). Thus one may presume that canopy photosynthesis is not light limited. However this is not necessarily so and unpublished data is presented (with the permission of Pammenter and Smith) which suggests that whole *S. plumieri* plants are light limited.

Maximisation of photosynthetically active area per total plant mass may be an important adaptation in environments where success relies on high productivity. Such maximisation can be achieved in a number of ways which include increases in specific leaf areas, increases in leaf weight ratios (Bjorkman, 1981) and alterations of canopy architecture (Jarvis and Leverenz, 1983).

Function of *A. populifolia* leaf hair layer

Leaf hairs have been shown to affect photosynthetic rate, degree of photoinhibition and water loss by plants. These effects have been shown to be the consequence of a reduction in light absorption (Ehleringer *et al.*, 1976; Ehleringer and Mooney, 1978; Ehleringer, 1984), decreased transmittance in the UV range (Lang and Schindler, 1994; Skaltsa *et al.*, 1994), altered leaf temperature (Ehleringer and Bjorkman, 1978a), and to a lesser degree, effects on the leaf boundary layer (Ehleringer and Mooney, 1978b).

The role of leaf hairs in a particular environment may involve a combination of the above. Ehleringer (1982) concluded that *Encelia farinosa* produces leaf pubescence under xeric conditions and optimises the reduction in photosynthesis and transpirational water loss such that pubescent leaves maintain higher carbon gain than leaves with less pubescence. Reduced photosynthetic rate in the less pubescent leaves was the result of leaf temperature exceeding the optimum temperature for photosynthesis (Ehleringer *et al.*, 1976). More recent work has shown that photoinhibition is higher in leaves without hairs. Lang and Schindler (1994) showed that in *Senecio medley* leaves without hairs the ratios of variable chlorophyll fluorescence (Fv/Fm and Fv/Fo) decreased and that there was a greater production of zeaxanthin following a 0.5 hr treatment with high light intensity. Similarly reductions in Fv/Fm ratios of *Olea europeae* leaves without hairs (Karabourniotis *et al*., 1993) and *Quercus ilex* leaves without hairs (Skaltsa *et al.*, 1994) were evident following a UV-B treatment.

Most studies have compared leaves with varying degrees of leaf pubescence (Ehleringer and Bjorkman, 1978a & b; Ehleringer, 1982), which has the disadvantage that the photosynthetic apparatus has acclimated to different light environments. This complicates the elucidation of the effects of leaf pubescence *per se*. Other studies have monitored the effects of various light treatments on leaves with and without hairs (Lang and Schindler, 1994; Skaltsa *et al.*, 1994; Karabourniotis *et al.*, 1993; Grammattikopoulos *et al.,* 1994) but have not quantified the reduction in incident PPFD caused by passage through the hair layer. Hence these results reflect merely the effect of a higher light intensity or spectrally different light.

Unlike *I. pes-caprae* and *S. plumieri* a thick layer of dead hairs covers the leaves *of A. populifolia*. Ripley *et al*. (1999) investigated the function of this hair layer. These findings are included as they represent a unique adaptation. *A. populifolia* leaf hairs can be removed easily as an intact layer and the light transmittance of the hair layer quantified. Then by supplying leaves with hairs and leaves without hairs appropriately different PPFD (accounting for the transmittance of the intact hair layer) comparisons could be made at the same PPFD incident on the epidermal surface. The diffusive resistance of the hair layer relative to stomatal conductance was measured and the influences of the hair layer on transpiration, leaf temperature, net CO_2 assimilation and photoinhibition were assessed.

METHODS

Light and CO_2 response curves

Gas exchange measurements were made on single leaves from four or five individual shoots or plants of each of the species. Leaves were selected and numbered as before (see methods Chapter 4). Gas exchange measurements were made using a Li-6400 photosynthesis system (LiCor, Lincoln, USA). The CO_2 reading on the Li-6400 was calibrated using certified calibration gas with CO_2 at a concentration of 406 µmol mol^{-1}. The calibration was checked periodically throughout the course of this study and no adjustments were required. Light was supplied by the red-blue LED internal light source. Atmospheric pressures measured during the experimental periods varied from 101 to 102.2 kPa.

For the construction of light response curves, leaf temperatures, water vapour partial pressures and ambient CO_2 concentrations (Ca) were maintained at 24^{o}C, 1.46 kPa, 350µmol mol^{-1} respectively. Light intensities were decreased from 2000 to 0 µmol m^{-2}s^{-1} via the intermediate intensities 1500, 1000, 800, 600, 400, 200 and 100 µmol m^{-2} s^{-1}.

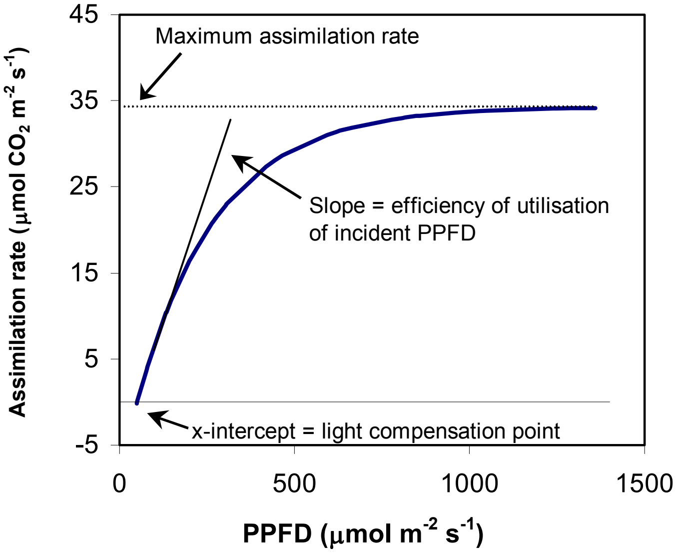


Figure 7.1: A theoretical light response curve (heavy solid line). The efficiency of utilisation of incident PPFD is defined as the initial linear portion of the response and the maximum assimilation rate as the saturated assimilation rate (A_{max}). The PPFD-axis intercept gives the light compensation point (a relative measure of dark respiration). Abbreviation: photosynthetic photon flux density (PPFD).

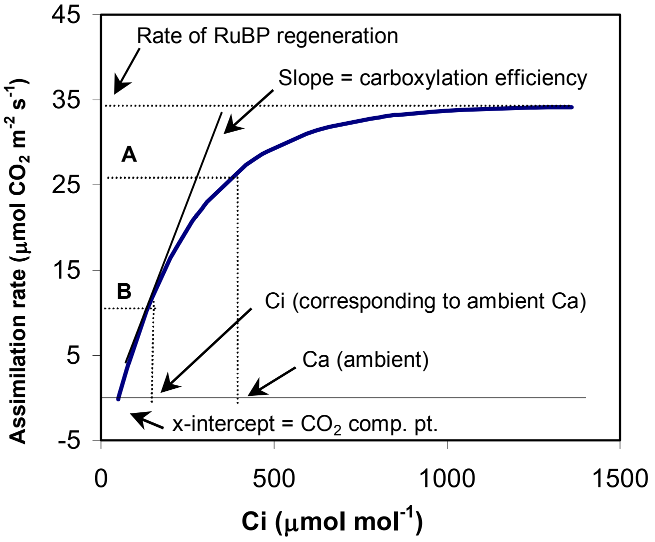


Figure 7.2: A theoretical CO_2 response curve (heavy solid line). The carboxylation efficiency is defined as the initial linear portion of the response and the rate of RuBP regeneration as the saturated assimilation rate. The Ci-axis intercept gives the CO_2 compensation point (a relative measure of photorespiration). The percent stomatal limitation is given by the assimilation rate (A - B)/A x100, where rate A is the rate that would be attained if the stomatal conductance at ambient CO_2 concentration was infinite (Ci=Ca). Rate B is the rate attained at the prevailing stomatal conductance where Ci< Ca due to the finite stomatal conductance. Abbreviations: intercellular CO_2 concentration (Ci), ambient CO_2 concentration (Ca).

For the CO_2 response curves leaf temperatures, water vapour partial pressures and incident PPFD were maintained at 24°C, 1.46 kPa and 1500 μmol mol^{-1} respectively. CO_2 concentrations were varied in the following sequence: 400, 300, 200, 100, 50, 400, 400, 600, 800, 1000, 1200 and 1500 μmol mol^{-1}. The repeated 400 μmol mol^{-1} measurement in the middle of the sequence ensured that the photosynthetic rates recovered following low CO_2 treatments.

Gas exchange parameters were recorded when the sum of the coefficients of variation for the difference in CO_2 and H_2O concentration between reference and analysis sources and the change in flow rate were less than 1%. The Li-6400 changes the air flow rate to control the water vapour concentration surrounding the leaf.

Using the program Statistica '99 Edition (© Statsoft, Inc., Tulsa, USA) individual replicate light and CO_2 response curves were fitted with the saturated exponential equation y = a + (b x (1-exponent (c x n))). Where y = CO_2 assimilation rate, a, b & c are constants and n = Ci or PPFD for CO_2 and light response curves respectively.

The efficiency of utilisation of incident PPFD, the light compensation point, light saturated photosynthetic rate, carboxylation efficiency, CO_2 compensation point, the stomatal limitation and the rate of RuBP regeneration were calculated or interpolated from light or CO_2 response curves (Figure 7.1 and 7.2). The efficiency of utilisation of incident PPFD and the carboxylation efficiency were taken as the initial linear slopes of the light and CO_2 response curves respectively. Each individual replicate was visually inspected and the initial points comprising the linear portion selected and fitted with a linear regression. The slopes of these regressions were quoted as efficiency of utilisation of incident PPFD or the carboxylation efficiency. Light saturated photosynthetic rate and the rate of RuBP regeneration were defined as the light and CO_2 saturated assimilation rates respectively. The light compensation point (a measure of dark respiration) and the CO_2 compensation point (a measure of photorespiration) were defined as the x-intercept of the light and CO_2 response curves respectively (Figure 7.1).

Percentage stomatal limitation is given by the assimilation rates (A-B)/A x100 (Figure 7.2) where assimilation rate A is the rate that would be attained if the stomatal conductance at ambient CO_2 concentrations was infinite (Ci=Ca). Assimilation rate B is the rate attained at the prevailing stomatal conductance where Ci< Ca due to the finite stomatal conductance.

In addition to fitting a curve to each individual replicate data from all the replicates for a particular species and on a particular experimental day were bulked and fitted with a single saturated exponential equation. These fits and corresponding r^2 values are given in the Figures 7.3 and 7.4.

Diurnal gas exchange measurements

Diurnal gas exchange was measured (see Chapter 4 for details) and the relevant parameters calculated according to the equations of von Caemmerer & Farquhar (1981). Leaf temperatures were calculated according to an energy budget equation (ADC, Hoddesdon, U.K.). Due to variation in the ambient CO_2 concentration (Ca) on different experimental days the intercellular CO_2 concentration (Ci) was expressed as the ratio Ci/Ca.

Relationship between VPD and incident PPFD

VPD was calculated from measurements of ambient temperature and relative humidity (see Chapter 5 for details). Incident radiation (MJ m^{-2} hr^{-1}) data was acquired from the SAWB and was converted to PPFD (see Chapter 3 for details).

Leaf age effects on gas exchange

Gas exchange measurements were made on every leaf (with the exception of leaves that were too small to fill the leaf chamber) of three to five individual shoots or plants of the three species. The small leaves are likely to be non-functional and Pammenter (*pers. com.*) using scanning electron micrographs (SEM) observed that the youngest leaves of *S. plumieri* had occluded stomata. Leaves were numbered with the smallest visible leaf being designated leaf 1. Gas exchange measurements were made using a Li-6400 photosynthesis system (see above). During the measurements leaf temperature, water vapour partial pressure, PPFD and ambient CO_2 concentrations were maintained at 24°C, 1.6 kPa, 1000 μmol m^2s^{-1} and 350 μmol mol^{-1} respectively. Stabilisation of gas exchange to the ambient chamber conditions was assessed using the criterion outlined above. The relationship between assigned leaf number (leaf age) and surface area was determined for six individual shoots or plants of each species.

The relationships between leaf age and leaf surface area and between leaf age and gas exchange rates were fitted with exponential or third order polynomial equations using the program Statistica '99 Edition (© Statsoft, Inc., Tulsa, USA). The type of equation selected was determined by the r^2 value, the equation with the highest r^2 value being preferred.

S. plumieri whole shoot response to light

This investigation was conducted by Pammenter and Smith in 1990 and the unpublished results are presented with the permission of the authors.

Study site and plant material

The study was undertaken at Mlalazi Nature Reserve, Mtunzini, KwaZulu Natal (see map, Chapter 3). Plants studied were growing on the foredunes and the study was undertaken in February 1990 (summer). Plant leaves were numbered sequentially with the youngest visible leaf being labelled as leaf 1. Shoots frequently had five leaves that were too small to be introduced into the leaf chamber and were not included for measurement.

Gas exchange measurements

Photosynthetic measurements were made using a LCA-II portable infrared gas analyser, mass flow controller and Parkinson broad-leaf chamber (Analytical Development Corporation, Hoddeson, U.K.). Once the leaf was introduced into the chamber, gas exchange values were allowed to stabilise for 30 to 45 seconds. See Chapter 4 for measurement, data logging and calculation details.

Daily course of gas exchange rates

Measurements were made on each leaf (from leaf 6 onwards) from a shoot at hourly intervals from sunrise to sunset. The proportion of each leaf surface shaded by other leaves varied during the day. PPFD was measured on both the shaded and unshaded areas of the leaf. The proportions of the shaded and unshaded areas were estimated and a weighted average PPFD calculated for the entire leaf. During the gas exchange measurements the proportions of shaded to unshaded parts of the leaf enclosed in the chamber were kept as close as possible to those of the whole leaf.

Leaf inclination angles

The angles between the leaf adaxial surface and the stem (perpendicular to the ground) were measured for each numbered leaf on ten individual stems.

A. populifolia **response to light and the role of the leaf hair layer**

This investigation was conducted by Ripley, Pammenter and Smith in 1998 and was subsequently published in the *Journal of Plant Physiology*, 1999, 155, 78-85.

Plant material

Plants of *A. populifolia* were collected daily from, or measurements were made on plants growing on the foredunes of Macassar Beach east of Cape Town, South Africa (34^{o}S,18^{o}30'E). Measurements were made on fully expanded but not senescing leaves. Leaf hairs were removed by gentle rubbing or intact hair layers were removed either with adhesive tape being applied to the leaf surface or by careful peeling with forceps. Laboratory gas exchange and fluorescence measurements were made on the same leaves before and after hair removal.

Hair layer characteristics

The reduction of incident light transmitted through the pubescent layer was estimated by removing the hair layer as an intact sheet and placing it over the probe of a Li-Cor, LI-188B integrating photometer (Li-Cor Inc., USA). The absorption spectrum was measured using a Beckman UV 5240 spectrophotometer with an integrating sphere attachment and comparisons were made with magnesium oxide standards (Beckman Instruments Inc., California).

The boundary layer was quantified by comparing water loss from a filter paper strip (Whatman #4) with and without a covering of a layer of leaf hairs. Water exchange was measured with an ADC Mark III infra-red gas analyser and measurements were made in an unstirred cuvette at a range of different flow rates. Air in the cuvette and filter paper temperature were measured with copper-constantan thermocouples and incoming air was humidified to 1.07 kPa.

Throughout this report the term 'leaf surface' refers to the upper epidermis below the indumentum.

Gas exchange

Laboratory gas exchange measurements were conducted using a CIRAS-1 Differential CO_2 / H_2O gas analyser connected to a PLC (B) Auto Cuvette (both from PP Systems, U.K.). Leaf temperatures were maintained at 25 ± 0.4 ^{o}C, incoming air was humidified to 0.8 kPa and air flow rates controlled at 400 ml min^{-1} during the construction of light response curves.

For field measurements the hair layer was removed from four leaves of similar size and orientation, on the evening prior to the day that measurements were taken. For temperature measurements copper-constantan thermocouples were inserted into the midrib of these leaves and four similar leaves with intact hair layers. Gas exchange was measured with a LCA-II portable infra-red gas analyser (ADC, England).

Fluorescence measurements

Fluorescence measures were made simultaneously with gas exchange measurements by inserting the probe of an FMS1 Fluorescence Monitoring System (Hansatech, Norfolk, U.K.) through a port in the side of the PLC (B) leaf cuvette. Non-photochemical quenching (q_{NP}) of maximal fluorescence was calculated according to Bilger and

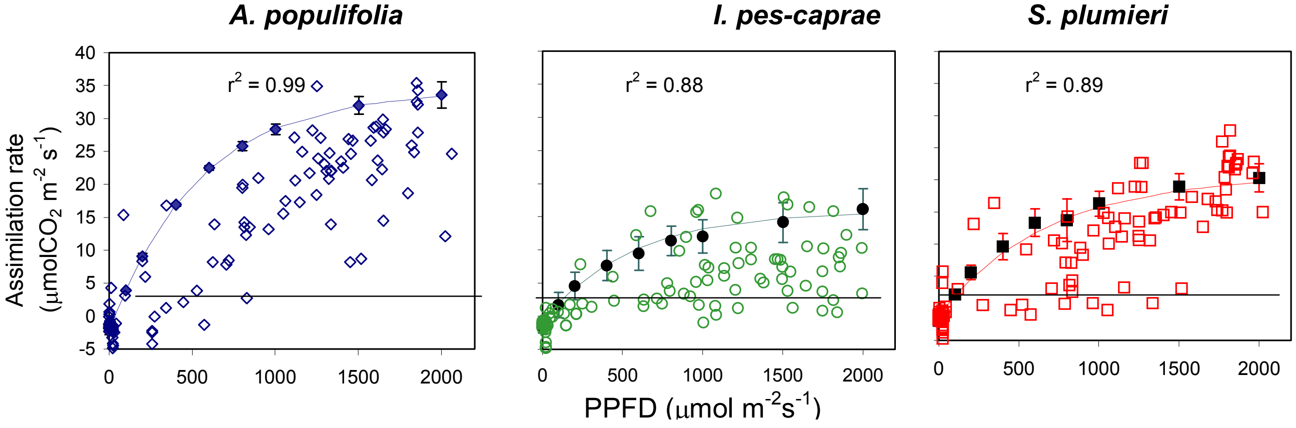
Bjorkman (1990). The coefficient of photochemical quenching (q_p) and PSII efficiency (Fv'/Fm') were calculated according to Schrieber *et al.* (1986). See Appendix C for calculation details.

RESULTS

Leaf light responses

Correlation between photosynthetic rate (measured in prevailing ambient conditions) and various abiotic factors showed that PPFD was the most important determinant of photosynthetic rate (Table 4.2). Not only was the relationship between photosynthetic rate and PPFD predictive but differences in peak assimilation rates measured on the various experimental days could be largely explained by differences in incident PPFD (Figure 4.2).

The relationship between assimilation rates and PPFD was further examined by constructing light response curves in the field under controlled conditions. These average curves superimposed on measurements made in the field under prevailing ambient conditions are given in Figure 7.3.



A. populifolia ***I. pes-caprae*** ***S. plumieri***

Figure 7.3: Light response curves for *A. populifolia*, *I. pes-caprae* and *S. plumieri*. Measurements made under controlled conditions (solid symbols) and under prevailing ambient conditions (open symbols). Standard deviations are represented by the vertical bars. N= 4 or 5. Abbreviation: photosynthetic photon flux density (PPFD).

For any particular PPFD the measurements made under controlled conditions appear similar to the highest assimilation rates measured at the prevailing ambient PPFD. Under controlled conditions none of the investigated species had assimilation rates that were saturated at PPFD values of 2000 μmol m^{-2} s^{-1}. *A. populifolia*, *I. pes-caprae* and *S. plumieri* photosynthetic rates increased by 5.2, 4.1 and 4.5 μmol m^{-2} s^{-1} when light intensity was increased from 1000 to 2000 μmol m^{-2} s^{-1}. The shape and magnitude of the light response curves of the three species were different as was evident when the derived significant points were compared (Table 7.1).

Table 7.1: Significant points extracted from light response curves. If values obtained from the same species on different days were significantly different this is indicated by the bracketed numbers. Species are numbered as (1) - *A. populifolia*, (2) - *I. pes-caprae* and (3) -*S. plumieri*. N=5.

	Efficiency of utilisation of incident PPFD (mmol mol^{-1})	**Light compensation point (μmol mol^{-1})**	**Light-saturated assimilation rates (μmol m^{-2} s^{-1})**
A. populifolia (1)	56 ± 5.0 (2 & 3)	36.4 ± 6.3 (3)	33.6 ± 2.0 (2 & 3)
I. pes-caprae (2)	39 ± 3.0 (1)	46.1 ± 13.4	16.4 ± 3.4 (1)
S. plumieri (3)	43 ± 12.0 (1)	54.9 ± 7.3 (1)	19.0 ± 3.6 (1)

Abbreviations: photosynthetic photon flux density, PPFD.

The efficiency of utilisation of incident PPFD and the maximum light-saturated assimilation rates of *A. populifolia* were significantly higher than those of either *I. pes-caprae* or *S. plumieri* (Table 7.1). These higher values are correlated with higher chlorophyll content per unit leaf surface area in *A. populifolia* (Figure 4.9) and may offer an explanation of the high light-saturated photosynthetic rates (Table 7.1) and the above-ground primary production rates (Table 4.6) measured in this species.

The light compensation point, a relative measure of dark respiration (glycolysis and the tricarboxylic acid cycle) was significantly lower in *A. populifolia* than in *S. plumieri* while values for *I. pes-caprae* were not significantly different from the other two species (Table 7.1). This data is in contrast to the above-ground shoot or plant respiration rates that were measured on the 30/1/98 and the 23/7/98 when *A. populifolia* had significantly higher respiration rates than either *I. pes-caprae* or *S. plumieri* (Table 4.3).

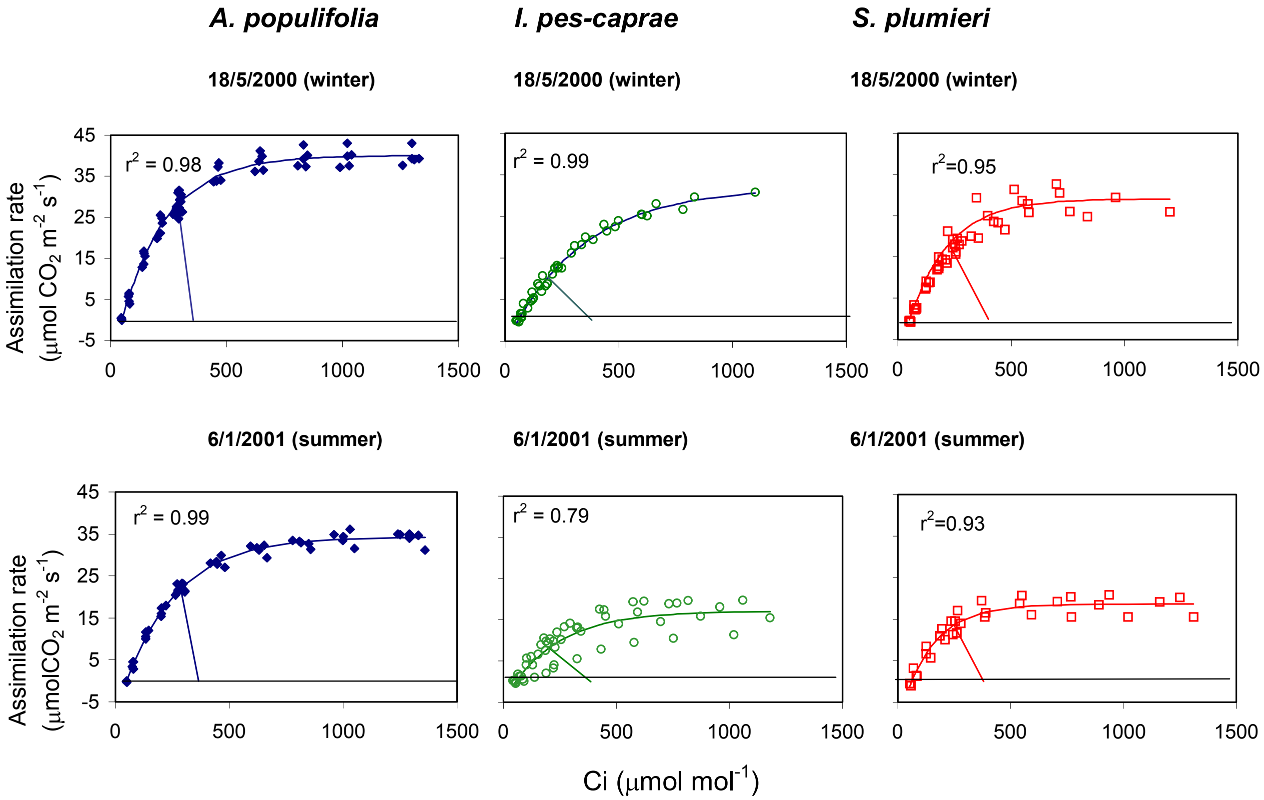


Figure 7.4: CO_2 response curves *for A. populifolia, I. pes-caprae* and *S. plumieri.* Measurements were made on the indicated experimental days. Straight lines show the stomatal effect on assimilation (A = G_{leaf} (Ca-Ci)). N= 4 or 5. Abbreviation: intercellular CO_2 concentration (Ci).

Leaf CO_2 responses

CO_2 response curves were different both among species and on the two experimental days (Figure 7.4). These differences are evident when comparisons are made between the significant points that can be derived from the curves (Table 7.2). These points were derived from each individual replicate CO_2 response curve and averages and standard deviations calculated for each species and experimental day.

A two-way ANOVA of carboxylation efficiency values (derived from individual replicate CO_2 response curves) indicated that differences among species ($p < 0.0001$), experimental days ($p<0.0001$) but not their interaction were significant. Carboxylation efficiencies averaged for both experimental days were 118.3 ± 20.6, 71.1 ± 14.4 and 95.8 ± 17.6 mmol m^{-2} s^{-1} for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. The differences in carboxylation efficiency offer a possible explanation for the interspecific differences in photosynthetic and production rates. Carboxylation efficiencies for all three species measured on the 6/1/2001 decreased in comparison to values measured on the 18/5/2000. Differences between experimental days were significant for *A. populifolia* and *S. plumieri* but not for *I. pes-caprae*. The efficiency of *A. populifolia*, measured on the 18/5/2000 (winter) was significantly higher than efficiency measured for both other species and on all experimental days.

Table 7.2: Significant points extracted from CO_2 response curves. If values obtained from the different species and from the same species on different days were significantly different this is indicated by the bracketed numbers. Species measured on the 18/5/2000 (winter) are numbered as (1) - *A. populifolia*, (2) - *I. pes-caprae* and (3) -*S. plumieri*. Species measured on the 6/1/2000 (summer) are numbered as (3) - *A. populifolia*, (4) - *I. pes-caprae* and (5). N=5.

	Carboxylation efficiency (mmol m^{-2} s^{-1})	**CO_2 compensation point (μmol mol^{-1})**	**Stomatal limitation (%)**	**RuBP regeneration rate (μmol m^{-2} s^{-1})**
18/5/2000 (winter)				
A. populifolia (1)	135.8 ± 8.7 (2, 3, 4, 5 & 6)	40.9 ± 5.0 (3)	7.1 ± 1.3 (2, 3, 4, 5 & 6)	39.8 ± 2.0 (2, 3, 4, 5 & 6)
I. pes-caprae (2)	79.5 ± 14.6 (1 & 3)	55.6 ± 6.5	48.3 ± 10.2 (1 & 3)	28.1 ± 2.6 (1, 4, 5 & 6)
S. plumieri (3)	106.6 ± 10.7 (1, 2, 5 & 6)	55.5 ± 2.7 (1)	28.1 ± 9.7 (1, 2 & 5)	28.7 ± 3.2 (1, 4, 5 & 6)
6/1/2001 (summer)				
A. populifolia (4)	100.8 ± 10.9 (1 & 5)	45.5 ± 5.7	36.4 ± 4.1 (1)	34.1 ± 1.5 (2, 3, 5 & 6)
I. pes-caprae (5)	62.8 ± 9.4 (1, 4 & 3)	48.1 ± 9.0	49.3 ± 2.6 (1, 3 & 6)	18.7 ± 2.0 (1, 2, 3 & 4)
S. plumieri (6)	77.8 ± 9.3 (1 & 3)	54.5 ± 13.0	32.1 ± 6.2 (1 & 5)	18.6 ± 2.7 (1, 2, 3 & 4)

Abbreviation: ribulose-1,6-bisphosphate (RuBP).

However *A. populifolia* efficiencies measured on the 6/1/2001 were only significantly higher than *I. pes-caprae* efficiencies measured on 6/1/2001. *S. plumieri* values measured on the 18/5/2000 were significantly higher than values measured for *I. pes-caprae* on the 18/5/2000 and those for *I. pes-caprae* and *S. plumieri* measured on the 6/1/2001.

The differences in CO_2 compensation points were significant both among species ($p<0.0001$) and experimental days ($p<0.0001$). This was only because values measured on the 18/5/2000 for *A. populifolia* and *I. pes-caprae,* were significantly different. Values averaged for both experimental days were 43.2 ± 5.6, 51.8 ± 8.3 and 55.1 ± 7.3 μmol mol^{-1} for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. These values are typical of values measured for C_3 plants (Coombs, 1985).

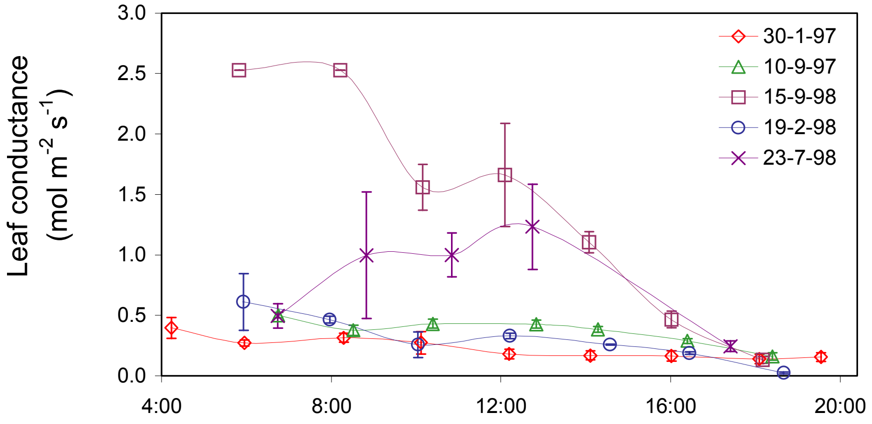
The differences in stomatal limitations among species ($p<0.0001$), between experimental days ($p<0.0005$) and their interaction ($p<0.0006$) were significant. *A. populifolia* stomatal limitations were significantly lower on 18/5/2000 than on 6/1/2001 while the values measured for each of the other two species were not significantly different on different experimental days. Values for *A. populifolia* were significantly different to values for *I. pes-caprae* and *S. plumieri* on 18/5/2000 but not on the 6/1/2001. *S. plumieri* stomatal limitations were significantly lower than values measured for *I. pes-caprae* on both experimental days. Differences in stomatal limitation offers an explanation of the differences in assimilation rates measured among the investigated species.

Differences in RuBP regeneration rates among species ($p<0.0001$) and between experimental days ($p<0.0001$) were significant but their interaction was not ($p=0.1388$). Unlike *S. plumieri* and *I. pes-caprae*, *A. populifolia* RuBP regeneration rates were not significantly different on different experimental days. They were however, significantly higher than the rates measured for the other two species on both experimental days. High rates of RuBP regeneration for *A. populifolia* correspond to high efficiency of utilisation of incident PPFD (Table 7.1) and to high leaf chlorophyll content (13.87 ± 3.82 mg cm^{-2} leaf area, Chapter 5). Values for *I. pes-caprae* and *S. plumieri* were not significantly different on either of the experimental days (Table 7.2).

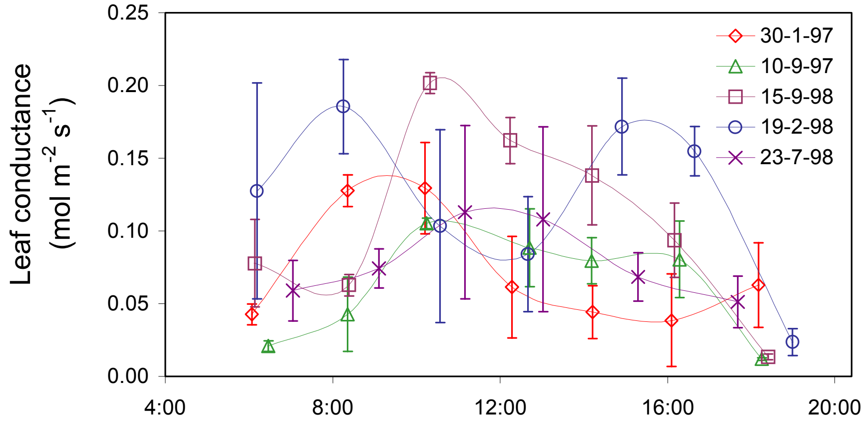
Stomatal control

The maximum leaf conductance (G_{Leaf}) extracted from each individual diurnal replicate and averaged for each species were 1.3 ± 1.1, 0.17 ± 0.05 and 0.33 ± 0.19 mol m^{-2} s^{-1} for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively.

A. populifolia



I. pes-caprae



S. plumieri

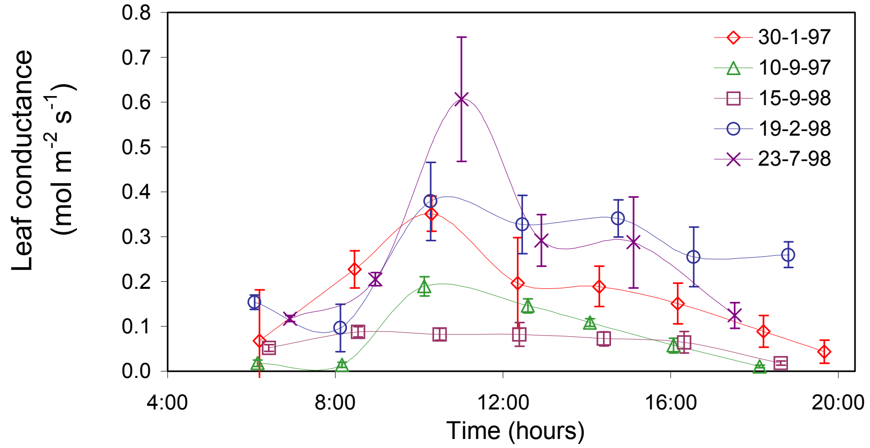


Figure 7.5: Diurnal leaf conductance responses for *A. populifolia, I. pes-caprae* and *S. plumieri* measured on the indicated dates. Standard deviations are represented by the vertical bars. N= 3. Note the scale differences for different species.

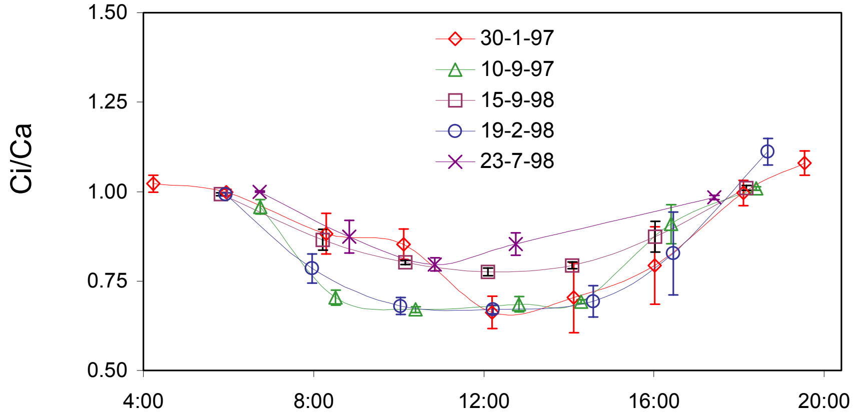
Values for *A. populifolia* were significantly higher than those for *I. pes-caprae* (p=0.0001) and *S. plumieri* (p=0.0002). The very high leaf conductances recorded for *A. populifolia* are likely to be due to the evaporation of dew trapped in the hair layer and may not represent stomatal conductance.

In addition to the differences in the extent to which stomata limit assimilation rates (see Table 7.2, above) diurnal stomatal responses were different in the three species (Figure 7.5). On four of the five experimental days (30/1/97, 19/2/98, 10/9/89 and 15/9/98) *A. populifolia* had high early morning leaf conductances that declined in an almost linear fashion throughout the day (Figure 7.5). These initially high values may have been the result of evaporating dew that was trapped in the leaf hair layer. The diurnal G_{Leaf} response on the 23/7/98 had a single peak with the highest values being attained at or just after midday.

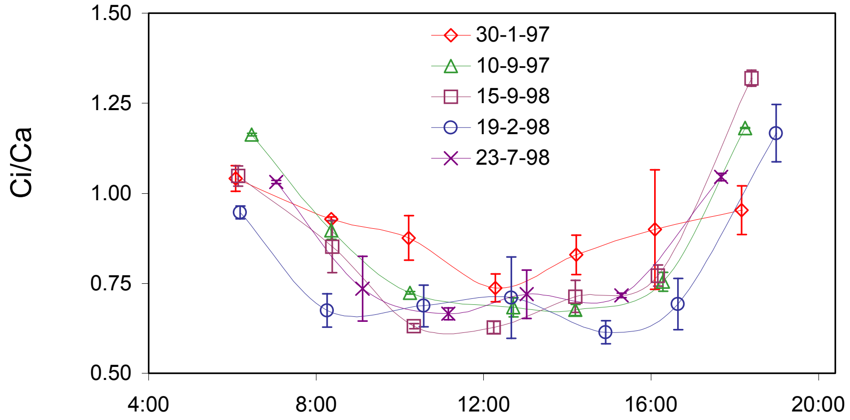
On several of the experimental days there was evidence of partial midday stomatal closure by *I. pes-caprae*. This was particularly evident on the 19/2/98 and less so on 30/1/97 and 10/9/98 (Figure 7.5). On the other experimental days typical single-peaked responses were recorded. Midday stomatal closure was accompanied by a decrease in assimilation rate (Figure 4.1) and an increase in the Ci/Ca (Figure 7.6). Ψ_{leaf} did not recover during this period of partial stomatal closure (Figure 5.4).

S. plumieri displayed single-peaked diurnal G_{Leaf} responses on all experimental days although like the other three species there were considerable differences in the magnitude of the response on different experimental days (Figure 7.5). Reciprocal to changes in the diurnal response of photosynthesis (Figure 4.1) the diurnal response of Ci/Ca^{2+}, for all three species declined during the morning to lows at or just after midday (Figure 7.6). The ratio increased during the afternoon (Figure 7.6). When early morning and evening values were excluded then the extent by which the Ci/Ca ratio changed during the day was small (as is evident from the range of Ci/Ca values recorded in Figure 7.7). The ratio measured in the early morning and after sunset exceeded 1 (Figure 7.6) due to the CO_2 generated within the leaf by dark respiration.

A. populifolia



I. pes-caprae



S. plumieri

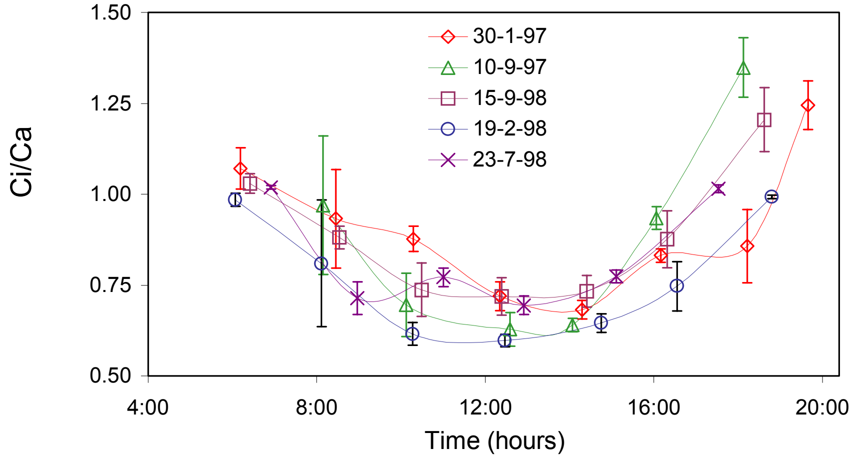


Figure 7.6: Diurnal intercellular to ambient CO_2 concentration ratio (Ci/Ca) responses for *A. populifolia*, *I. pes-caprae* and *S. plumieri* measured on the indicated dates. Standard deviations are represented by the vertical bars. N= 3.

I. pes-caprae and *S. plumieri* had the highest ratios after sun-set indicating that these leaves had higher dark respiration rates than *A. populifolia*. This was in agreement with the light compensation point data (Table 7.1, above).

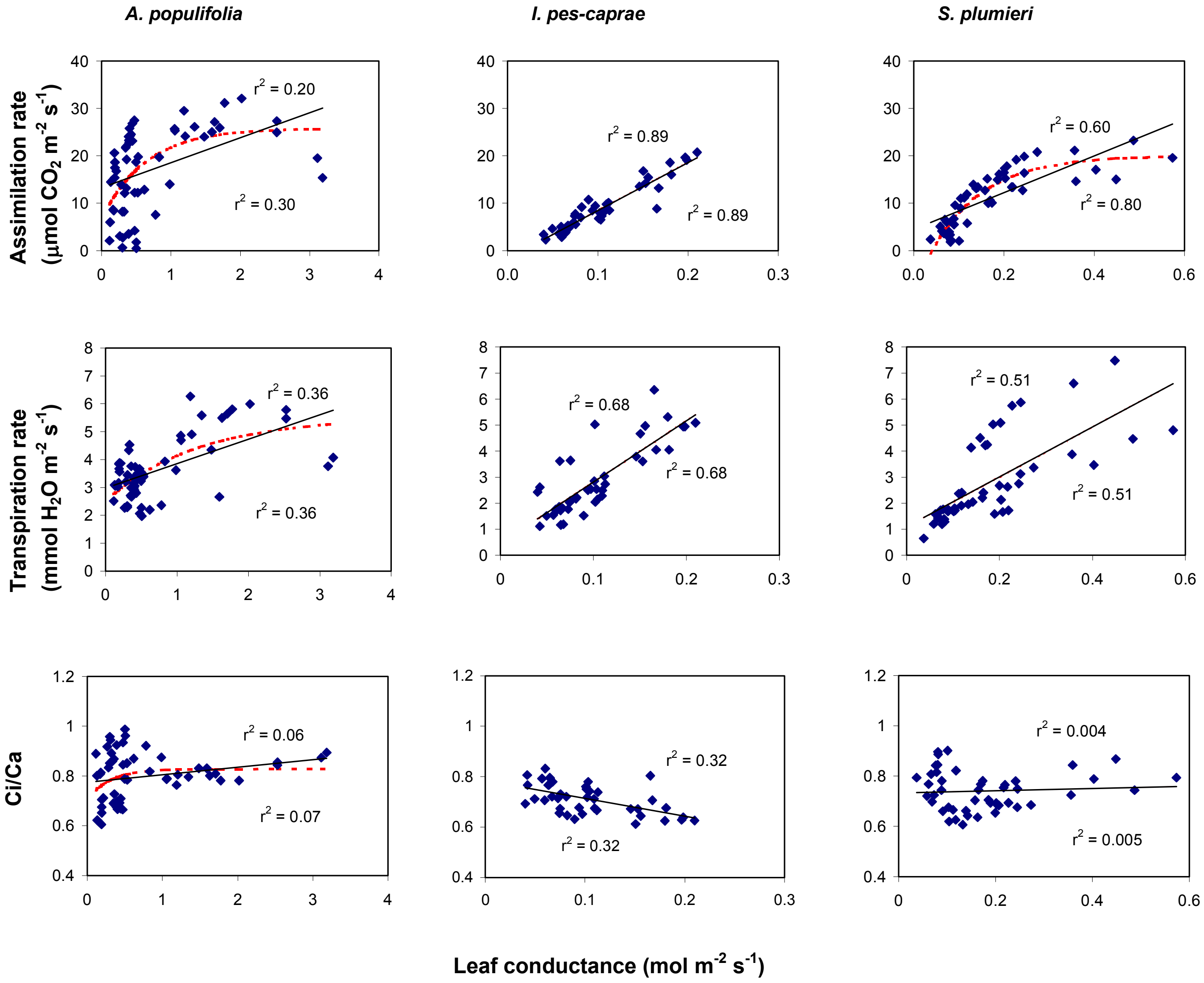


Figure 7.7: Assimilation rates, transpiration rates and Ci/Ca response to leaf conductance for the indicated species. Data where CO_2 assimilation rates were less than 1 μmol m^{-2} s^{-1} were omitted from the analysis (see text for explanation). Data was fitted with both linear (solid lines) and saturated exponential (dotted lines) equations. The r^2 values for the linear equations are given at the top of each figure and the r^2 values for the saturated exponential equations are given below. Note the different x-axis scale for each species. Abbreviation: intercellular to ambient CO_2 concentration ratio (Ci/Ca).

Integrated stomatal response

Stomata respond to plant water relations, photosynthetic rates, light and other ambient conditions making determination of the major factor to which stomata are responding difficult. A comparative consideration of how assimilation rate, transpiration rate and Ci/Ca change in response to changes in G_{Leaf} (Figure 7.7) gives some insight into which of these is most affected by changes in G_{Leaf}. For the construction of Figure 7.7 all data points which had corresponding assimilation rates below 1 μmol m^{-2} s^{-1} were omitted because it was difficult to get reliable leaf conductance measurements in the early morning and late afternoon due to dew formation on the leaves. Furthermore the measured difference between reference and analysis CO_2 concentrations are low under such conditions and minor errors result in big differences in the calculated assimilation rates and Ci values.

The response of *A. populifolia* assimilation rate to increasing G_{Leaf} was better described by a saturated exponential equation than by a linear equation although neither produced high r^2 values (0.2 & 0.3 respectively).

The saturated exponential response suggests that maximal CO_2 assimilation rates were limited by some factor other than stomatal conductance. Furthermore the Ci/Ca ratios at these high G_{Leaf} values were not reduced below the Ci/Ca values noted at lower G_{Leaf}. This supports the above suggestion. The apparently very high G_{Leaf} noted for *A. populifolia* are probably the result of evaporation of dew trapped in the leaf hair layer. For the majority of the data, leaf conductances were below 1 mol m^{-2} s^{-1} (Figure 7.7). Measurements made under controlled conditions (CO_2 and light response curves, Figures 7.3 and 7.4), neither early in the morning nor late in the evening, showed a range of leaf conductances from 0.6 to 1.6 mol m^{-2} s^{-1}.

Transpiration rate was used in the calculation of G_{Leaf} and hence these two parameters are not independent (see the equations of von Caemmerer & Farquhar, 1981). Hence conclusions about the dependence of transpiration rate on G_{Leaf}, based on calculations made in this way, are not valid.

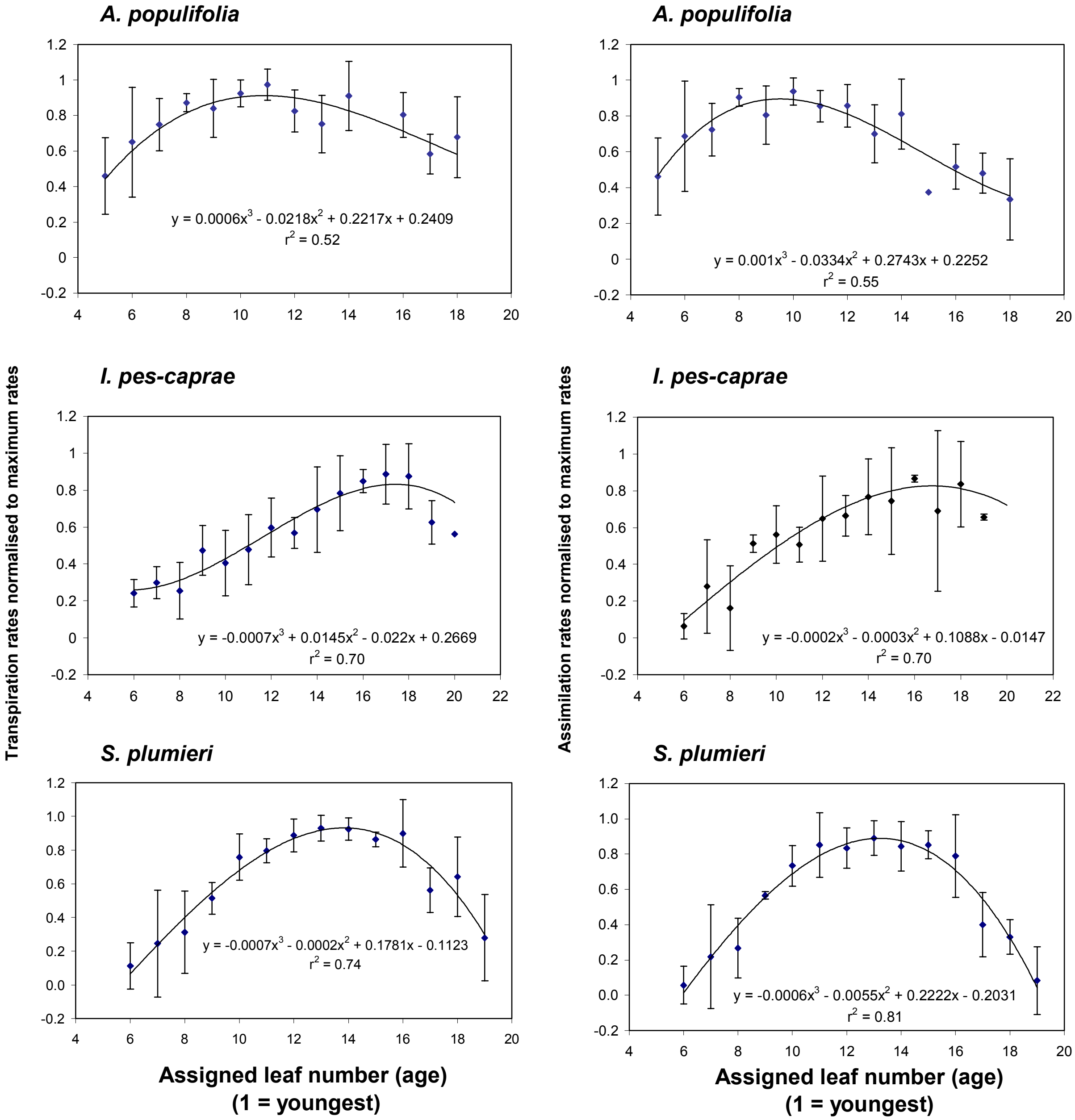


Figure 7.8: Relative changes in assimilation and transpiration rates with leaf number for the indicated species. Standard deviations are represented by the vertical bars. Equations for polynomial fits and r^2 values are given on the figure. N= 3.

Unlike *A. populifolia* the response of *I. pes-caprae* assimilation rate, transpiration rate and Ca/Ci to changes in G_{Leaf} were equally well described by either linear or saturated exponential equations. Both assimilation and transpiration rates increased with increasing stomatal conductance and yielded r^2 values of 0.89 and 0.68 respectively. The slopes of these relationships were similar so that the ratio of A/E with changing G_{Leaf} increased slightly, suggesting a deviation from optimal stomatal behaviour (Farquhar and Sharkey, 1982). Both maximal assimilation and transpiration rates appeared to be limited by maximal G_{Leaf} suggesting that under normal conditions the stomata regulate these processes. This is in agreement with the large degree of stomatal limitation indicated for *I. pes-caprae*, by CO_2 response curves (Figure 7.4 and Table 7.2). Furthermore the limitation of assimilation rate by G_{Leaf} appears to be imposed by internal CO_2 concentration limitations. This was evident because the Ci/Ca ratio declined with increasing G_{Leaf} (Figure 7.7). It is possible that the stomata limited the entry of CO_2 which in turn limited assimilation rate.

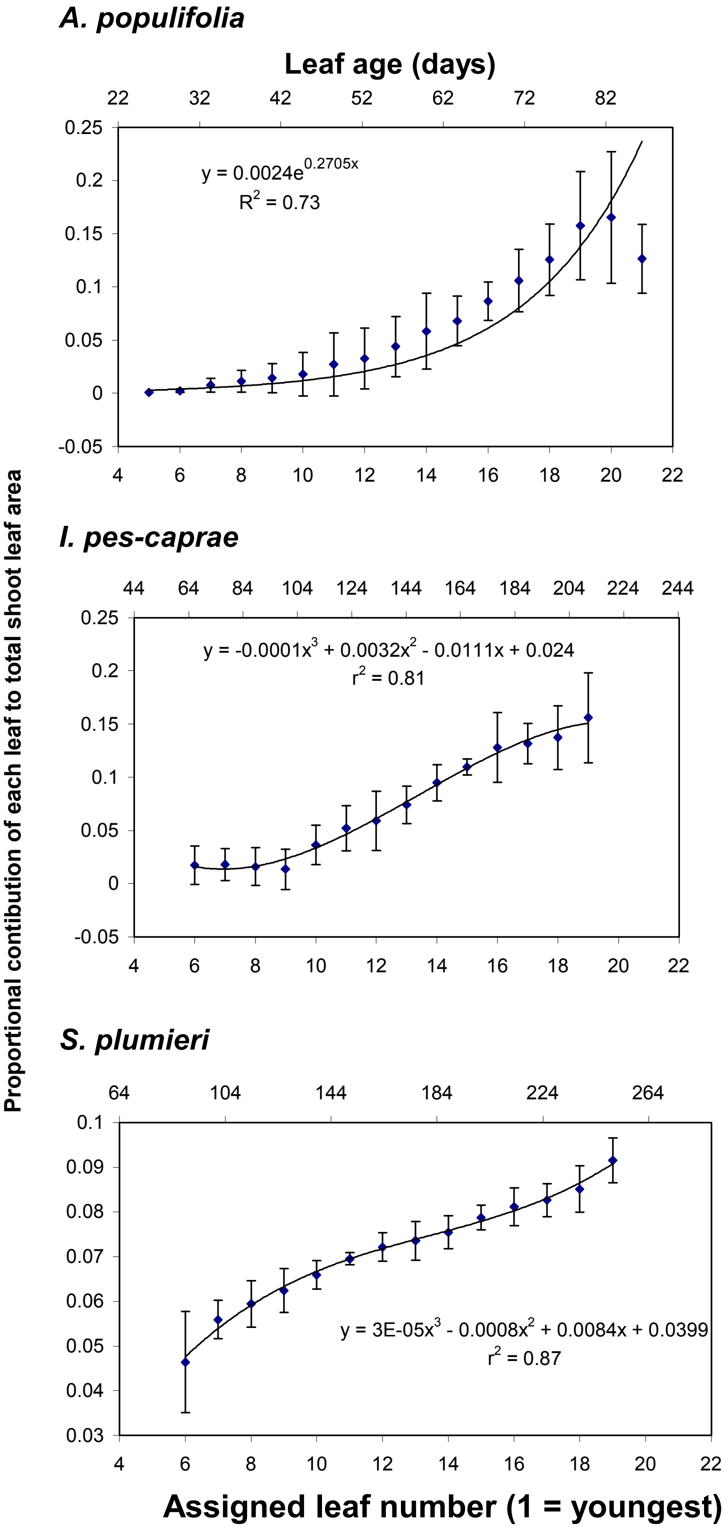
The response of *S. plumieri* assimilation rate to G_{Leaf} was best described by a saturated exponential equation (r^2 = 0.80). Like *A. populifolia* when assimilation rates were saturated there was no decline in the Ci/Ca ratio suggesting that some factor other than maximum stomatal conductances limited CO_2 assimilation. *S. plumieri* transpiration rate response to G_{Leaf} was equally well described by either linear or saturated exponential equations ($r^2 = 0.51$ for both).

Leaf age effects

A. populifolia, I. pes-caprae and *S. plumieri* assimilation and transpiration rate increased with increasing leaf age from youngest leaves, to peak at leaf number 10 to 16 and subsequently declined in the older leaves (Figure 7.8). The responses of both assimilation and transpiration rates to leaf age are adequately described by third order polynomial equations (given on the figure). The differences in the shape of these curves indicates less of a difference between leaves of differing age (number) for *A. populifolia* than was evident for *I. pes-caprae* or *S. plumieri*.

Leaves of different number also contribute differently to the proportions of the leaf area for the whole shoot (Figure 7.9). The youngest leaves are the smallest and the oldest leaves being largest. This relationship between leaf area and leaf age (number) was not linear and was best described by an exponential equation for *A. populifolia* (r^2 = 0.72), and polynomials for *I. pes-caprae* (r^2 = 0.81) and *S. plumieri* (r^2 = 0.87) (equations included on the figure). Leaf longevity estimates (Chapter 4) and the average number of leaves on the plants or shoots used for these measurements were used to approximate leaf ages and these are given on the secondary x-axes (Figure 7.9). These can only be considered as approximations as leaf longevity changes with season as has been shown for *S. plumieri* by Steinke and Lambert (1986) and Pammenter (unpublished data).

Water use budgets (Chapter 5) and consequent nutrient supply (Chapter 6) were based on the assumption that in response to changing VPD, whole shoots (or plants) and leaves (typically leaf 10 to 13) respond in the same way. This is problematic for *A. populifolia* and *I. pes-caprae* budgets but not for *S. plumieri* budgets as the data from Peter (2000) was included. This data was collected from leaves with a wide range in ages (Peter, 2000). Furthermore Peter (2000) showed that the response of leaf and whole shoot transpiration rate, to increasing VPD were similar. Values measured for whole shoot water loss (as estimated by weight loss) agreed with values predicted from the regression of leaf E to VPD (Peter, 2000). However *A. populifolia* and *I. pes-caprae* water budgets were based on data originating from only leaf 10 to 13 (Figure 5.2) and so the response of whole shoots to increasing VPD was not established. *A. populifolia* leaves numbered 10 to13 had the highest transpiration rates while *I. pes-caprae* leaves of these numbers had intermediate transpiration rates (Figure 7.6). Hence water loss and nutrient supply are likely to be over-estimated for *A. populifolia* and under-estimated for *I. pes-caprae*.


A. populifolia

I. pes-caprae

S. plumieri

Figure 7.9: Proportional contribution of each leaf to the total shoot leaf area for the indicated species. Standard deviations are represented by the vertical bars. N= 6.

However as leaves 10 to 13 are large and represent a high proportion of the shoot or plant leaf surface area relative to the leaves with lower leaf numbers (Figure 7.9) this potential error is somewhat reduced. The young leaves contribute little to the total shoot leaf surface area and volume of water transpired.

Annual water budgets for *A. populifolia* and *I. pes-caprae* can be recalculated allowing for the effect of leaf age on transpiration rates per unit leaf area. This is shown below:

$$\text{Recalculated transpired volume} = \sum\left\{\frac{\text{Original transpired volume} \times \text{S.A.} \times \text{Norm E}}{\text{Norm E of leaves \# 10 to 13}}\right\}$$

Where:

The original transpired volume (Table 5.8) was calculated from the relationship of E to VPD (Figure 5.2) and was extrapolated to the shoot leaf surface area per unit dune surface area (see Chapter 5 for details).

Proportional surface area of each leaf (S.A.) is derived from regressions of the proportional contribution of each leaf to the total shoot surface area vs. leaf number (Figure 7.9).

Normalised transpiration rate for each leaf (Norm E) is derived from the regression of normalised transpiration rates vs. leaf number (Figure 7.8).

Average normalised transpiration rate for leaves # 10 to 13 (Norm E of leaves # 10 to 13) is derived from regression of normalised transpiration rates vs. leaf number (Figure 7.8) using a leaf number of 11.5.

The volumes of water transpired by *A. populifolia* and *I. pes-caprae,* per unit dune surface area, corrected for leaf age effects were lower than the original estimates for *A. populifolia*, and higher than estimates for *I. pes-caprae* (Table 7.3). However a comparison of the *S. plumieri* data collected from leaves 10-13 (this study) with data collected for a whole range of leaves (Peter, 2000) were not dissimilar (Figure 5.2) and questions the necessity for leaf age corrections. Furthermore when the relationships between leaf age and transpiration rates were determined the microclimatic conditions for each leaf were maintained constant (PPFD, leaf temperature, ambient relative humidity and CO_2 concentration). Under natural conditions mutual shading and leaf orientation (see below) are such that the conditions for each leaf could be significantly different. This would certainly make the response of transpiration to leaf age somewhat different from the response shown in Figure 7.6. It would require extensive experimentation to elucidate such effects.

Table 7.3: A comparison of the annual volumes of water transpired by *A. populifolia, I. pes-caprae* and *S. plumieri* in an average rainfall year (Average of 1978 to 1998 data). Values were calculated either accounting for the effects of leaf age, or not. Volumes are calculated per unit dune surface area.

	Volume of water transpired (l m^{-2} yr^{-1})		
	A. populifolia	***I. pes-caprae***	***S. plumieri***
Originally calculated volume of water transpired (Chapter 6)	208	593	430
Re-calculated volume of water transpired	175	628	—
Difference	**33**	**- 35**	—

S. plumieri whole shoot response to light

Data for *S. plumieri* from Pammenter and Smith showed that due to the random arrangement of leaves on a stem there was considerable mutual shading (Figure 7.10). Older leaves that are at the base of the stem receive less light than younger leaves higher up the stem. Additionally there are differences in light interception according both to leaf inclination angles (Figure 7.11) and to the leaf positions on the stem relative to the passage of the sun across the sky during the day (Figure 7.12). Leaf inclination angles measured between the stem and the adaxial leaf surfaces vary according to assigned leaf number (Figure 7.11). The majority of the light intercepted by leaves occurred in (i) the eastern quadrant in the morning, (ii) the western quadrant in the afternoon, (iii) the northern quadrant throughout the day and (iv) the southern quadrant in the morning and afternoon but not in the middle of the day (Figure 7.12).

Individual leaves are not light saturated by midday light intensities (Figure 5.2) and due to mutual shading are often illuminated by intensities lower than those measured at midday.

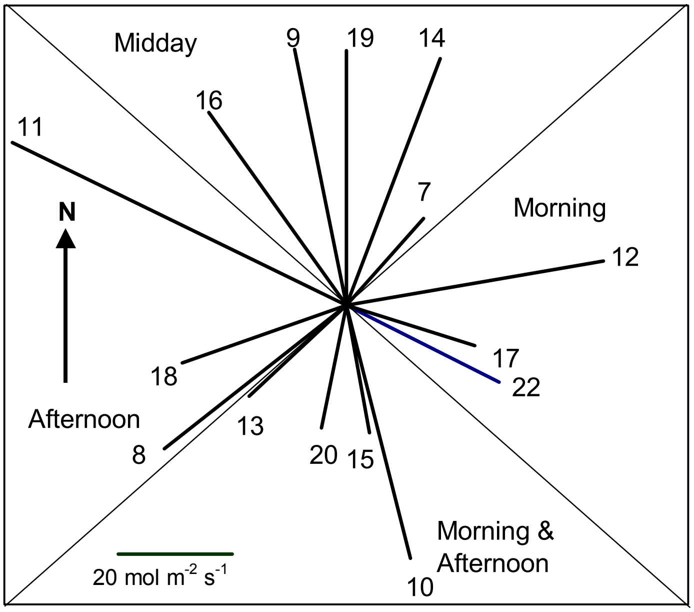


Figure 7.10: Photosynthetic photon flux density (PPFD) intercepted by leaves in different positions on a stem. Assigned leaf numbers are indicated with leaf one being the youngest. The orientation of each line represents the azimuth angle of that particular leaf and its length is proportional to the intercepted PPFD. The figure is divided into four quadrants and the period of the day when illumination was maximal in each quadrant is indicated. Data with permission from Pammenter and Smith (unpublished).

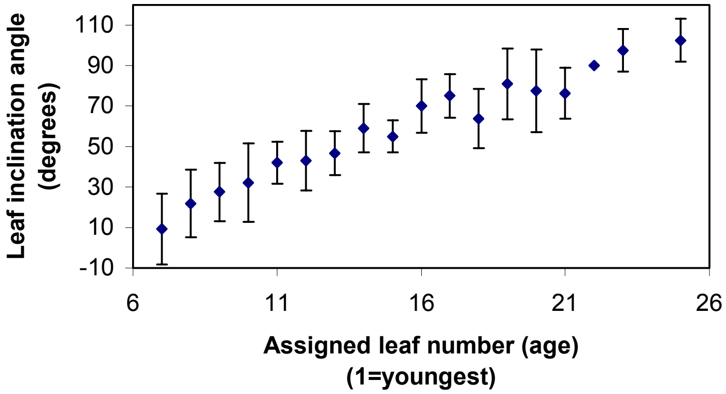


Figure 7.11: Leaf inclination angles (measured between the perpendicular stem and the leaf adaxial surface) related to assigned leaf number (age). Data with permission from Pammenter and Smith (unpublished).

Hence the amount of CO_2 assimilated by whole shoots was dependent on the total amount of PPFD intercepted by the shoots (Figure 7.13).

Table 7.4: A comparison of the average monthly leaf production rates of *S. plumieri* growing at Mtunzini, Durban and Old Woman's River and incident PPFD measured at Port Elizabeth and Durban.

Location	**Monthly leaf production rate (leaves month$^{-1)}$**	**Average monthly PPFD (mol m^{-2} month^{-1})**
Old Woman's River [*1]	2.1 ± 0.9	9040
Mtunzini [*2]	3.8	—
Durban [*3]	2.8	6217

Abbreviation: photosynthetic photon flux density (PPFD). [*1] present study; [*2] Pammenter, 1983; [*3] Steinke and Lambert, 1986. Average monthly PPFD calculated from average monthly solar radiation data (W m^{-2}) supplied by the SAWB. See chapter 4 for conversion details.

This apparent light limitation suggests that at locations receiving greater amounts of incident light, the growth and productivity of *S. plumieri* would be higher. Table 7.4 lists the average monthly leaf production rates for *S. plumieri* at Mtunzini, Durban and Old Woman's River and the average monthly incident PPFD at Durban and Port Elizabeth.

Durban receives less incident PPFD than does Port Elizabeth mainly due to shorter day length and yet it sustains higher leaf production rates than does Old Woman's River (Table 7.4). This apparent contradiction may be the result of several factors. Firstly the incident PPFD was calculated from direct solar radiation data (see Chapter 4 for details) measured at standard instrument height (SAWB) which is unaffected by orientation or shading. The leaf production rates were measured at specific locations on dunes and the daily amounts of incident light are affected by dune orientation and shading. Hence the amounts of light intercepted by the plants growing in the respective locations may be dissimilar to the amounts of incident solar radiation. Furthermore growth is affected by other abiotic factors such as ambient temperature, water availability etc. and these are different at Old Woman's River, Mtunzini and Durban.

Unfortunately data comparable to that presented above, is unavailable for either *A. populifolia* or *I. pes-caprae*.

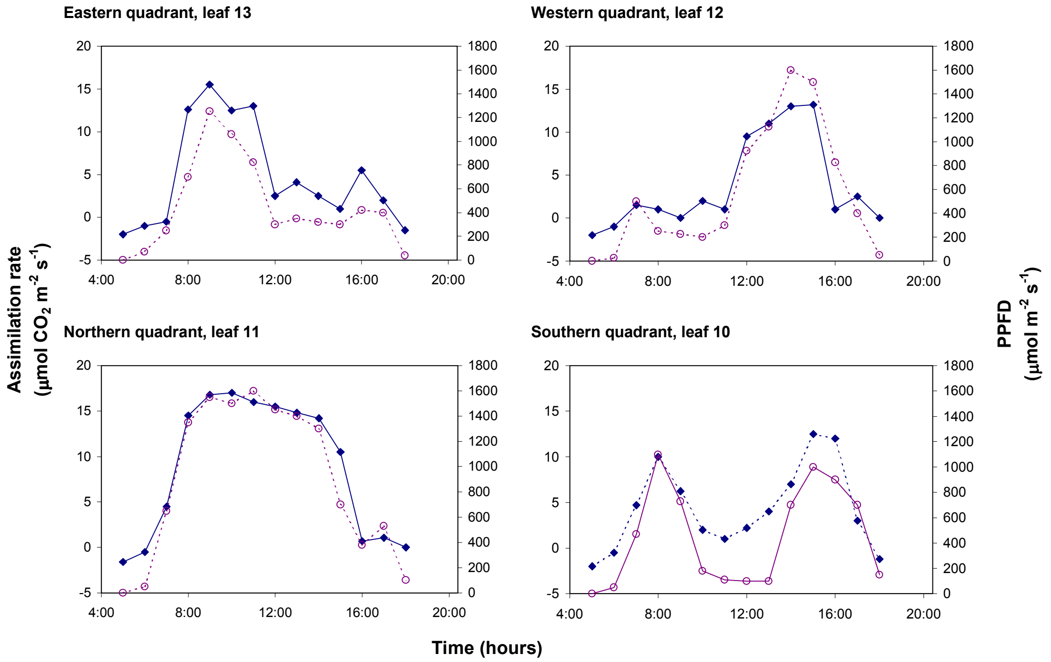


Figure 7.12: Daily course of CO_2 assimilation (solid symbols & lines) and photosynthetic photon flux density (PPFD; open symbols & broken lines) of four leaves from different quadrants around the stem. Data with permission of Pammenter and Smith.

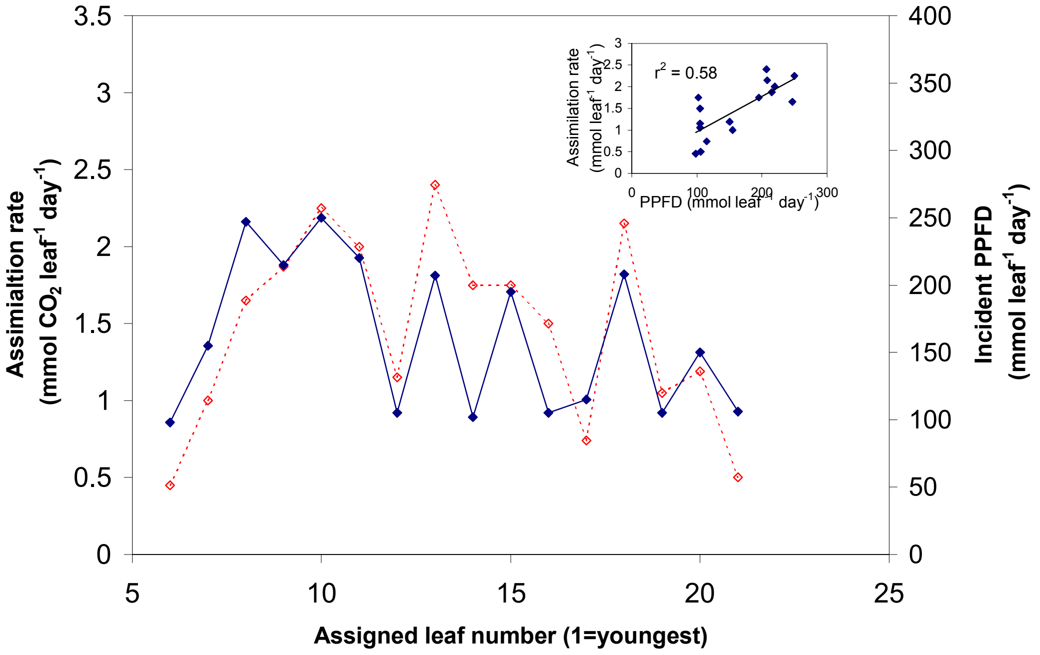


Figure 7.13: Total daily CO_2 assimilated by *S. plumieri* shoots (solid symbols & lines) and photosynthetic photon flux density (PPFD) intercepted (open symbols & broken lines) by leaves of different assigned number (age) on one stem. Inset shows the linear relationship between intercepted PPFD and CO_2 assimilation. Data with permission from Pammenter and Smith.

A. populifolia response to light and the role of the leaf hair layer

These results are presented here as they are relevant to describing the unique adaptations that *A. populifolia* possesses.

Characteristics of hair layer

Microscopic examination (Plate 7.1) shows that the hairs consist of two water-filled basal cells and a string of dead cells. When the hair layer was removed, fracture occurred at a dead cell junction, the water-filled basal cells remaining undamaged. Leaf pubescence (five different plants) reduced transmitted PPFD to 83% ± 2% of incident light. This effect was constant over light intensities ranging from 300 to 2200 μmol m^{-2} s^{-1}. The absorption of light by the hair layer was not uniform across the entire spectrum measured. Hair absorption over the photosynthetically active wavelengths was uniform and low. Absorption increased below 400nm and peaked in the UV range (Figure 7.14). Conductance to the diffusion of water by the isolated hair layers (measured in an unstirred chamber in which air-flow rates were varied) ranged from 1.7 to 3.3 mol m^{-2} s^{-1} (raw data not shown).

Laboratory gas exchange studies

Light response curves for leaves with hairs were plotted as a function of the intensity incident on the leaf surface (by multiplying the intensity of incident light on the hair layer by 0.83). These were not identical to curves plotted for leaves without hairs (Figure 7.15). At values between 0 and 1000 μmol m^{-2} s^{-1} for PPFD incident on the leaf surface, leaves with and without hairs had similar assimilation rates. At higher light intensities leaves without hairs showed slightly lower assimilation rates than leaves with an intact hair layer. This was the case for each individual leaf studied. The mean assimilation rates of leaves with hairs in 1700 μmol m^{-2} s^{-1} of incident light were significantly different from mean rates for leaves without hairs in incident light at 1660 μmol m^{-2} s^{-1} (t-test for dependent correlated samples, $p = 0.013$). Leaves without hairs had higher leaf conductances (Figure 7.16) and higher intercellular CO_2 concentrations (Ci) (Figure 7.17) than leaves with hairs over the entire range of PPFD measured. The differences were less at high light intensities. At the same PPFD incident on the leaf surface the reduced assimilation rates of leaves without hairs relative to leaves with hairs were not the consequence of limited CO_2 supply, as leaves without hairs had higher Ci values.

Higher leaf conductances in leaves without hairs resulted in higher transpiration rates, an effect that decreased at higher light intensities as leaf conductance differences decreased at these intensities (Figure 7.18). The combined effect of the hair layer on photosynthetic performance and transpiration rate resulted in higher instantaneous water-use efficiencies for the leaves with hairs over the entire range of light intensities incident on the leaf surface (data not shown).

Laboratory chlorophyll fluorescence studies

At low light intensity most absorbed radiant energy was dissipated photochemically but as light intensity increased there was a decrease in photochemical energy dissipation (q_P) and an increase in non-photochemical energy dissipation (q_{NP}).

This was apparent for all the replicates of this experiment. One representative replicate is shown in Figure 7.19. As the light intensity increased, q_P of the leaves without hairs decreased below the values measured for leaves with hairs (Figure 7.19). Similarly the leaves without hairs showed greater energy dissipation by non-photochemical quenching than did leaves with hairs. This effect was evident only at intensities above 1300 μmol m^{-2} s^{-1} suggesting more than simply a light intensity effect. Regardless of treatment leaves showed reversible reductions in PSII efficiency even after long (90 -175 min) exposure to high light (Figure 7.20). Fv/Fm values of dark-adapted hairy leaves were in the region of 0.88. This was reduced to a relatively constant Fv'/Fm' of 0.75 on illumination at 2000 μmol m^{-2} s^{-1}. After dark adaptation for 30 minutes Fv/Fm values were restored to 0.88. Removal of leaf hairs and illumination at 2000 μmol m^{-2} s^{-1} reduced Fv'/Fm' to 0.6. Reduction of light intensity to 1660μmol m^{-2} s^{-1} (a value similar to the light intensity transmitted through the hair layer to the leaf surface) increased the Fv'/Fm' ratio to 0.65 (in contrast to the values of 0.75 for leaves with hairs). Assimilation rates showed similar responses to altered light intensity and hair removal but did not stabilise as quickly as did measured fluorescence.

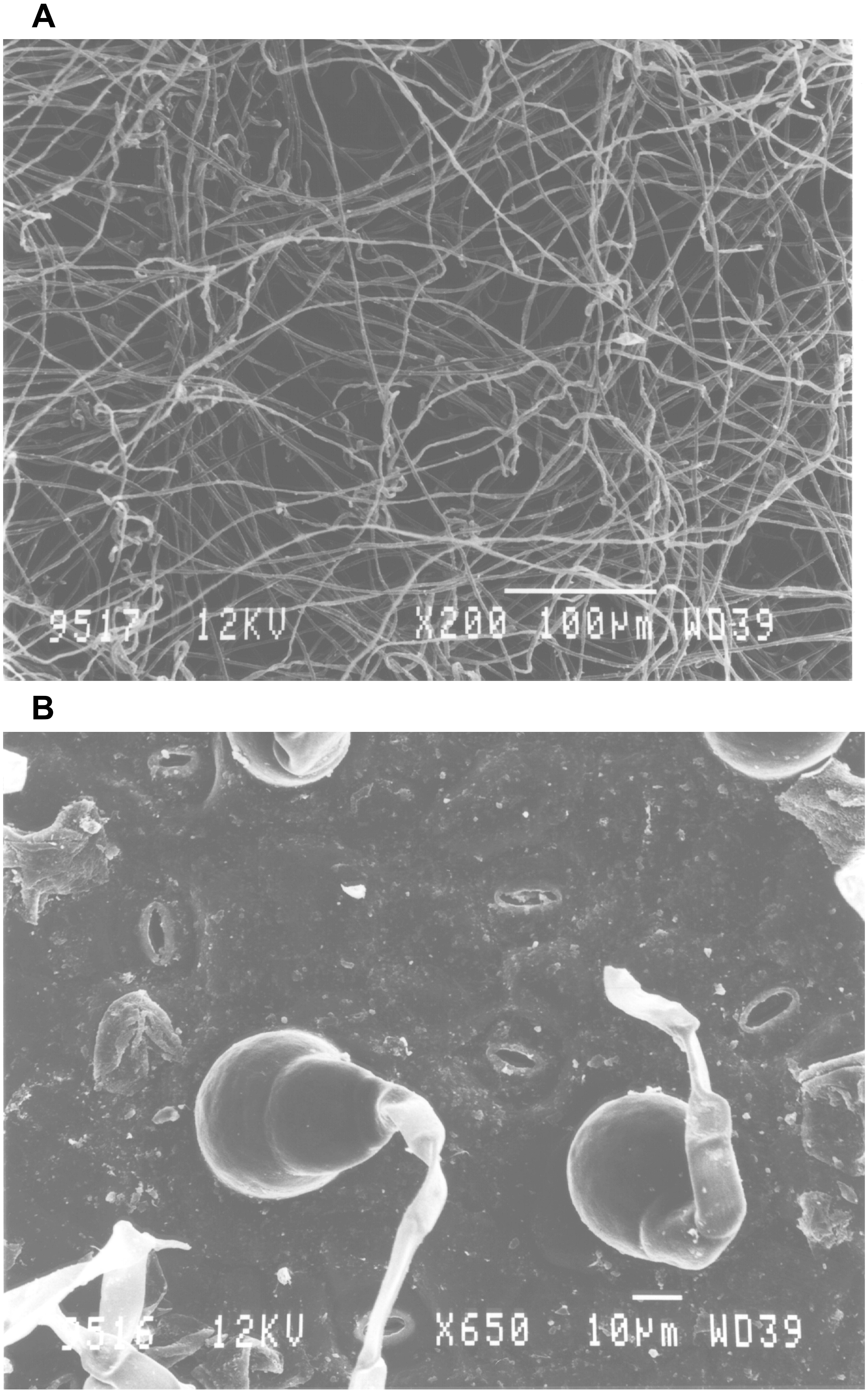


Plate 7.1 A and B: SEM micrographs of the *A. populifolia* leaf hair layer x 200 (A) and water filled basal cells x 600 (B). When the hair layer was removed fracture occurred at a dead cell junction and the water-filled basal cells were not damaged.

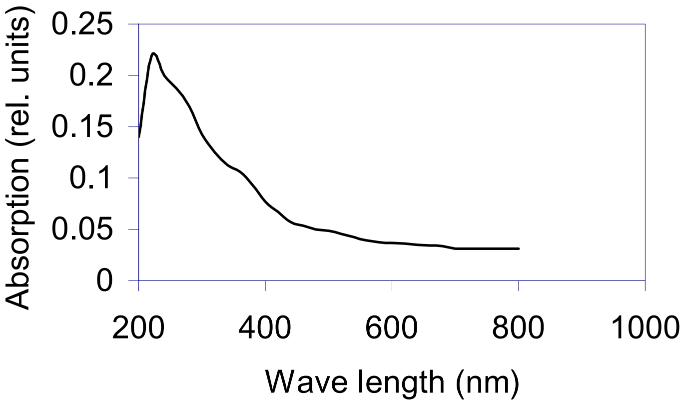


Figure 7.14: Absorption spectrum of a complete layer of leaf hairs.

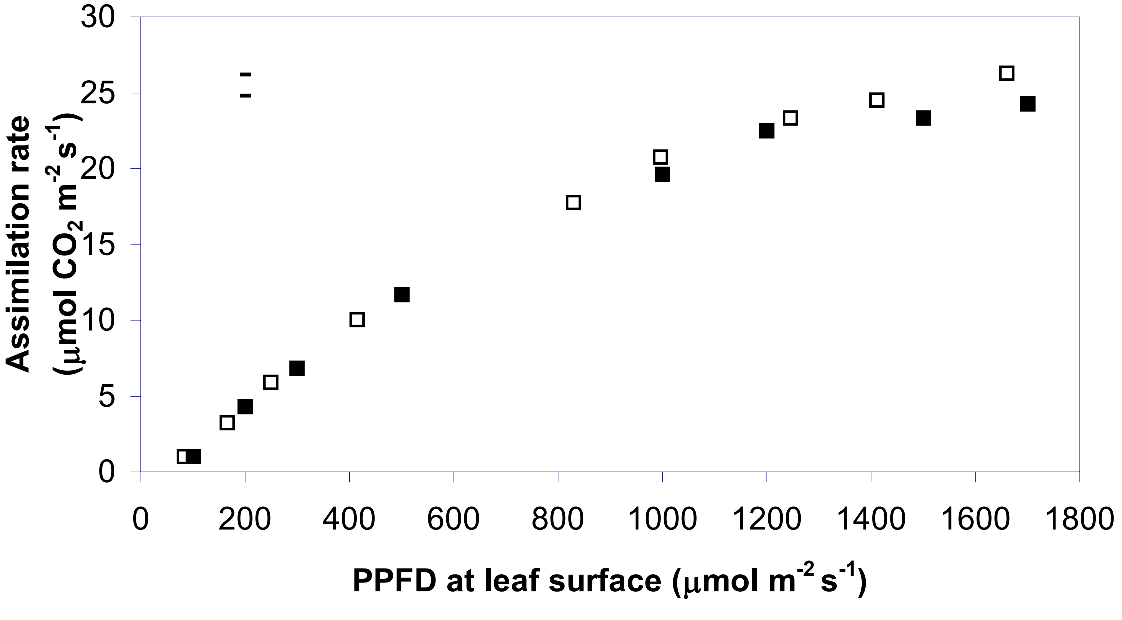


Figure 7.15: Mean assimilation rates of leaves with ( ) and without (■) hairs in response to increasing photosynthetic photon flux density (PPFD). Light intensities for leaves with hairs have been corrected to PPFD transmitted through the hair layer. The maximum standard deviation of replicate leaves is indicated in the figure. (N=4).

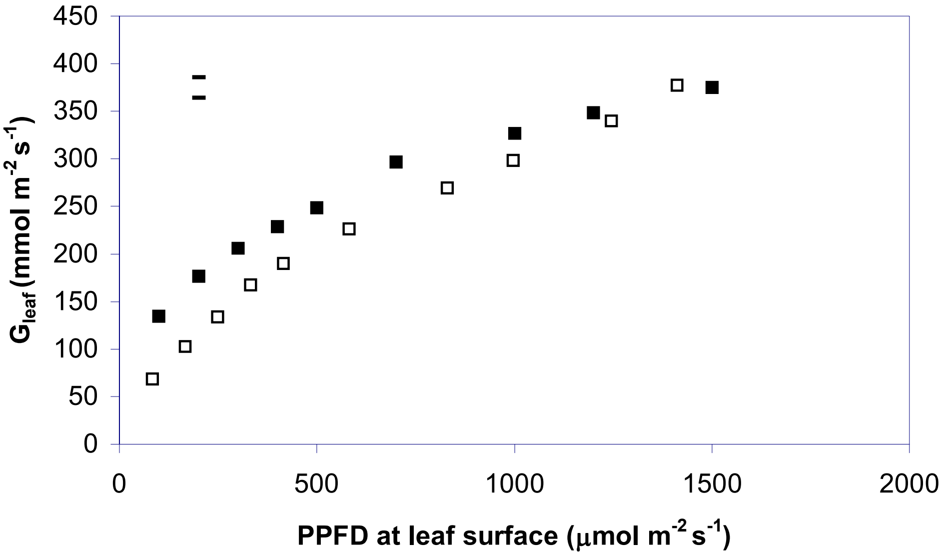


Figure 7.16: Mean conductances (G_{Leaf}) of leaves with ( ) and without (■) hairs in response to increasing photosynthetic photon flux density (PPFD). Light intensities for leaves with hairs have been corrected to PPFD transmitted through the hair layer. The maximum standard deviation is indicated in the figure. (N=4).

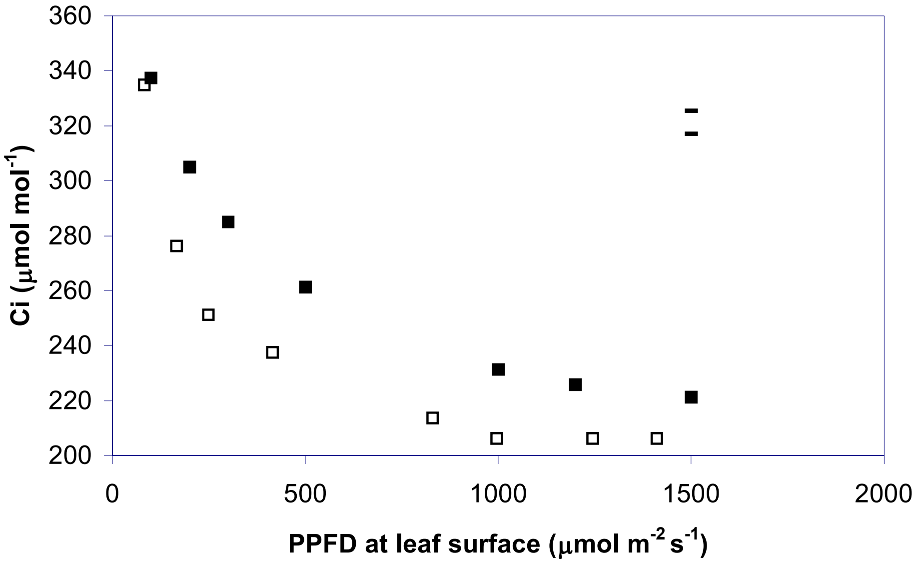


Figure 7.17: Mean intercellular CO_2 concentration (Ci) in leaves with ( ) and without (■) hairs in response to increasing photosynthetic photon flux density (PPFD). Light intensities for leaves with hairs have been corrected to PPFD transmitted through the hair layer. The maximum standard deviation is indicated in the figure. (N=4).

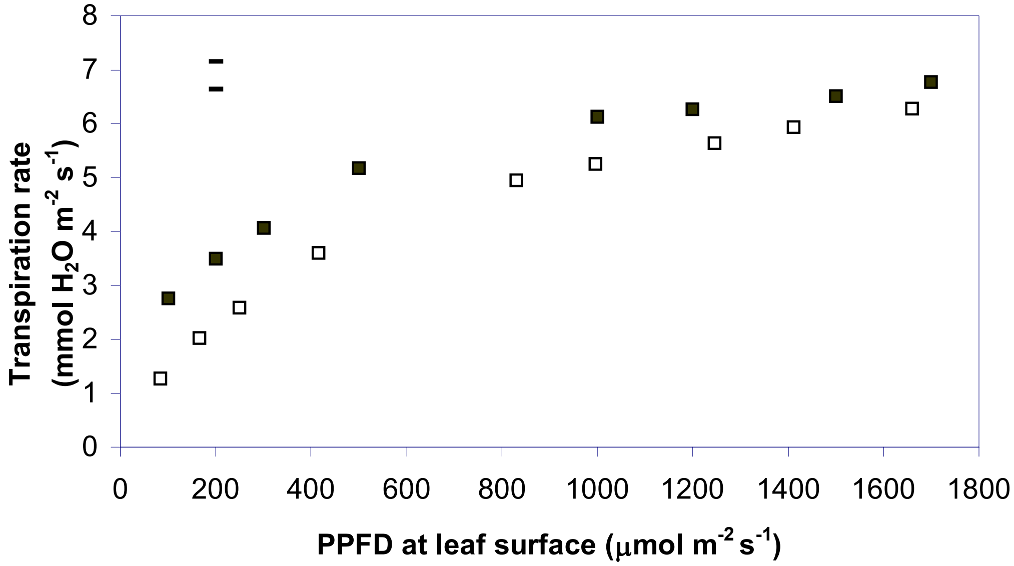


Figure 7.18: Mean transpiration rates of leaves with ( ) and without (■) hairs in response to increasing photosynthetic photon flux density (PPFD). Light intensities for leaves with hairs have been corrected to PPFD transmitted through the hair layer. The maximum standard deviation is indicated in the figure. (N=4).

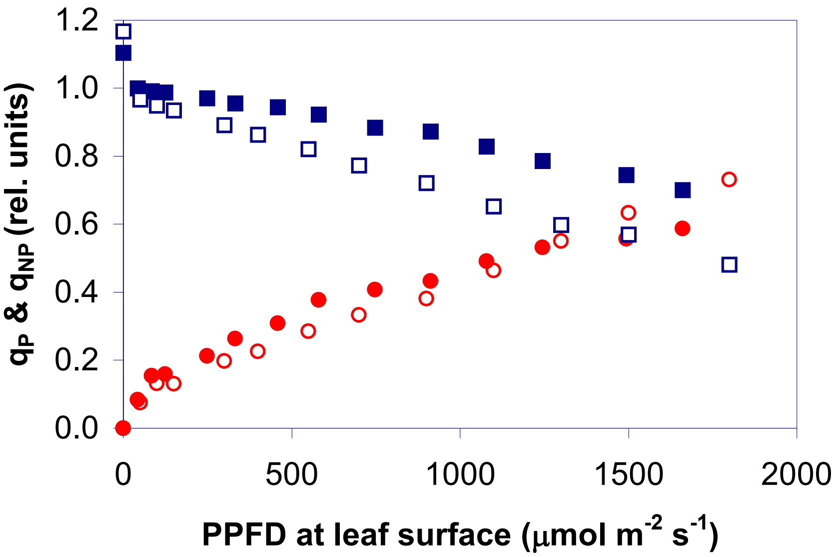


Figure 7.19: Effect of increasing photosynthetic photon flux density (PPFD) on the photochemical (q_P) (squares) and non-photochemical (q_{NP}) (circles) energy dissipation of a leaf with hairs (open symbols) and without hairs(closed symbols). Light intensities for leaves with hairs have been corrected to PPFD transmitted through the hair layer. This figure shows the data for a representative leaf. In all cases (N=4) the differences between leaves with and without hairs showed the same pattern.

Field studies

Assimilation rates of leaves with or without hairs were the same over the range of PPFD intensities encountered in the field and are comparable to rates measured in the laboratory (Figure 7.21). Differences between leaves with and without hairs (at the same PPFD incident on the leaf surface) which were encountered at high light intensities in the laboratory, were not obvious in the field. This may have been because of the variability in the data resulting from measurements of leaves with and without hairs not being made on the same leaf.

As in the laboratory the lower leaf conductances of leaves with hairs resulted in lower transpiration rates (Figure 7.21) in the field. The transpiration rates at light intensities higher than 1000 μmol m^{-2} s^{-1} were significantly different to those for leaves with hairs (t-test for independent samples, $p < 0.01$). Lower leaf conductances and transpiration rates resulted in higher water use efficiencies at all light intensities measured.

Higher transpiration rates in leaves without hairs lowered the leaf temperature in comparison with controls. This difference was reduced in the afternoon on which measurements were made. This was due to cloud cover which affected both the thermal loading and evaporative cooling of the leaves (Figure 7.22). Lower leaf temperature for leaves without hairs was also evident in the laboratory study where leaf temperatures were kept constant. The cuvette temperature required to maintain constant leaf temperature was consistently higher for leaves without hairs. Hence the reduction in temperature due to the increased evaporative cooling in leaves without hairs is greater than potential increase in temperature which is avoided by increased reflectance of incident PPFD by the leaves with hairs.

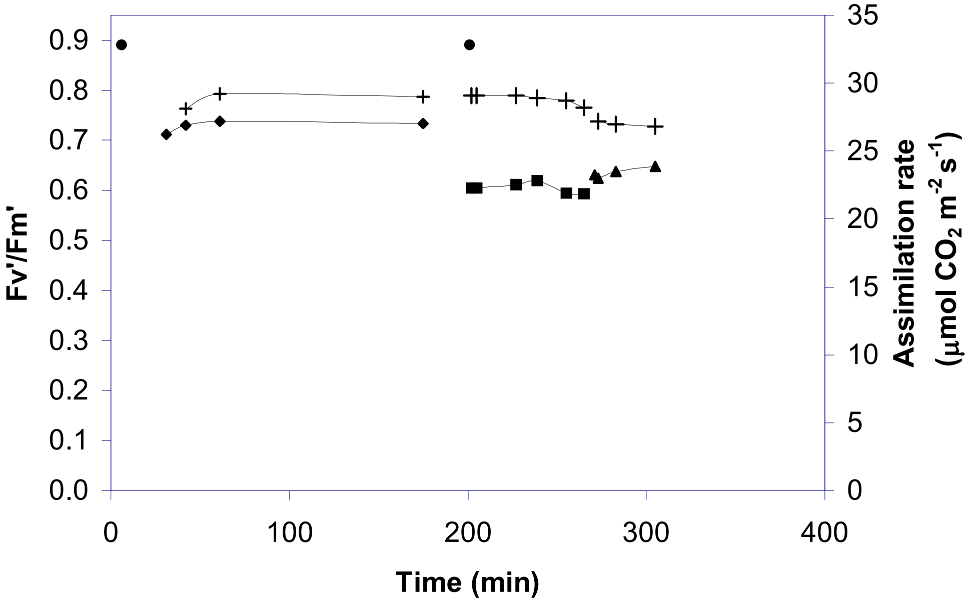


Figure 7.20: Quantum yield of PSII (Fv'/Fm') and assimilation rate of a leaf before and after hair removal. A leaf with hairs was dark adapted for 30 minutes and Fv/Fm measured. The leaf was then illuminated at 2000 μmol m^{-2}s^{-1} for 175 minutes after which the hairs were removed. After 30 minutes dark adaptation Fv/Fm was re-measured and the leaf was then again illuminated for a further 90 minutes at 2000 μmol m^{-2}s^{-1}. Light intensity was then reduced to 1660 (=2000 x 0.83) μmol m^{-2}s^{-1} for a further 40 minutes. •Fv/Fm of a dark-adapted leaf, ♦Fv'/Fm' of a leaf with hairs illuminated at 2000 μmolm^{-2}s^{-1}, ■ Fv'/Fm' of the same leaf with hairs removed and illuminated at 2000 μmolm^{-2}s^{-1}, ▲ Fv'/Fm' of the hairless leaf illuminated at 1660 μmolm^{-2}s^{-1}, ✚ Assimilation rate.

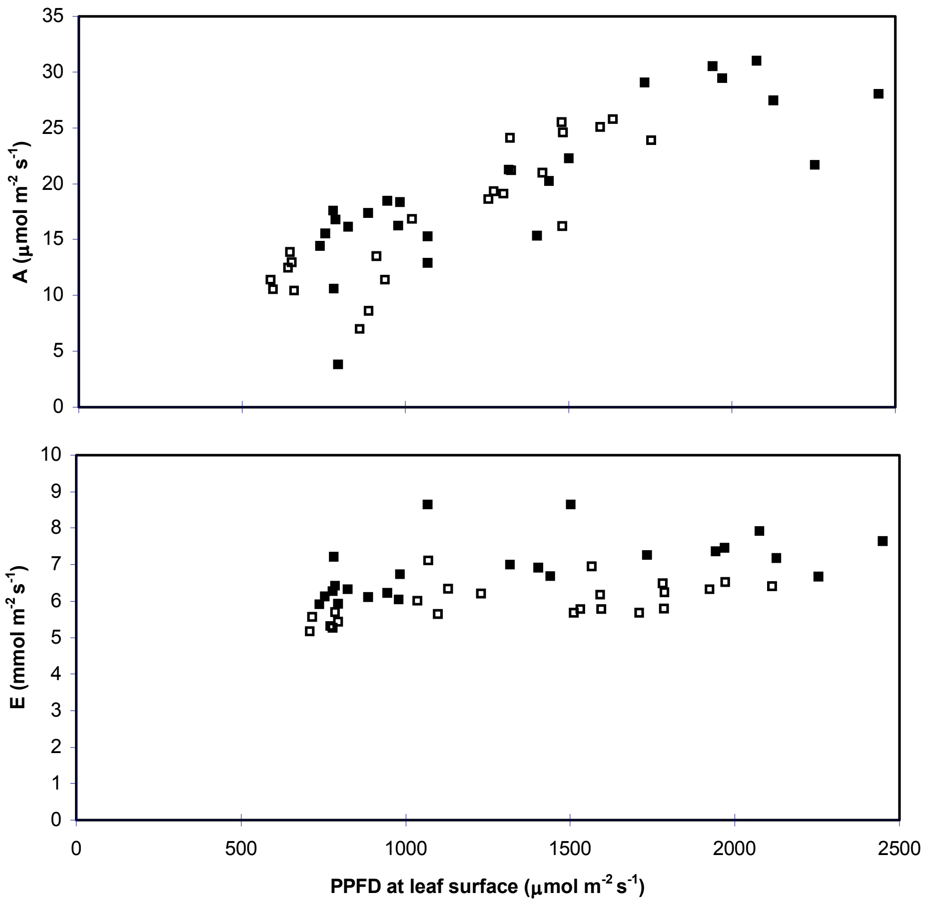


Figure 7.21: Assimilation (A) and transpiration (E) of leaves with ( ) or without (■) hairs, measured in the field in response to increasing photosynthetic photon flux density (PPFD). Light intensities for leaves with hairs have been corrected to PPFD transmitted through the hair layer. (N=4).

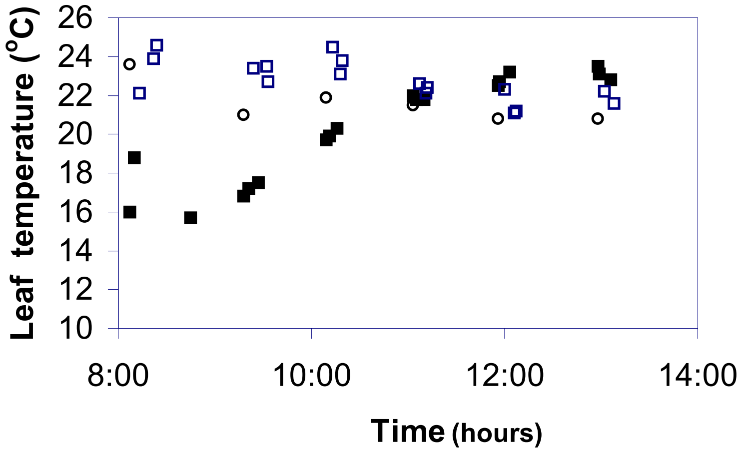


Figure 7.22: Air temperature (o) and leaf temperatures of leaves with ( ) or without (■) hairs measured in the field at various times of the day.

DISCUSSION

Light responses

The fact that none of the three species investigated showed light-saturated photosynthesis (A_{max}) even at PPFDs equivalent to full sunlight (Figure 7.3) is indicative of plants that are adapted to high light environments (Bjorkman, 1981). The light intensity under which plants develop is known to affect both their quantum yield and maximal photosynthetic rates. An example of such effects is the response of *Atriplex triangularis* to different light intensity growth regimes (Bjorkman, 1981; in Table 7.5). Correlated with this is the acclimation in the leaf pigment concentrations, ratios of PSI:PSII and pool sizes of electron transport intermediates (Bjorkman, 1981; Gabrielsen, 1948). However despite all three of these species having developed in similar light conditions there were significant differences in their light responses (Figure 7.3). *A. populifolia* had significantly higher efficiencies of utilisation of incident PPFD and A_{max} (Table 7.1) while *I. pes-caprae* and *S. plumieri* efficiencies of utilisation of PPFD and A_{max} were not significantly different. The high efficiency of utilisation of incident PPFD measured for *A. populifolia* may result from the possession of high leaf chlorophyll content per unit leaf surface area and insusceptibility to photoinhibition as a result of its leaf hair layer (Chapter 4, Productivity; and below). Although the chlorophyll content per unit leaf area was different among the investigated species the ratio of chlorophyll a:b was not significantly different ($p=0.225$) among species (Figure 4.9, Chapter 4), probably indicating similar PSI:PSII ratios (Bjorkman, 1981). The high photosynthetic rates recorded for *A. populifolia* (Figure 4.1, Chapter 4) are the result of the high efficiency of utilisation of incident PPFD.

The efficiency of utilisation of incident PPFD for *A. populifolia* exceeded those measured for dune grasses and were more like those of the C_3 dicotyledonous dune and under-story forest species (Table 7.5). Efficiencies of all three species investigated far exceed those measured for fynbos species (Table 7.5).

Table 7.5: Significant points extracted from light response curves for indicated dune and other plant species.

Species	**Efficiency of utilisation of incident PPFD (mmol mol^{-1})**	**Light compensation point (μmol mol^{-1})**	**Photosynthetic rate at light saturation (A_{max}) (μmol m^{-2} s^{-1})**
A. populifolia *1	56 ± 5.0	36.4 ± 6.3	33.6 ± 2.0
I. pes-caprae *1	39 ± 3.0	46.1 ± 13.4	16.4 ± 3.4
S. plumieri *1	43 ± 12.0	54.9 ± 7.3	19.0 ± 3.6
Ammophila arenaria *2	32.3	± 47	—
Elymus mollis *2	26.2	± 47	—
Atriplex leucophylla *3	26.2	172.4	35.0
Ambronia maritima *3	44.8	34.5	28.0
Ambrosia chamissonis *2	57.1	34.5	42.5
Cakile maritima *3	57.1	34.5	39.0
Euphorbia forbesii *4	62.5	—	6.5
Caloxylon sandwicense *4	60.0	—	3.9
Protea laurifolia *5	14.1	± 50	12
Elytropappus gnaphaloides *5	7.5	± 50	7.2
Rhus dissecta *5	8.57	± 50	7.0
Atriplex triangularis *6 (low light)	47	±40	5.0
Atriplex triangularis *6 (high light)	40	±40	33.0

*1 This study; *2 Pavlik, 1983(a), laboratory response of temperate dune grasses supplied with high nutrients; *3 De Jong, 1979, North American beach plants grown in growth chambers; *4 Pearcy and Calkin, 1983, naturally growing forest under-story; *5 von Willert *et al.,* 1989, naturally growing fynbos. Bjorkman *et al.*, 1972, cited in Bjorkman, 1982, plants grown under different light intensity regimes (low light = 92 μmol m^{-2} s^{-1}; high light = 920 μmol m^{-2} s^{-1}).

Light compensation points indicate that for the mature fully expanded leaves used for these measurements *A. populifolia* had the lowest rates of dark respiration (Table 7.1). These data are in contrast to the measurements on whole shoots where *A. populifolia* had the highest nocturnal respiration rates (Table 4.3). These nocturnal measurements include the CO_2 production by fully expanded mature leaves, by young developing leaves and by stems and reproductive structures. Hence it is apparent that although this species invests the least respiratory energy on the maintenance of fully expanded leaves whole shoot respiration rates were the highest. This indicates a large proportion of respiratory CO_2 production associated with growth. Furthermore *A. populifolia* above-ground shoot production was the highest of the three species investigated (Table 4.6). *S. plumieri* had the highest light compensation points indicative of a high respiratory rate and a higher investment of energy in the maintenance of fully expanded mature leaves (Table 7.1). *I. pes-caprae* rates were intermediate between those of *A. populifolia* and *S. plumieri.* The high between replicate variation for these two species (as indicated by the large standard deviations) resulted in these rates being insignificantly different from the rates measured for the other two species (Table 7.1).

CO_2 responses

In *A. populifolia, I. pes-caprae* and *S. plumieri* CO_2 saturated photosynthesis occurred at Ci values of approximately 500, 500 and 400 μmol m^{-2} s^{-1} respectively (Figure 7.4). Furthermore the range of Ci values measured at ambient conditions when photosynthetic rates were greater than 1 μmol m^{-2} s^{-1} ranged from 218 to 355, 226 to 308 and 224 to 334 μmol m^{-2} s^{-1} for *A. populifolia I. pes-caprae* and *S. plumieri* respectively. Hence all three species under usual ambient conditions are not CO_2 saturated and the carboxylation efficiencies become important determinants of measured photosynthetic rate. *A. populifolia* had the highest carboxylation efficiency (Table 7.2) and could thus attain the highest photosynthetic rate (Figure 4.1). Carboxylation efficiencies (Table 7.2) and photosynthetic rates (Tables 4.1 & 4.4) for *I. pes-caprae* were the lowest and those of *S. plumieri* were intermediate.

A. populifolia had significantly higher rates of CO_2 saturated photosynthesis than did *I. pes-caprae* or *S. plumieri* indicating higher rates of RuBP regeneration and the capacity for higher rates of ATP and NADPH production (Table 7.2). This is in agreement with the higher efficiency of utilisation of PPFD measured for this species (Table 7.1) and is possibly the result of the greater chlorophyll content per unit leaf surface area than the other two species. Furthermore stomatal limitations and photorespiratory rates in this species were lower than those measured for *I. pes-caprae* or *S. plumieri* although this was significant only on the 18/5/2000 (Table 7.2). A combination of these low stomatal limitations and photorespiratory rates, high carboxylation efficiencies and rates of RuBP regeneration collectively explain the high photosynthetic rates measured for this species.

The differences in CO_2 response curves determined on different experimental days may be the result of several factors. For *A. populifolia* which is short-lived (Tinely, 1980; *pers. obs.*) plant age may have been a contributory factor. The same plants or at least plants belonging to the same cohort were used for measurements on both dates. Although leaves of similar assigned number (age) were selected on each occasion, the age of the plants and not leaf age may affect leaf photosynthetic performance. *A. populifolia* plants certainly appear to have declining vigour when they get old (*ca.* 2 years; *pers. obs.*). This is unlikely to be as important for *I. pes-caprae* and *S. plumieri,* which are long-lived species. In addition to plant age the prevailing conditions and the recent history of prevailing conditions may be important considerations. An example of such effects is to be found in the work of Ishikawa *et al*. (1991) who investigated three Japanese coastal dune species and noted changes in carboxylation efficiency, leaf conductance (and hence Ci) in response to changes in soil-water salinity. Factors such as growth light intensity, nutrient status, water status and ambient temperature also affects light and CO_2 response curves (Farquhar and Sharkey, 1982; and references cited therein).

Carboxylation efficiencies, CO_2 compensation points and rates of RuBP regeneration measured for *A. populifolia*, *I. pes-caprae* and *S. plumieri* fall within the range measured for C_3 species from dune and other environments (Table 7.6). Carboxylation efficiencies of the listed C_4 species are far higher than those of the C_3 species due to the high affinity of phosphoenolpyruvate carboxylase for CO_2.

Stomatal limitations of about 30% are typical of C_3 plants in ambient CO_2 conditions (Jones, 1983). *A. populifolia* limitations measured on the 6/1/2000 and for those *S. plumieri* measured on both experimental days were similar to this typical value. However *I. pes-caprae* limitations measured on both experimental days exceeded this typical value and *A. populifolia* values measured on the 18/5/2000 were lower than this typical value (Table 8.2). *S. plumieri* stomatal limitations measured in this investigation were similar to values measured by Pammenter and Smith (unpublished, see Table 7.6). The values measured for *A. populifolia* and for *Calystegia soldanella* and those for *I. pes-caprae* and *Carex kobomugi* were similar (Table 7.6). However the latter comparison must be treated cautiously as the investigation by Ishikawa *et al.*(1991) was performed on saline-treated hydroponically grown material which had access to unlimited water.

Table 7.6: Significant points extracted from CO_2 response curves for the indicated dune and other plant species.

Species	**Carboxylation efficiency (mmol m^{-2} s^{-1})**	**CO_2 compensation point (μmol mol^{-1})**	**Stomatal limitation (%)**	**RuBP regeneration rate (μmol m^{-2} s^{-1})**
A. populifolia [*1]	118.3 ± 20.6	43.2 ± 5.6	23.4 ± 15.7	37.0 ± 3.4
I. pes-caprae [*1]	71.1 ± 14.4	51.8 ± 8.3	48.8 ± 6.9	23.4 ± 5.4
S. plumieri [*1]	95.8 ± 17.6	55.1 ± 7.3	29.6 ± 8.3	24.9 ± 6.0
S. plumieri [*2]	133	60	33 ± 3	39
Atriplex leucophylla [*3] (C_4)	345	± 0	—	± 28
Abronia maritima [*3]	137	30-40	—	± 36
Ambrosia chamissonis [*3]	233	30-40	—	± 58
Cakile maritima [*3]	208	30-40	—	± 56
Scaevola coriacea **[*4]**	130 ± 10.8	—	—	24.8 ± 2.6
Scaevola gaudichaudii [*4]	86.9 ± 6.5	—	—	19.3 ± 1.3
Scaevola mollis [*4]	65.2 ± 10.8	—	—	13.6 ± 1.4
Ischaemum anthephoroides[*5] (C_4)	2292 ± 158	—	9.1	21.8 ± 0.3
Carex kobomugi [*5]	116 ±22	—	54.2	25.1 ± 1.4
Calystegia soldanella [*5]	118 ± 85	—	23.8	20.5 ± 2.2

Abbreviation: ribulose-1,6-bisphosphate (RuBP).[*1] present study- values averaged over both experimental days. [*2] Pammenter and Smith (unpublished), naturally growing plants, measurements made at high light intensity, values are from one typical plant with the exception of the stomatal limitation value which is the average of 6 replicates. [*3] De Jong (1978), North American beach plants grown in growth chambers and measurements made at temperature optima. [*4] Robichaux and Pearcy (1984), greenhouse grown material that originated from different habitats, *Scaevola coriacea* from coastal strand, *Scaevola gaudichaudii* from dry scrub and *Scaevola mollis* from wet forest habits; measurements made at 27°C, and 1500 – 1800 μmol m^{-2} s^{-1} PPFD. [*5] Ishikawa *et al.* (1991) growing plants transferred to a 10mM NaCl + nutrient hydroponic solution; measurements made at 500-600 μmol m^{-2} s^{-1} PPFD, 40-50% relative humidity and ambient temperatures of 25°C after two weeks.

Stomatal control

Differences in the ranges of leaf conductances measured for the three investigated species at ambient conditions were considerable (Figure 7.7) with *A. populifolia* having large leaf conductances in comparison to *S. plumieri* and *I. pes-caprae* (Figure 7.5). This may have in part been due to the high transpiration rates measured for *A. populifolia* resulting from the evaporation of dew from the leaf hair layer. The evaporation of dew from *A. populifolia* leaves did not appear to limit CO_2 entry into the leaves as was evident from the diurnal Ci/Ca responses (Figure 7.4). The Ci/Ca values were highest in morning and evening, periods that coincide with dew deposition. The capture of dew and its subsequent evaporation may represent considerable water saving for *A. populifolia*.

Leaf conductances recorded during the construction of CO_2 response curves when ambient CO_2 concentrations were 360 µmol mol^{-1} and leaves were not wet with dew were significantly different among species ($p<0.0001$, N=10). Average leaf conductances of 0.85 ± 0.29, 0.08 ± 0.04,and 0.22 ± 0.09 mol m^{-2} s^{-1} were recorded for *A. populifolia*, *I. pes-caprae* and *S. plumieri* respectively. Leaf conductances for *A. populifolia* are much higher than the values measured for a range of fynbos species where the highest leaf conductances of 0.176 mol m^{-2} s^{-1} were measured for *Diosma oppositifolia* (von Willert *et al*., 1989). The majority of the fynbos species measured by these researchers had leaf conductances that were comparable to the values measured for *I. pes-caprae*. *A. populifolia* leaf conductances also exceeded values (0.21 – 0.50 mol m^{-2} s^{-1}) measured for a range of North American coastal foredune species (De Jong, 1979). This range is more like the leaf conductances measured for *S. plumieri*. The relatively low leaf conductances measured for *I. pes-caprae* suggest that stomata limit CO_2 assimilation and transpiration. This was confirmed by the assessment of stomatal limitations from CO_2 response curves (Figure 7.4). *I. pes-caprae*, *S. plumieri* and *A. populifolia* had average stomatal limitations of 48.8 ± 6.9, 29.6 ± 8.3 and 23.4 ± 15.7 % respectively.

The degree to which stomata of *I. pes-caprae* control water loss and CO_2 gain was evident when responses of CO_2 assimilation, transpiration and Ci/Ca to leaf conductance were considered (Figure 7.7). The responses of both assimilation and transpiration for *I. pes-caprae* were linearly related to G_{Leaf} and their slopes were such that A/E increased only slightly over the range of leaf conductances measured. Similar linear responses of assimilation and transpiration to leaf conductance have been measured for the fynbos species *Rafnia capensis, Leucadendron pubescence* and *Paranomus bracteolaris* (von Willert *et al*., 1989). This was not the case for *A. populifolia* and *S. plumieri* where the response of CO_2 assimilation to G_{Leaf} was curvilinear (Figure 7.7) and did not result in a constant A/E ratio for the range of leaf conductances measured.

Reasons for the differences in the degree to which stomata control gas exchange were not apparent but it is possible that *I. pes-caprae* has lower hydraulic conductance and therefore may be more susceptible to declining plant water status at comparable transpiration rates. This suggestion is indirectly supported by the finding that on several occasions *I. pes-caprae* showed midday reductions in leaf conductance (Figure 7.5), a phenomenon related to water saving.

The other two species may not be as sensitive to declining water status and have developed similar (*A. populifolia*) or lower (*S. plumieri*) midday Ψ_{leaf} than *I. pes-caprae* (Figure 5.4) yet have maintained higher transpiration rates (Figure 5.1). It is possible that both *A. populifolia* and *S. plumieri* have higher hydraulic conductance and can hence recover plant water status without the need for limiting transpiration losses. With such adaptations it is not surprising that the major stomatal responses of *A. populifolia* and *S. plumieri* are to light and the supply of CO_2 and not to the maintenance of plant water status. Further research is required to investigate the plant hydraulic conductances in these species.

The degree to which these three species control transpiration rates may also offer an explanation for the differences in the responses of E to VPD. This is best described by saturated exponential equations. However the values of the fitted parameters for these equations differed considerably among species (Figure 5.2). Little stomatal control of water loss or positive stomatal responses to increasing VPD would result in a predictable response of E to VPD as was noted for *A. populifolia* and *S. plumieri*. Stomatal control particularly to changing plant water status would result in deviation from the predicted response of E to VPD a phenomenon that was noted for *I. pes-caprae* (Figure 5.2). Hence the leaf water potentials of *I. pes-caprae* that varied to no greater extent, nor reached more negative values than those of *A. populifolia* and *S. plumieri* (Figure 5.4), are likely to have been the result of stomatal regulation.

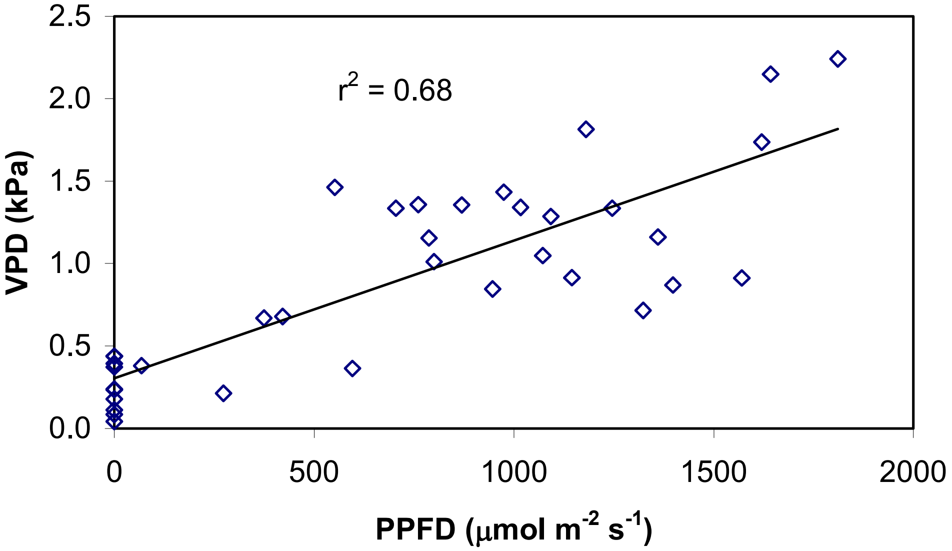


Figure 7.23: The relationship between atmospheric vapour pressure deficit (VPD) and photosynthetic photon flux density (PPFD). VPD was calculated from ambient temperatures and relative humidity measured on the five experimental days. Incident PPFD data is from the SAWB, Port Elizabeth.

During the initial linear increase in E with increasing VPD, transpiration was not limited by leaf conductance (G_{Leaf}) nor did G_{Leaf} increase in a linear fashion in response to increasing VPD. Alternatively G_{Leaf} may have increased linearly in response to one of the variants of VPD (e.g. PPFD). VPD was linearly related to incident PPFD (Figure 7.23) and as PPFD increased so did photosynthesis (Figure 4.2) and the demand for Ci. The ratio Ci/Ca remained relatively constant over all G_{Leaf} values measured (Figure 7.7) with the exception of ratios for *I. pes-caprae* which declined in a linear fashion. In response to increasing PPFD stomata may be opening and at the same time VPD may also be increasing as air temperature increases. As a result of both of these changes E may respond linearly to changes in VPD.

The response of E to VPD saturates when maximum leaf conductance is attained. This limits both the entry of CO_2 into the leaf (and hence Ci) and the exit of water vapour (transpiration) (Figure 5.2). Maximum G_{Leaf} values for the three species were considerably different (Figure 7.7).

Leaf age

The effects of leaf age complicate the scaling of leaf gas exchange measurements to the canopy level (Norman, 1993). One of the simplest solutions to this problem, as shown by Peter (2000) and Peter and Ripley (2001), is to include measurements from leaves of all ages when attempting such an exercise. Corrections made using data obtained from leaves of different age maintained under identical conditions may not be appropriate because of the importance of microclimatic and shading conditions that occur under natural conditions. Further investigation is required to resolve these issues.

S. plumieri whole shoot response to light

Considerable evidence from crops with high LAI shows that the CO_2 assimilation of the canopies due to mutual shading is determined more by the quantum efficiency and intercepted PPFD (Hesketh, 1980; Jarvis and Leverenz, 1983), than by the light saturated assimilation rates. Similarly the data for *S. plumieri* showed that leaves were not light saturated at midday PPFD values (Figure 7.3) and because of mutual shading many leaves on the stems are subject to low PPFD (Figure 7.10). Leaves occupying different positions on the stems relative to the sun's passage across the sky receive different amounts of light at different times of the day (Figure 7.12). Therefore under field conditions CO_2 assimilation is limited more by incident PPFD than any other factor so that on a daily basis the carbon gain by individual leaves was closely correlated with total daily intercepted PPFD (Figure 7.12 & 7.13). Even though *S. plumieri* vegetated dunes have low LAIs (Table 3.6) they are apparently light limited. However geographic dune locations that potentially receive higher solar radiation did not have higher leaf production rates (Table 7.4). These comparisons did not take into account dune orientation and whether solar radiation measured at standard conditions was representative of the amounts of light incident on the plants growing at the different locations.

Light limitations would be increased at higher LAI and it is possible that low LAIs are the result of maintaining resource availability including light. Barbour *et al.* (1985) considers the dense vertically orientated swards of *Ammophila arenaria* with high LAI to be an advantage increasing the efficiency of utilisation of direct and diffuse radiation. However when reduction in PPFD with decreasing height from the top of the canopy (Pavlik, 1982) is considered in conjunction with the *ca.* 1800 µmol $^{-2}$ s^{-1} PPFD required to saturate CO_2 assimilation (Pavlik. 1983) it is apparent that *Ammophila arenaria* photosynthetic rate is dramatically reduced (by 30%) 50cm below the upper canopy surface. Presumably some optimum LAI and canopy architecture exists that maximises light interception for the amount of carbon invested. Hesp (1991) attributes the changes in the packing density of shoots of *Spinifex sericeus* occupying an incipient foredune in N.S.W. Australia, to variations in seasonal temperature. Seasonal temperature and incident PPFD are co-variants and it is possible that the observed result is in response to changes in PPFD.

Comparable data for canopy light responses of *A. populifolia* and *I. pes-caprae* are unavailable. The arrangement of *A. populifolia* leaves is not unlike that of *S. plumieri* and similar patterns of mutual shading may exist. *I. pes-caprae* leaves are frequently v-shaped in cross section when cut transversely through the mid-rib so that at certain inclinations the one side of the leaf shades the opposite side (*pers. obs.*). Adams *et al*. (1988) measured similar light responses for both adaxial and abaxial *I. pes-caprae* leaf surfaces. They attributed this result to the fact that both surfaces received similar direct illumination due to their folded nature. Further investigation of *A. populifolia* and *I. pes-caprae* whole shoot and canopy light interactions is required.

A. populifolia **response to light and the role of the leaf hair layer**

Minimum leaf resistances measured in the stirred chamber used for gas exchange measurements were 2.7 and 2.5 s m^2 mol^{-1} for leaves with and without hairs respectively (Figure 7.16). These values for *A. populifolia* are comparable to values measured for *Olea europaea* (Grammatikopoulos *et al.*, 1994) and *Encelia farinosa* (Ehleringer and Mooney, 1978) where minimum resistances of 3.58 and 1.12 s m^2 mol^{-1} were measured respectively. For *A. populifolia* the difference between leaves with and without hairs suggests a diffusion resistance of the hair layer of 0.2 s m^2 mol^{-1}. This compares well with values ranging from 0.3 to 0.6 s m^{-2} mol^{-1} measured directly on isolated hair layers.

Although these values are lower than the minimum leaf resistances measured in well stirred chambers they are adequate to account for the increase in transpiration by leaves without hairs observed in the field under high light conditions (Figure 7.21). The increased transpiration observed in leaves with the hairs removed is not likely to be the result of injury to the epidermal cells as the basal cells of the hairs were not damaged on hair removal.

Reduced water loss from leaves with hairs despite, their having higher albedo in comparison with leaves without hairs, resulted in higher leaf temperatures both in the laboratory and in the field (Figure 7.22). This finding is contrary to that of Ehleringer *et al.* (1976) who showed that leaf hairs of *Encelia farinosa* reduced leaf temperatures to temperatures optimal for photosynthesis. The effect on water loss from *A. populifolia* leaves with hairs was greater than the effect on photosynthesis and resulted in increased WUE.

Up to an incident PPFD of 1000 μmol m^{-2} s^{-1}, the reductions in *A. populifolia* photosynthetic rates of leaves with hairs could be accounted for by the 0.17 reduction in incident light transmitted through the hair layer (Figure 7.15). Above this intensity photosynthetic rates of leaves without hairs were lower than those of leaves with hairs. This was so even when differences in transmitted light were accounted for. Unlike in the work of Ehleringer *et al.* (1976) reduction in photosynthetic rates were not found to be the result of leaf temperature exceeding the optimum for photosynthesis as the temperature in the laboratory study was maintained at 25 ± 0.3 ^{o}C. Nor were they the consequence of stomatal limitations. When compared to leaves with hairs leaves without hairs maintained higher leaf conductances and consequently higher Ci at all light intensities measured (Figure 7.16).

Reductions of photosynthetic rate in leaves without hairs were accompanied by greater dissipation of energy non-photochemically (Figure 7.19). A phenomenon that has been documented for *Senecio medley*. In this plant the non-photochemical heat was accompanied by a greater production of zeaxanthin in leaves without hairs following a 30 minute high light intensity treatment (Lang and Schindler, 1994). In *A. populifolia* leaves despite correction of PPFD values transmitted through the hair layer q_{NP} values of leaves without hairs remained higher than those with an intact hair layer showing that these differences are not merely due to the effect of the differing light intensities incident on the leaf surfaces. This effect was also noted for differences in Fv'/Fm' values for leaves with hairs illuminated at 2000 μmol m^{-2} s^{-1} and for leaves without hairs illuminated at 1660 μmol m^{-2} s^{-1} (a light intensity comparable to that which would be transmitted through the hair layer) (Figure 7.20).

Karabourniotis *et al.* (1993), Lang and Schindler (1994) and Skaltas *et al.* (1994) showed that leaf hairs are an effective barrier against UV radiation and the absorption spectrum of the hair layer measured for *A. populifolia* (Figure 7.14) suggests it serves the same function. Selectively removing the UV radiation from the transmitted light would account for the observed differences in photosynthetic rate, q_{NP}, q_P and Fv'/Fm' at high light intensities. Unlike the response of *Olea europaea* (Grammatikopoulos *et al.*, 1994) *A. populifolia* did not exhibit stomatal closure in response to the higher incident UV radiation in leaves without hairs. Removal of the hair layer subjected leaves to higher UV intensities increasing the q_{NP} values and decreasing both q_P and rate of photosynthesis.

The hair layer on the leaves of *A. populifolia* a coastal dune pioneer species effectively reduces water loss with minimal reduction in photosynthetic rate resulting in higher WUE but paradoxically higher leaf temperature. The hair layer also decreases photoinhibitory reduction in photosynthetic carbon gain at high light intensities by selectively removing UV radiation transmitted to the photosynthetic apparatus.

Summary of inter-species comparison

Table 7.7 gives a summary of the data presented in Chapter 7 that can be used in comparing the three species *A. populifolia*, *I. pes-caprae* and *S. plumieri*. As discussed above there are differences in the photosynthetic, respiratory and stomatal physiology which account for some of the differences in gas exchange and production. Furthermore certain species have unique adaptations, including mutual leaf shading and the presence of leaf hairs.

Table 7.7: Comparison of data presented for *A. populifolia*, *I. pes-caprae* and *S. plumieri* in Chapter 7. The number of symbols indicates the magnitude of the indicated parameter relative to the smallest value measured for any one of the three species.

Measured parameter:	***A. populifolia***	***I. pes-caprae***	***S. plumieri***
Significant points from light and CO_2 response curves			
Efficiency of utilisation of incident PPFD	♦½	♦	♦
Light compensation point	♦	♦¼	♦½
A_{max}	♦♦	♦	♦
Carboxylation efficiency	♦½	♦	♦¼
CO_2 compensation point	♦	♦¼	♦¼
RuBP regeneration rate	♦½	♦	♦
Stomatal measurements or descriptions			
Stomatal limitation	♦	♦♦	♦¼
Average peak stomatal conductance*[1]	♦♦♦♦♦♦♦♦♦♦½	♦	♦♦¾
One or two-peaked diurnal stomatal responses*[2]	♦	♦♦	♦
A response to G_{Leaf}	Linear or curvilinear $r^2 = 0.36$	Linear $r^2 = 0.89$	Linear or curvilinear $r^2 = 0.51$
Ci/Ca response to G_{Leaf}	Linear $r^2 = 0.07$	Linear $r^2 = 0.32$	Linear $r^2 = 0.005$
Plant features			
Leaf age effect *[3]	♦	♦¼	♦½
Whole shoot light response	—	—	Light limited
Leaf hairs	Reduce photoinhibition and transpiration	—	—

Abbreviations: photosynthetic photon flux density (PPFD), photosynthetic rate at light saturation (A_{max}), ribulose-1,6-bisphosphate (RuBP), leaf conductance (G_{Leaf}), intercellular to ambient CO_2 ratio (Ci/Ca).*[1] Calculated from average leaf conductances recorded during the measurement of CO_2 responses when ambient CO_2 concentration was 360 μmol mol^{-1} and leaves were not wet with dew. *[2] calculated from the maximum number of peaks recorded from diurnal variations in G_{Leaf}. *[3] Calculated from the differences in minimum and maximum transpiration rates from plots of transpiration rate to increasing assigned leaf number.

CONCLUSIONS

Examination of the measured physiological characteristics allowed certain explanations to be presented concerning the productivity, water and nutrient relations of the three species investigated.

- Differences in photosynthetic physiology including efficiency of utilisation of incident PPFD, carboxylation efficiencies, rates of RuBP regeneration, and light and CO_2 compensation points offer explanations for the differences in measured photosynthetic and respiratory productivity.

- Diurnal stomatal behaviour, stomatal limitations and integrated responses offer additional explanations for observed differences in CO_2 exchange and offer explanations for the observed differences in transpiration rates and plant water relations.

- The measured differences in photosynthetic and respiratory rates correlates with the differences measured in both shoot production and above-ground primary production.

- Whole shoot photosynthetic responses of *S. plumieri* because of leaf dispositions and mutual shading are closely correlated with the total amount of light incident on the shoots and this suggests that whole shoot productivity is light limited.

- *A. populifolia* leaf hair layer reduced photoinhibition by reducing the amount of UV transmitted through to the leaf surface and increased WUE by decreasing transpiration relative to photosynthesis.

CHAPTER 8
HABITAT LIMITATIONS AND PLANT PERFORMANCE

INTRODUCTION

Dune pioneer plant species may be adapted to avoid light, water and nutrient stress (resource stresses). The possession of such adaptations for acquiring and utilising resources that are either limiting or are in excess may determine which species can inhabit the foredunes. Species without such adaptations may be limited by resource stresses and consequently be excluded from the foredunes. Furthermore success in the foredunes may not simply be the result of possessing particular adaptations but may include the ability of a species, because of its adaptations, to compete with others for resources.

Alternatively it may be the adaptation to disturbance that determines which species can inhabit the foredunes. Disturbance within the context of coastal foredunes includes environmental factors such as wind, salt-spray, soil-salinity, sand-burial, sand-blasting and the occasional inundation of the plants with seawater. For a review of such factors and adaptations see Chapter 1.

Further complication arises because adaptations may be in response to a combination of both resource stress and disturbance. Different species may possess different adaptations and thus be limited by different resources or disturbances. Limitations may affect seedlings and establishing plants rather than established adult plants. Limitations at any stage in a species' life cycle may be effective in excluding that species from the foredunes. As was explained in Chapter 1 gradients in both resource availability and the magnitude of disturbances exists within the foredunes (Brown and McLachlan, 1990) creating various microhabitats, to which different species become adapted.

The principal question posed by this thesis (*Why and what allows the foredunes to be inhabited by only specific plant species and be dominated by even fewer?*) is unlikely to have a simple answer. This study investigated the resource requirements of various species, the acquisition and use of these resources by established plants and the response of these plants to natural environmental stresses. Implicit in this direction of investigation is that the principal question posed could be answered only if the resources investigated proved to be limiting to adult plants and if adaptations to these limitations, both physiological and other, were evident. Nevertheless even if the resource stresses were shown not to be limiting, the elimination of these as potential limitations would certainly forward our understanding of the functioning of coastal dune systems. In addition this line of investigation would go a long way in explaining how the selected species survive and thrive on the foredunes.

This investigation did not attempt to quantify either the disturbances characteristic of the foredune environment nor the response of the plants to these disturbances. However the plant performances and growth characteristics that were measured allowed for certain explanations to be offered as to how the investigated foredune species may be able to withstand disturbances such as sand burial and blasting, mechanical damage and salt spray.

The research direction chosen appears justified by the findings of and discussion raised by researchers of both dune and other ecosystems. Research has shown that coastal dunes are poor in nutrients (Willis, 1965; Salisbury, 1952; Harte and Pammenter, 1983; Barbour *et al*., 1985; Ehrenfeld, 1990; Cain *et al*., 1999) and that dune species respond positively to nutrient addition (Willis, 1965; Pavlik, 1983b; Dougherty *et al*., 1990; Zhang, 1996). Water availability has also been considered limiting (Rozema *et al*., 1985; Barbour *et al*., 1985; Barbour, 1990; Hesp, 1991; Davy and Figueroa, 1993) due to soil characteristics, ambient temperatures and the osmotic effects of accumulating salts (Barbour *et al*., 1985). Beach plants are potentially susceptible to light stress. This beach environment has both high incident (Barbour *et al.*, 1985) and high reflected light intensities (Chapman, 1976). Susceptibility may be exacerbated by potential water stress (Hesp, 1991) and low winter temperatures. A more complete discussion of the research findings and current understanding of coastal dune-plant water relations, nutrient relations, light stress and the potential effects of these on productivity are given in Chapters 4, 5 and 6. In light of these findings it is not unreasonable to hypothesise that water-, nutrient- and light-stress may limit the particular species that can inhabit the foredunes and that these limited species have adaptations to these stresses.

Further justification for the chosen direction of the research arises from research on other ecosystems which has shown that resource acquisition and use, resulting from particular adaptations, determines whether a particular species can survive in a particular environment. Examples include desert species that are adapted for the acquisition and conservation of water (Ehleringer, 1983) and legumes which have evolved symbiotic relationships for acquiring nitrogen in nutrient deficient environments (Marschner, 1995). Other researchers have shown that adaptations to disturbances allow particular species to inhabit some environments. Examples include savanna species that can withstand defoliation by herbivory (Scholes and Walker, 1993), arctic species that avoid freezing (Burke and Stushoff, 1979) and species that can survive fire by either resprouting or by re-establishing from seed (Bowen and Pate, 1991).

To identify conclusively the unique environmental factor(s) to which only particular species have adapted would require the combined assessment of plant response and adaptation to both the resource stresses and disturbances. Thus in the context of this thesis an attempt to answer the principal question (*Why and what allows the foredunes to be inhabited by only specific plant species and be dominated by even fewer?*) required that several other questions needed to be answered. Included are: Is the foredune environment limited by resource availability or are some resources present in excess? How does resource availability affect plant function and do the species studied respond in the same ways? Is adaptation to acquiring limited or coping with excess resources, the key to understanding which plant species can thrive in the foredune environment or does the key lie with plant adaptations to disturbance? It is these questions that this chapter addresses and in doing so integrates the findings of the entire study. Answering these questions requires a synthesis of the answers to the specific questions pertaining to plant productivity, water and nutrient relations and associated physiology which were raised in

Chapter 1 and were addressed in Chapters 4 to 7. The integration of such findings was conducted by the construction of a conceptual model.

A CONCEPTUAL MODEL OF RESOURCE LIMITATIONS AND PLANT PERFORMANCE

The model presented here is based on a model developed for desert species (Ehleringer, 1993) in which interspecific differences in plant productivity and growth are explained in terms of differences in soil-water availability among species growing in different microhabitats. Ehleringer's (1993) model accounts for the effects of soil-water status and water stress on stomatal conductance which influences the Ci/Ca ratio and hence photosynthesis and productivity. The latter determining growth and reproductive output (Figure 8.1). The effect of water stress on photosynthesis as affected by stomatal conductance was assessed by measuring carbon isotope discrimination values. The model showed that the carbon isotope discrimination values could be used to distinguish both different life-forms and the micro-habitat preferences among plant species from the Sonoran Desert.

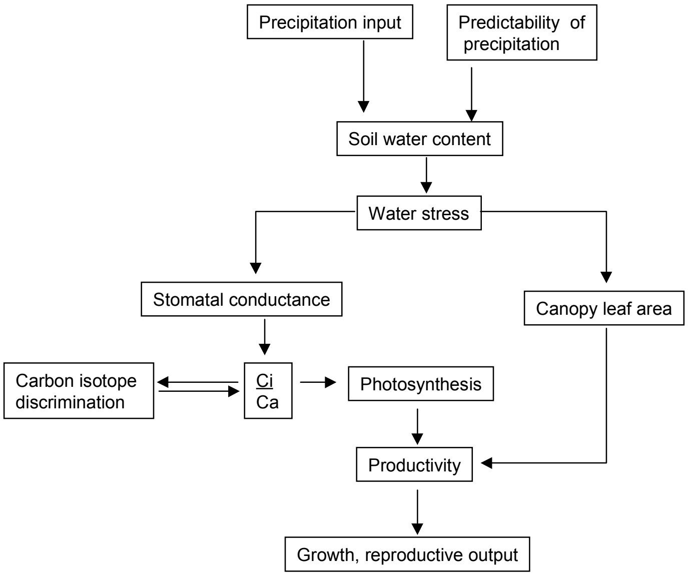


Figure 8.1: A conceptual model of how plant productivity and ultimately growth, reproductive output and plant fitness are affected by water stress and several of the gas exchange characteristics that influence photosynthetic rate (After Ehleringer, 1993).

Carbon isotope discrimination was positively correlated with expected soil-water availability suggesting that species in wetter habitats had higher Ci values. Discrimination values were also inversely related to the plant life expectancy. Long-lived species had lower Ci values than did shorter-lived species indicating more conservative water use efficiency than the shorter-lived species. Hence the model correlates the acquisition and subsequent use of water with the species' microhabitats. When the resource water is limiting then species with particular adaptations, in this case conservative water use, are predominant. Water availability in this ecosystem was the controlling factor.

Using a similar rationale a model was developed that takes into account the relationship between the supply of resources from the environment, plant function in relation to the acquisition and use of these resources and the effect this has on the resultant productivity (Figure 8.2). This gives insight into the potential limitations created by the particular resources. The resultant description of plant function emphasises the adaptations that plants develop in response to resource stress and/or disturbance. Furthermore plant function and productivity and the differences in these among species were used to explain why these species survive on the foredunes and why particular species are more abundant within the particular microhabitats of the foredunes.

The assessment of resource limitations included the calculation of simple water and nutrient budgets and the assessment of plant function in response to natural conditions. The former is self explanatory and the latter is clarified by a simple example: if plants maintain relatively high transpiration rates yet do not develop very negative leaf water potentials then it is reasonable to conclude that water is not limiting. The confidence in such conclusions is increased if similar results are obtained on repeating this assessment in different climatic seasons.

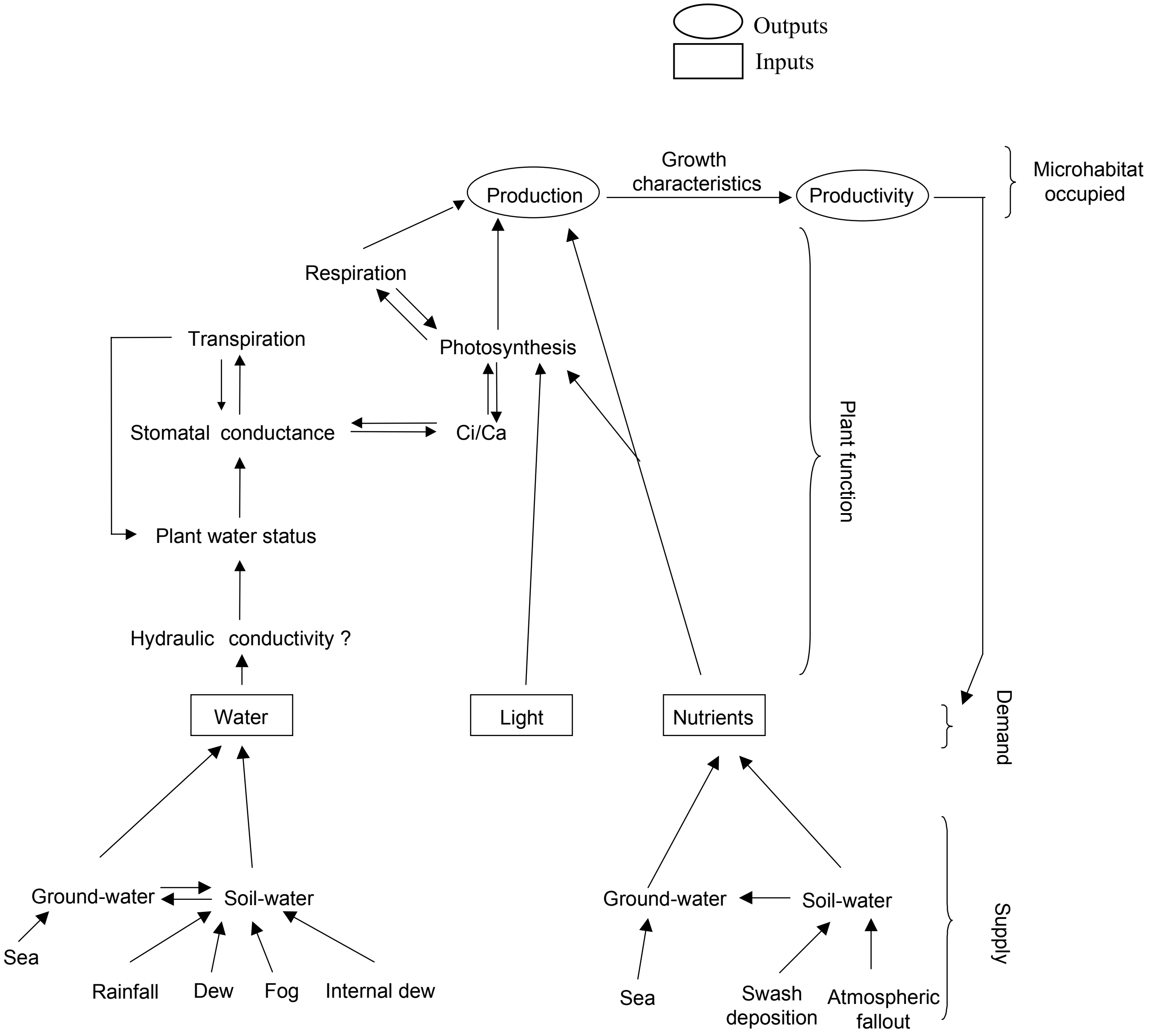


Figure 8.2: A conceptual model of how plant production and ultimately productivity (the model's outputs) are related to the acquisition and use of water, nutrients and light (the model's inputs) and how in turn productivity determines the demand for resources. The environment supplies resources and the possible origins of these resources are indicated (Refer to Chapters 4, 5, & 6 for the details of these sources). Production, productivity and growth characteristics offer some explanation of why certain species are more abundant in certain microhabitats found within the pioneer zone of the foredunes.

The model considers the effects of water, nutrients and light availability and use, on the photosynthetic performance and productivity of the three species investigated (Figure 8.2). Water availability and transpiration rate in conjunction with plant hydraulic conductance determines the leaf water status (Passioura, 1982) which through well-documented mechanisms effects stomatal conductance (Mansfield and Davies, 1981). Stomatal conductance effects the diffusion of CO_2 into the leaf and influences Ci/Ca ratio (Farquhar and Sharkey, 1982). Ci in conjunction with light absorption and the intrinsic capabilities of the photosynthetic apparatus determine the photosynthetic rate. Photosynthetic production and respiration determine net carbon gain. Carbon gain, carbon allocation patterns and the acquisition of mineral nutrients determine production. Production per unit land surface area as determined by the species growth characteristics determines productivity. Important components of growth and productivity are carbon-sink strengths and resultant carbon allocation. These are likely to differ among species and are also modified by the prevailing stress and disturbance conditions. Production and productivity may be considered the outputs of the conceptual model.

Plant growth determines the demand for resources and thus may be limited by the supply of such resources. If resources are available in excess then other growth limitations are implicated. The environment determines the supply of resources and the various sources are indicated in Figure 8.2. Plants have no control over the supply of resources. Adaptations that enable more efficient resource acquisition or reduce demand may be evident and are good indicators of whether resources are excessive or limiting. A few examples of such adaptations include the reallocation of nutrients from old to young leaves which reduces overall nutrient demands; osmotic adjustment that allows water to be acquired under conditions of reduced water availability; stomatal behaviour that reduces water loss and leaves with high concentrations of xanthophylls that may be de-epoxidated to reduce photoinhibition. Growth characteristics may also represent adaptations to limited resources and it is possible that low biomass and growth may be associated with ensuring resource availability by reducing demand. Such adaptations may also be in response to disturbance for example such adaptation may reduce potential sand-trapping and hence sand burial.

Measurement of model inputs and outputs

Quantitative or relative measurements of many of the components of the model were made in this investigation and included the following:

Plant water status was assessed directly and diurnal plant water potential and its components were measured (Chapter 5). Transpiration rates in relation to environmental factors were determined and this allowed volumes of water transpired over periods of changing environmental conditions to be determined. These were compared to measured rainfall inputs accounting for beach-sand water holding capacities (Chapter 5). Stomatal conductances were measured under prevailing ambient, under controlled conditions and as stomatal limitations (Chapter 7). Plant hydraulic conductivity has not been quantified and remains an important question to be answered in the future.

Intercellular CO_2 concentrations and photosynthetic rates were measured both under natural and controlled conditions (Chapters 4 & 7). Intrinsic photosynthetic capabilities were assessed and carboxylation efficiencies and rates of RuBP regeneration determined (Chapter 7). Leaf and stem storage of photosynthate, either as soluble sugars or as starch were assessed (Chapter 4).

Light incident on leaves and the gas exchange response to this light was measured (Chapters 4 & 7). Utilisation efficiencies of incident PPFD and light saturated photosynthetic rates were measured (Chapter 7). Correlated leaf properties such as the sensitivity to light stress and pigment concentrations were measured (Chapter 4). Canopy architecture and its effects on light interception and photosynthesis were investigated in *S. plumieri*. Comparable data for the other two species are unfortunately lacking.

Respiration was measured both directly (Chapter 4) for whole plants or shoots and relative measurements of dark and photorespiration were determined for fully expanded mature leaves (Chapter 7).

Above-ground plant nutrient content was measured and in conjunction with above-ground growth (Chapter 4) was used to define above ground nutrient demand (Chapter 6). Nutrient supply was estimated using predicted volumes of water transpired and water nutrient content (Chapter 6). The reallocation of nutrients among leaves was measured. Above-ground production, productivity and growth characteristics were determined (Chapter 3 & 4) and growth preferences were determined both by mapping (Chapter 3) and from the documentation of other researchers (Avis, 1992; Lubke, 1983).

Absolute and relative interspecific differences in model components

As the empirical relationships between the model inputs and outputs have not been determined it is not possible to input empirical data and to calculate a final answer for each species. However it is possible to compare the relative magnitudes of the inputs in different species and to make comparisons with the measured outputs. This allows conclusions to be drawn about potential limitations of plant performance. For example *A. populifolia* has high transpiration rate, high leaf-water potential and an apparently unlimited supply of water. This allows for the maintenance of high stomatal conductance and Ci values and results in high photosynthetic and respiratory rates which translate into high production rates. These may allow this species to grow closest to the high tide line where sand burial is probable. However these conclusions are only obvious when these rates are compared to the relatively lower rates and lower growth that was measured for *I. pes-caprae* and the rates for species from other ecosystems. The example illustrates how this model allows consideration of resource limitations, plant adaptations as related to potential limitations and offers explanations for how these plants may cope with disturbance. This type of assessment was conducted for each individual species and is presented below.

A. populifolia

Of the three species studied *A. populifolia* had the highest leaf conductances (Figure 8.5) and hence had the highest average peak transpiration rate (Table 5.1) and daily amounts of water transpired (Table 5.3). The possession of a leaf hair layer reduced transpiration rates (Figure 7.18) more than CO_2 assimilation rate (Figure 7.15) and resulted in increased WUE (Table 5.5; and see: *A. populifolia* response to light and the role of the leaf hair layer, Results, Chapter 7). Furthermore the hair layer appears to trap dew which is subsequently evaporated without reducing CO_2 assimilation, constituting a potential water saving. The role of the hair layer requires further research.

Despite moderate to high transpiration rates when compared with other plant species (Table 6.9), *A. populifolia* did not develop very negative midday leaf water potentials (Figure 6.4) suggesting efficient water conduction. This species showed no evidence of osmotic adjustment (Table 7.7). *A. populifolia* appears unlimited by the supply of water.

The calculated water budgets support this suggestion (Table 5.8, Figures 5.9 & 5.10). The capacitance budget accounted for the volume of sand potentially exploited by the species. Due to shallow rooting *A. populifolia* was assumed to utilise the lowest volume of sand. Despite this, and due to the low LAI measured for *A. populifolia*, water supply exceeded water loss at field capacities of 4% and estimated water supply was greater than that for the other species investigated (Figure 5.10). Modelled sand-water contents were reduced to the minimum (0.5%) on only a single occasion in 1994. This was achieved by either reducing the field capacity to 1.5 % (Figure 5.10) or by increasing the LAI from 0.23 to 0.42. This demonstrates the dramatic effect that increasing the LAI has on resource requirements and that low biomass is an effective means of ensuring resource availability. Alternatively, this could be viewed as low resource availability limiting biomass production.

In addition to possessing the highest leaf conductances *A. populifolia* had the highest efficiencies of utilisation of incident PPFD (Table 7.1), highest carboxylation efficiencies and lowest stomatal limitations (Table 7.2). As a result of this the light saturated (Figure 7.3), CO_2 saturated (Figure 7.4), ambient photosynthetic rates (Figure 4.1 & Table 4.2) and daily amounts of CO_2 assimilated (Table 4.4) were higher than those of the other species investigated. Photosynthetic rates were also high in comparison with other coastal and non-coastal species (See: Photosynthetic rates, Discussion, Chapter 4). The high light and CO_2 saturated photosynthetic rates indicate a high capacity for the regeneration of

RuBP and electron transport and hence high rates of ATP and NADPH production (Farquhar and Sharkey, 1982). The high efficiency of utilisation of incident PPFD may have been due to higher chlorophyll content per unit leaf surface area than that measured for the other species (See: Chlorophyll concentration, Results, Chapter 4). High light utilisation efficiencies were unlikely to have resulted from higher leaf nitrogen contents as leaves from *A. populifolia* contain less nitrogen per unit dry mass than do leaves from *I. pes-caprae* (Figure 6.3). If nitrogen content per unit dry mass (Figure 6.3) were converted to content per unit leaf area (using the conversions in Table 3.4) the leaf nitrogen contents for *A. populifolia* were not the highest of the three investigated species.

The apparent insensitivity of *A. populifolia* to photoinhibition may contribute to the high photosynthetic production. This species showed the smallest diurnal reductions in photochemical efficiency (φ_{P0}, Figure 4.6) and the most rapid recovery of φ_{P0} following light treatment (Figure 4.8). The hair layer on *A. populifolia* leaves contributes to the lack of sensitivity to light stress by removing a larger proportion of the UV wavelengths than the photosynthetically active wavelengths (Figure 7.14) thus reducing photoinhibition. This decreases the amount by which CO_2 assimilation (Figure 7.15) and q_P value (Figure 7.19) are reduced in comparison to the reductions that would have resulted if the hair layer had decreased light intensity of all wavelengths equally (See: *A. populifolia* response to light and the role of the leaf hair layer, Discussion, Chapter 7). High light intensities certainly appear not to limit the photosynthetic productivity of this species and the adaptations associated with high light intensity may well contribute to the success of *A. populifolia* on the foredunes.

A. populifolia had both the lowest measured light (Table 7.1) and CO_2 compensation points (Table 7.2) indicating the lowest rates of dark respiration (Bjorkman, 1981) and photorespiration (Farquhar and von Caemmerer, 1982) for fully expanded mature leaves. However whole shoot dark respiration rates were the highest of the three species investigated (Table 4.3). This suggests that although little energy is invested in the maintenance of mature leaves greater amounts are expended on growth.

Photosynthetic rates of *A. populifolia* like *S. plumieri*, showed a marked dependence on incident PPFD (Figure 4.2), with 80% of the variability in assimilation rates being accounted for by PPFD (Table 4.2). The rate did not saturate even at light intensities measured at midday (Figure 7.3). Transpiration rate was most dependent on the ambient relative humidity (Table 5.2) with variations in this parameter accounting for 66% of the variability in transpiration rates. However variation in the abiotic parameters relative humidity, temperature, PPFD and VPD were not independent (Figure 8.23) and the relationship of E to VPD (Figure 5.2) was predictive ($r^2 = 0.51$). The WUE measured for *A. populifolia* was more dependent on incident PPFD than on the other abiotic factors measured (adjusted $r^2 = 0.62$; Table 5.4). This was a result of the greater variation in photosynthetic rate and its dependence on PPFD rather than of the variation in transpiration rates. The apparent dependence of unsaturated photosynthesis and transpiration on PPFD suggests that light may in fact limit the productivity of this species particularly if mutual shading of leaves reduces incident light intensity. Further research is required to determine these effects.

High photosynthetic and respiratory rates may account for the high growth rates of *A. populifolia.* It produces the greatest number of leaves (Table 4.5), the greatest weight of above-ground stems and leaves per shoot (Table 4.5) and has the highest above-ground shoot primary production (Table 4.6). The high growth rate correlated well with the high respiratory rate measured (Table 4.3). Photosynthetic and respiratory rate expressed per unit leaf area have been shown to be related to relative growth rate (Pereira, 1995) although the correlation between the two is rather poor. However the correlation improves if rates are expressed per unit leaf dry mass (Poorter, 1989).

The growth of *A. populifolia*, unlike that of *I. pes-caprae* and *S. plumieri*, was relatively uniform throughout the year (Figure 4.5). *A. populifolia* accumulated less leaf and stem sugar than *S. plumieri,* less leaf starch than *I. pes-caprae* and like *I. pes-caprae* had lower total non-structural carbohydrates than *S. plumieri* (Figures 4.3, 4.4 & Table 4.8) suggesting that the acquired photosynthate is rapidly invested in growth rather than being stored. *A. populifolia*, unlike the linear growth forms of *I. pes-caprae* and *S. plumieri*, branched extensively in response to burial.

A. populifolia had the shortest leaf life-span and the highest biomass turnover (See: Leaf production and dry weight accumulation, Results, Chapter 4) possibly as a result of the low respiratory expenditure in mature leaves (Figure 7.4 and Table 7.2). Furthermore *A. populifolia* plants appear to be short-lived (c*a.* 2 years, Tinely, 1985; *pers. obs.*). Leaf longevity does not appear to be related to foliar sodium or chloride accumulation as *A. populifolia* had the shortest-lived leaves and did not accumulate the highest sodium and chloride ion concentrations (Figure 6.9). Of the two succulent species investigated *A. populifolia* had the higher SLA indicating a lower investment of carbon per unit leaf area.

The maintenance of high growth rates requires good availability of resources which are largely determined by the resource inputs and turnover per unit land surface area. Some resources such as nitrogen and potassium are particularly scarce in the dune environment (Table 6.1). *A. populifolia* had the lowest LAI such that despite having the highest production rates (Table 4.6), water losses (Table 5.3) and nutrient requirements per shoot, these become the lowest of the three investigated species when expressed per unit dune surface area (Table 4.6; Table 5.8 & Figure 5.9; Figure 6.4 & 6.5). An exception was the *A. populifolia* demand for nitrogen which remained higher than that of the other two species even when expressed in this way (Figure 6.4 & 6.5). This may represent a controlling resource stress. Hesp (1983 & 1989) proposed a positive feed-back mechanism between plant growth and sand trapping. Accreting sand results in the deposition of nutrients which stimulate growth and increased growth increases sand trapping. Although this explanation may apply to *Ammophila arenaria* and may offer an explanation of the differences in vigour observed between seaward and landward marran stands, the lower LAI and above-ground biomass measured for *A. populifolia* relative to values for *I. pes-caprae* and *S. plumieri* (and hence the reduced sand trapping ability despite potential nitrogen limitation) is not reconcilable with Hesp's feed-back mechanism.

If nutrients are available in sufficient concentrations then the redistribution of nutrients internally is reduced (Marschner, 1995). With the exception of nitrogen *A. populifolia* has the lowest demand for nutrients per unit land area (Figures 6.4 & 6.5) and although potassium and phosphorus were initially imported into developing leaves this species showed the least reallocation (export) of nutrients from the older leaves (Table 6.4 & 6.5, summarised in 6.6). It appears that nutrients are not limiting *A. populifolia* growth although more information on nitrogen availability and reallocation is required.

I. pes-caprae

I. pes-caprae has the lowest stomatal conductance (Figure 7.5) and has the highest stomatal limitation (Table 7.2) exerting more stomatal control over water loss than was apparent for the other two species (See: Stomatal control, Discussion, Chapter 7). As a result of these factors *I. pes-caprae* has the lowest peak transpiration rates (Table 5.1) and volumes of water transpired per day (Table 5.3). The reason why *I. pes-caprae* exerted greater stomatal control over gas exchange was not apparent but it could be genetically determined and may be related to plant hydraulic conductivity and rooting patterns. *I. pes-caprae* appears limited by water availability and hence displays physiological adaptations that reduce water loss. Despite each shoot having adventitious roots, this species requires below-ground stem connections to maintain plant water status (Figure 5.7) and like *A. populifolia* this species showed no evidence of osmotic adjustment (Table 5.7).

Calculated water budgets indicated that of the three species investigated, *I. pes-caprae* was the most likely to be limited by water availability (Table 5.8; Figures 5.9 & 5.10). This apparent water limitation arises not from high transpiration rates but because *I. pes-caprae* has the highest LAI of the three species investigated (Tables 3.6). Modelled budgets for *I. pes-caprae* that account for the water capacitance of dune soils, approached the minimum sand-water content on several occasions even at a field capacitance of 4% and using the measured LAI (Figure 5.10).

I. pes-caprae had the lowest efficiencies of utilisation of incident PPFD (Figure 7.3 & Table 7.1), carboxylation efficiencies and highest stomatal limitations (Figure 7.4 & Table 7.2). As a result these and low stomatal conductances, light-saturated photosynthetic rates, CO_2-saturated rates, ambient rates and daily amounts of CO_2 assimilated (Figures 4.1, 7.3, 7.4; Table 4.2, 4.4) were lower than those of the other investigated species. Photosynthetic rates were similar to rates measured for temperate coastal species (See: Photosynthetic rates, Discussion, Chapter 4). The relatively lower light and CO_2 saturated photosynthetic rates indicate a lower capacity for the regeneration of RuBP and hence lower rates of electron transport and ATP and NADPH production (Farquhar and Sharkey, 1982). The lower efficiency of utilisation of incident PPFD in this species may be as a result of a lower chlorophyll content per unit leaf surface area (See: Chlorophyll concentration, Results, Chapter 4). As with *A. populifolia* there appeared to be no correlation between shoot nitrogen content and photosynthetic rate.

Of the three species investigated *I. pes-caprae* displayed the highest diurnal reduction in φ_{P0} (Figure 4.6) and had the slowest recovery of φ_{P0} following a high light treatment (Figure 4.8). *I. pes-caprae* also displayed the largest increases in k_N and decreases in k_P, following incubation in natural light (See: Recovery of φ_{P0} following incubation in natural incident light, Results, Chapter 4). This relative susceptibility may have contributed to the lower photosynthetic rates measured. *I. pes-caprae* appears to be sensitive to the high light intensity characteristic of foredunes possibly because water conservation involves reduced stomatal conductance that results in reduced photosynthetic CO_2 supply. A condition that has been recognised as contributing to photoinhibition (Powles and Osmond, 1979).

I. pes-caprae and *S. plumieri* have similar light (Table 7.1) and CO_2 compensation points (Table 7.2). These were higher than those for *A. populifolia.* These data indicate similar rates of dark and photorespiration in the two species but rates lower than those measured for *A. populifolia*. *I. pes-caprae* has the lowest whole shoot respiration rate (Table 4.3).

Photosynthetic CO_2 assimilation, transpiration and WUE were more dependant on PPFD than on other abiotic factors measured (Table 4.2; Table 5.2 & 5.4 respectively) although variation in PPFD accounted for less of the variation in the measured gas exchange parameters than was noted for *A. populifolia*. As a result of stomatal regulation the relationship between E and VPD was the least predictive ($r^2 = 0.42$) of the three species investigated (Figure 5.2).

The relatively low photosynthetic and respiratory rates measured for this species may account for the low growth rates and *I. pes-caprae* although producing an intermediate number of leaves per month (Table 4.5) had the lowest above-ground primary shoot production (Table 4.6). Furthermore the contribution of dry mass to monthly stem production was the lowest of all three species (Table 4.5). *I. pes-caprae* accumulated on average the highest concentrations of leaf starch. Such reserves could be necessary for supporting seasonal changes in growth rate (Figure 4.5).

I. pes-caprae had leaf longevity intermediate between that of *A. populifolia* and *S. plumieri* (See: Leaf production and dry weight accumulation, Results, Chapter 4). *I. pes-caprae* mature leaf respiration rates were not different from those for *S. plumieri* but both species had higher rates than those of *A. populifolia* (Table 7.2). Both species apparently invested more energy in the maintenance of leaves than was invested by *A. populifolia*. *I. pes-caprae* accumulated less sodium than *A. populifolia* or *S. plumieri* (Figure 6.9) possibly due to the lower transpiration rate measured for this species (See: Effect of leaf age on nutrient concentration and nutrient remobilization, Discussion, Chapter 6). The lack of hypertrophy in this species may be as the result of the lower leaf sodium concentration. The fact that *S. plumieri* accumulated more sodium than did *I. pes-caprae* yet had longer-lived leaves, suggests that sodium accumulation does not determine leaf longevity. This conclusion is complicated by differences in the relative water contents of the leaves from these species. *I. pes-caprae* had the highest SLA of the three species investigated indicating that its non-succulent leaves contained less carbon per unit surface area than the other species.

The lower production rates of *I. pes-caprae* affects the demand for resources (Table 4.6). Hence it is possible for the LAI of *I. pes-caprae* to increase above that of *A. populifolia* or *S. plumieri* (Table 3.6) and yet maintain similar resource requirements per unit dune surface area. In fact the higher LAI for *I. pes-caprae* results in greater volumes of water being transpired per unit dune surface area than was the case for the other two species (Table 5.8 & Figure 5.9). However the caution concerning water budgets as stated previously (Annual and monthly budgets, Results, Chapter 5) remains applicable.

I. pes-caprae nutrient demands per unit dune surface area were similar or perhaps marginally lower than those of *S. plumieri* but potassium and phosphorus demands were higher than those of *A. populifolia.* Both potassium and phosphorus were initially imported into expanding leaves of *I. pes-caprae* and were then subsequently exported from older leaves (Table 6.4 & 6.5, summarised in 6.6). The extent of this potassium export was similar for *I. pes-caprae* and for *S. plumieri* but the export of phosphorus from older *I. pes-caprae* leaves exceeded that from *S. plumieri* leaves. The higher demand for nutrients per unit dune surface area may well result in a higher internal cycling of nutrients than was achieved by *A. populifolia*.

S. plumieri

S. plumieri has leaf conductances that are intermediate between those of *A. populifolia* and *I. pes-caprae* (Figure 8.5). This resulted in peak transpiration rates (Table 5.1) and daily volumes of water transpired (Table 5.3) that were intermediate between those of *A. populifolia* and *I. pes-caprae* (Table 5.1). Transpiration rates in combination with intermediate CO_2 assimilation rates resulted in WUE which were higher than those for *I. pes-caprae* but not dissimilar to those of *A. populifolia* (Table 5.5).

As a result of transpiration rate and the apparent lack of stomatal response to the plant water status *S. plumieri* developed the most negative midday leaf water potential (Figure 5.4). These remained in the range that can be considered typical of mesic plants (Table 5.10). Furthermore water budgets although not as positive as those for *A. populifolia* indicated that water supply was not limiting (Table 5.8; Figures 5.9 & 5.10). The lack of water conserving stomatal behaviour and the adequate water supply suggests that the magnitude of reduction in midday leaf-water potential is the result of limiting hydraulic conductance. This is supported by the finding that leaf-water potentials recovered to pre-dawn values during the late afternoons (Figure 5.4). As with both other species there was no evidence of osmotic adjustment (Table 5.7). Despite each shoot of this species having adventitious roots, like *I. pes-caprae*, it requires below-ground stem connections to maintain plant water status (Figure 5.7).

The water capacitance budgets (Figure 5.10) that accounted for the volume of sand potentially exploited by the species predict that water supply exceeds water loss at field capacities of 4%. Estimated compounded sand-water content remained intermediate to that of the other two species. Modelled sand-water contents were reduced to the minimum sand-water content (0.5%) only by either reducing the field capacity to 1.5 % (Figure 5.10) or by increasing the LAI from 1.05 to 1.45.

Of the three species investigated *S. plumieri* had intermediate stomatal conductances (Figure 7.5), efficiencies of utilisation of PPFD (Figure 7.3 & Table 7.2), carboxylation efficiencies and stomatal limitations (Figure 7.4 & Table 7.2). As a result the light-saturated photosynthetic rates (Figure 7.3), CO_2-saturated rates (Figure 7.4), ambient rates (Figure 4.1 & Table 4.2) and daily amounts of CO_2 assimilated (Table 4.4) were intermediate between those of the *A. populifolia* and *I. pes-caprae*. Furthermore *S. plumieri* also showed diurnal reductions in φ_{P0} (Figure 4.6) and recovery rates of φ_{P0} following exposure to sunlight (Figure 4.8) that were intermediate between those of *A. populifolia* and *I. pes-caprae* suggesting an intermediate sensitivity to high light intensity stress.

S. plumieri had intermediate whole shoot respiration rates (Table 4.3) but mature leaf respiration rates were the highest of the three species investigated (Table 7.1). Apparently *S. plumieri* invests the greatest proportion of energy on the maintenance of mature leaves which are both the longest lived (See: Leaf production and dry weight accumulation, Results, Chapter 4) and of the succulent species contained the greater proportions of carbon per unit leaf area.

Leaves and stems from *S. plumieri* had the highest sugar but not starch contents (Figures 4.3 & 4.4) indicating that sugar was the preferred storage or transport carbohydrate in this species. As with *I. pes-caprae* it is possible that the storage of carbohydrates is necessary for supporting seasonal growth. Alternatively this large clonal species may transport carbohydrates between shoots possibly supporting the reproductive effort in which some of the shoots are engaged. The exchange of resources between *S. plumieri* shoots has not been investigated. Intermediate photosynthetic and respiratory rates translate into intermediate above-ground production rates (Table 4.6). When production was converted to productivity because of the LAI, *S. plumieri* had the highest productivity of the three species (Table 4.6).

Leaf conductance of *S. plumieri* increased with increasing VPD (Peter, 2000) probably due to the stomatal response to increasing PPFD and the increased demand for CO_2 (See: Discussion, Chapter 7). Of the environmental factors monitored, assimilation rate (Table 4.2) and WUE (Table 5.4) were most strongly correlated with PPFD. Differences in PPFD accounting for 83 and 48 % of the variation in assimilation rate and WUE. Transpiration was most strongly correlated with VPD and the saturated exponential response yielded a r^2 value of 0.78. In addition to PPFD being identified as the major determinant of leaf assimilation rate, the whole shoot assimilation rates were limited by incident PPFD (Figure 7.13). This was due to mutual shading (Figure 7.10) and unsaturated light responses (Figure 7.3).

S. plumieri leaves accumulated the most sodium possibly due to persistent transpiration by leaves that were the longest lived (Figure 6.9). Like both *A. populifolia* and *I. pes-caprae, S. plumieri* leaves initially accumulated both potassium and phosphorus and subsequently exported potassium from its older leaves. However unlike the other two species *S. plumieri* continued to import phosphorus into all its leaves (Tables 6.4 & 6.5, summarised in Table 6.6).

A combination of production rates and LAI resulted in potassium and nitrogen demands that were not dissimilar to those of *I. pes-caprae* (Figure 6.4 & 6.5). Phosphorus demands were somewhat less than those of *I. pes-caprae* but not as low as those of *A. populifolia*. The estimated supply of nutrients from either sand-water and ground-water approximated the demands created by above-ground production. Isotopic studies could not identify either of these as the preferential water source (Figure 5.8).

LIMITATIONS OF AND ADAPTATIONS TO THE FOREDUNE ENVIRONMENT

Limitations

It is evident from the discussion above that each of the three species investigated has unique adaptations and that different resources may be limiting to different species.

A. populifolia appears unlimited by water availability and light stress but may be limited by nitrogen availability. Further research is required to clarify the latter point particularly as nitrogen has been shown to be heterogeneously distributed above the swash zone (Cain *et al*., 1999), a region in which the abundance of *A. populifolia* is frequently the highest (see discussion below).

S. plumieri also appears unlimited by water availability and although there was more evidence of the mechanisms associated with photoinhibition than was evident for *A. populifolia,* both leaves and whole shoots appear light limited. Nutrient demand created by above-ground growth was adequately met by the supply of nutrients from transpired water but the supply was not excessive and the potential limitation of these resources cannot be ruled out. The finding that nutrients were reallocated from old to younger leaves supports this suggestion.

I. pes-caprae appears limited by water and evidence of both conservative water use and insufficient supply was, on occasion, apparent. However the calculated water budgets for *I. pes-caprae* must be interpreted with a certain amount of caution due to the weak predictive relationship between E and VPD (see, Results, Chapter 5). Stomatal limitation, possibly as a result of the water limitation, reduced photosynthetic rate and may have resulted in the sensitivity of this species to light stress. Possibly because of lower volumes of water transpired calculated nutrient supply was sufficient but not in excess of nutrient demand. This and the evidence of nutrient reallocation from older to younger leaves suggests that like *S. plumieri*, *I. pes-caprae* may be nutrient limited.

Adaptations to resource stress and disturbance

The resource demand generated by plant growth cannot exceed supply. The demand can be generated in several different ways. Species can have low biomass and high biomass turnover (growth) or can have a higher biomass and a lower turnover and still have similar resource demands. These two parameters are components of primary productivity and hence it is primary productivity that must be suited to resource supply. Interestingly the primary productivity (Table 4.6) despite considerable differences in biomass (Table 4.6) and growth (Tables 4.5 & 4.6) among the investigated species was not that dissimilar and this was particularly so for *A. populifolia* and *S. plumieri*. Biomass, biomass turnover and the resultant primary productivity appear adapted to the resources available in the foredune environment. Differences in primary productivity that do exist may result from differences in the adaptations that each of the three species possess such that each species resource acquisition or demand is not identical. The unique adaptations possessed by each of the three species were discussed above and included features such as leaf hairs, nutrient reallocation and gas exchange physiology. Other potential adaptations such as rooting patterns, volumes of soil exploited and rooting densities require investigation. Different microhabitats occur even within the pioneer zone of the foredunes and these create differential selective pressures.

The primary productivity, growth characteristics and adaptations of plants are not likely to have evolved as a result of resource limitations alone and may be related to disturbance. As stated previously, disturbance includes the effects of abiotic factors such as salt spray, sand burial, sand blasting and the inundation of plants with seawater. Hence in addition to the resource stresses certain disturbances (abiotic factors) are common to the foredunes and the investigated species appear to possess some common adaptations.

These are particularly evident when comparisons are made with plants from other ecosystems.

All three species have relatively high photosynthetic rates characteristic of early successional species (See: Photosynthetic Rates, Discussion, Chapter 4). However dark respiration rate expressed as a proportion of maximum photosynthetic rate was low in comparison to those for woody savanna species (See: Respiration Rates, Discussion, Chapter 4). This suggest that these dune pioneers expend relatively little energy on growth and maintenance. Furthermore the fact that the above-ground primary productivity of the investigated dune species was higher than that of the woody savanna species (Table 4.7) suggests that it was the maintenance component of respiratory energy that was reduced and not the expenditure on growth.

Transpiration rates (Table 5.9) and WUE of the three species investigated can be considered moderate to high and not untypical of mesic species (See: Transpiration rates and Water use efficiencies, Discussion, Chapter 5). Similarly plant water relations and particularly the magnitude of the negative water potentials developed at midday (Table 5.10) were not unlike those of species from mesic environments (See: Leaf Water Potential, Discussion, Chapter 5). Apparently water availability in the dunes (Table 5.8; Figure 5.9 & 5.10) in conjunction with sufficiently high hydraulic conductances or regulated transpiration rates resulted in plant water status typical of mesophytes. Furthermore none of the species displayed osmotic adjustment (Table 5.7).

Biomass and LAI of the species investigated were low in comparison with savanna and fynbos ecosystems but were similar to temperate dune ecosystems (Table 4.7). They may be correlated to the resource paucity of the dune systems system (Table 6.1). Associated with resource paucity was the reallocation of selected nutrients from older leaves for which there was at least some evidence in all three species (Table 6.4 & 6.5, summarised in 6.6).

Despite having considerably different shoot production and growth forms the above-ground primary production rates were not dissimilar and were higher than the primary productivity of savanna and fynbos ecosystems (Table 4.7). Such productivity may be necessary to survive both the sand burial and mechanical damage that is characteristic of the foredune environment.

Adaptations to microhabitats within the pioneer zone of the foredunes

All three species investigated occur within the pioneer zone but even within this zone there are sites where each species is more abundant (Lubke, 1983). *A. populifolia* is frequently found growing closest to the high-tide line whereas *I. pes-caprae* and *S. plumieri* are found slightly further inland on the sea-ward and land-ward faces of the foredunes (Figure 3.3 & 3.4). Within this pioneer zone gradients in abiotic factors have been recognised. These gradients have as yet been inadequately quantified with the possible exception of soil salinity (Avis, 1992), salt spray deposition (Barbour and De Jong, 1977; Boyce 1954; Oosting and Billings, 1942; Lubke and Avis, 1982; Donnelly and Pammenter, 1983), nutrient build-up and sand burial (van der Valk, 1974). Brown and McLachlan (1990), in a conceptual model of the gradients across coastal dune fields considered physical and chemical status including sand transport, sand calcium carbonate content, sand alkalinity, salt spray and ambient temperature to be highest at the high-tide line and to decline with distance inland. Ecosystem characteristics including physical stress and turnover rates (e.g. nutrients and biomass) are also considered highest at the high-tide line and decrease inland. On the other hand soil nitrogen, soil organic matter, biomass and plant density are lowest at the high tide line and increase with distance inland (Brown and McLachlan, 1990). In addition to this conceptual model the work of Cain *et al*. (1999) has shown that resources such as nitrogen are heterogeneously distributed above the high-tide line and become more uniform with distance inland. Furthermore the probability of localised environmental disturbance such as inundation or erosion by large seas or burial with sand, is largest closest to the sea. Thus of the three species investigated *A. populifolia* is subject to the severest and most unpredictable conditions but the measured production, productivity, growth characteristics and related physiology could confer selective advantage to life under these conditions.

A. populifolia

The measured physiological parameters show that *A. populifolia* has high productivity and growth rates and invests a large proportion of its assimilated carbon into stem growth. Growth is unaffected by season. Collectively these may be adaptations for dealing with sand-burial and the unpredictable nature of the habitat just inland from the high-tide line. Such high growth rates would require the availability of resources. Resource constraints may be one of the reasons for the low biomass measured for *A. populifolia.* High transpiration rate and related physiology may be associated with ensuring the adequate supply of dissolved nutrients via the transpiration stream. Alternatively or in addition to which, high transpiration rate may be the result of maintaining high leaf conductances to CO_2, conditions necessary for supporting high photosynthetic rates.

A. populifolia low biomass and an open canopy may reduce the frictional interference with sand-laden wind and hence reduce the potential for sand-burial. Equally the open canopy would allow accumulated sand to be removed. Reduced sand trapping may also reduce the acquisition of wind-borne resources. In response to this the branching growth form may enhance the exploration for resources that are heterogeneously distributed. Short life-spans and life-cycles in conjunction with physiology that appears not to be associated with maintenance would be advantageous in environments that are subjected to unpredictable crises. Hence *A. populifolia* has many attributes that classify it as a ruderal according to Grime's (1977, 1979) functional classification. Such species occupy favourable environments characterised by high levels of disturbance and low biotic competition.

I. pes-caprae and S. plumieri

Both *I. pes-caprae* and *S. plumieri* are found further inland from the high-tide line and occupy a habitat that is less likely to be disturbed. Possibly as a result of less sand transport and burial these species can afford to have lower growth rates than *A. populifolia.* They invest proportionately less carbon in stem growth. However under conditions of sand burial both *I. pes-caprae* and *S. plumieri* appear to allocate more resources to stem growth and such stems have longer internodes (*pers. obs.*; Pammenter, *pers. comm.*). Research on the response of growth and carbon allocation patterns of these species in response to alterations of carbon source and sink strength is required.

Both *I. pes-caprae* and *S. plumieri* are longer lived than *A. populifolia* and expend more energy (dark respiration) on the maintenance of fully expanded mature leaves. They also invest more carbon in their leaves. When the succulent species (*A. populifolia* and *S. plumieri*) are compared the lower SLA of *S. plumieri* indicates a greater investment of carbon per unit leaf area. Furthermore the lower growth rates of *I. pes-caprae* and *S. plumieri* result in a smaller demand for resources per shoot and hence more shoots can be produced per unit land surface area. This may also be related to sand-burial. Sand transport decreases with increasing distance from the high-tide line. This decreases the potential for sand-burial and may allow these two species to have higher biomass and LAI than was measured for *A. populifolia*.

The more predictable or evenly distributed resources found away from the high-tide line may allow *S. plumieri* and *I. pes-caprae* to have more seasonal growth patterns possibly related to favourable ambient conditions or to seasonal reproductive requirements.

Although occupying the same habitat within the foredune *I. pes-caprae* and *S. plumieri* differ in their physiological responses to the environment. *S. plumieri* has higher photosynthetic, transpiration and growth rates with stomatal behaviour that appears to be in response largely to photosynthetic demand rather than in controlling water loss. *I. pes-caprae* has lower photosynthetic, transpiration and growth rates and showed a greater degree of stomatal regulation with stomata apparently limiting both the CO_2 and water exchange. These differences could result from differences in plant hydraulic conductances and below-ground rooting patterns, issues that will be resolved only with further research.

In Grime's (1977; 1979) functional classification both *I. pes-caprae* and *S. plumieri* would shift along the disturbance axis indicating a habitat less disturbed than that occupied by *A. populifolia*. This shift would indicate species with reduced ruderal and increased competitive strategies, conditions favoured by perennial herbs.

The reproductive strategies adopted by *A. populifolia*, *I. pes-caprae* and *S. plumieri* as related to the physiology and growth data presented in this investigation would be a fascinating study. I eagerly anticipate the findings of my fellow researcher Irma Knevel (Botany Department, Rhodes University) who is in the process of preparing a thesis on the phenology of certain Eastern Cape dune pioneer plants.

CLASSIFICATION OF THE SPECIES IN TERMS OF FUNCTIONAL TYPES

Identifying characteristics that are common to foredune species is not unlike the recent trend in ecological research in which identified plant attributes are used to define plant functional types (Grime, 1977). This discussion would be incomplete without such consideration. Functional plant ecology has arisen from the need to condense the enormous diversity of individual species and populations into a relatively small number of generally recurrent patterns (Woodward and Cramer, 1996; Grime *et al*., 1997). This grouping of plants according to non-phylogenetic characters, using structural and functional characters, summarises the performance of the plants in ecosystem processes by their functionally different responses to environmental change (Garcia-Mora *et al.*, 1999). Such classifications have been performed for many ecosystems including coastal foredunes (Garcia-Mora *et al*., 1999). These researchers used seven attributes (persistence, canopy height, below-ground storage organs, below-ground structures, leaf characteristics, capability of withstanding sand burial and dispersal by sea water) to classify three functional types. Type I consisted of winter annuals of moderate size with soft leaves with no apparent adaptation to the coastal environment. Type II were mostly perennials with below-ground spreading root networks and leaves with assumed adaptations to coastal environmental stress. Type III included plants capable of being dispersed by sea-water and of withstanding sand-burial.

With respect to this classification and using the attributes cited by Garcia-Mora *et al.* (1999) the three species studied would certainly have grouped with Types II and III. However such functional groupings detract from the underlying differences in plant function that species may possess. The three species studied in this investigation were shown to have considerably different physiology which considered together with growth habit made it possible to explain how all three species live and thrive within the same foredune habitat. Hence although a functional-type approach may be useful in identifying general recurrent patterns in plant function when large numbers of species are considered, in-depth investigations may reveal that different mechanisms have evolved to overcome environmental stress common to a particular environment. Plants that are grouped together by these generalisations do not necessarily function in the same way.

CONCLUSIONS

The fundamental question asked in this thesis was "W*hy and what allows only particular species to flourish in the foredune environment?"* This question was addressed by attempting to answer the following: Is the foredune environment limited by resource availability or are resources present in excess? How does the resource availability affect plant function and do the investigated species respond in the same way? Is the possession of adaptations for acquiring limited or coping with excess resources, the key to understanding which plant species can thrive in the foredune environment or does the key lie with plant adaptations to disturbance? Answers to these questions have been presented in this chapter and the following conclusions can be drawn.

- With respect to the three species investigated no single resource limitation could be shown to be the controlling factor in the foredune environment.

- Different resources appear to be limiting to the different species investigated and this was related to the specific adaptations possessed by each species.
- Only nutrient limitations appear to be common to all three species. However insufficient data on nutrient demand and the spatial and temporal distribution of nutrients is available to make this finding conclusive.
- Water availability appears not to limit *A. populifolia* or *S. plumieri* but limits *I. pes-caprae* and may result in the lower productivity measured for this latter species.
- In addition to being limited by water availability and possibly as a result of this, *I. pes-caprae* was more sensitive to light stress than either *A. populifolia* or *S. plumieri*.
- Some of the adaptations that the three species possess were shown to affect resource acquisition and use. The function of some of these and the possession of other adaptations conferred advantage to coping with disturbance.
- Some of the investigated adaptations affect resource use (demand) although the particular resource affected may not have been limiting. For example the leaf hair layer possessed by *A. populifolia* increased WUE although this species appears not to be limited by water supply. The layer also functions to reduce potential light stress and this suggests that a single adaptation may be in response to multiple factors.
- To occupy and be successful in the foredune environment appears to require adaptation to both resource stress and disturbance.
- Although some plant functions could be identified as adaptations to the foredune environment, in general there was also evidence of individual species adaptations to the microhabitats found within the pioneer zone. General adaptations are evident when comparisons are made with species from other ecosystems and include features such as high photosynthetic rates and productivity, a low expenditure of respiratory energy of on maintenance and a low biomass. Comparisons of the species investigated reveals specific adaptations such as the differences in photosynthetic, respiratory and growth characteristics; the role of the hair layer of *A. populifolia* leaves and the water conserving stomatal behaviour of *I. pes-caprae*.

- The key to understanding what limits a particular species to the foredunes where there is no single factor or limitation is understanding the functioning of that species in relation to both resource stress and disturbance. Furthermore the function of each particular species needs to be considered in relation to the specific abiotic and biotic factors that are prevalent in the microhabitat occupied by that species.
- Although this investigation gives some explanation of why the studied species can inhabit the foredunes it does not offer reasons why these species are absent on the dunes further inland. Such explanations would require ecophysiological studies of the species that inhabit these areas so that comparisons could be made. However it is reasonable to hypothesise that plant adaptations, to the reduced disturbances and changed stresses characteristic of inland microhabitats, would convey competitive advantage to the species found occupying these microhabitats. This would exclude the species that are adapted to the conditions specific to the pioneer zone of the foredunes.

Appendix

Appendix A

JIP analyses of chlorophyll fluorescence induction curves

On illumination, fluorescence signals follow a polyphasic rise that is typical of oxygenic photosynthetic tissue and these phases have been labelled O, J, I and P (Kitajima and Butler, 1975; Strasser *et al.*, 1995; Kruger *et al.*, 1997). In the method of Strasser and Strasser (1995) the fluorescence intensity after illumination at 50 μs (F_0), 150 μs, 300 μs and 2ms (F_J), the maximal fluorescence intensity (F_M), the time to reach maximal fluorescence intensity (t_{Fmax}) and the area between the fluorescence transient and the level of the maximal fluorescence intensity are retained as original data. These data are used for the subsequent calculation of phenomenological and biophysical expressions giving a dynamic description of a photosynthetic sample in a given physiological state. The method employs a simplified model of energy fluxes in a photosynthetic apparatus (Strasser and Strasser, 1995). Based on the theory of energy fluxes in biomembranes (Sironval *et al.*, 1981; Strasser, 1981), Strasser *et al.* (1995) derive formulae for energy fluxes (both per reaction centre and per cross-sectional area) and the flux ratios or yields using the experimental values that were defined as the original data above.

According to this model most of the excitation energy from the photons absorbed (ABS) by the antennae pigment chlorophylls is dissipated as heat, less as fluorescence emission and the rest is channelled as trapping flux (TR) to the reaction centre (RC). The excitation energy is converted to redox energy when Q_A is converted to Q_A^-. Q_A^- is subsequently reoxidised to Q_A initiating electron transport (ET).

The specific energy (biophysical) fluxes per RC at time zero, ABS/RC, TR_0/RC and ET_0/RC can be derived from experiments as shown below. The maximum quantum yield of primary photochemistry $TR_0/ABS = \varphi_{P0,}$ the efficiency by which a trapped exciton can move an electron into the electron transport chain $ET_0/ TR_0 = \Psi_0$, or the probability that an absorbed photon will move an electron into the electron transport chain $ET_0/ABS = \varphi_{E0}$, are all directly related to the three fluxes by the ratios of any two of them. TR/RC expresses the rate at which an exciton is trapped by the RC resulting in the reduction of

Q_A to Q_A^-. The maximum rate of this is given by TR_0/RC because at time zero all the reaction centres are open. The expression used by Strasser and Strasser (1995) to derive this maximal rate utilises the initial slope of the induction curve between 50 and 150 µs, normalised to the maximum variable fluorescence $F_V=F_M-F_0$ and the relative variable fluorescence at 2ms (V_J; where $V_J = (F_J- F_0)/(F_M-F_0)$). This normalised slope (M_0) indicates the real trapping rate only in DCMU-treated samples where the re-oxidation of Q_A^- is blocked. Otherwise the initial slope indicates the net rate of closure of the reaction centres where trapping decreases the number of open centres and electron transport increases it ($M_0 = TR_0/RC - ET_0/RC$). Strasser and Strasser (1995) have shown experimentally that M_0 of DCMU-treated samples can be simulated in untreated samples by multiplying the measured M_0 by a factor reciprocal to V_J. Therefore

$TR_0/RC = M_{0,DCMU} = M_0/V_J$

And thus

$ET_0/RC = TR_0/RC - M_0 = (M_0/V_J - M_0) = (M_0/V_J) \times (1-V_J) = (TR_0/RC) \times (1-V_J)$.

Hereafter

$\Psi_0 = ET_0/TR_0 = 1-V_J$

and

$\varphi_{E0} = ET_0/ABS = (TR_0/ABS) \times (ET_0/TR_0) = \varphi_{P0} \times \Psi_0$

where φ_{P0} was calculated (Kitajima and Butler, 1975) from F_0 and F_M as

$\varphi_{P0} = (F_M-F_0)/F_M = 1-(F_0/F_M)$

So

$\varphi_{E0} = [1-(F_0/F_M)]- (1-V_J)$

Concerning ABS/RC it was derived as follows:

$TR_0/RC = (TR_0/ABS) \times (ABS/RC) = \varphi_{P0} \times (ABS/RC)$

Hence

$ABS/RC = (TR_0/RC)/\varphi_{P0} = (M_0/V_J)/[1- (F_0/F_M)]$

Kruger *et al.* (1997) point out that TR_0/RC, ABS/RC and ET_0/RC refer only to active reaction centres and not to centres that have transformed to quenching centres as can occur under stress conditions.

The phenomenological fluxes are ABS/CS, TR_0/CS and ET_0/CS where CS stands for the cross section of the tested sample. Strasser and Strasser (1995) and Kruger *et al.* (1997) found that the chlorophyll content of test samples did not change during their experiments and therefore regarded ABS/CS as a constant. Similarly chlorophyll content remained constant on individual experimental days in the present study. The above authors consider that F_0 of dark-adapted samples provides a reasonable measure of ABS/CS (in arbitrary units) and therefore TR_0/CS and ET_0/CS can also be expressed in these units. Further heterogeneity between samples due to differences in pigment concentrations were excluded by normalising all fluorescence values by F_0 of dark adapted leaves. Therefore ABS/CS once normalised equals 1 and thus values of TR_0/CS and ET_0/CS equal $TR_0/ABS = \varphi_{P0}$ and $ET_0/ABS = \varphi_{E0}$ respectively.

The concentration of reaction centres RC/CS was derived as:

$RC/CS = (ABS/CS)/(ABS/RC)$

And by assumption ABS/CS remained constant throughout the experiment and by normalisation equals 1. Therefore:

$RC/CS = (ABS/RC)^{-1} = [1- (F_0/F_M)]/(M_0/V_J)$

Conformational parameters relating biophysical and phenomenological parameters were also derived. Conformational parameters include photochemical (k_P) and non-photochemical (k_N) and fluorescence (k_F) rate constants. The following expressions were derived assuming that k_F is constant and this assumption has been extensively discussed by these authors (Havaux *et al.*, 1991):

$k_N = (ABS/CS) \times k_F \times (1/F_M)$

$k_P = (ABS/CS) \times k_F \times [(1/F_0) - (1/F_M)]$

Appendix B

Changes in the normalised leaf weight and phosphorus and potassium concentrations in response to increasing leaf age.

Figures 6.10, 6.11 and and 6.12 show the responses of leaf weights and nutrient concentrations to increasing assigned leaf number (age). Leaf weights and nutrient concentrations have been normalised. Linear regressions were fitted to entire or portions of the data in such a way as to maximise the r^2 values.

Calculation of the relative comparative data in Table 6.6
Average shoot nutrient concentrations were calculated as the average of values for plants or shoots harvested on the 21/2/98 and the 25/7/98 (Figure 6.3). Values for each measurement were expressed relative to the smallest value for that measurement and hence comparisons can be made between species but not between the measurements of potassium, nitrogen and phosphorus.

Nutrient budgets were calculated from the average values shown in Figures 6.4 and 6.5. Values for each measurement were expressed relative to the smallest value recorded for that measurement and hence comparisons can be made between species but not between the different measurements.

Nutrient accumulation was calculated as the average slope of the linear regressions of leaf nutrient concentrations (plants or shoots sampled on both the 21/2/98 and 25/7/98) related to assigned leaf number (Tables 6.3). Nutrient accumulation was expressed relative to the smallest value obtained from calculations for calcium, magnesium and sodium for all three species. Hence comparisons can be made both between species and between the values calculated for calcium, magnesium and sodium.

The import or export of potassium and phosphorus were calculated as the average sum of the slopes of the linear regressions of leaf nutrient concentrations (plants or shoots, sampled on both the 21/2/98 and 25/7/98) and the slopes of leaf weight changes as related to assigned leaf number (Tables 6.4 & 6.5). This was repeated for both portions of the responses to increasing assigned leaf number. Phosphorus and potassium import or export values are expressed relative to the smallest values from calculations for phosphorus or potassium respectively. Hence comparisons can be made both between species and between portions 1 and 2 but not between values for phosphorus and potassium.

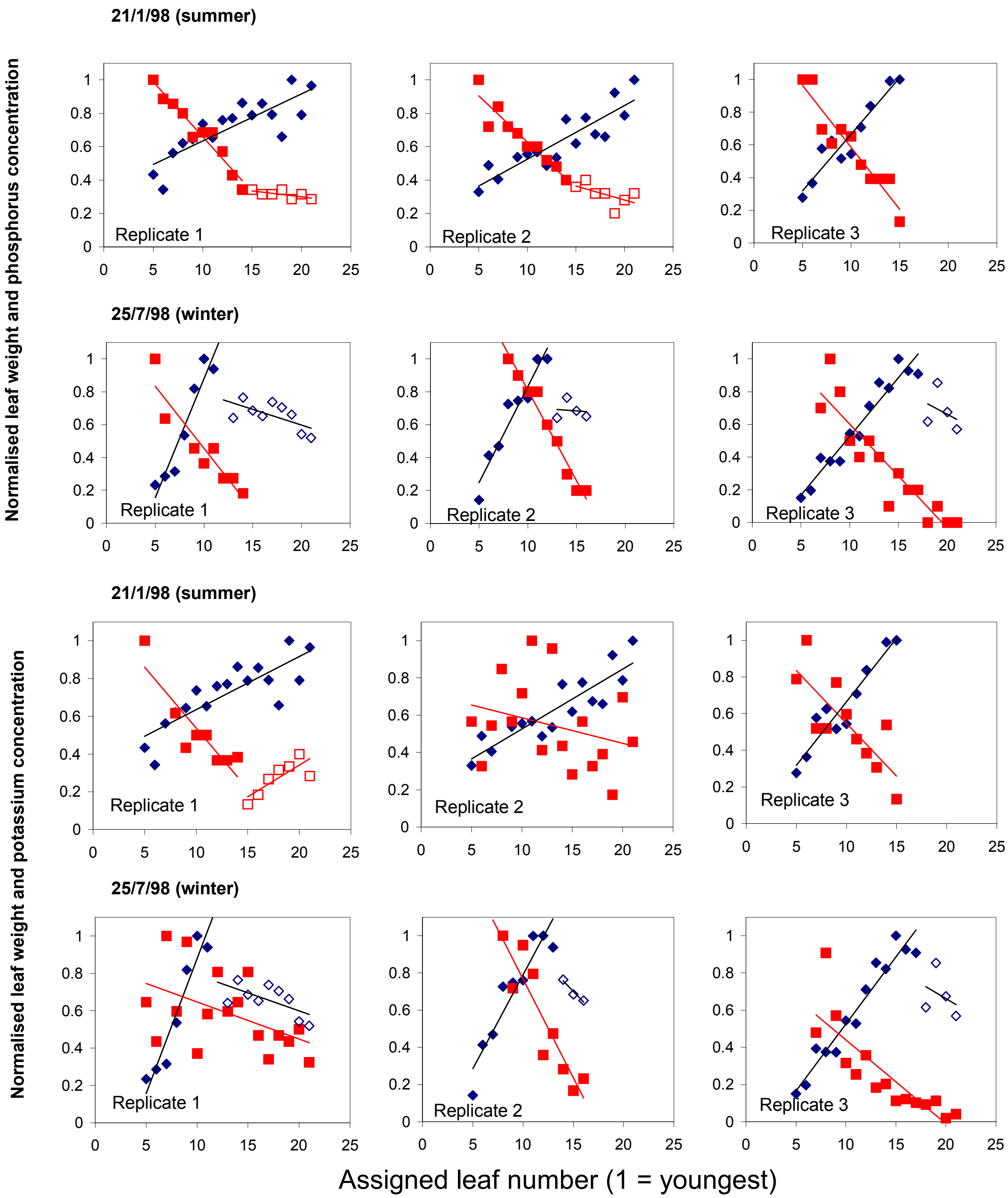


Figure 6.10: The relationship between normalised phosphorus and potassium concentrations (squares), normalised leaf weight (diamonds) and leaf number for individual *A. populifolia* replicates sampled on the 21/1/98 (summer) and on the 25/7/98 (winter). Monophasic curves were fitted with a single linear regression and biphasic curves fitted with two linear regressions. Monophasic and portion one of the biphasic responses are shown with soild symbols. Portion two of the biphasic responses is shown with open symbols. Decisions as to which point should be included in the particular regression were based on maximising the r^2 values.

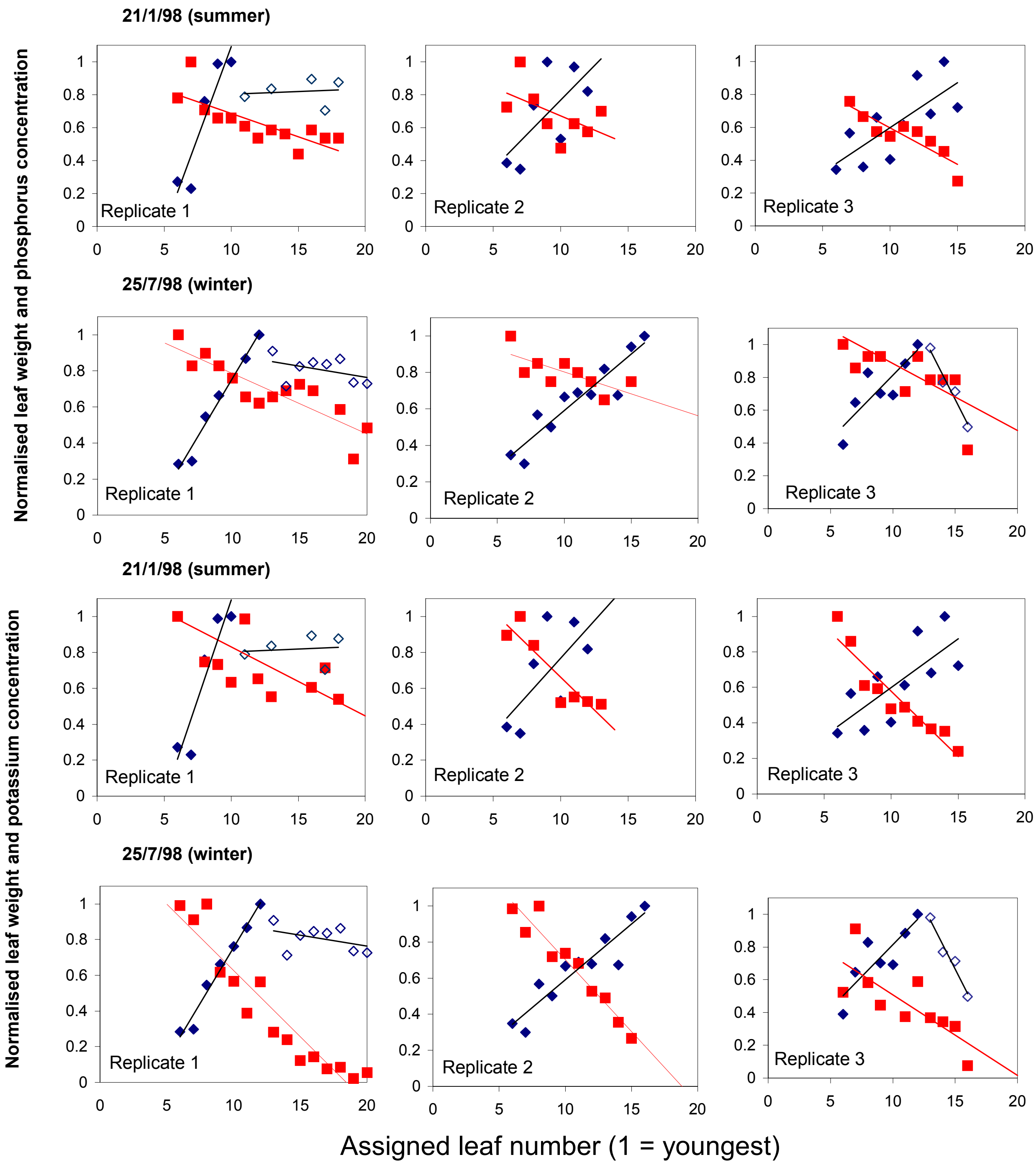


Figure 6.11: The relationship between normalised phosphorus and potassium concentrations (squares), normalised leaf weight (diamonds) and leaf number for individual *I. pes-caprae* replicates sampled on the 21/1/98 (summer) and on the 25/7/98 (winter). Monophasic curves were fitted with a single linear regression and biphasic curves fitted with two linear regressions. Monophasic and portion one of the biphasic responses are shown with soild symbols. Portion two of the biphasic responses is shown with open symbols. Decisions as to which point should be included in the particular regression were based on maximising the r^2 values.

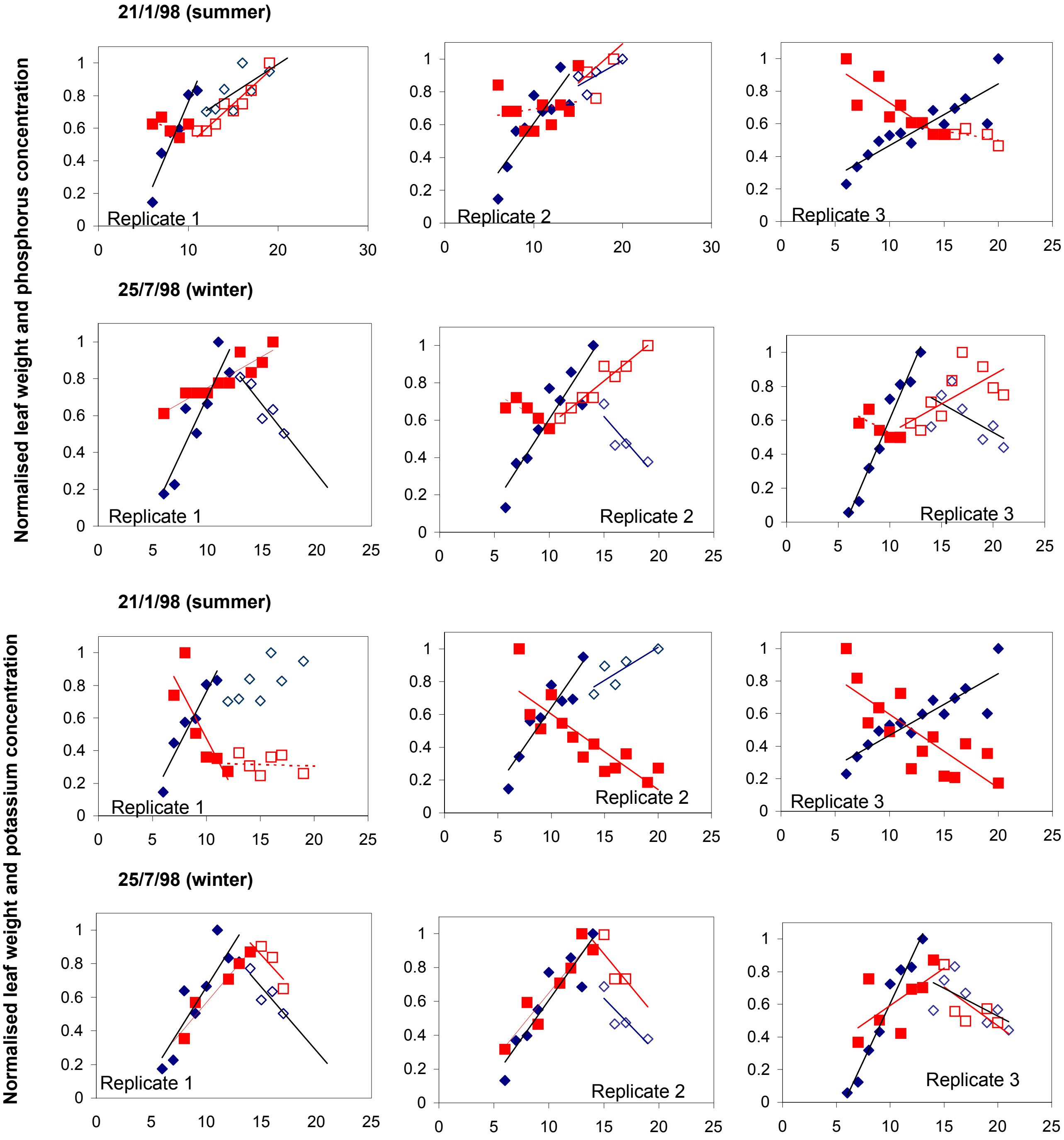


Figure 6.12: The relationship between normalised phosphorus and potassium concentrations (squares), normalised leaf weight (diamonds) and leaf number for individual *S. plumieri* replicates sampled on the 21/1/98 (summer) and on the 25/7/98 (winter). Monophasic curves were fitted with a single linear regression and biphasic curves fitted with two linear regressions. Monophasic and portion one of the biphasic responses are shown with soild symbols. Portion two of the biphasic responses is shown with open symbols. Decisions as to which point should be included in the particular regression were based on maximising the r^2 values.

Appendix C

Non-photochemical (q_{NP}), photochemical quenching (q_p) and PSII efficiency (Fv'/Fm').

These parameters (q_{NP}, q_P and Fv'/Fm') were measured using the pulse-amplitude modulated method. The rationale of this is simple: on application of a sufficiently intense pulse of light, Q_A is fully reduced and hence photochemical fluorescence quenching becomes suppressed; the remaining quenching is non-photochemical.

In practice the leaf was dark adapted to ensure complete oxidation of Q_A and in this state the minimal and maximal yields Fo and Fm were determined. During illumination (in this case at the intensities used for the gaseous exchange measurements) steady state fluorescence was measured (Fs). Then with the help of superimposed saturating pulses the changed levels of maximum yield (Fm') were detected. Fo is affected by the processes of non-photochemical quenching (Bilger and Schreiber, 1986) and this altered parameter is termed Fo'. Fo' was determined upon sample darkening and the application of weak far-red background light for PSI driven Q_A oxidation.

Then:

$$q_{NP} = \frac{Fm - Fm'}{Fm - Fo}$$

$$q_P = \frac{Fm' - Fs}{Fm' - Fo}$$

$$Fv'/Fm' = \frac{Fm' - Fo'}{Fm'}$$

References

Abrahamson WG and Caswell H (1982) On the comparative allocation of biomass, energy and nutrients in plants. *Ecology*, 63, 982-991.

Adams WW III (1988) Photosynthetic acclimation and photoinhibition of terrestrial and epiphytic CAM tissues growing in full sunlight and deep shade. *Australian Journal of Plant Physiology*, 15, 123-134.

Adams WW III and Barker DH (1998) Seasonal changes in xanthophyll cycle-dependent energy dissipation in *Yacca glauca,* Nattall. *Plant, Cell and Environment*, 21, 501-511.

Adams WW III and Demmig-Adams B (1992) Operation of the xanthophyll cycle in higher plants in response to diurnal changes in incident sunlight. *Planta*, 186, 390-398.

Adams WW III, Demmig-Adams B, Barker DH and Kiley S (1996) Carotenoids and photosystem II. characteristics of upper and lower halves of leaves acclimated to high light. *Australian Journal of Plant Physiology*, 23, 669-677.

Adams WW III, Terashima I, Brugnoli E and Demmig B (1988) Comparisons of photosynthesis and photoinhibition in the CAM vine *Hoya australis* and several C_3 vines growing on the coast of eastern Australia. *Plant, Cell and Environment*, 11, 173-181.

Ahmad MH and Neckelmann J (1978) N_2-fixation by the roots and rhizosphere of sand dune plants. *Zeitschrift fuer Planznernaehrung und Bodenkunde*, 141, 171.

Allen SE (1974) Chemical analysis of ecological materials. Blackwell, Oxford.

Antonovics J, Clay K and Schmitt J (1987) The measurement of small scale environmental heterogeneity using clonal transplants of *Anthoxanthum odoratum* and *Danthonia spicata. Oecologia*, 71, 601-607.

Art HW, Bornmann FH, Voigt GK and Woodwell GM (1974) Barrier island forest ecosystem: Role of meteorological inputs. *Science*, 184, 60-62.

Avis AM (1992) Coastal dune ecology and management in the Eastern Cape. Ph.D. thesis, Rhodes University, Grahamstown, South Africa.

Avis AM and Lubke RA (1985) The effect of wind-borne sand and salt spray on the growth of *Scirpus nodulosus* in a mobile dune system. *South African Journal of Botany*, 51, 100-110.

Bach CE (1998) Interactive effects of herbivory and sand burial on growth of a tropical dune plant, *Ipomoea pes-caprae. Ecological Entomology,* 23, 238-245.

Bach CE (2000) Effects of clonal integration on response to sand burial and defoliation by the dune plant *Ipomoea pes-caprae* (Convolvulaceae). *Australian Journal of Botany*, 48, 159-166.

Baines KA (1989) The water use, growth and phenology of four savanna grass species in response to changing soil moisture availability. M.Sc. thesis, University of Witwatersrand, Johannesburg, South Africa.

Balarin M (1996) The effect of sand burial on seed germination and seedling establishment on foredune systems of three dune pioneer species. B.Sc. (Honours) thesis, Rhodes University, Grahamstown, South Africa.

Baldocchi DD (1993) Scaling water vapour and carbon dioxide exchange from leaves to a canopy: Rules and tools. In: Scaling physiological processes. JR Ehleringer and CB Field (eds). Academic Press, Inc., San Diego, USA, 77-114.

Barbour MG (1978) Salt spray as a microenvironmental factor in the distribution of beach plants at Point Reyes, California. *Oecologia*, 32, 213-224.

Barbour MG (1990) The coastal beach plant syndrome. *Proceedings Canadian Symposium on coastal Sand Dunes*. 197-214.

Barbour MG (1992) Life at the leading edge: The beach plant syndrome. In: Coastal plant communities of Latin America. V. Seeliger (ed). Academic Press Inc., San Diego, 291- 306.

Barbour MG, Craig RB, Drysdale FR and Ghiselin MT (1973) Coastal ecology: Bodega Head. University of California Press, Berkeley, CA.

Barbour MG and DeJong TM (1977) Response of west coast beach taxa to salt spray, seawater inundation and soil salinity. *Bulletin of the Torrey Botanical Club,* 104, 2-34.

Barbour MG, DeJong TM and Pavlik BM (1985) Marine beach and dune plant communities. In: Physiological ecology of North American plant communities. BF Chabot and HA Mooney (eds). Chapman and Hall, New York.

Barbour MG, Rejmanek M, Johnson AF and Pavlik BM (1987) Beach vegetation and plant distribution patterns along the northern Gulf of Mexico. *Phytocoenologia,* 15(2), 201-233.

Barbour MG and Robichaux RH (1976) Beach phytomass along the Californian coast. *Bulletin of the Torrey Botanical Club*, 103, 16-20.

Baroli I and Melis A (1998) Photoinhibitory damage is modulated by the rate of photosynthesis and by the photosystem II light-harvesting chlorophyll antenna size. *Planta*, 205, 288-296.

Bazzaz FA (1993) Scaling in biological systems: Population and community perspectives. In: Scaling physiological processes. Leaf to globe. JR Ehleringer and CB Field (eds). Academic Press Inc., San Diego.

Bazzaz FA and Carlson RW (1982) Photosynthetic acclimation to variability in the light environment of early and late sucessional plants. *Oecologia*, 54, 313-316.

Beadle CL, Ludlow MM and Honeysett JL (1993) Water relations. In: Photosynthesis and production in a changing environment. DO Hall, JMO Scurlock, HR Bolhar-Nordenkampf, RC Leegoed and SP Long (eds). Chapman and Hall, London, 113-128.

Beale CV and Long SP (1995) Can perennial grasses attain high efficiencies of radiant energy conversion in cool climates? *Plant Cell and Environment*, 18, 641-650.

Bewley JD and Krochko JE (1982) Desiccation tolerance. In: Encyclopedia of Plant Physiology. New series12B. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 325-378.

Bielher K and Fock H (1996) Evidence for the contribution of the Mehler-peroxidase reactions in dissipating excess electrons in drought stressed wheat. *Plant Physiology*, 112, 265-272.

Bilger W and O Bjorkman (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynthesis Research* 25, 173-185.

Bilger W and Schrieber U (1986) Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. *Photosynthesis Research*, 10, 303-308.

Bjorkman O (1973) Comparative studies on photosynthesis in higher plants. In: Current topics in photobiology, photochemistry and photophysiology. A Giese (ed). Vol 8, Academic Press, New York, 1-63.

Bjorkman O (1981) Responses to different quantum flux densities In: Encyclopedia of Plant Physiology. New series12A. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 57-107.

Blackmore AC (1992) The functional classification of South African savanna plants based on their ecophysiological characteristics. M.Sc. thesis, University of Witwatersrand, Johannesburg, South Africa.

Bliss LC (1985) Alpine. In: Physiological ecology of North American plant communities. BF Chabot and HA Mooney (eds). Chapman and Hall, New York, 41-65.

Bolhar-Nordenkampf HR, Hofer M and Lechner EG (1991) Analysis of light-induced reduction of the photochemical capacity in field-grown plants. Evidence for photoinhibition? *Photosynthesis Research*, 27, 31-39.

Bowen BJ and Pate JS (1991) Adaptations of S.W. Australian members of the Proteaceae: allocation of resources during early growth. In: Proceedings of the International Protea Association sixth biennial conference, Perth: Promaco Conventions, 289-301.

Boyce SG (1954) The salt spray community. *Ecological Monographs*, 24, 29-69.

Boyer JS (1995) Measuring the water status of plants and soils. Academic Press, USA.

Bradford KJ and Hsiao TC (1982) Physiological responses to moderate water stress. In: Encyclopedia of Plant Physiology. New series 12B. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 263-324.

Bray RH and Kurtz LT (1945) Determination of total organic and available forms of phosphorus in soils. *Soil Science*, 59, 39-45.

Brestic M, Cornic G, Fryer MJ and Baker NR (1995) Does photorespiration protect the photosynthetic apparatus in French bean leaves from photoinhibition during drought stress? *Planta*, 196, 450-457.

Brown JF (1997) Effects of experimental burial on survival, growth and resource allocation of three species of dune plants. *Journal of Ecology,* 85, 151-158.

Brown AC and McLachlan A (1990) Ecology of sandy shores. Elsevier, Amsterdam.

Buckman HO and Brady NC (1969) The nature and properties of soils. Seventh edition, Collier-Macmillan Limited, USA.

Bunce JA, Miller LN and Chabot BF (1977). Competitive exploitation of soil reserves by five eastern North American tree species. *Botanical Gazette*, 138, 168-173.

Burke MJ and Stushnoff C (1979) Frost hardiness: A discussion of possible molecular causes of injury with particular reference to deep supercooling of water. In: Stress physiology in crop plants. H Mussell and RC Staples (eds). Wiley, New York, 197-225.

Burns MER and Lubke RA (1986) Succession and zonation in the East London dune forests. *The Naturalist*, 30(3), 38-44.

Buysse J and Merckx R (1993) An improved colourimetric method to quantify sugar content in plant tissue. *Journal of Experimental Botany*, 44, 1627-1629.

Cain ML, Subler S, Evans JP and Fortin M-J (1999) Sampling spatial and temporal variation in soil nitrogen availability. *Oecologia*, 118, 397 – 404.

Caldwell D (1985) Cold desert. In: Physiological and ecology of North American plant communities. BF Chabot and HA Mooney (eds). Chapman and Hall, London, 198-212.

Caldwell MM, White RW, Moore RT and Camp LB (1977) Carbon balance, productivity and water use of cold-winter desert shrub communities dominated by C_3 and C_4 species. *Oecologia,* 29, 275-300.

Clayton JL (1972) Salt spray and mineral cycling of two Californian coastal ecosystems. *Ecology*, 53, 74-81.

Campbell EE, Parker-Nance T and Bate GC (1992) A compilation of information on the magnitude, nature and importance of coastal aquifers in Southern Africa. Water Research Commission, WRC Report No 370/1/92.

Chapman VJ (1976) Coastal vegetation. Second edition, Pergamon Press, Oxford, England.

Chapin FS (III) and van Cleve K (1989) Approaches to studying nutrient uptake, use and loss in plants. In: Plant physiological ecology. RW Pearcy, J Ehleringer, HA Mooney and PW Rundel (eds). Chapman Hall, London, 185-208.

Cheplick GP and Demetri H (1999) Impact of saltwater spray and sand deposition on the coastal annual *Triplasis purpurea* (Poaceae). *American Journal of Botany,* 86(5), 703-710.

Clements FE (1916) Plant succession. Carnegie Inst. Washington. Pub. 242.

Clifford SC, Arndt SK, Corlett JE, Joshi S, Sankhla N, Popp M and Jones HG (1998) The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Zizphus mauritiana* (Lamk.). *Journal of Experimental Botany*, 49, 967-977.

Clifton-Brown JC and Jones MB (1999) Alteration of the transpiration rate by changing air vapour pressure deficit, influences leaf extension rate transiently in *Miscanthus*. *Journal of Experimental Botany*, 50, 1393-1401.

Coombs J (1985). Carbon metabolism. In: Techniques in bioproductivity and photosynthesis. J Coombs, DO Hall, SP Long and JMO Surlock (eds). Pergamon Press, Oxford, 139-157.

Corkidi L and Rincon E (1997) Arbuscular mycorrhizae in tropical sand dune ecosystem on the Gulf of Mexico. *Mycorrhiza*, 7, 17-23.

Cowan IR (1982) Regulation of water use in relation to carbon gain in higher plants. In: Encyclopedia of Plant Physiology. New series 12B. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 589-613.

Cowan IR and Farquhar GB (1977) Stomatal function in relation to leaf metabolism and environment. In: Integration of the activity in the higher plant. DH Jennings (ed). Cambridge University Press, Cambridge, 471-505.

Cowles HC (1899) The ecological relations of the vegetation on the sand dunes of Lake Michigan. *Botanical Gazette*, 27, 95-117; 167-202; 281-308; 361-391.

Critchley C and Smillie RM (1981) Leaf chlorophyll fluorescence as an indicator of high light stress (Photoinhibition) in *Cucumis sativus* L. *Australian Journal of Plant Physiology*. 8, 133-141.

Critchley C (1988) The molecular mechanism of photoinhibition - Facts and fiction. *Australian Journal of Plant Physiology*, 15, 27-41.

Dainty J (1977) The ionic and water relations of plants which adjust to a fluctuating saline environment. In: Ecological processes in coastal environments. RL Jefferies and AJ Davy (eds). Blackwell Scientific Publications, UK, 201-210.

Davis Instrument Corporation (1994) Weatherlink version 3.01. Davis Instruments Corporation, Hayward, California.

Davy AJ and Figueroa ME (1993) The colonization of strandlines. In: Primary succession on land. J Miles and DWH Walton (eds). Special publication series of the British Ecological Society, Number 12, Blackwell Scientific, 113 –131.

Day JM, Neves MCP and Dobereiner J (1975) Nitrogenase activity of the roots of tropical forage grasses. *Soil Biology and Biochemistry*, 7, 107-112.

Dawson TE and Ehleringer JR (1991) Streamside trees that do not use stream water. *Nature*, 350, 335-337.

De Jong TM (1978) Comparative gas exchange of four California beach taxa. *Oecologia*, 34, 343-351.

De Jong TM (1979) Water and salinity relations of Californian beach species. *Journal of Ecology*. 67, 647-663.

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. *Biochimica et Biophysica Acta*, 1020, 1-14.

Demmig-Adams B and Adams WW III (1996) Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. *Planta*, 198, 460 – 470.

Demmig-Adams B and Adams WW III (1992) Photoprotection and other responses of plants to high light stress. *Annual Review of Plant Physiology and Plant Molecular Biology,* 43, 599-626.

Demmig B and Bjorkman O (1987) Comparisons of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O_2 evolution in leaves of higher plants. *Planta*, 171, 171-184.

Demmig B, Winter K, Kruger A and Czygan F-C (1988) Zeaxanthin and the heat dissipation of excess light energy in *Nerium oleander* exposed to a combination of high light and water stress. *Plant Physiology,* 87, 17-24.

Devall MS (1992) The biological flora of coastal dunes and wetlands.2. *Ipomoea pes-caprae* (L.) Roth. *Journal of Coastal Research*, 8,442-456.

Devall MS and Thien LB (1989) Factors influencing the reproductive success of *Ipomoea pes-caprae* (Convulvaceae) around the Gulf of Mexico. *American Journal of Botany,* 76, 1821-1831.

Doing H (1985) Coastal foredune zonation and succession in various parts of the world. *Vegetatio,* 65, 65-75.

Drennan P and Pammenter NW (1982) Physiology of salt excretion in the mangrove *Avicennia marina* (Forsk.) Vierh. *New Phytologist,* 91, 597-606.

Deshmukh IK (1977) Fixation, accumulation and release of energy by *Ammophila arenaria* in a sand dune succession. In: Ecological processes in coastal environments in a sand dune succession. RL Jefferies and AJ Davy (eds). Blackwell Scientific Publications, London, 353-362.

Donnely FA and Pammenter NW (1983) Vegetation zonation on a Natal coastal sand-dune system in relation to salt spray and soil analysis. *South African Journal of Botany*, 2, 46-51.

Dougherty KM, Mendelssohn IA and Monteferrante FJ (1990) Effect of nitrogen, phosphorus and potassium additions on plant biomass and soil nutrient content of a swale barrier strand community in Louisiana. *Annals of Botany*, 66, 265-271.

Dyer RA (1967) The genera of southern African flowering plants, volume 1, dicotyledons. Department of Agriculture and Technical Services.

Ehleringer JR (1982) The influence of water stress and temperature on leaf pubescence development in *Encelia farinosa*. *American Journal of Botany,* 69(5), 670-675.

Ehleringer JR (1983) Ecophysiology of *Amaranthus palmeri*, a Sonoran Desert ephemeral. *Oecologia*, 57,107-112.

Ehleringer JR (1984) Ecology and ecophysiology of leaf pubescence in North American desert plants. In: Biology and chemistry of plant trichomes. E Rodriguez, PL Healey and I Mehta (eds). Plenum Press, London, 132-133.

Ehleringer JR (1993) Gas exchange implications of isotopic variation in arid land plants. In: Plant responses to water deficit. Environmental biology series. H Griffiths and J Smith (eds). BIOS Scientific Publishers, London, 265-284.

Ehleringer JR and Bjorkman O (1978a) Pubescence and leaf spectral characteristics in a desert shrub, *Encelia farinosa. Oecologia,* 37, 183-200.

Ehleringer JR and Bjorkman O (1978b) A comparison of photosynthetic characteristics of *Encelia* species possessing glabrous and pubescent leaves*. Plant Physiology,* 62, 185-190.

Ehleringer JR, Bjorkman O and Mooney HA (1976). Leaf pubescence: Effects on absorptance and photosynthesis in a desert shrub. *Science,* 192, 376-377.

Ehleringer JR and Cooper TA (1988) Correlations between carbon isotope ratio and microhabitats in desert plants. *Oecologia*, 76, 562-566.

Ehleringer JR and Dawson TE (1992) Water uptake by plants: Perspectives from stable isotope composition. *Plant, Cell and Environment*, 15, 1073-1082.

Ehleringer JR and Mooney HA (1978) Leaf hairs: Effects on physiological activity and adaptive value to a desert shrub. *Oecologia,* 37, 183-200.

Ehrenfeld JG (1990) Dynamics and processes of barrier island vegetation. *Review in Aquatic Sciences*, 2, 437-480.

Elfman B, Maun MA and Hopkins WG (1986) Population biology of *Ammophila breviligulata* and *Calamovilfa longifolia* on Lake Huron sand dunes. II. Ultra structure of organelles and photosynthetic properties. *Canadian Journal of Botany*, 64, 2151-2159.

Ernst WHO (1983) Element nutrition of two contrasted dune annuals. *Journal of Ecology*, 71, 197-209.

Falkowski PG, Fujita Y, Ley A and Mauzerall D (1986) Evidence for cyclic electron flow around Photosystem II in *Chlorella pyrenoidosa*. *Plant Physiology*, 81, 310-312.

Farage PK and Long SP (1987) Damage to maize photosynthesis in the field during periods when chilling is combined with high photo fluxes. In: Progress in photosynthesis research. Vol IV. J Biggins (ed). Marius Nijhoff, Dordrecht, 139-142.

Farage PK and Long SP (1991) The occurrence of photoinhibition in an over-winter crop of oil-rape (*Brassica napus* L.) and its correlation with changes in crop growth. *Planta*, 185, 279-286.

Farquhar GD and Caemmerer von S (1982) Modelling of photosynthetic response to environmental conditions. In: Encyclopedia of Plant Physiology. New series12B. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 549-587.

Farquhar GD, Caemmerer von S and Berry JA (1980) A biochemical model of photosynthetic CO_2 fixation in leaves of C_3 species. *Planta*, 149, 78-90.

Farquhar GD and Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology,* 33, 317-45.

Fay PJ and Jeffrey DW (1992) The foreshore as a nitrogen source for *A. arenaria* grass. In: Coastal dunes. RWG Carter, TFG Curtis and MJ Sheehy-Skeffington (eds). Balkema, Rotterdam, 177-188.

Fernandez-Baco L, Figueroa ME, Luque T and Davy AJ (1998) Diurnal and seasonal variations in chlorophyll fluorescence in two Mediterranean-grassland species under field conditions. *Photosynthetica*, 35(4), 535-544.

Fink BH and Zedler JB (1990) Maritime stress tolerance studies in California dune perennials. *Madrono*, 37, 200-213.

Fitter AH and Hay RKM (1987) Environmental physiology of plants. Second edition. Academic Press, UK.

Flanagan LB, Erhleringer JR and Marshall JD (1992) Differential uptake of summer precipitation among co-occurring trees and shrubs in a pinyon-juniper woodland. *Plant, Cell and Environment*, 15, 831-836.

Gabrielsen EK (1948) Effects of different chlorophyll concentrations on photosynthesis in foliage leaves. *Plant Physiology*, 1, 5-37.

Garcia-Mora MR, Gallego-Fernandez JB and Garcia-Novo F (1999) Plant functional types in coastal foredunes in relation to environmental stress and disturbance. *Journal of Vegetation Science*, 10, 27-34.

Garcia-Novo F (1976) Ecophysiological aspects of the distribution of *Elymus arenarius* and *Cakile maritima* on the dunes of Tents-Muir point (Scotland). *Oecologia Plantarum*, 11(1), 13-24.

Garcia-Novo F (1979) The ecology of vegetation of the dunes in Donana National Park (South-West Spain). In: Ecological processes in coastal environments. RL Jefferies and AJ Davy (eds). Blackwell, Oxford, 571-592.

Garcia-Plazaola JI, Artetxe U and Becerril JM (1999) Diurnal changes in antioxidant and carotenoid composition in the Mediterranean scherophyll tree *Quercu ilex* (L) during winter. *Plant Science*, 143, 125-133.

Gat JR (1980) The isotopes of hydrogen and oxygen in precipitation. In: Handbook of environmental isotope geochemistry. Volume 1. The terrestrial environment. P Fritz and JC Fontes (eds). Elsevier, Amsterdam, 21-47.

Genty B, Briantais J-M and Baker NR (1989) The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta*, 990, 87-92.

Gerlach A (1993) Biogeochemistry of nitrogen in a coastal dune succession on Spiekeroog (Germany) and the impact of climate. *Phytocoenologia*, 23, 115- 127.

Gerlach A, Albers EA and Broedlin W (1994) Development of the nitrogen cycle in the soils of a coastal dune succession. *Acta Botanica Neerlandica*, 43(2), 189-203.

Gilmore AD, Hazlett TL Debrunner PG and Govindjee (1996) Photosystem II chlorophyll a fluorescence lifetimes and intensity are independent of the antenna size differences between barley wild-type and chlorina mutants: Photochemical quenching and xanthophyll cycle-dependent non-photochemical quenching of fluorescence. *Photosynthesis Research*, 48, 171-187.

Gilmore AM and Yamamoto HY (1991) Zeaxanthin formation and energy-dependent fluorescence quenching in pea chloroplasts under artificially mediated linear and cyclic electron transport. *Plant Physiology*, 96, 635-643.

Goff JA and Gatch S (1946) Low-pressure properties of water from –160^{o} F to 212^{o} F. *Transactions of the American Society of Heating and Ventilation Engineers*, Vol. 52, 95-121.

Goldberg ED (1963) Chemistry. In: The sea. Ideas and observations on progress in the study of seas. John Wiley Interscience. New York, 4-5.

Gorham J, Hughes LI and Wyn- Jones RG (1981) Low-molecular-weight carbohydrates in some salt stressed plants. *Physiologica Plantarum*, 53, 27-33.

Grace J and Russel G (1982) The effect of wind and reduced water supply on the growth and water relations of *Festuca arundinacea* (Schreb). *Annals of Botany*, 49, 217-225.

Grammattikopoulos G, Karabourniotis G, Kyparissis S, Petropoulou Y and Manetas Y (1994) Leaf hairs of Olive (*Olea europaea*) prevent stomatal closure by ultraviolet-B radiation. *Australian Journal of Plant Physiology, 21*, 293-301.

Grier CC and Running SW (1977) Leaf area of mature northwestern coniferous forests: Relation to site water balance. *Ecology*, 58, 83-899.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and animals and its relevance to ecological and evolutionary theory. *American Naturalist*, 111, 1169-1194.

Grime JP (1979) Plant strategies and vegetation processes. Wiley, Chichester.

Grime JP (1998) Report of the unit of comparative plant ecology. Unit of Comparative Plant Ecology, Department of Comparative Plant Sciences, The University of Sheffield, Sheffield, U.K.

Grime JP, Hodgson JG, Hunt R, Thompson K, Hendry GAF, Cambell BD, Jalili A, Hillier SH, Diaz S and Burke MJW (1997) Functional types: Testing the concept in Northern England. In: Plant functional types. TM Smith, HH Shugart and FI Woodward (eds). Cambridge University Press, Cambridge, 123-151.

Groom QJ and Baker NR (1992) Analysis of light induced depressions of photosynthesis in leaves of a wheat crop during the winter. *Plant Physiology*, 22, 261-266.

Grootjans AP, Ernst WHO and Stuyfzand PJ (1998) European dune slacks: strong interactions of biology, pedogenesis and hydrology. *Tree*, 13, 96-100.

Guppy HB (1917) Plants, seeds and currents in the West Indies and Azores. Williams and Norgate, London.

Haldimann P (1997) Chilling-induced changes to carotenoid composition, photosynthesis and the maximum quantum yield of photosystem II photochemistry in two maize genotypes differing in tolerance to low temperature. *The Journal of Plant Physiology*, 151, 610-619.

Hall AE and Schultze E-D (1980) Stomatal response to environment and a possible interrelation between stomatal effects on transpiration and CO_2 assimilation. *Plant Cell Environment*, 3, 467-474.

Haller AHA (2000) The presence and role of arbuscular mycorrhizal fungi in coastal sand dune systems. M.Sc. Thesis, Rhodes University, Grahamstown, South Africa.

Hanson AD and Hitz WD (1982) Metabolic responses of mesophytes to plant water deficits. *Annual Review of Plant Physiology*, 33, 163-203.

Harman KT (2000) The genetic diversity of *Scaevola plumieri* L. (Goodeniaceae), an indigenous South African dune coloniser, as revealed by Inter-Simple Sequence Repeat (ISSR) Fingerprinting. B.Sc. (Honours) thesis. Rhodes University, Grahamstown, South Africa.

Harte JM and Pammenter NW (1983) Leaf nutrient content in relation to senescence in the coastal sand dune pioneer *Scaevola thunbergii* Eckl. & Zeyh. *South African Journal of Science*, 79, 420-422.

Harris D and Davy AJ (1988) Carbon and nutrient allocation in *Elymus farctus* seedlings after burial with sand. *Annals of Botany*, 61, 147-157.

Havaux M, Strasser RJ and Greppin H (1991) A theoretical and experimental analysis of the qp and qn coefficients of chlorophyll fluorescence quenching and their relation to photochemical and non-photochemical events. *Photosynthesis Research*, 27, 41-55.

Haxeltine A and Prentice IC (1996) A general model for the light-use efficiency of primary production. *Functional Ecology*, 10, 551-561.

Heber U, Bligny R, Streb P and Douce R (1996) Photorespiration is essential for the protection of the photosynthetic apparatus of C3 plants against photoinactivation under sunlight. *Botanica Acta*, 109, 307-315.

Hertling UM (1997) *Ammophila arenaria* (L.) Link (Marram grass) in South Africa and its potential invasiveness. Ph.D. thesis, Rhodes University, Grahamstown, South Africa.

Hesketh JD (1980) Predicting canopy photosynthesis from gas exchange studies in controlled environments. In: Predicting photosynthesis for ecosystem models Vol I. JD Hesketh and JW Jones (eds). CRC Press, Boca Raton, 37-50.

Hesp PA (1983) Morphodynamics of incipient foredunes in N.S.W. Australia. In: Eolian sediments and processes. ME Brookfield and TS Ahlbrandt (eds). Academic Press, London, 325-342.

Hesp PA (1989) A review of the biological and geomorphological processes involved in the initiation and development of incipient foredunes. In: Coastal sand dunes. CH Gimingham, W Ritchie, BB Silletts and AJ Willis (eds). Proceedings of the Royal Society, Edinburgh, 96, 181-202.

Hesp PA (1991) Ecological processes and plant adaptations on coastal dunes. *Journal of Arid Environments,* 21, 165-191.

Hesp PA and McLachlan A (2000) Morphology, dynamics, ecology and fauna of *Arctotheca populifolia* and *Gazamia rigens* nabkha dunes. *Journal of Arid Environments*, 44, 155-172.

Hinckley TM, Dougherty PM, Lassoie JP, Roberts JE and Tesky RO (1979) A severe drought: Impact on tree growth phenology, net photosynthetic rate and water relations. *American Midland Naturalist*, 102, 307-316.

Holton B (1980) Some aspects of the nitrogen in a Californian coastal dune ecosystem, with emphasis on C*akile maritima.* Ph.D. thesis, University of California, Davis, California.
Holton B and Johnson AF (1979) Dune scrub communities and their correlation with environmental factors at Point Reyes National Seashore, California. *Journal of Biogeography*, 6, 317-328.

Hsiao TC, Acevedo E, Fereres E and Henderson DW (1976) Stress metabolism: Water stress, growth and osmotic adjustment. *Philosophical Transactions Royal Society London,* Series B, 273, 479-500.

Huiskes AHL (1979) *Ammophila arenaria* (L.) Link. biological flora of the British Isles. *Journal of Ecology*, 67, 363-382.

Huiskes AHL(1980) The effects of habitat perturbation on leaf populations of *Ammophila Arenaria* (L.) link. *Acta Botanica Neerlandica*, 29(5/6), 443-450.

Huiskes AHL and Harper JL (1979) The demography of *Ammophila arenaria* in a dune sere*. Oecologia Plantarum,* 14, 435-446.

Hyde R and Wasson RJ (1983) Radiative and meteorological control on the movement of sand at Lake Mungo, NSW, Australia. *Aeolian Processes and Sediments*, 38, 311-323.

Ishikawa S, Oikawa T and Furukawa A (1991) Responses of photosynthesis, leaf conductance and growth to different salinities in three coastal dune plants. *Ecological Research*, 6, 217 – 226.

Jarvis PG and Leverenz JW (1983). Productivity of temperate, deciduous and evergreen forests. In: Encyclopedia of Plant Physiology. New series12D. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 233-280.

Jefferies RL and Davy AJ (1977) Ecological processes in coastal environments. Blackwell Scientific Publications, London, 353-363.

Jefferies RL, Davy AJ and Rudmik T (1977) The growth strategies of coastal halophytes. In: Ecological processes in coastal environments. RL Jefferies and AJ Davy (eds). Blackwell Scientific Publications, London, 243-268.

Jeffrey C (1979) On the nomenclature of the strand *Scaevola* species (Goodeniaceae). *Kew Bulletin* 34(3), 537-545.

Jeschke WD and Pate JS (1991) Cation and chloride partitioning through xylem and phloem within the whole plant of *Ricinus communis* L. under conditions of salt stress. *Journal of Experimental Botany*, 42, 1105-1116.

Jones HG (1983) Plants and microclimate. Cambridge University Press, Cambridge, UK.

Judd FW, Lonard RI and Sides SL (1977) The vegetation of South Padre Island, Texas in relation to topography. *The Southwestern Naturalist*, 22, 31-48.

Karabourniotis G, Kyparissis A and Manetas Y (1993) Leaf hairs of *Olea europaea* protect the underlying tissues against ultraviolet-B radiation damage. *Environmental and Experimental Botany,* 33 (2), 341-345.

Ketelaar R (1998) Sand trapping capacity of different vegetation types and patterns of sand transport in the foredunes of the south eastern Cape, South Africa. M.Sc. thesis. University of Amsterdam, Netherlands.

Kitajima M and Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochimica et Biophysica Acta*, 375, 105-115.

Knevel I - Ph.D. student researching the phenology of coastal dune pioneers, Botany Department, Rhodes University, Grahamstown, South Africa.

Koide RT, Robichaux RH, Morse SR and Smith CM (1989) Plant water status, hydraulic resistance and capacitance. In: Plant physiological ecology. RW Pearcy , J Ehleringer, HA Mooney and PW Rundel (eds). Chapman Hall, London, 161-184.

Koontz JA, Guttman IS and Eshbaugh WH (1996) *Scaevola plumieri* and *S. taccada* on Andros Island: Is it hybridization or morphological plasticity? In: Proceedings of the 6th Symposium of the natural history of the Bahamas. NB Elliot, DC Edwards and PJ Godfrey (eds). Bahamian Field Station, San Salvador, Bahamas.

Kozaki A and Takeba G (1996) Photorespiration protects C3 plants from photooxidation. *Nature*, 384, 557-560.

Kramer PJ (1983) Water relations of plants. Academic Press, London.

Krause GH (1988) Photoinhibition of photosynthesis: An evaluation of damaging and protective mechanisms. *Physiologica Plantarum*, 74, 566-574.

Krause GH and Cornic G (1987) CO_2 and O_2 interactions in photoinhibition. In: Photoinhibition. DJ Kyle, CB Osmond and CJ Arntzen (eds). Elsevier, Amsterdam, 169-196.

Kruger FJ (1977) A preliminary account of aerial plant biomass in fynbos communities of the mediterranean-type climate zone of the Cape Province*. Bothalia*, 12, 301-307.

Kruger GHJ, Tsimilli-Michael M and Strasser RJ (1997) Light stress provokes plastic and elastic modifications in structure and function of Photosystem II in camellia leaves. *Physiologica Plantarum*, 101, 265-277.

Lambers H (1985) Respiration in intact plants and tissues: Its regulation and dependence on environmental factors, metabolism and invaded organisms. In: Encyclopedia of Plant Physiology. New series18. R Douce and DA Day (eds). Springer-Verlag, Germany, 418-473.

Lammerts EJ and Grootjans AP (1997) Nutrient deficiency in dune slack pioneer vegetation: A review. *Journal of Coastal Conservation,* 3, 87-168.

Lang M and Schindler C (1994) The effects of leaf hairs on blue and red fluorescence emission and zeaxanthin cycle performance of *Senecio medley* L. *Journal of Plant Physiology*, 144, 680-685.

Lang A and Thorpe MR (1989) Xylem, phloem and transpiration flows in grape: Application of a technique for measuring the volume of attached fruits to high resolution using Archimedes' principal. *Journal of Experimental Botany,* 40, 1069-1078.

Lechowicz MJ, Hellens LE and Simon JP (1980) Latitudinal trends in the response of growth respiration and maintenance respiration to temperature in the beach pea, *Lahyrus japonicus. Canadian Journal of Botany*, 58, 1521-1524.

Lee JA, Hermer R and Ignaciuk R (1983) Nitrogen as a limiting factor in plant communities. In: Nitrogen as an ecological factor. JA Lee, S McNiel and IH Rorison (eds). Blackwell Scientific Publications, Oxford, 94 –112.

Leopald AC and Kriedeman PE (1975) Plant growth and development. Second Edition. McGraw Hill, New York.

Lichtenhaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology,* 148, 349-382.

Little LR and Maun MA (1996) The '*Ammophila* problem' revisited: a role for mycorrhizal fungi. *Journal of Ecology*, 84, 1-7.

Long SP and Hallgren J-E (1985) Measurement of CO_2 assimilation by plants in the field and the laboratory. In: Photosynthesis and production in a changing environment. DO Hall, JMO Surlock, HR Bolhar-Nordenkampf, RC Leegoed and SP Long (eds). Chapman and Hall, London, 129-167.

Long SP and Humphries S (1994) Photoinhibition and photosynthesis in nature*. Annual Review of Plant Physiology and Plant Molecular Biology*, 45, 633-62.

Long SP and Woolhouse HW (1979) Primary production in *Spartina* marshes. In: Ecological processes in coastal environments. RL Jefferies and AJ Davy (eds). Blackwell Scientific, Oxford, 333-352.

Lubke RA- Associate Professor, Botany Department, Rhodes University, Grahamstown, South Africa.

Lubke RA (1983) A survey of the coastal vegetation near Port Alfred, Eastern Cape. *Bothalia,* 14, 725-738.

Lubke and Avis (1982) Factors effecting the distribution of *Scirpus nodosus* plants in a dune slack community. *South African Journal of Botany*, 1, 163-164.

Lubke RA, Avis AM, Steinke TD & Boucher C (1997) Coastal vegetation. In: Vegetation of southern Africa. RA Cowling, DM Richardson and SM Pierce (eds). Cambridge University Press, Cambridge, 300-321.

Lubke RA, Tinley KL and Cowling RM (1988) Vegetation of the Eastern Cape. Towards an environmental plan for the Eastern Cape. MN Bruton and FW Gess (eds). Rhodes University Press, 68-87.

Lubke RA and de Villiers G (1981). The vegetation of the Joan Muirhead Nature Reserve, Kenton-on-Sea. *The Naturalist*, 35, 21-30.

Lubke RA and van Wijk Y (1988) Terrestrial plants and coastal vegetation. In: A field guide to the Eastern Cape coast. RA Lubke, FW Gess and MN Bruton (eds). Wildlife Society of South Africa, 167-240.

Lubke RA and van Wijk Y (1998) Terrestrial plants and coastal vegetation. In: Field guide to the Eastern and Southern Cape coasts. RA Lubke and I de Moor (eds). University of Cape Town Press, Cape Town, 289-343.

Mansfield TA and Davies WJ (1981) Stomata and stomatal mechanisms. In: The physiology and biochemistry of drought resistance. LG Paleg and D Aspinall (eds). Academic Press, London, 315-347.

Matrolab (PTY., LTD.) 4 Harbot Close, Brackenfell, Capetown, South Africa.

Martinez ML and Moreno-Casasola P (1996) Effects of burial by sand on seedling growth and survival in six tropical sand dune species from the Gulf of Mexico. *Journal of Coastal Research*, 12(2), 406-419.

Martinez ML and Moreno-Casasola P (1998) The biological flora of coastal dunes and wetlands: *Chamaecrista chamaecristoides* (Colladon) I. and B. *Journal of Coastal Research*, 14(1), 162-174.

Martinez ML, Moreno-Casasola P and Vazquez G (1997) Effects of disturbance by sand movement and inundation by water on tropical dune vegetation dynamics. *Canadian Journal of Botany*, 75, 2005-2014.

Martinez ML, Valverde T and Moreno-Casasola P (1992) Germination response to temperature, salinity, light and depth of sowing of ten tropical dune species. *Oecologia*, 92(3), 343-353.

Marschner H (1995) Mineral nutrition of higher plants. Second Edition. Academic Press, London.

McLachlan A, Ascaray C and Dutoit P (1987) Sand movement, vegetation succession and biomass spectrum in a coastal dune slack in Algoa Bay, South Africa. *Journal of Arid Environments*, 12, 9-25.

Medina E and Klinge H (1983) Productivity of tropical forests and tropical woodlands In: Encyclopedia of Plant Physiology. New series12D. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 281-304.

Miller PC, Miller JM and Miller PM (1983) Seasonal progression of plant water relations in fynbos in the Western Cape Province, South Africa. *Oecologia*, 56, 392-396

Miller RE, ver Hoef JM and Fowler NL (1995) Spatial heterogeneity in eight central Texas grasslands. *Journal of Ecology*, 83, 919-928.

Momen B and Helms JA (1996) Osmotic adjustment induced by elevated ozone: Interactive effects of acid rain and ozone on water relations of field grown seedlings and mature trees of *Pinus ponderosa*. *Tree Physiology*, 15, 799-805.

Momen B, Menke JW and Welker JM (1992) Tissue water relations *Quercus wislizenii* seedlings: Drought resistance in a California evergreen oak. *Acta Oecologia*, 13(1), 127-136.

Mooney HA (1980) Photosynthetic plasticity of populations of *Heliotropium curassavicum* L. originating from differing thermal regimes. *Oecologia,* 45, 372-376.

Mooney HA and Billings WD (1960) The annual carbohydrate cycle of alpine plants as related to growth. *American Journal of Botany*, 47, 594-598.

Mooney HA, Kummerow J, Johnson AW, Parsons DJ, Hays RI, Giliberto J and Chu C (1977) The producers- their responses and adaptive responses. Dowden, Hutchinson and Ross, Stroudsburg, PA.

Morgan JM (1984) Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology*, 94, 1781-1787.

Morris M, Eveleigh DE, Riggs SC and Tiffney WN (1974) Nitrogen fixation in the Bayberry (*Myrica pennsylvanica*) and its role in coastal succession. *American Journal of Botany*, 61, 867-870.

Morrison RG and Yarranton GA (1973) Diversity, richness and evenness during a primary dune succession at Grand Bend, Ontario. *Canadian Journal of Botany*, 51, 2401-2411.

Morrison RG and Yarranton GA (1974) Vegetational heterogeneity during a primary dune succession at Grand Bend, Ontario. *Canadian Journal of Botany*, 52, 397-410.

Niinemets U, Bilger W, Kull O and Tenhunen JD (1998) Acclimation to high irradiance in temperate deciduous trees in the field: Changes in xanthophyll cycle pool size and in photosynthetic capacity along a canopy light gradient. *Plant, Cell and Environment*, 21, 1205-1218.

Nobel PS (1981) Wind as an ecological factor. In: Encyclopedia of Plant Physiology. New series12A. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 475-500.

Nobel JS and Marshall C (1983) The population biology of plant with clonal growth. II. The nutrient strategy and modular physiology of *Carex arenaria*. *Journal of Ecology*, 71, 865-877.

Noctor G and Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology*, 49, 249-79.

Norman JM (1993) Scaling processes between leaf and canopy levels. In: Scaling physiological processes leaf to globe. JR Ehleringer and CB Field (eds). Academic Press, Inc. San Diego, USA, 41-76.
Odum EP (1971) Fundamentals of ecology. Third edition. W.B. Saunders, Philadelphia.

Ogren E (1988) Photoinhibition of photosynthesis in willow leaves under field conditions. *Planta*, 175, 229-236.

Olson JS (1958) Rates of succession and soil changes on southern Lake Michigan sand dunes. *Botanical Gazette*, 119, 125-170.

Olsson-Seffer P (1909) Hydrodynamic factors influencing plant life on sandy sea shores. *New Phytologist*, 8, 37-49.

Oosting HJ (1954) Ecological processes of vegetation of maritime strand in the south eastern United States. *Botanical Review,* 20, 226-262.

Oostings HJ and Billings WD (1942) Factors effecting vegetational zonation on coastal dunes. *Ecology*, 23, 131-142.

Osmond CB (1981). Photorespiration and photoinhibition. Some implications for the energetics of photosynthesis. *Biomedica et Biophysica Acta*, 639, 77-98.

Osmond CB, Bjorkman O and Anderson DJ (1980) Physiological processes in plant ecology. Springer, New York.

Packham JR and Willis AJ (1997) Ecology of dunes, salt marsh and shingle. Chapman and Hall, London.

Pammenter NW – Professor, School of Life and Environmental Science, University of Natal, Durban, South Africa.

Pammenter NW (1983) Some aspects of the ecophysiology of *Scaevola thunbergii*, a subtropical dune pioneer. In: Sandy beaches as ecosystems. DH Swart, GA Eagle, A McLachlan , AC Brown, AEF Heydorn and F Herbst (eds). DR W. Junk Publishers, The Hague, 675-698.

Pammenter NW (1985) Photosynthesis and transpiration of the subtropical coastal sand dune pioneer *Scaevola plumieri* under controlled conditions. *South African Journal of Botany,* 51(6), 421-424.

Pammenter NW and Smith VR (unpublished manuscript) Carbon assimilation by a plant in the high-light coastal dune environment is apparently light limited.

Park Y, Anderson JM and Chow WS (1996) Photoinactivation of functional photosystem II and D1-protein synthesis in vivo are independent of the modulation of the photosynthetic apparatus by growth irradiance. *Planta*, 198, 300- 309.

Parker J (1968). Drought-resistance mechanisms. In: Water deficits and plant growth. TT Kozlowski (ed). Academic Press, New York, London, 195-234.

Passioura JB (1982) Water in the soil-plant-atmosphere-continuum. In: Encyclopedia of Plant Physiology. New series12B. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 5-33.

Pavlik BM (1982) Nutrient and productivity relations of the dune *grass Ammophila arenaria* and *Elymus mollis*. Ph.D. thesis, University of California, Davis.

Pavlik BM (1983a) Nutrient and productivity relations of the dune grass *Ammophila arenaria* and *Elymus mollis*.I. Blade photosynthesis and nitrogen use efficiency in the laboratory and field. *Oecologia*, 57, 233-238.

Pavlik BM (1983b) Nutrient and productivity relations of the dune grass *Ammophila arenaria* and *Elymus mollis*.II. Growth and patterns of dry matter and nitrogen allocation as influenced by nitrogen supply. *Oecologia*, 57, 233-238.

Pavlik BM. (1983c) Nutrient and productivity relations of the dune grass *Ammophila arenaria* and *Elymus mollis*. III. Spatial aspects of clonal expansion with reference to rhizome growth and the dispersal of buds. *Bulletin of the Torrey Botanical Club*, 110(3), 271-279.

Pavlik BM (1984) Seasonal changes of osmotic pressure, symplastic water content and tissue elasticity in the blades of dune grasses growing *in situ* along the coast of Oregon. *Plant, Cell and Environment*, 7, 531-539.

Pavlik BM (1985) Water relations of the dune grasses *Ammophila arenaria* and *Elymus mollis* on the coast of Oregon, USA. *Oikos*, 45, 197-205.

Pearcy RW and Calkin HC (1983) Carbon dioxide exchanges of C_3 and C_4 tree species in the understory of a Hawaiian forest. *Oecologia*, 55, 333-341.

Pearcy RW, Harrison AT, Mooney HA and Bjorkman O (1974) Seasonal changes in net photosynthesis of *Atriplex hymenelytra* shrubs growing in Death Valley, California. *Oecologia*, 17, 111-121.

Pemadasa MA and Lovell PH (1974) The mineral nutrition of some dune annuals. *Journal of Ecology*, 62, 855-868.

Peter CI (2000) Water requirements and distribution of *Ammophila arenaria* and *Scaevola plumieri*. MSc. thesis, Rhodes University, Grahamstown.

Peter CI and Ripley BS (2001) An empirical formula for estimating the water use of *Scaevola plumieri. South African Journal of Science,* 96, 593-596.

Powles SB and Osmond CB (1979) Photoinhibition of intact attached leaves of C_3 plants illuminated in the absence of both carbon dioxide and of photorespiration. *Plant Physiology*, 64, 982 - 988.

Rabionwitch EI (1951) The light factor. Photosynthesis II. Wiley-Interscience, New York.

Ranwell DS (1972) Ecology of salt marshes and sand dunes. Chapman and Hall, London, UK.

Ranwell DS, Newborough W and Anglesey I (1959) The dune system and dune slack habitat. *Journal of Ecology*, 47, 571-601.

Rey MEC (1982) Epidemiological, morphological and physiological studies of selected plant diseases at Nylsvley. Ph.D. thesis. University of Witwatersrand, Johannesburg.

Richter H (1997) Water relations of plants in the field: some comments on the measurement of selected parameters. *Journal of Experimental Botany*, 48, 306, 1-7.

Ridley HN (1930) The dispersal of plants throughout the World. L. Reeve & Co. Ltd. Kent.

Ripley BS, Pammenter NW and Smith VR (1999) Function of leaf hairs revisited: The hair layer on leaves *Arctotheca populifolia* reduces photoinhibition, but leads to higher leaf temperatures caused by lower transpiration rates. *The Journal of Plant Physiology*, 155, 78-85.

Robichaux RH and Pearcy RW (1984) Evolution of C_3 and C_4 plants along an environmental moisture gradient: Patterns of photosynthetic differentiation in Hawaiian *Scaevola* and *Euphorbia* species. *American Journal of Botany*, 7(1), 121-129.

Rosa LM and Forseth IN (1995) Diurnal patterns of soybean leaf inclination angles and azimuthal orientation under different levels of ultraviolet-B radiation. *Agricultural and Forest Meteorology*, 78, 107 – 119.

Rozema JP, Bijwaard G, Prast G and Broekman R (1985) Ecophysiological adaptations of coastal halophytes from foredunes and salt marshes. *Vegetatio,* 62, 499-521.

Salisbury E (1952) Downs and Dunes. Their plant life and its environment. G. Bell and Sons, LTD. London.

Schiegl WE and Vogel JC (1969) Deuterium content of organic matter. *Earth and Planetary Science Letters*, 7, 307-313.

Schindler C and Lichtenthaler HK (1996) Photosynthetic CO_2 assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field grown maple trees in the course of a sunny and a cloudy day. *The Journal of Plant Physiology*, 148, 399-412.

Scholes RJ and Walker BH (1993) An African savanna: Synthesis of the Nylsvley study. Cambridge University Press, Cambridge, UK.

Schonherr J (1982) Resistance of plant surfaces to water loss: Transport properties of cutin, suberin and associated lipids. In: Encyclopedia of Plant Physiology. New series 12B. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 153-179.

Schrieber U, Schliwa U and Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorimeter. *Photosynthesis Research,* 10, 51-62.

Schultze E -D (1982) Plant life forms and their carbon, water and nutrient relations In: Encyclopedia of Plant Physiology. New series12B. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 615-676.

Schultze E-D and Hall AE (1982) Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. In: Encyclopedia of Plant Physiology. New series12B. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 181-230.

Schultze E-D, Lange OL, Kappen L and Buschbom U (1974) The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. I. A simulation of the daily course of stomatal resistance. *Oecologia*, 17, 159-170.

Schultze E-D, Lange OL, Kappen L, Evenari M and Buschbom U (1975) The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. II. The significance of leaf water status and internal carbon dioxide concentration. *Oecologia*, 18, 219-233.

Schultze E-D, Lange OL, Kappen L, Evenari M and Buschbom U (1980) Long-term effects of drought on wild and cultivated plants in the Negev desert. I. Maximal rates of photosynthesis. *Oecologia*, 45, 11-18

Schultze RE, Maharaj M, Lynch SD, Howe BJ and Melville-Thomas B (1997) South African atlas of agrohydrology and climatology. Water Research Commission report, TT82/96.

Seliskar DM (1990) The role of waterlogging and sand accretion in modulating the morphology of the dune slack plant *Sirpus americanus*. *Canadian Journal of Botany,* 68, 1780 – 1787.

Seliskar DM (1994) The effect of accelerated sand accretion on growth, carbohydrate reserves and ethylene production in *Ammophila breviligulata*. *American Journal of Botany*, 81(5), 536-541.

Sêstak Z, Câtsky J and Jarvis PG (1971) Plant photosynthetic production: Manual of methods. Junk Publishers, The Hague.

Simon J-P (1979) Adaptation and acclimation of higher plants at the enzyme level: Latitudinal variations of thermal properties of NAD malate dehydrogenase in *Lathyrus japonicus* Willd. (Leguminosae). *Oecologia*, 39, 273-287.

Simpsom RJ, Lambers H and Dalling MJ (1982) Translocation of nitrogen in a vegetative wheat plant (*Triticum aestivum*). *Plant Physiology*, 56, 11-17.

Sims PL and Singh JS (1978) The structure and function of ten western American grasslands, III. Net primary production, turnover and efficiencies of energy capture and water use. *Journal of Ecology*, 66, 673-697.

Sinclair R and Venables WN (1983) An alternative method for analysing pressure-volume curves produced with the pressure chamber. *Plant, Cell and Environment*, 6, 211-217.

Sironval C, Strasser RJ and Brouers M (1981) Equivalence entre la theorie des flux et la theorie des relations entre proportions de pigments pour la description de la repartition de l'energie lumineuse absorbee par les membranes photoactives. *Bulletin Academie Reyale de Belgique*, 67, 248-259.

Sival FP and Grootjans AP (1996) Dynamics of seasonal bicarbonate supply in a dune slack: Effects on organic matter, nitrogen pool and vegetation succession. *Vegetatio*, 126, 39-50.

Skaltsa H, Verykokidou E, Harvala C, Karabourniotis G and Manetas Y (1994) UV-B protective potential and flavonoid content of leaf hairs of *Quercus ilex. Phytochemistry,* 37(4), 987-990.

Skiba U and Wainwright M (1984) Oxidation of elemental-S in coastal-dune sands and soils*. Plant and Soil*, 77, 87-95.

Slayter RO and Taylor SA (1960) Terminology in plant-and soil water-relations. *Nature*, 187, 145-168.

Smith WK (1985) Western montane forests. In: Physiological and ecology of North American plant communities. BF Chabot and HA Mooney (eds). Chapman and Hall, London, 95-126.

Smirnoff N and Stewart GR (1985) Stress metabolites and their role in coastal plants*. Vegetatio*, 62, 273-278.

South African Weather Bureau (SAWB) The Forum Building, 159 Struben Street, Pretoria, South Africa.

Steinke TD and Lambert G (1986) A preliminary study of the phenology of *Scaevola plumieri*. *South African Journal of Botany*, 52 (1), 43-46.

Stewart GR, Larher F, Ahmad I and Lee JA (1979) Nitrogen metabolism and salt tolerance in higher plant halophytes. In: RL Jefferies and AJ Davy (eds). Ecological processes in coastal environments. Blackwell Oxford, 211-228.

St. John H (1970) Classification and distribution of the *Ipomoea pes-caprae* group (Convolvulaceae). *Botanische Jahrbuecher fuer Systematik Pflanzengeschichte und Pflanzengeographie*, 89, 563-583.

Stock WD and Allsop N (1992) Functional perspective of ecosystems. In: The Ecology of Fynbos. R Cowling (Ed). Oxford University Press, Capetown, 241-259.

Stocker O (1956) Die abhangigkeit der transpiration von den umweltfaktoren In: Encyclopedia of Plant Physiology. Vol III. W Ruhland (ed). Springer-Verlag, Germany, 436-488.

Stone AW, Weaver AvB and West WO (1998) Climate and weather. In: Field guide to the Eastern and Southern Cape coasts. R Lubke and I de Moor I. (eds). UCT Press, Capetown, 41-49.

Strasser RJ (1981) The grouping model of plant photosynthesis: Heterogeneity of photosynthetic units in thylakoids. In: Photosynthesis. III. Structure and molecular organisation of the photosynthetic apparatus. G Akoyunoglou (Ed.). Balaban International Science Services, Philadelphia, PA, 727-737.

Strasser BJ and Strasser RJ (1995) Measuring fast fluorescence transients to address environmental questions: The JIP-Test. Photosynthesis: From light to biosphere. Proceedings of the 10th International Congress on Photosynthesis, 977-980.

Strasser RJ, Srivastava A and Govindjee (1995) Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochemistry and Photobiology*, 61, 32-42.

Stroud LM and Cooper AW (1968) Colour-infrared aerial photographic interpretation and net primary productivity of a regularly flooded North Carolina salt marsh. Water Resource Res. Inst., Univ. of N.C., Rept. No. 14.

Svoboda J (1977) Ecology and primary production of raised beach communities, Truelove Lowland. In: Truelove Lowland, Devon Island Canada: High arctic ecosystem. Bliss L.C. (Ed). University Alberta Press, Edmonton, 185-216.

Szarek SR and Ting IP (1975) Photosynthetic efficiency of CAM plants in relation to C_3 and C_4 plants. In: Environmental and biological control of photosynthesis. R Marcelle (ed). W Junk, The Hague, 289-297.

Taiz L and Zeiger E (1991) Plant Physiology. Benjamin/Cummings Publishing, California.

Thomas DS, Eamus D and Bell D (1999) Optimisation theory of stomatal behaviour. I. A critical evaluation of five methods of calculation. *Journal of Experimental Botany*, 50(332), 385-392.

Tinley KL (1985) Coastal dunes of South Africa. South African National Scientific Programmes Report No 109, Foundation for Research Development, Council for Scientific and Industrial Research, Pretoria.

Tyree MT (1999) The forgotten component of plant water potential: A Reply - Tissue pressures are not additive in the way M.J. Canny suggests. *Plant Biology*, 1, 598-601.

Tyree MT and Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany,* 23, 267-282.

Tyree MJ and Jarvis PG (1982) Water in tissues and cells In: Encyclopedia of Plant Physiology. New series12B. OL Lange, PS Nobel, CB Osmond and H Zeigler (eds). Springer-Verlag, Germany, 35-77.

van der Heen (2000) Competition in coastal sand dune succession cause or mechanism. Ph.D. thesis, University of Groningen, The Netherlands.

van der Maarel E and van der Maarel-Versluys M (1996) Distribution and conservation status of littoral vascular plant species along the European coasts. *Journal of Coastal Conservation*, 2, 73-92.

van der Valk AG (1974) The environmental factors controlling foredune plant communities in Cape Hatteras National Seashore, *Ecology*, 55, 1349-1358.

van der Valk AG (1977) The role of leaves in the uptake of nutrients by *Uniola paniculata* and *Ammophila breviligulata. Chesapeake Science*, 18, 77-79.

van Dijk HWJ and Grootjans AP (1993) Wet dune slacks: Decline and new opportunities. *Hydrobiologia*, 265, 281-304.
Venkatesan A and Chellappan KP (1999) Salinity effect on the activities of certain antioxidant enzymes in *Ipomoea pes-caprae-* sweet potato, A. Halophyte. *Indian Journal of Plant Physiology*, 4(1), 40-42.

von Caemmerer S and Farquhar CD (1981) Some relationships between the biochemistry of photosynthesis and the gaseous exchange of leaves. *Planta*, 153, 376-387.

von Willert DJ, Herppich M and Miller JM (1989) Photosynthetic characteristics and leaf water relations of mountain fynbos vegetation in the Cedarberg area (South Africa*). South African Journal of Botany*, 55 (3), 288-298.

Wallen B (1980) Changes in structure and function of *Ammophila* during primary succession. *Oikos*, 34, 227-238.

Walter H and Leith H (1967) Klimmatdiagramm-Weltatlas. V.E.B. Gustav. Fischer-Verlag, Jens, East Germany.

Waring RH, Emmingham WH, Gholz HL and Grier CC (1978) Variation in maximum leaf area of coniferous forests in Oregon and its ecological significance. *Forest Science,* 24. 131-140.

Watkinson AR, Huiskes AHL and Nobel JC (1979) The demography of sand dune species with contrasting life-cycles. In: Ecological processes in coastal environments. RL Jefferies, AJ Davy (eds). Blackwell Scientific Publications, Oxford, 95-112.

White JWC (1988) Stable hydrogen isotopic ratios in plants: A review of current theory and some potential applications. In: Stable isotopes in ecological research. PW Rundel, JR. Ehleringer and KA Nagy (eds). Springer-Verlag, 142-162.

White JWC, Cook ER, Lawrence JR and Broeckner WS (1985) The D/H ratio of sap in trees: Implications for water sources and tree ring D/H ratios. *Geochimica et Cosmochimica Acta,* 49, 237 – 246.

Willis AJ (1965) The influence of mineral nutrients on the growth of *Ammophila arenaria. Journal of Ecology*, 53, 735-745.

Willis AJ and Jefferies RL (1963) Investigations on the plant water relations of sand-dune plants under natural conditions. In: The water relations of plants. AJ Rutter and FH Whitehead (eds). Blackwell Scientific Publications, Oxford, 168-189.

Willis AJ and Yemm EW (1961) Braunton Burrows: Mineral nutrient status of the dune soils. *Journal of Ecology,* 49, 377-390.

Wilson DE (1977) Ecological observations on the tropical strand plants *Ipomoea pes-caprae* (L.) R. Br. (Convolvulaceae) and *Canavalia maritima* (Aubl.) Thou. (Fabaceae). *Brenesia*, 31-42.

Woledge J (1977) The effects of shading and cutting treatments on the photosynthetic rate of ryegrass leaves. *Annals of Botany*, 41, 1276-1286.

Woodward FI and Cramer W (1996) Plant functional types and climate change: Introduction. *Journal of Vegetation Science*, 7, 306-38.

Yaun T, Maun MA and Hopkins WG (1993) Effects of sand accretion on photosynthesis, leaf-water potential and morphology of two dune grasses. *Functional Ecology*, 7, 676-682.

Young MM (1987) The Alexandria dunefield vegetation. MSc. Thesis, University of Port Elizabeth, Port Elizabeth, South Africa.

Zhang J (1996) Interactive effects of soil nutrients, moisture and sand burial on the development, physiology, biomass and fitness of *Cakile edentula*. *Annals of Botany*, 78, 591-598.