ASPECTS OF THE PHYSIOLOGY OF THE RESURRECTION PLANT *XEROPHYTA HUMILIS* UNDER DIFFERENT ENVIRONMENTAL CONDITIONS.

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"What would the world be, once bereft
Of wet and wildness? Let them be left
O let them be left, wildness and wet;
Long live the weeds and the wilderness yet."

Gerard M. Hopkins

PREFACE

Assoc. Prof. J. M. Farrant and Dr C. Vander Willigen supervised this thesis. It is hereby declared that this thesis, submitted for the degree of Master of Science at the University of Cape Town, is the result of my own investigation, except where the work of others is acknowledged.

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LIST OF ABBREVIATIONS

A_{net}	Net photosynthetic CO_2 assimilation rate
ANOVA	Analysis of variance
AOS	Active oxygen species
ATP	Adenosine tri-phosphate
Ca	Chlorophyll a concentration
Ca+b	Total chlorophyll a+b concentration
Cb	Chlorophyll b concentration
Chl a	Chlorophyll a
Chl a/b	Ratio of chlorophyll a to chlorophyll b
Chl b	Chlorophyll b
C_i	Internal CO_2 concentration in leaves
Cx+c	Total carotenoid x+c concentration
DM	Dry mass
E	Transpiration rate
e_a	Vapour pressure of ambient air
e_s	Vapour pressure of saturated air
FM	Fresh mass
Φ_{PSII}	Quantum yield of photosystem two
Ft	Real time chlorophyll fluorescence
Fv/Fm	Maximal quantum efficiency; variable fluorescence/ maximum fluorescence
g_s	Stomatal conductance
g. g^{-1} DM	Grams H_2O per gram dry mass

IRGA	Infra-red gas analyser
NADP+	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NS	Not significant
PAR	Photosynthetic active radiation
PSI	Photosystem one
PSII	Photosystem two
Q_A	Initial electron-accepting plastoquinone in PSII
qN	Non photosynthetic fluorescence quenching
qP	Photosynthetic fluorescence quenching
R_D	Dark respiration rate
RH	Relative humidity
Rubisco	Ribulose-1,5-bisphosphate carboxylase-oxygenase
RuBP	Ribulose-1,5-bisphosphate
RWC	Relative water content
S	Significant
VPD	Vapour pressure difference
VP_{leaf}	Vapour pressure in substomatal spaces
WC	Water content
(x+c)	Xanthophylls and carotenes
X. humilis	*Xerophyta humilis*
X. scabrida	*Xerophyta scabrida*
X. villosa	*Xerophyta villosa*
Ψ	Water potential

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ABSTRACT

Xerophyta humilis is a resurrection monocot that is able to survive drying of its tissues to an air-dry state. In order to assess the validity of laboratory-based experimentation, and the results obtained from such studies, a comparison of aspects of *X. humilis* physiology under different environmental conditions was undertaken. Analyses of chlorophyll fluorescence, gas exchange parameters, pigment concentrations and electrolyte leakage were performed on plants dehydrated under laboratory conditions differing with respect to light intensity ("high light" (*ca.* 1400 μmol. m^{-2}. s^{-1}) and "low light" (*ca.* 400 μmol. m^{-2}. s^{-1})). Physiological parameters were also examined during rehydration under high and low light laboratory conditions, and natural field conditions. Plants maintained fully hydrated for some time were also examined under laboratory conditions for the occurrence of rhythmic patterns in their daily physiology, and for any evidence of acclimatisation to the different environments.

Plants dehydrated under high and low light underwent metabolic changes at the same water contents, regardless of treatment. The time taken to dry once drying was initiated was also the same in both treatments, but drying was initiated more rapidly in plants under high light. However, the more rapid initiation of drying in this case was probably due to differing air-movement characteristics in the controlled environment room, rather than the higher light intensity. Plants drying under higher light intensities experienced higher levels of stress, most likely light induced, as expressed by the significantly greater upregulation or maintenance of protective pigments and the higher levels of membrane damage. Plants rehydrated under low light, high light and field

conditions varied little with respect to rate of rehydration and recovery of pigments and photosystem efficiency. However, unlike either of the laboratory treatments, field rehydrated plants had lower levels of membrane damage and maintained higher levels of anthocyanin throughout the recovery period. Field rehydrated plants, and to a lesser extent laboratory hydrated plants, also showed large cyclic variations in chlorophyll fluorescence parameters that were not correlated to cyclic changes in water content. Studies of fully hydrated plants that had not recently undergone dehydration and rehydration revealed that the cyclic or rhythmic variations evident in field rehydrated plants were not a consequence of rehydration. The rhythmic phenomena occurred on a daily cycle, correlated to the photoperiod, and were evident in certain chlorophyll fluorescence parameters (qN, qP and Φ_{PSII}) as well as certain gas exchange parameters (net photosynthetic CO_2 assimilation, transpiration and stomatal conductance). Rhythmic phenomena did not appear to differ between high and low light treatments, and neither did any apparent adaptation in chlorophyll fluorescence or gas exchange parameters occur in plants moved into the high and low light environments.

Although differences between high and low light treatments were minimal, differences did occur in levels of membrane damage and protection mechanisms. Thus consideration of light intensity under which experiments are performed is of importance if damage and protective mechanisms during dehydration of *X. humilis* are to be understood holistically. The presence of rhythmic phenomena is also of importance in the design of experiments and interpretation of results, due to large variations in gas exchange and chlorophyll fluorescence parameters that occur daily.

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Chapter 1

INTRODUCTION

1. Water deficit

When plants moved out of the aquatic habitat of their origins and colonised the land they became exposed to a drying environment. Under normal conditions, vascular plant tissues consist of 60 – 95% water (Monneveux and Behassen, 1996) with intercellular spaces containing air that is saturated with water vapour. Sufficiently irrigated plants seldom have a water potential (Ψ) less than -1.5 MPa (Gaff, 1989; Salisbury and Ross, 1992; Beckett, *et al.*, 2000). Air, however, even at 98% RH has a significantly lower Ψ than this and thus exerts a drying pressure on hydrated plant tissues. Although plants have evolved to live and reproduce successfully in such an environment, when water deficit increases plants can become stressed, i.e. experience drought. 'Drought' has been defined in many ways (Iljin, 1957) but will be defined here according to Jones (1992) as "a restricted water supply or enhanced water loss that tends to reduce plant productivity". Since 'plant stress' can be defined as any unfavourable substance or condition that affects a plant's metabolism, growth or development, (Lichtenthaler, 1996) drought as defined here is synonymous to water-deficit stress.

Plants that survive drought have been catagorised into drought avoiders, drought evaders and drought tolerators (Bewley, 1979; Jones, 1992). Drought

or water-stress avoiders are those that prevent water loss from tissues and store water, such as succulents. Evaders usually have rapid life cycles and escape drought by growing only during moist periods and existing in a desiccated, metabolically quiescent form during the dry periods. Desert ephemerals are evaders, existing in the seed-form during drought. Drought tolerators exist in a vulnerable hydrated form during drought, and although they do have many mechanisms to limit water loss and to efficiently utilise available water, their survival strategy involves protection against the deleterious effects of water-deficit (Jones, 1992; Monneveux and Belhassen, 1996). Pammenter and Berjak (1999) suggest that the intermediate water contents (-1.5 to -11 MPa) are when most damage occurs in drying tissue. Some drought tolerators can survive drying to Ψ of –3 MPa for short periods (Mittler *et al.*, 2001; Walters *et al.*, 2002).

Desiccation-tolerant or 'resurrection' plants conversely, are able to survive drying to an air-dry state (Gaff, 1977; Bewley 1979). This can be a Ψ of less than –100 MPa (Gaff, 1997), or even –300 MPa in some bryophytes (Proctor, 2000) and a relative water content (RWC – water content as a percentage of that at full turgor) of less than 5% (Gaff, 1989). They are found almost exclusively in habitats or microhabitats with shallow soils that rapidly dry out after rain and thus experience frequent cycles of insufficient available water punctuated by periods of plenty (Child, 1960; Gaff, 1977; Porembski and Barthlott, 2000). It is tempting to refer to desiccation-tolerant plants as purely extreme drought tolerators, but in fact desiccation-tolerant plants are drought evaders, as has also been suggested by Proctor (2000), in reference to

bryophyte desiccation-tolerant plants, and Mittler *et al.*, (2001). Like desert ephemerals, they exist in a thoroughly air-dry metabolically quiescent state (Alpert and Oliver, 2002) during dry periods to avoid irreparable damage. However, because the tissues that are metabolically active during unstressed periods are the same tissues that become dormant, the plants do experience brief periods of water-deficit stress (passing quickly from mild to severe) during the transition phases of intermediate water contents during drying down and rehydrating. Consequently angiosperm desiccation-tolerant plants are transient drought tolerators, although they do not have many mechanisms to maintain their water content as true drought tolerators do, during their brief periods of intermediate water content.

1.1 Water-deficit stress

The role of water in the vegetative plant body is manifold (Meidner and Sheriff, 1976): structurally it is incompressible and involved in maintaining turgor pressure, and in the stability of membranes. It is a solvent and the medium for most cellular chemical reactions. It is the transport medium of inorganic substances and organic products throughout the plant. It is involved in temperature regulation through transpiration and it is involved in metabolism as both a substrate (e.g. photosynthesis) and a product (e.g. photosynthesis!) (Monneveux and Belhassen, 1996; Walters *et al.*, 2002). Almost all abiotic stresses (freezing, heat, salt etc.) result ultimately in water-deficit stress (Holmberg and Bulow, 1998).

1.1.1 Mechanical/ physical stress

In plants intolerant of desiccation, loss of RWC reduces cellular turgidity, directly effecting growth on a cellular level (Bray, 1997). Once turgor pressure is lost the plasmalemma retracts from the cell wall and tearing can occur. In this state cell walls are subjected to increasing tension and undergo cytorrhysis (collapse) (Iljin, 1957; Farrant and Sherwin, 1998).

Rehydration of tissue that has been dried results in further mechanical disruption of membranes and cellular integrity as water rushes in to the cells and exacerbates any existing damage (Iljin, 1957; Bewley, 1979; Kaiser, 1987; Bewley and Oliver, 1992; Farrant and Sherwin, 1998).

One of the first signs of abiotic stress is, in fact, membrane damage (Holmberg and Bulow, 1998). This can also be a consequence of chaotic biochemical interactions and metabolism.

1.1.2 Biochemical and metabolic stress

Concentration of cellular contents occurs on water loss. At RWCs of less than 20 to 30%, lipoprotein associations in membranes cannot be maintained and lipid bilayers become rearranged into a hexagonal phase (Bewley, 1979; Vertucci and Farrant, 1995). Hydrophilic and hydrophobic interactions stabilise macromolecules, and water removal can thus also cause conformational changes in these molecules (Vertucci and Farrant, 1995). Concentration of cellular contents can cause deleterious interactions between molecules otherwise kept separate (Walters *et al.*, 2002), and hazardous molecules such

as free radicals and certain by-products of nitrogen-metabolism can become concentrated and increasingly hazardous (Wallsgrove and Lea, 1985).

Under water deficit plant metabolism can become chaotic, and this is most pronounced at intermediate water contents (Pammenter and Berjak, 1999). Mechanical and biochemical cellular damage disrupts many metabolic processes. Increasing viscosity of a dehydrating cytoplasm affects the rates of diffusion and movement of molecules (Farrant and Sherwin, 1998; Lawlor and Cornic, 2002) and toxic molecules can inhibit enzymes and denature proteins.

One of the consequences of chaotic metabolism is the production of active oxygen species (AOS) (reviewed by Larson, 1988; Smirnoff, 1993). These can form in mitochondria by reduction of oxygen (Vertucci and Farrant, 1995), as well as in the chloroplasts as a consequence of light-chlorophyll interactions (discussed below in section 2.3). AOS further damage cellular constituents exacerbating metabolic perturbations caused by water stress. They are potentially one of the largest metabolic contributors that cause damage to cells during desiccation.

1.2 Aspects of desiccation tolerance

In order to survive the lethal array of stresses associated with water-deficit, desiccation-tolerant plants have evolved a complex suite of protection and repair mechanisms (Oliver *et al.*, 1998; Farrant, 2000).

The loss of turgor, and associated tension within cells, is counteracted in various ways. Some species (e.g. *Craterostigma wilmsii*) exhibit extensive

controlled folding of the cell wall closely associated with the plasmalemma (Hartung *et al.*, 1998; Vicré *et al.*, 1999, Farrant, 2000). Others, (e.g. *Xerophyta humilis*) accumulate many small vacuoles that become filled with a non-aqueous substance, possibly disaccharides, amino acids or proteins (Farrant and Sherwin, 1998; Farrant, 2000), which are accumulated in high quantities during desiccation (eg, Leopold and Vertucci, 1986; Crowe *et al.*, 1992).

Sugars are also suggested to be involved in stabilising proteins and membranes (Bewley 1979; Oliver and Bewley, 1997). Although it is currently under debate (Buitnik *et al.*, 2002). They have been thought to stabilise the general sub-cellular milieu by forming highly viscous 'glasses' (Vertucci and Farrant, 1995; Ingram and Bartels, 1996; Pammenter and Berjak, 1999) and can also scavenge free radicals (Vertucci and Farrant, 1995). The various proteins that are upregulated function in stabilising membranes and other cellular components (Bray, 1993; Oliver *et al.*, 1998; Franco and Melo, 2000) and may be involved as enzymes in protective and repair pathways (Franco and Melo, 2000). Accumulation of compatible solutes such as sugars and proteins may also have minor roles in desiccation-tolerant plants in osmotic adjustment or free radical scavenging (Ingram and Bartels, 1996; Franco and Melo, 2000).

2. Photosynthetic systems and water-deficit

Photosynthesis is the single most important biochemical process on earth. Without it, light energy could not be converted into chemical energy and consequently, chemically-based life as we know it would not be possible.

Photosynthesis is the process whereby energy-containing photons of light are 'captured' by a plant and converted into energy stored in the form of carbohydrates. The process of conversion of radiation energy to chemical energy in an unstressed system is orderly and controlled; largely due to the order and structure of the photosynthetic machinery itself.

2.1 Photosynthetic machinery

Photosynthesis occurs in the chloroplasts. These specialised organelles have a number of structures that provide the physical frame for the biochemical process of photosynthesis.

Light energy interception is the function of pigments, specifically chlorophyll a and b (Chl a and Chl b) as well as various carotenoid pigments. The molecular structures of these pigments allows them to absorb light maximally in the 650-700 nm and 400-475 nm wavelength ranges for the chlorophylls a and b and around 460 nm for carotenoids (Hendry, 1993; Bryce and Hill, 1999; Cooper-Driver, 2001; Salibury and Ross, 1992). The rest is reflected as the green colour we perceive. Chlorophyll molecules that initially intercept the photons of light and 'gather' the associated energy are 'antennae' chlorophyll molecules. These occur in greater proportions than the more specialised chlorophyll molecules in the core complexes of the photosystems (Hopkins, 1995) to which they eventually pass some of the energy from the photons. The energy not passed onto the photosystems is emitted as light (fluorescence) or heat (non-photochemical fluorescence quenching - qN) (Demmig-Adams and Adams, 1992; Hopkins, 1995; Lawlor and Cornic, 2002). Other than acting as accessory light capture pigments, carotenoids are also

involved in qN, through de-epoxidation of the xanthophyll pigment violanxanthin to zeaxanthin; (Demmig-Adams and Adams, 1992; Munne-Bosch and Alegre, 2000a,b,c; Deltoro *et al.*, 1998).

Relative and absolute quantities of the pigments directly interfacing with the light energy (chlorophylls and carotenoids) are variable. Variation occurs across species and individuals, as well as within a single individual over time, as a direct result of changing environmental conditions (Lichtenthaler, 1987; Demmig-Adams and Adams, 1992), developmental stage, or phenology.

For example, the ratio of chlorophyll a to chlorophyll b (Chl a/ b) is about 3.2-4 in sun-adapted plants, and 2.5- 2.9 in shade-adapted plants (Lichtenthaler, 1987; Ayari *et al.,* 2000). Relative quantities of Chl b also decline during the chlorophyll breakdown process as Chl b is first converted to Chl a (Matile *et al.*, 1996; Matile and Hörtensteiner, 1999) before being broken down further into colourless catabolites. They are also lower during chlorophyll synthesis as Chl a is made first and accumulates before being converted into Chl b (Król *et al.*, 1999).

Variation in the levels, absolute and relative, of various carotenoids such as carotenes and xanthophylls also occur in response to changing environmental conditions. Sun-adapted plants have larger absolute quantities of carotenoids than shade-adapted plants (Demmig-Adams and Adams, 1992). Because of the adjustable and variable nature of the photo-pigment make-up of plants, pigment-composition analysis can reveal much about physiological status of plants.

It must also be noted that other, non-photosynthetic, pigments are present in plant tissues. These pigments occur in both photosynthetic and non-photosynthetic organs. The anthocyanins are such a pigment-group occurring in 'higher' plants (Hendry, 1993). Anthocyanins reflect light of a similar wavelength to that absorbed by chlorophylls, appearing as reds, purples and blues. They have thus long been suggested to act as 'sunscreen' pigments protecting the plant against absorbing too much energy (Hendry, 1993; Matile, 2000; Cooper-Driver, 2001), which has recently been shown to be the case in senescing Red-Osier Dogwood (Feild *et al.*, 2001). They are water soluble, occur mainly in the vacuoles, and can be upregulated during times of light-related stresses.

The photosynthetic pigments are bound to proteins located in a system of interconnected membranes that form sack-like thylakoids. The thylakoids are stacked into grana, and this system of appressed membranes is the location of many membrane-bound enzymes, proteins and complexes, including Photosystems I and II (PSI and PSII) (Hopkins, 1995). The interconnected membranes of the thylakoids act as channels for lipid-soluble molecules such as some electron-carriers, and as a stabilising structure for many potentially reactive enzymes and proteins (Hopkins, 1995; Koonjul, *et al.*, 2000). This membrane system is surrounded by the stroma in which the Calvin cycle enzymes occur and function, and a double membrane envelope surrounds the entire organelle. Electron transport, light capture, enzyme functioning and almost all metabolic processes of photosynthesis cannot occur without the membranes and membrane-formed structures within the chloroplast. Disruption of these membranes, as can occur under water-deficit, whether by

separation of stacks and appressed regions, or by destruction of the membranes themselves, results in chaotic metabolism and cessation of photosynthesis.

2.2 Relevant aspects of photosynthetic biochemistry

Within the framework of the thylakoid membranes in the chloroplast, the antennae chlorophyll, and carotenoid molecules, intercept photons of light. Any extra energy that is not passed from these molecules to the reaction centre of PSII is re-emitted into the atmosphere as light of a longer wavelength to that intercepted (fluorescence), or as heat (Demmig-Adams and Adams, 1996a; Lichtenthaler and Miehé, 1997; Maxwell and Johnson, 2000.)

It is in the PSII reaction centre that the radiant energy is first changed into chemical energy in the form of a redox potential. This is due to the oxidation of H_2O to H^+ ions and O_2. The electron taken from water is then passed along a series of electron acceptors via the protein complexes, cytochromes *b* and *f*, to PSI. The first electron acceptors are plastoquinones, Q_A and Q_B. The final light-phase fate of the electron is in PSI, where it is accepted by $NADP^+$. $NADP^+$ is reduced to NADPH, which diffuses into the stroma to participate in the dark phase (Calvin cycle) reactions of photosynthesis. This entire process is known as linear, or non-cyclic, electron transfer. Some of the electrons passing through PS I become involved in *cyclic* electron transfer around the photosystem. Reactions at both PSII and PSI result in a proton gradient across the thylakoid membrane, which drives the production of ATP. The

strong reductant NADPH is produced as a consequence of linear electron flow, for use in the Calvin cycle (Hopkins, 1995).

Atmospheric CO_2 diffuses down a concentration gradient, through the stomata on the leaf-surface, and into the stroma of the thylakoids. It is in the stroma that the NADPH and ATP from the light reactions of photosynthesis energise the photosynthetic carbon reduction cycle, or Calvin cycle. During this reaction CO_2 is added to the sugar ribulose-1,5-bisphosphate (RuBP) by the enzyme ribulose- 1,5-bisphosphate carboxylase-oxygenase (Rubisco), and dissacharides and certain complex sugars are eventually formed (Salisbury and Ross, 1992). Another function of Rubisco is oxygenation of RuBP. This occurs when oxygen binds to the reactive site of Rubisco instead of CO_2, and photorespiration occurs. Photorespiration is the evolution of CO_2 associated specifically with the functioning of photosynthesis, and can result in the production of hydrogen peroxide (Ingram and Bartels, 1996), an active oxygen species.

2.3 Stressed photosystems

Even under ideal conditions plants in bright sunlight never use the full amount of energy that is available to them. Some plants will even use as little as 10% of the photon energy gathered under full sunlight (ca. 2000 μmol. m^{-2}. s^{-1}). The rest is for the most part harmlessly dissipated via mechanisms such as the xanthophyll cycle (as heat), photorespiration and the water-water cycle (Asada, 1999). Thus, when a plant experiences stressful conditions this proportion of unusable excess energy can become damaging as dissipation mechanisms reach capacity.

Although various environmental factors such as pollution and temperature extremes can interact with high light levels to cause stress to photosystems, the effects of water-deficit stress will be discussed here.

Under conditions of water-deficit stress photosynthesis is inhibited by a combination of low CO_2 levels and various metabolic factors (Kaiser, 1987; Lawlor and Cornic, 2002). A water-stressed plant will react initially by closing stomata to prevent further water loss (Kaiser, 1987; Hopkins, 1995; Lawlor and Cornic, 2002). Consequently stomatal conductance declines and internal CO_2 levels (C_i) drop. Because CO_2 is the final dark-phase electron acceptor, decreased levels of C_i result in decreased net photosynthesis, known as photoinhibition, under the first stages of water deficit (Lawlor and Cornic, 2002). Although photosynthetic reduction of CO_2 is restricted when stomata close, the electron supply from oxidised water is not restricted. The oxidation of water at PSII is a very stable reaction that can persist under levels of severe water-stress (Eickmeier *et al.*, 1992). If photon interception persists energised electrons will continue to enter the electron transport chain. Because CO_2 is not available the electron carriers, especially Q_A, can become 'blocked'; unable to oxidise fast enough to accept electrons at the rate at which they are provided. Consequently high-energy electrons can be passed instead to oxygen, forming hazardous active oxygen species (AOS) such as singlet oxygen, superoxide and hydrogen peroxide, amongst others (Smirnoff, 1993). It is well known that an environmental stress such as water deficit can cause an otherwise non-stressful level of light to become stressful and this seems to be predominantly due to the production of AOS.

The initial cause of photoinhibition is the decrease in C_i due to stomatal closure, but as leaf relative water content decreases below 75% metabolic inhibition of photosynthesis begins to play more and more of a role (Lawlor and Cornic, 2002). Metabolic photoinhibition is suggested to be a result of both damage to metabolic systems and an active down regulation of photosynthesis for protection (Demmig-Adams and Adams, 1992), but whatever the cause it is a universal symptom of stressed photosystems.

2.3.1 Photoprotection

The significant involvement of AOS in photosystem stress is indicated by the multitude of enzymes, scavengers and protective systems in place to both prevent their formation and to deactivate them once formed (reviewed by Larson, 1988; Smirnoff, 1993). AOS production is in fact a natural part of normal non-stressed plant metabolism (such as during the Mehler reaction and water-splitting at PSII); mild increases in AOS levels can even stimulate resistance (Noctor *et al.*, 2002). However, as mentioned previously (section 1.1.2) corrupt and chaotic metabolism – such as can occur during water-deficit stress – causes severe elevations in AOS production. AOS also accumulate in desiccation-tolerant plants (e.g. Navari-Izzo *et al.*, 1994). The high-energy, oxygen-rich environment of chloroplasts makes them particularly susceptible to the rapid formation of AOS (Larson, 1988).

In order to prevent or decrease light-chlorophyll interactions plants employ mechanical and chemical shading mechanisms. Leaf rolling and folding occurs in desiccation-tolerant plants (Sherwin and Farrant, 1998; Farrant *et al.*, 1999) for the purpose self-shading, as well as in some other species (Gaff,

1989; Muslin and Homman, 1992; Lawlor and Cornic, 2002). A few species also change leaf orientation relative to the sun to minimise photon-interception (Eickmeier *et al.*, 1992; Demmig-Adams and Adams, 1992; Van Rense and Curwiel, 2000), and many species occurring in sunny conditions accumulate anthocyanins during water-deficit, and hence light, stress (Larson, 1988; Sherwin and Farrant, 1998). Not only do anthocyanins reflect the wavelengths of light preferentially absorbed by the chlorophylls (Hendry, 1993), thereby reducing light-chlorophyll interactions, they can also act as antioxidants (Larson, 1988). Other pigments that can play an antioxidant role are the carotenoids. As mentioned previously, carotenoids also function in photoprotection by the dissipation of energy as heat. The xanthophyll cycle dissipates intercepted photon-energy by the de-epoxidation of violaxanthin to antheraxanthin and zeaxanthin (non-photosynthetic fluorescence quenching) (Demmig-Adams and Adams, 1996a, 1996b). This is a very important mechanism for safely getting rid of excess energy and up to 97% of absorbed energy can be emitted in this way (Bolhár and Öquist, 1993). In conditions of high light and light stress, as well as increasing the relative proportions of antheraxanthin and zeaxanthin, plants can increase the total size of their carotenoid pool (Demmig-Adams and Adams, 1996a).

Aside from the preventative mechanisms of light avoidance and qN, plants also contain a host of AOS scavenging enzymes and substrates. Some examples of components of scavenging systems are glutathione, ascorbate, tocopherol, and enzymes such as super-oxide dismutase and peroxidases. Upregulation of such systems occurs during water-deficit stress (Kranner and Grill, 1997; Farrant, 2002; Kranner, 2002).

Resurrection plants are known to increase anthocyanin contents (Sherwin and Farrant, 1998; Farrant, 2000; Vander Willigen *et al.*, 2001), fold and curl their leaves (Sherwin and Farrant, 1998; Muslin and Homman, 1992), and upregulate AOS scavenging metabolism (Farrant, 2000). The 'poikilochlorophyllous' resurrection plants (Hambler, 1961) even actively dismantle their entire photosynthetic apparatus and breakdown their chlorophyll to prevent the hazard of chlorophyll-energization under water stress (eg. Tuba *et al.*, 1998).

3. Rhythmic phenomena

Plants, like many other organisms, exhibit cyclic phenomena. Some of these rhythms occur on a seasonal scale, such as flowering, and the rhythmic changes are cued by environmental factors such as temperature and day length (or more correctly, night length). Plants also go through rhythmic daily changes, and rhythmic changes that perambulate about significantly shorter periods. Some leaf and petal movements follow daily rhythms, as can photosynthesis, stomatal opening and gas exchange (Hopkins, 1995). Rhythmic phenomena are not very well understood and there is much variation between plants in both the phenomena and the possible causes thereof. It is known, however, that some rhythms are exogenously stimulated by e.g. light and some are endogenous (known as circadian rhythms). Endogenous rhythms can continue when changing light stimuli are removed indicating that plants have a 'biological clock' that 'measures' time independent of rhythmic stimuli (McClung, 2001). That said, endogenous

rhythms can also be influenced by exogenous stimuli, which often act as regulating mechanisms.

To date no investigations into any endogenous or exogenous rhythms of any angiosperm resurrection plants has been done, except for a study by Schneider *et al.* (1999) on diurnal xylem pressure changes of the resurrection plant, *Myrothamnus flabellifolius*.

4. This study

In this study some physiological parameters of the desiccation-tolerant 'resurrection' plant *Xerophyta humilis* (Bak.) Dur. and Schinz (Velloziaceae). were compared between natural field and artificial laboratory conditions. Comparison between laboratory and field studies have been reported for lichens (Lange *et al.*, 2001), but apart from a few simple observations and superficial explorations (Gaff, 1977; Schneider *et al.*, 1999) no field-based investigations into the physiology or biochemistry of angiosperm desiccation tolerance have previously been done. Experimentation is carried out under laboratory conditions, occasionally in controlled environment chambers, but often simply on laboratory bench-tops, (e.g. Child, 1960; Gaff and Ellis, 1974; Gaff, 1977; Sherwin and Farrant, 1996; Farrant and Sherwin, 1998; Cooper and Farrant, 2002 *inter alia*), and frequently on tissue-cultured material (Bartels, *et al.*, 1990; Norwood *et al.*, 2000) or excised leaves or twigs instead of whole plants (eg; Heatherington *et al.*, 1982; Muslin and Homman, 1992; Tuba *et al.*, 1993a; Navari-Izzo *et al.*, 1994; Dace *et al.*, 1998; Cooper and Farrant, 2002l reviewed Pammenter et al., 2002). Needless to say , the light, humidity, temperatures, day-legth and hosts of other environmental

variables differ vastly between the extreme conditions of the natural habitats of desiccation-tolerant plants and the artificial environment of a laboratory.

X. humilis is a diminitutive resurrection monocot belonging to the Velloziaceae family (Figure 1.1). All members of the Velloziaciae are desiccation tolerant. *Xerophyta* spp. occur in tropical Africa and Madagascar (Porembski and Barthlott, 2000), *X. humilis* being found in the north-western regions of South Africa, and Namibia. It lives in shallow rocky soils often as a matt of closely packed individuals in shallow depressions (Figure 1.1; Porembskii and Barthlott, 2000). *X. humilis* survives drying to a RWC of less than 5%, and is completely dependant on *de novo* gene transcription and synthesis of new proteins for rehydration (Dace *et al.*, 1998). Many small vacuoles filled with non-aqueous substance develop during drying, which are suggested to provide mechanical stability (Farrant and Sherwin, 1998), and photosynthetic pigments and apparatus undergo controlled dismantling (Dace *et al.*, 1998). Anthocyanins accumulate (Farrant, 2000) and can appear as an intense blue-black colour in field-dried plants (Gaff, 1977). Some aspects of the transcription of PSII in *X. humilis* genes have been examined by Collet *et al.* (2002). All experiments reported to date on this species have been laboratory based. As yet, this data has not been intergrated with the plants in their natural environment.

The aims of this study were a) to investigate if the physiology of rehydration differed between field and laboratory conditions, and b) to investigate how differing light levels might affect the physiology of desiccation tolerance (dehydration and rehydration) under otherwise identical laboratory conditions. The apparatus and functioning of the photosynthetic systems were examined for this purpose. Fluorescence induction kinetics were examined and photosynthetic and protective pigments were quantified in all experiments, as was membrane integrity. In addition, gas exchange parameters were recorded in laboratory-based experiments.

Further investigations into aspects of photosynthesis of non-stressed plants were carried out to assist interpretation of results. Fluorescence induction kinetics and gas exchange parameters of fully hydrated plants were examined in the laboratory for plants under the two different light levels.

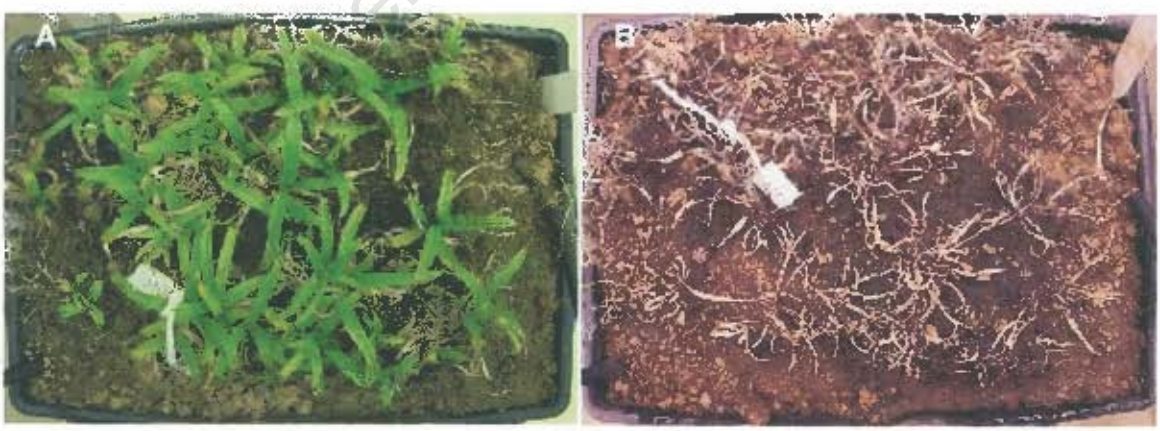

Figure 1.1. Hydrated (A) and desiccated (B) *Xerophyta humilis* plants in seedling trays.

Chapter 2

MATERIALS AND METHODS

Experiments were performed both on plants collected in the field but maintained under laboratory conditions, and on plants in the field. The same general measurements were taken during various different experiments; the methods of which are presented below. Experiment-specific variations to these materials and methods will be discussed in the appropriate chapters.

1. Plant material and treatments

1.1. Laboratory-based experiments

Plants rehydrated for *in situ* experiments in Borakalalo National Park, North West Province, South Africa (27,8^0 S; 25,15^0 E), were collected directly after the completion of the experiments described below (section 1.2), during September 2001. Whole plants were dug up in clods of soil, and were immediately transplanted directly into seedling trays (16 cm x 23 cm, 10 cm deep) containing soil from the surrounding area. The plants were then transported to the University of Cape Town where they were maintained at field capacity, by watering, in a glasshouse for six to nine months. There was no supplementary lighting, and average maximum temperatures were 28^{0}C in summer and 15^{0}C in winter.

Hydrated plants were then transferred to "phytotrons" - constant environment chambers (Environmental Growth Chambers, Ohio) in which laboratory-based experiments were conducted. Phytotrons were set up with a controlled day: night photoperiod of 14h: 10h, daytime temperatures of 25^{0}C and night time temperatures of 20^{0}C. In the phytotrons plants were subjected to either relatively high light intensities to simulate their natural environment, or lower light intensities to simulate

the conditions frequently used in laboratory-based experiments. Air movement in the phytotrons was recorded with an analogue anemometer, and was *ca.* 1.3 m. s^{-1} in the high light phytotron and *ca.* 0.07 m. s^{-1} in the low light phytotron. Daily maximum light intensity at plant height for the "high light" treatments (henceforth referred to as high light) was *ca.* 1200 μmol. m^{-2} .s^{-1}. Daily maximum light intensity for the "low light" treatments (henceforth referred to as low light) was *ca.* 320 μmol. m^{-2} .s^{-1}. Plants subjected to high and low light treatments are referred to as "high light plants" and "low light plants", respectively. Levels of photosynthetically active radiation (PAR) were measured using a LI-250 light meter (LI-COR Biosciences, inc. Nebraska) and are shown in Figure 2.1.

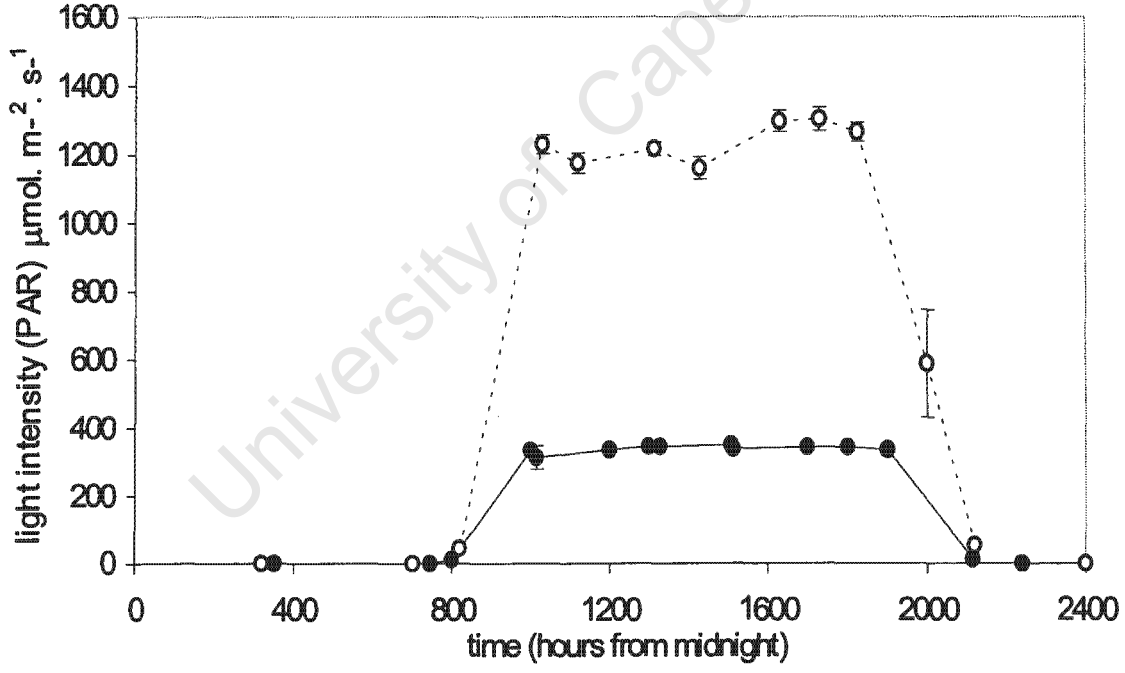

Figure 2.1. Daily photoperiod and light intensities for high light (- - o - -) and low light (—•—) treatments.

1.1.1. Laboratory dehydration

Plants were maintained hydrated in the phytotrons for at least two weeks to allow for any acclimation. They were then dehydrated, by withholding water and allowing the soil to dry. Chlorophyll fluorescence and gas-exchange parameters were measured during dehydration. The same leaves were used for chlorophyll fluorescence and gas-exchange measurements at each sampling period. They were 'tagged' by loosely tying string around the leaf-bases. Leaves with similar appearances to the tagged leaves were detached for measuring other parameters: detached leaves were transported from the phytotrons to the laboratory in sealed 1.5 ml eppendorf tubes where they were cut longitudinally into three sections. The first section was used to determine water content and membrane integrity (by measuring electrolyte leakage rates); the second to determined contents of chlorophylls and carotenoids, and the third, contents of anthocyanins. The fresh mass of each leaf section was promptly determined, using a 5 place Mettler AG-135 balance (Mettler Toledo, Greifensee). Sampling was continued until plants were air-dry (water content less than 0.5 g. g^{-1} DM).

1.1.2. Laboratory rehydration

Plants were left in an air-dry state for 17 – 25 d, after which daily watering was resumed. Watering was initiated at dusk, thereafter watering was continued at dawn and dusk. The same parameters measured during dehydration were also investigated during rehydration.

1.1.3. Acclimatisation and daily rhythms

To determine if 'acclimatisation' or daily rhythmic changes occurred, measurements of pre-dawn chlorophyll fluorescence induction kinetics, and gas-exchange parameters were performed on hydrated plants for the initial 2 week-period in the phytotrons. Light-

response curves were drawn during the initial week, at predawn with increasing light intensities, using the same leaves throughout. At least eight leaves were sampled during each sampling period.

1.2. Field-based experiments

Investigations were conducted in Borakalalo National Park on naturally occurring *Xerophyta humilis*. Experiments were performed in the first half of September 2001 before the beginning of the summer rainfall season, after the plants had been dry for at least 2 months (9.5 mm of rain fell in Borakalalo during June 2001, and thereafter no rain fell until after experimentation was complete). Average maximum and minimum temperatures during September 2001 were 27°C and 9.6°C respectively, with minimum night time temperatures frequently below 3°C during the sampling period (Climatic data provided by the South African Weather Bureau). Daily average light intensity was measured at *ca* 1500 μmol. m^{-2}. s^{-1}, using the LI-250 light meter, during sampling periods. Monthly mean light intensity for Borakalalo during 2001, in MJ. m^{-2}, is presented in Figure 2.2A. For comparative purposes this data is converted from MJ. m^{-2} to μmol. m^{-2}. s^{-1} using the approximate conversion factor provided by Ting and Giacomelli (1987) and presented with monthly mean rainfall during 2001, in Figure 2.2B.

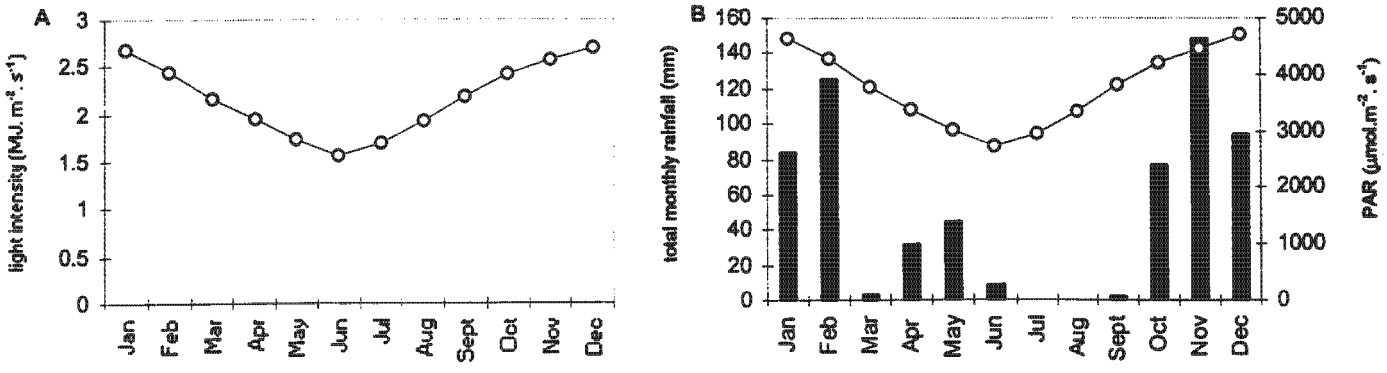

Figure 2.2. Average monthly light intensity (A). Total monthly rainfall (solid bars) and photosynthetic active radiation (PAR, —o—) (B) for Borakalalo Game Reserve during 2001.

1.2.1. Field rehydration

Plants were artificially hydrated *in situ*, by the application of 40 L of tap water to an area of approximately 3 m^2. The initial application of water was in the late afternoon. Thereafter, 40 L of water was applied at each sampling period (three to four times a day), during which chlorophyll fluorescence induction-kinetics parameters were recorded on leaves of intact plants. The same leaves used for chlorophyll fluorescence measurements were subsequently harvested and taken back to the field-laboratory, in sealed 1.5 ml eppendorf tubes, where they were cut longitudinally into three sections (as in laboratory based experiments – section 1.1.1) and subjected to water content, membrane integrity and pigment analyses. Leaf sections were weighed on a 4 place DE-100A balance (Denver Instrument Company, Colorado). At least 15 leaves were sampled during each sampling period.

2. Methods

2.1. Water content and membrane integrity

2.1.1. Water content

Subsequent to conductivity measurements (described in section 2.1.2.), leaf sections were oven-dried for 48 h at 70°C to determine dry mass. Water content (WC), expressed as g H_2O per g dry mass (g. g^{-1} DM), was determined gravimetrically using the standard formula:

$$WC = (\text{fresh mass} - \text{dry mass}) / \text{dry mass} \quad \ldots\ldots\ldots\ldots \quad (2.1)$$

Relative water contents (RWC) were determined as a percentage of the water content at full turgor. Full turgor, measured on at least 30 leaves, was obtained by hydrating

the plants overnight sealed in a plastic bag (to raise the humidity to 100%) during two experiments.

$$RWC = WC_{(sample)} / WC_{(full\ turgor)} \quad \text{.....(2.2)}$$

Water content of the soil was assessed on a mass-basis. Weight of water lost from drying plant-tissue was assumed to contribute insignificantly to total pot weight. Pots were weighed on a Mettler PE 11 Balance (Mettler Toledo, Greifensee) during each sampling period. Pot mass was represented as a percent of mass at field capacity.

2.1.2. Membrane integrity

Membrane integrity was inferred from the rate at which electrolytes leaked from the leaf sections. Prior to oven drying, leaf sections were placed in 1 ml of distilled, deionised water, further purified with a Milli-Q ultra-pure water filter (Millipore Corporation, Massachusetts), and conductivity of the resulting solution was measured over at least 100 min on a CM100 conductivity meter (Reid and Associates cc., Durban). Electrolyte leakage rates were calculated as the average increase in steady state leakage over time, expressed as $\mu S.\ cm^{-1}.\ ml^{-1}.\ min^{-1}.\ g^{-1}$ dry mass.

2.2. Chlorophyll fluorescence and gas exchange analysis

Chlorophyll fluorescence induction-kinetics measurements were made using an OS-500 modulated fluorometer (Opti-sciences, Tyngsboro). Measurements were done on individual leaves, dark-adapted for 10 minutes, with a saturating light intensity of 7500 $\mu mol.\ m^{-2}\ .s^{-1}$. Actual quantum yield of PSII (Φ_{PSII}), maximal quantum efficiency of PSII (Fv/Fm), photochemical fluorescence quenching (qP) and non-photochemical fluorescence quenching (qN), calculated automatically by the fluorometer according to the equations below, were assessed.

$$Fv/ Fm = (Fm - Fo)/ Fm \quad \text{..... (2.3)}$$

$$Y = (Fms - Fs)/ Fms \quad \text{..... (2.4)}$$

$$qP = (Fm - Fs)/ (Fms - Fo) \quad \text{..... (2.5)}$$

$$qN = (Fm - Fms)/ (Fm - Fo) \quad \text{..... (2.6)}$$

Gas exchange measurements were performed on the same leaves as the fluorometry, with an LI-6400 IRGA, (LI-COR Biosciences, inc. Nebraska) using an "arabidopsis chamber" leaf cuvette. Dark respiration rates were determined from predawn and night-time gas-exchange measurements. Measurements were taken with ambient CO_2 and relative humidity levels, and an external light source (Phytotron light, which was provided by a combination of 400W sodium Osram vialox NAV-T, 400W metalhalide Osram powerstar HQI-T and 500W incandescant 230V ES light globes). Light-response curves were measured using the Li-6400 IRGA with a standard LI-COR cuvette and an attached red light source (Li-6400-02).

In addition to gas exchange parameters the LI-6400 calculated leaf-temperature, using an energy budget equation, and measured air temperature. Vapour pressure difference (VPD, in KPa) between substomatal spaces and ambient air was calculated according to the following formula:

$$VPD = VP_{leaf} - e_a \quad \text{..... (2.7)}$$

Where VP_{leaf} is the vapour pressure of water in substomatal air spaces, assuming a relative humidity of 100%, and e_a is the vapour pressure of the ambient air. Saturated vapour pressure values (e_s) were obtained from Pearcy *et al.* (1992), according to leaf

temperature for VP_{leaf} and air temperature for e_a. e_a was calculated according to the following equation, derived from Pearcy *et al.* (1992):

$$e_a = (RH/ 100) * e_s \quad \text{..... (2.8)}$$

As the mature leaves of the *X. humilis* specimens were typically between 1.5 cm and 3 cm long, positioning of the IRGA cuvette and fluorometer dark-adaption clips was restricted. For consistency the cuvette and clips were hence placed as close to the leaf-bases as possible for each leaf, care being taken not to damage the plants.

2.3. Pigment analysis

2.3.1. Chlorophylls and carotenoids

Chlorophylls a + b and carotenoids x + c were extracted from finely chopped leaf sections in 1 mL of 100% pure acetone according to Lichtenthaler (1987). Samples were kept in the dark at 4^0C for at least one week to ensure complete extraction of pigments into the solvent. Samples were centrifuged at 13900 *g* at 4^0C for 10 min, and pigment concentrations in the supernatant were determined spectrophotometrically using a Beckman DU 650 (Beckman Coulter, Inc., California). Extracts were measured at wavelengths of 470, 644.8 and 661.6 nm, and pigment concentrations (mg pigment. mL solution^{-1}) were determined using the following formulae (adapted from Lichtenthaler, 1987):

$$C_a = (11.24A_{661.6} - 2.04A_{644.8})/ 1000 \quad \text{..... (2.9)}$$

$$C_b = (20.13A_{644.8} - 4.19A_{661.6})/ 1000 \quad \text{..... (2.10)}$$

$$C_{a+b} = (7.05A_{661.6} + 18.09A_{644.8})/ 1000 \quad \text{..... (2.11)}$$

$$C_{x+c} = (1000A_{470} - 1.90C_a - 63.14C_b)/ 214)/ 1000 \ldots\ldots (2.12)$$

2.3.2. Anthocyanins

The procedure for extraction of anthocyanins was the same as that for chlorophylls and carotenoids, except acidified methanol (100% MeOH: H_2O: HCl, 79: 20: 1 v/v) was the solvent used (Mancinelli *et al.*, 1975). Centrifuged samples were read at wavelengths of 530 and 657 nm on the Beckman DU 650 Spectrophotometer, and anthocyanin concentration (μmol anthocyanins. mL^{-1} solution) was determined by the following equation, adapted from Mancinelli *et al.* (1975) and Sims and Gamon (2002):

$$[\text{Anthocyanins}] = (A_{530} - 1/3A_{657})/ 0.3 \ldots\ldots\ldots\ldots\ldots (2.13)$$

All pigment contents were finally expressed per gram dry mass of leaf tissue. The dry mass (DM) values of the leaf sections used for pigment analysis were calculated from fresh masses (FM) of the sections, and the WC determined for each leaf according to equation 2.1 (section 2.1.1). DM was calculated according to the following equation:

$$DM = FM / (WC+1) \ldots\ldots\ldots\ldots\ldots (2.14)$$

2.4. Statistical analyses

Means and standard deviations (in all figures represented as error bars) were calculated at each sample period, using Microsoft Excel 97 software, (Microsoft Corporation, USA). Selected data were analysed using Scheffe's one-way Analysis of variance (ANOVA) or Students t-test (95% confidence levels), unless otherwise stated. Statgraphics Plus 7.0 data analysis software (Manugistics, Inc., Rockville) was used for all analyses.

Chapter 3

LABORATORY DEHYDRATION

1. Introduction

Aspects of the physiology of dehydrating *Xerophyta humilis* have been examined in studies by Farrant *et al.* (1999) and Farrant (2000). In these experiments plants were maintained under shaded conditions (in a greenhouse under 30% shade-cloth, with no supplementary lighting), and were dried by withholding water. Plants were dried in the shaded greenhouse in which they were maintained in experiments by Farrant *et al.* (1999), and under light intensities that reached a daily maximum of 1200 μmol. m^{-2}. s^{-1} in experiments by Farrant (2000). The light levels experienced by these plants in their natural habitats, and thus the light levels under which they naturally dehydrate, exceed 2000 μmol. m^{-2}. s^{-1} (Figure 2.2). Many of the changes that occur when a poikilochlorophyllous desiccation-tolerant plant, such as *X. humilis*, dehydrates are thought to relate to protection against light stress and light-induced damage (e.g. Sherwin and Farrant, 1998). Desiccation-tolerant plants that are adapted to dehydrating in a shaded environment, such as the resurrection fern *Polypodium polypodioides*, experience much greater levels of damage if forced to dry while exposed to direct sunlight (Muslin and Homman, 1992). Comparison of the physiological changes occurring in a species adapted to a *high* light environment, during drying in high and low light environments, has not been investigated prior to this study.

Plants collected in the field were used in laboratory studies of dehydration. Due to the scope of this project an examination of dehydration of plants in the field was not possible, as drying is unpredictable and uncontrollable, being reliant on local weather. Laboratory-based dehydration studies were conducted under high light conditions to simulate the field environment, and under low light conditions such as have been commonly used in physiological examinations of resurrection plant physiology.

2. Methods

Plants were collected and maintained, and then subjected to drying in high light and low light phytotrons as described in chapter 2, section 1.1. Plants were closely monitored after cessation of watering. Measurements were taken during ten sampling periods. An initial sample was taken before watering was ceased, and thereafter sampling was done from the point when the soils were no longer at field capacity until the plants themselves were air-dry.

3. Results

3.1. Water contents

Mean water contents (WC) prior to the start of tissue-drying were similar for both high light and low light treated plants at about 2.4 g H_20. g^{-1} DM, or 95% RWC. Thereafter WC declined to statistically similar levels of 0.48 (± 0.4) g. g^{-1} DM and 0.54 (± 0.1) g. g^{-1} DM respectively (20 and 22%, RWC) (Figure 3.1A and 3.1B). The mean WC of high light plants was very variable (1.8 to 2.8 g. g^{-1} DM) before decreasing to 0.48 g. g^{-1} DM in air-dry leaves. Mean WCs of low light plants varied less prior to the drying-induced decline. Both

high and low light plants took *ca.* 50 h from time of maximum water content to reach an air-dry state. However, high light treatment plants began drying 40 h after cessation of watering, while low light plants began drying 80 h after cessation of watering. Thus, although in both high and low light treatments plants dried to the same WC and took the same length of time to dry once drying of the plants had started, high light plants reached an air-dry state sooner (*ca.* 75 h) than low light plants (*ca.* 122 h). Because of the variation in WC, data are represented with respect to time, hereafter.

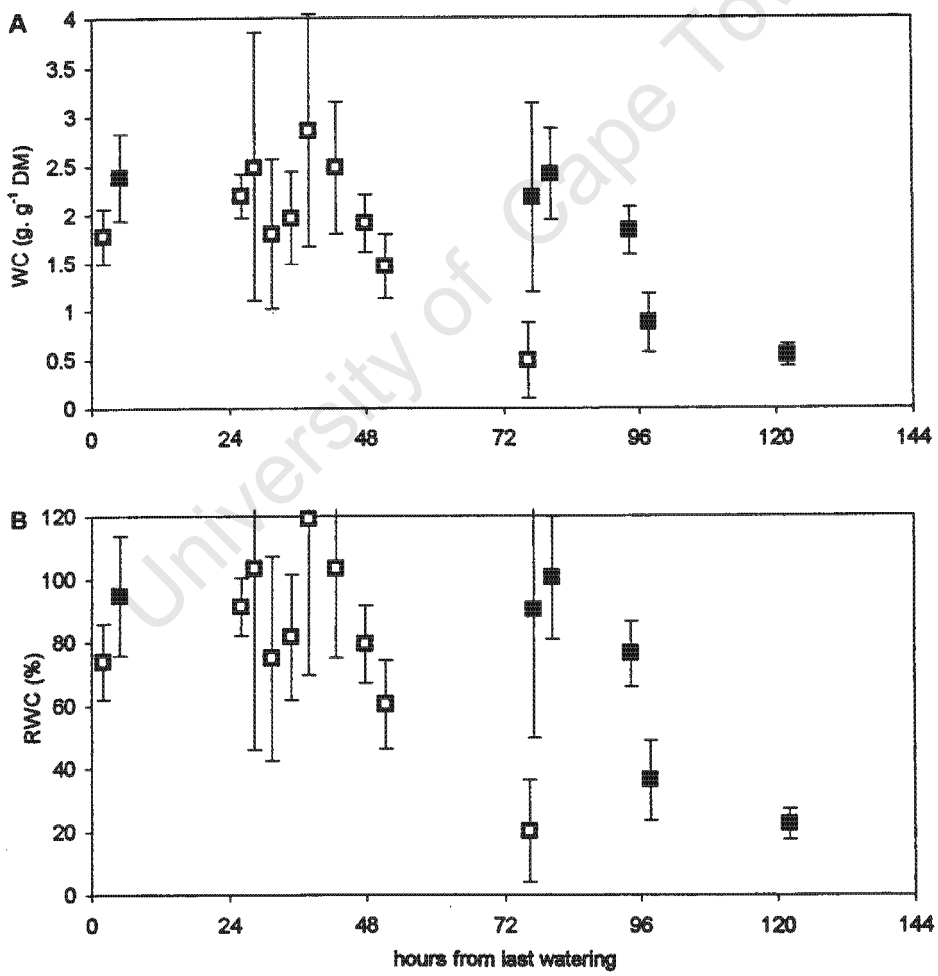

Figure 3.1. Change in water content (WC), (A), and relative water content (RWC) (B), of *Xerophyta humilis* leaves drying (initiated by cessation of watering) under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

The rate of decrease in pot mass, representing water loss from soil, was the same for high light and low light treatments, Figure 3.2 (n = 14, p = 0.7, Analysis of Covariance performed on regressions through mean values). The gradient of the linear regression (through data points after 24 h) for high light pots was –0.0996, (R^2 = 0.919) and through data points after 45 h for low light pots, was –0.1093 (R^2 = 0.7991). High light pot-masses had only declined to 95% of that at field capacity when high light plants were air-dry. Low light pot-masses had declined further, to 90% of that at field capacity, when low light plants were air-dry, indicating relatively drier soils.

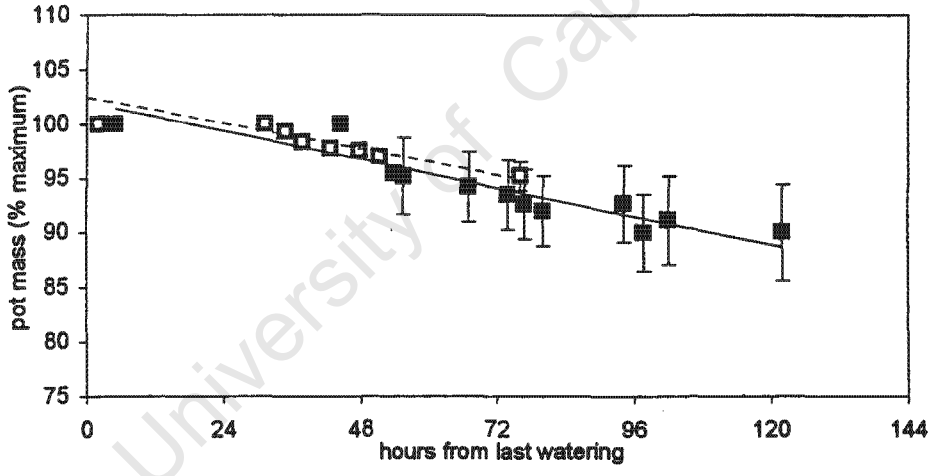

Figure 3.2. Decrease in mass of plant-pots over time, indicating soil water loss, during drying (initiated by cessation of watering) of *Xerophyta humilis* in high light (*ca.* 1200 μmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 μmol. m^{-2}. s^{-1}; ■) in constant environment chambers. (----) Represents the linear regression through high light mass-means; (—) represents the linear regression through low light mass-means. n = 8

3.2. Membrane integrity

Electrolyte leakage (Figure 3.3), representative of membrane integrity, was initially 8.35 (± 4.1) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM increasing to very high levels

(55 (± 26) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM) after 26 h in high light leaves. Electrolyte leakage declined to 4 (± 3) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM , and remained low until increasing slightly to 13 (± 5) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM in air-dry leaves. Electrolyte leakage in low light leaves did not rise initially, remaining around 6 μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM, and rose to 9.5 (± 3) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM in air-dry low light leaves, indicative of disrupted membranes. The difference between initial and final electrolyte leakage rates was not significant in high or low light leaves. At 98 h and a water content of 0.9 g. g^{-1} DM, an anomalous increase of electrolyte leakage to 36 (± 19.5) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM occurred in low light leaves, decreasing again in dry leaves.

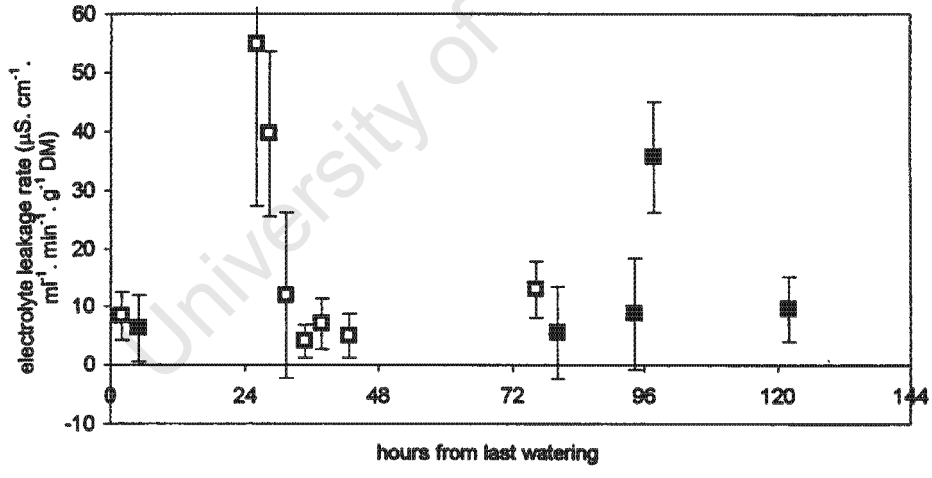

Figure 3.3. Change in electrolyte leakage rate over time during drying (initiated by cessation of watering) of *Xerophyta humilis* under high light (*ca.* 1200 μmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 μmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

3.3. Chlorophyll fluorescence

Actual quantum yield (or efficiency) of PSII (Φ_{PSII}), indicative of the actual proportion of absorbed light energy that is used in photochemistry, varied

between 0.25 and 0.55 in high light leaves until the final decline to 0 began after 52 h (Figure 3.4.). Φ_{PSII} had reached 0 in air-dry plants at 76 h after cessation of watering. In low light plants Φ_{PSII} varied between 0.75 and 0.3, with large variation within the data, until 80 h after cessation of watering. At this point Φ_{PSII} was 0.7, declining thereafter to reach 0 at 102 h. Φ_{PSII} was 0 in air-dry plants (Figure 3.4). It must be noted that the OS-500 modulated fluorometer cannot measure chlorophyll fluorescence induction kinetics if real-time fluorescence values are below F_t = 50. In such instances F_t values frequently are 0, and fluorescence parameters are recorded as 0.

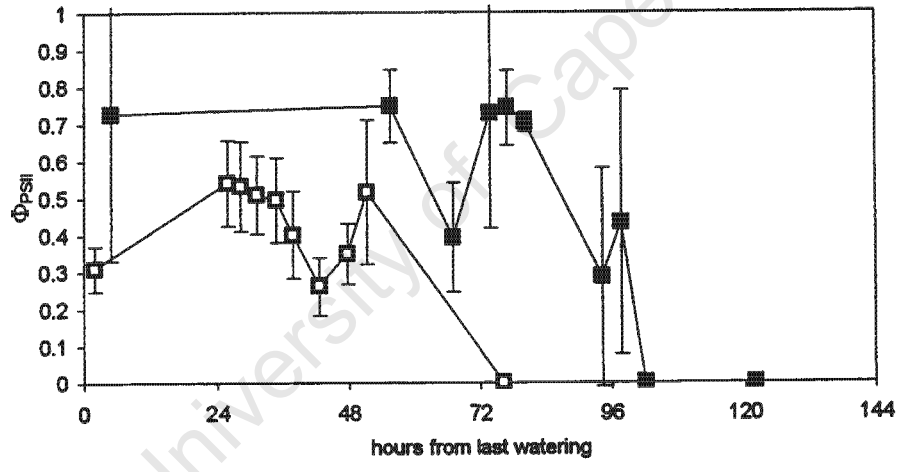

Figure 3.4. Change in quantum yield of photosystem II (Φ_{PSII}) over time during drying (initiated by cessation of watering) of *Xerophyta humilis* under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

Maximal quantum efficiency (Fv/Fm) provides information about the intrinsic efficiency of PSII, indicative of the proportion of light that PSII can use if all reaction centres are open. Fv/ Fm results are represented in Figure 3.5. The mean initial Fv/Fm value in high light leaves was 0.69 (± 0.05). Between 25 to 48 h after cessation of watering, Fv/Fm, varied between 0.5 and 0.6. After

about 48 h, and below a WC of 2 g. g^{-1} DM (RWC of *ca.* 80%), Fv/Fm declined rapidly reaching 0 by 76 h when the plants were air dry. Mean Fv/Fm in hydrated low light leaves was *ca.* 0.75 from initial measurements until about 80 h after cessation of watering (WC of 2.4 g. g^{-1} DM). After 98 h when WC had declined to 1.8 (± 0.25) g. g^{-1} DM (RWC of *ca.* 75%), Fv/Fm had declined to 0.65 (± 0.07). Thereafter Fv/Fm declined rapidly, as in the high light treatment, reaching 0 after 102 h. Fv/Fm was 0 when low light plants were air dry.

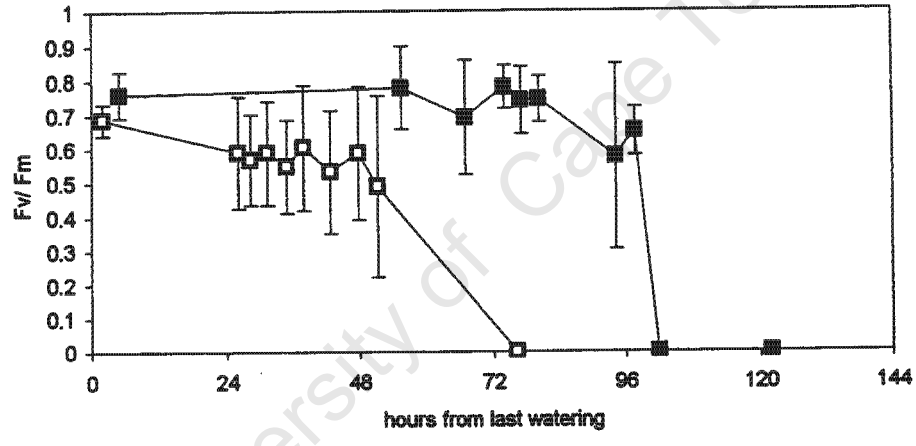

Figure 3.5. Change in optimal quantum efficiency (Fv/Fm) over time during drying (initiated by cessation of watering) of *Xerophyta humilis* under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

Photosynthetic fluorescence quenching (qP; representing proportion of open PSII reaction centres) was similar at the start of drying in high and low light plants (Figure 3.6A). In contrast, mean non-photosynthetic fluorescence quenching (qN; dissipation of energy as heat, relative to the dark-adapted state), was initially significantly higher in high light plants than in low light plants, (Figure 3.6B; n = 16, $p < 0.0001$). Mean qP for high light leaves varied

between 0.45 and 1.75 until 52 h after cessation of watering. At 52 h qP was 1.2 (± 1.2), after which it declined to 0 in air-dry plants. There was much variability within sampling periods in high light leaves, with standard deviations of up to ± 1.2. Mean qP for low light leaves varied between 0.55 and 1.2 until 94 h after cessation of watering; standard deviations remained below ± 0.33. At 98 h low light qP was 0.9 (± 3.2), but declined to 0 by 102 h. qP was 0 in air-dry plants.

Mean qN in high light plants varied between 0.58 and 0.98 with considerable variability within the samples. High light qN was 0.7 (± 0.33) at 52 h, declining to 0 in air dry plants at 76 h. Mean qN in low light plants varied between 0.28 and 0.5 with less variation than high light plants. Low light qN was 0.44 (± 0.29) at 98 h, declining to 0 by 102 h. qN was 0 in air-dry plants.

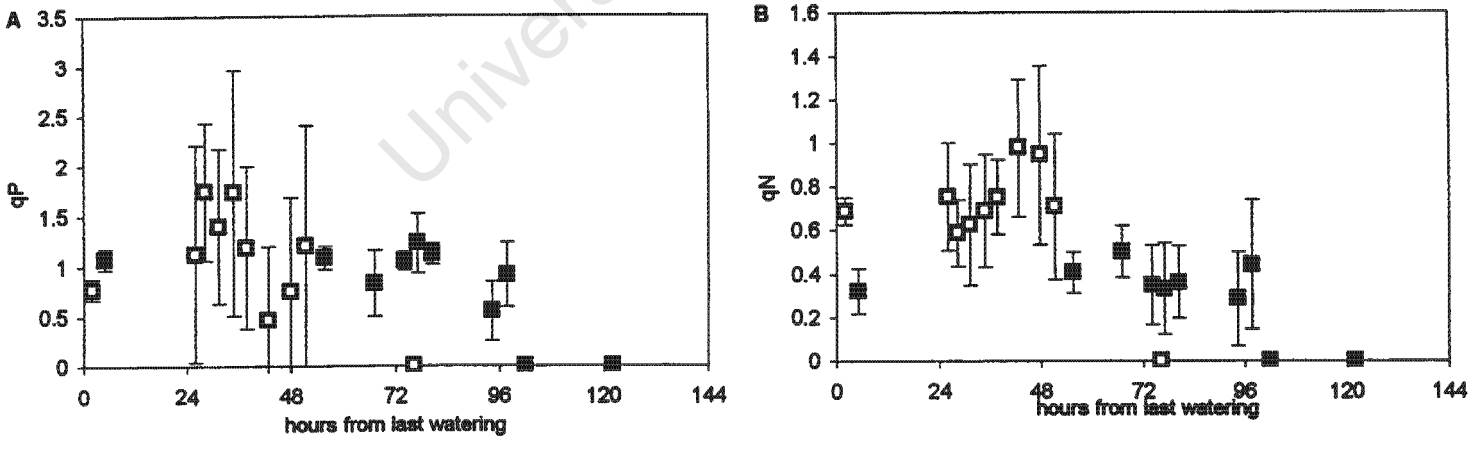

Figure 3.6. Change in photosynthetic (qP) (A), and non-photosynthetic (qN) (B), chlorophyll fluorescence quenching over time, during drying (initiated by cessation of watering) under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

3.4. Gas exchange

Net photosynthetic CO_2 assimilation rates (A_{net}, Figure 3.7) in high light plants declined continuously during drying, from 9.4 (± 6.6) μmol. m^{-2}. s^{-1} at 26 h after cessation of watering (and a WC of *ca.* 2 g. g^{-1} DM), to –0.74 (± 1.4) μmol. m^{-2}. s^{-1} in air-dry plants at 76 h. A_{net} in low light plants were more variable during drying, varying between 1.3 and 6.5 μmol. m^{-2}. s^{-1} until 80 h after cessation of watering (and a WC of *ca.* 2 g. g^{-1} DM), where after it declined to 0.12 (± 1.5) μmol. m^{-2}. s^{-1} after 98 h. In air-dry low light A_{net} was 0.7 (± 1.65) μmol. m^{-2}. s^{-1}.

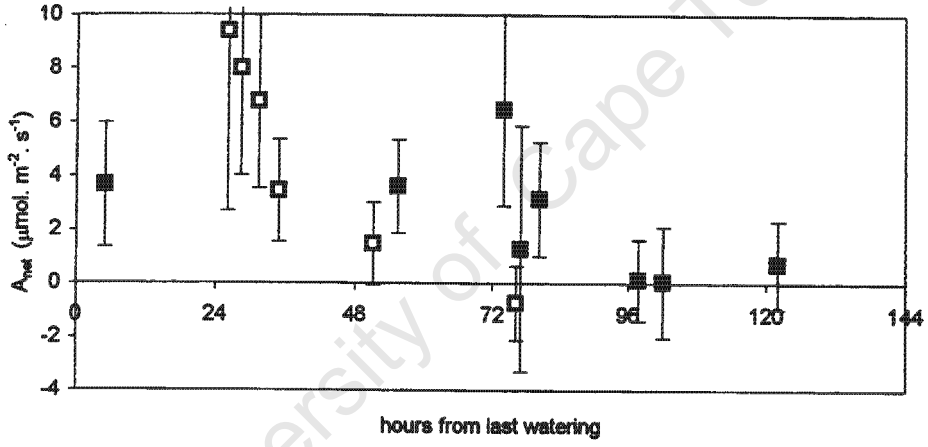

Figure 3.7. Change in net photosynthetic CO_2 assimilation rate (A_{net}) over time during drying (initiated by cessation of watering) of *Xerophyta humilis* under high light (*ca.* 1200 μmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 μmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

A_{net} in high light plants were significantly logarithmically correlated to stomatal conductance (g_s) (Figure 3.8A; R^2 = 0.96). As stomatal conductance declined, net photosynthetic assimilation declined. This also occurred in low light plants, but a logarithmic regression in these plants only had a correlation coefficient of 0.35. A_{net} also declined as Fv/Fm declined (Figure 3.8B) but there was no change in Fv/Fm when A_{net} declined. In addition there was no change in A_{net}

when Fv/Fm declined (in both treatments), and there was hence no dependant relationship between Fv/Fm and carbon fixation.

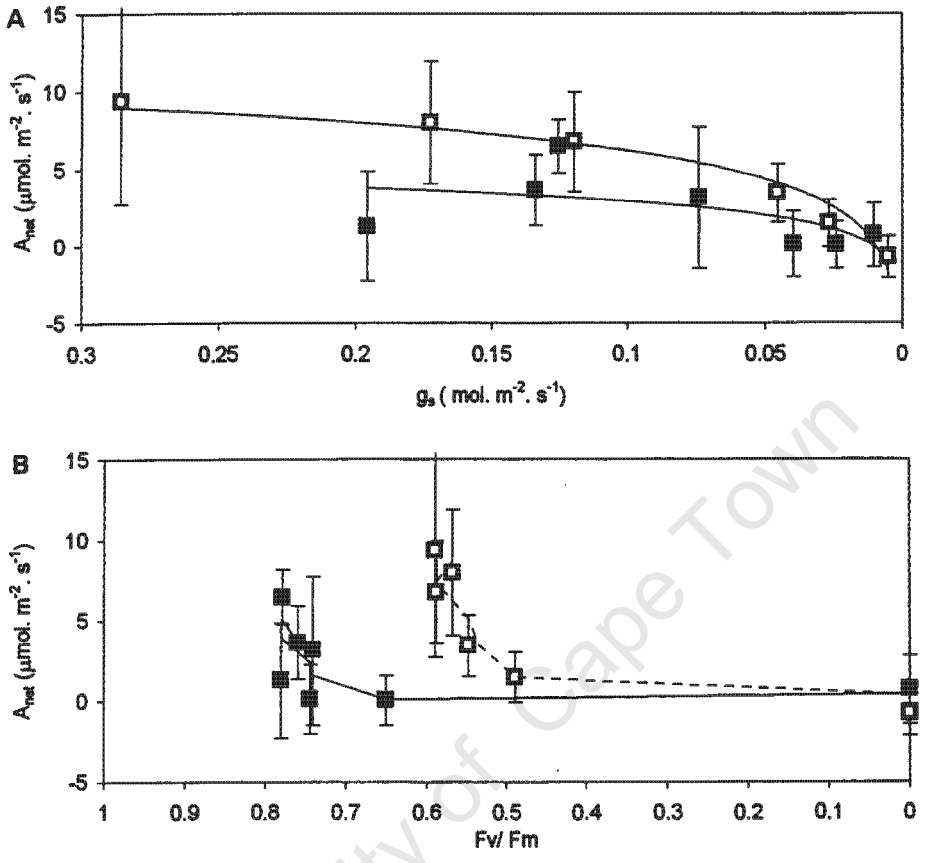

Figure 3.8. Change in net photosynthetic CO_2 assimilation rate, (A_{net}), with change in stomatal conductance (g_s) (A), and Fv/Fm (B), during drying (initiated by cessation of watering) of *Xerophyta humilis*) under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

Dark respiration rates (R_D) varied between 2.3 (± 0.73) and –0.63 (± 0.67) µmol. m^{-2}. s^{-1} in high light plants. R_D in low light plants was 0.52 (± 0.9) µmol. m^{-2}. s^{-1} at 67 h and 2.35 (± 1.6) µmol. m^{-2}. s^{-1} at 94 h after cessation of watering. R_D data did not show any apparent trend in either high light or low light plants (Figure 3.9), and, unlike net assimilation rates, continued at high levels until plants had lost up to at least 40% of their RWC.

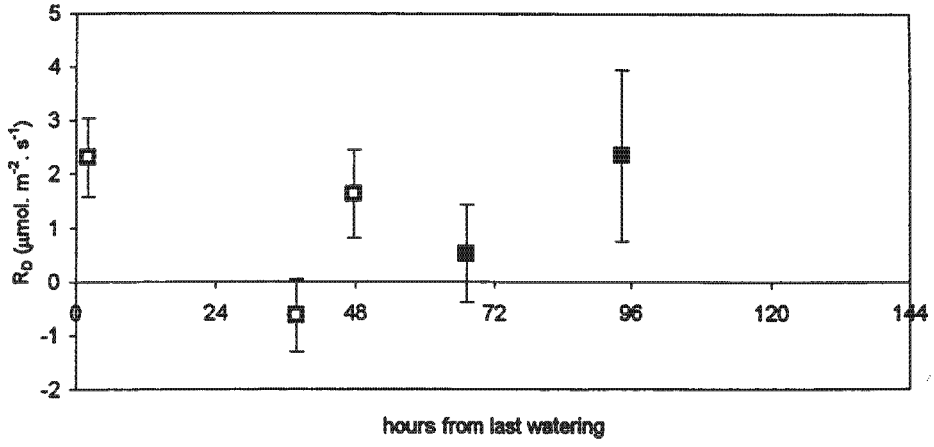

Figure 3.9. Change in dark respiration rates (R_D) over time, during drying (initiated by cessation of watering) of *Xerophyta humilis* under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

Vapour pressure difference (VPD) between leaf substomatal air spaces and ambient air is represented in Figure 3.10. These calculations are based on the assumption that air in substomatal air spaces is saturated with water vapour, which is unlikely in leaves with very low water contents. Subtomatal air spaces of such leaves are likely to contain air of a similar relative humidity to that of ambient air, and hence this data is probably not reflective of the true VPD at low RWC's. There were no apparent trends in VPD during drying in either high or low light plants, but day time VPD was significantly higher (n = 14, p = 0.019) in high light plants. Mean day time VPD in high light plants varied between 2.8 and 3.9 KPa and mean day time VPD in low light plants varied between 2.2 and 3.4 KPa. Mean night time VPD varied between 1.5 and 1.7 KPa in high light plants, and was 1.1(± 0.04) KPa at 67 h and 0.92 (± 0.1) KPa at 94 h in low light plants. Overall VPD (day and night) did not differ significantly between high light and low light plants.

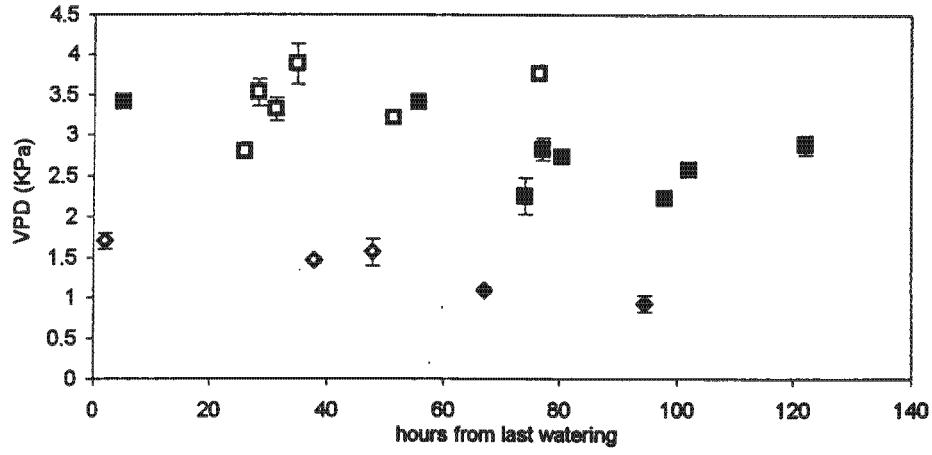

Figure 3.10. Vapour pressure difference (VPD) between leaf and air, during drying of *Xerophyta humilis* under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; day: □, night: ◇) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; day:■, night:◆) in constant environment chambers. n = 8.

Day time leaf temperature was higher in high light plants than in low light plants during drying (Figure 3.11). No significant changes in leaf temperature occurred consequent of drying; leaf temperature was directly linearly correlated (R^2 = 0.99) with air temperature (data not shown) throughout drying in high and low light plants. Mean leaf temperature was higher in high light plants (n = 14, p = 0.0018), however this did not cause a higher transpiration rate (E) (Figure 3.12).

During the day, E in high light plants was initially 9.8 (± 4.7) mmol. m^{-2}. s^{-1}, and was 6.97 (± 0.93) mmol. m^{-2}. s^{-1} in low light plants. The differences were not significant (n = 11, p = 0.14). In both treatments E declined rapidly over time; the decline started after 26 h in high light plants and after 74 h in low light plants. Both high and low light plants reached a final E of about 0.2 mmol. m^{-2}. s^{-1}.

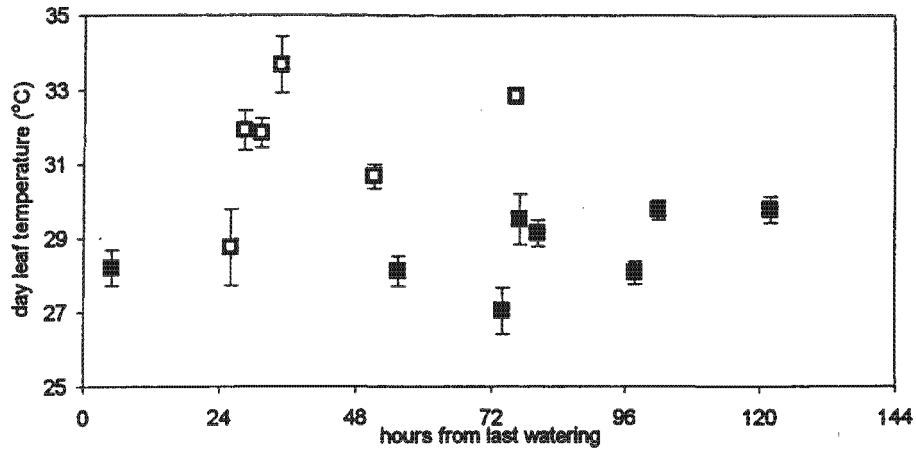

Figure 3.11. Leaf temperature of *Xerophyta humilis* during day time, during drying (initiated by cessation of watering) under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

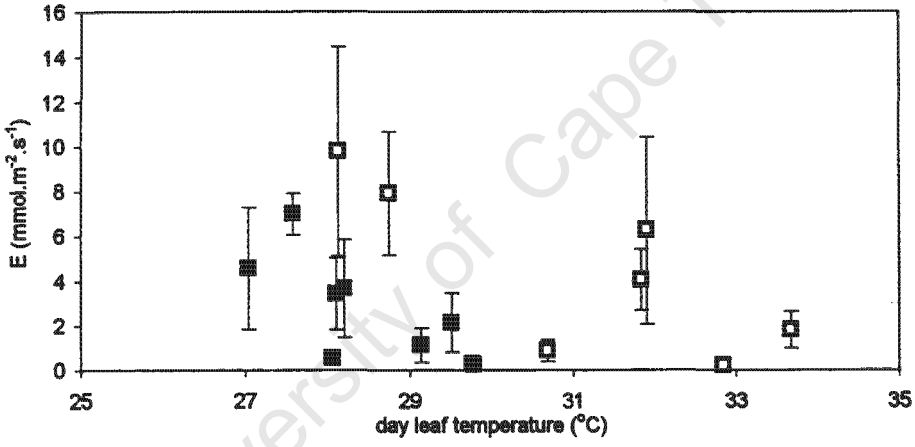

Figure 3.12. Variation of transpiration rate, E, with respect to leaf temperature of *Xerophyta humilis* during drying (initiated by cessation of watering) under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

Daytime stomatal conductance (g_s) during drying did not differ significantly between high and low light plants, but was significantly higher in fully hydrated plants compared with the dry plants in the final sampling periods (Figure 3.13)

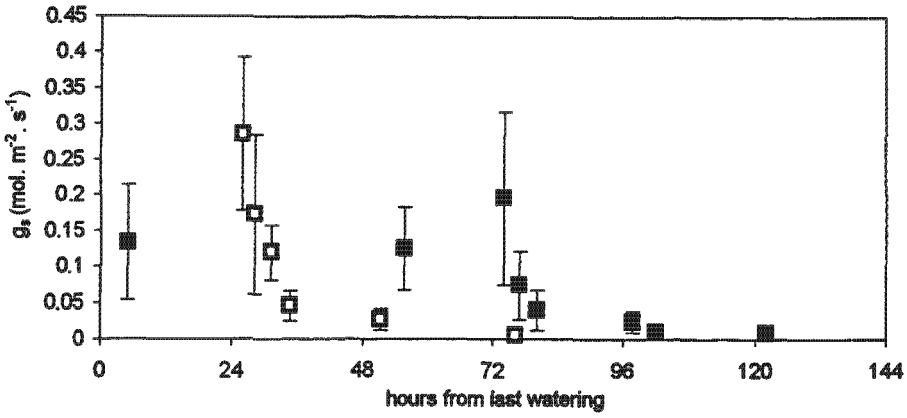

Figure 3.13. Change in stomatal conductance (g_s) over time, during drying (initiated by cessation of watering) of *Xerophyta humilis* under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

3.5. Pigment concentrations

Total chlorophyll (a+b) concentration in high and low light plants (Figure 3.14A) declined significantly from 3.6 mg. g^{-1} DM in both treatments, to 0.33 (± 0.8) mg. g dm^{-1} and 0.94 (± 1.24) mg. g^{-1} DM, respectively, in air-dry plants (Figure 3.14A). Final chlorophyll concentrations were not significantly different between the treatments, but both differed significantly from initial levels ($n \geq 11$, $p < 0.0009$). Carotenoids (x+c) in high and low light plants were also similar at the beginning of drying, at 0.94 (± 0.23) and 0.97 (± 0.095) mg. g^{-1} DM respectively. Carotenoid (x+c) concentrations declined significantly ($n \geq 11$, $p < 0.0002$) to 0.33 (± 0.12) and 0.44 (± 0.18) mg. g^{-1} DM in air-dry high and low light plants, respectively (Figure 3.14B).

Although concentrations of both chlorophylls and carotenoids decreased significantly during drying, carotenoids did not decrease to the same extent. Consequently, the proportion of carotenoids to chlorophylls increased from 0.25 mg. g^{-1} DM in both high and low light plants at the start of drying, to 13.8

(± 3.5) mg. g^{-1} DM in air-dry high light plants and 1.36 (± 1.8) mg. g^{-1} DM in air-dry low light plants (Figure 3.14C).

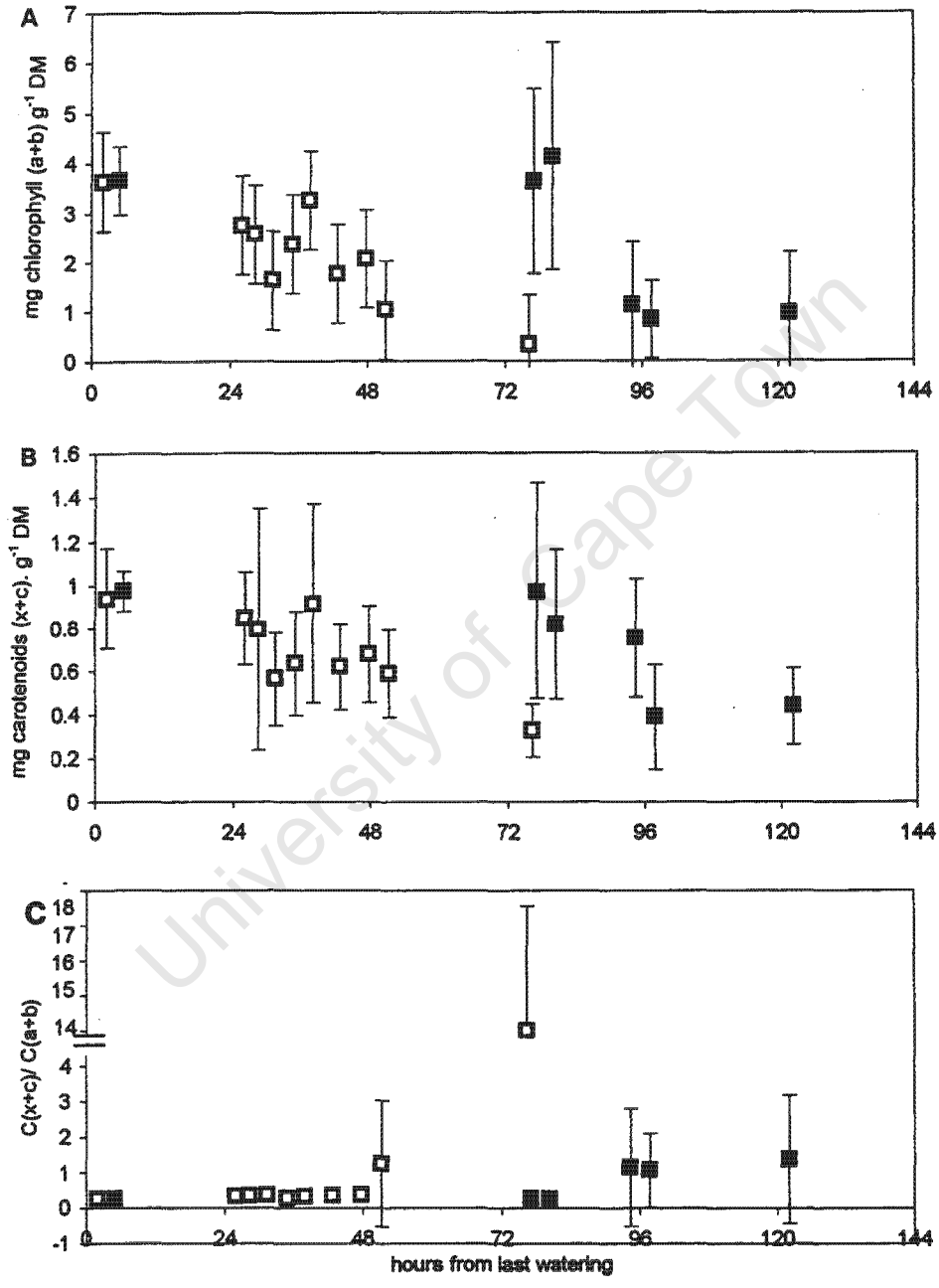

Figure 3.14. Change in chlorophyll (a+b) content (A), carotenoid (x+c) concentrations (B), and the proportion of carotenoids to chlorophylls (C) over time during drying (initiated by cessation of watering) of *Xerophyta humilis* plants under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

Anthocyanin concentration per gram dry mass of tissue was below 20 μmol. g^{-1} DM in both high and low light plants at the start of drying (Figure 3.15). In air dry plants, anthocyanin levels increased significantly in both treatments ($n \geq 11$, $p \leq 0.015$), to 165 (± 80) μmol. g^{-1} DM in high light plants, and to 52 (± 19) μmol. g^{-1} DM in low light plants. Although anthocyanin levels increased significantly in dry plants of both treatments, this increase was significantly greater in high light plants ($n = 15$, $p = 0.0029$).

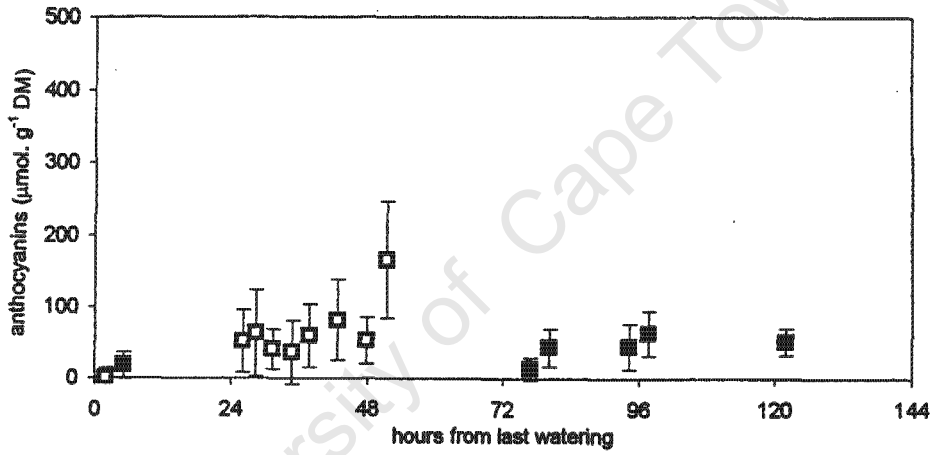

Figure 3.15. Change in concentration of anthocyanins over time during drying (initiated by cessation of watering) of *Xerophyta humilis* under high light (*ca.* 1200 μmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 μmol. m^{-2}. s^{-1}; ■) in constant environment chambers. n = 8.

4. Discussion

Although plants dried more quickly *over all* in the high light treatment (i.e. time taken to dry after cessation of watering), they took the same time to dry (*ca.* 50 h) from start of actual drying of leaf tissue in both high and low light treatments (Figure 3.1). In addition, drying-related phenomena tended to occur at the same WC in both treatments. Thus, although all drying-related

phenomena occurred *ca.* 40 h sooner in high light plants, there were no differences between rates of many drying related phenomena in high light and low light plants on a water content basis (e.g. Fv/Fm, Figure 3.5; Φ_{PSII}, Figure 3.6; chlorophyll and carotenoid concentration, Figure 3.14; *inter alia*).

Resurrection plants are found in shallow soils and on rocky outcrops, which dry rapidly (Porembski and Barthlott, 2000). Drying of resurrection plant tissue begins once soil water is no longer available, and it might be expected that the high light plants dried sooner because their soils dried sooner. There was, however, no difference in the rate of total soil drying in either treatment (Figure 3.2). Interestingly, high light plants reached an air-dry state when soils had lost only half the amount of water lost in the low light treatment, by the time low light plants had reached an air-dry state. Transpiration from plants contributes to soil drying, and as high light plants ceased transpiration sooner after cessation of watering than low light plants (Figure 3.12), transpiration in the high light treatment would cease to contribute to soil drying sooner than in the low light treatment. Soil drying rate is thus not the controlling factor responsible for plant drying rate for *X. humilis*.

Leaves of *Polypodium polypodioides* have been found by Muslin and Homann (1992) to lose more water when exposed to high light than when shaded. In their experiments the role of leaf temperature was excluded. It was, however, not reported if leaf temperature differed between the 'bright light' and 'shaded' treatments, only that it did not rise above 33°C. In the study on *X. humilis*, marginally higher leaf temperatures occurred in high light plants than in low

light plants (Figure 3.11). Leaf temperatures up to 10°C greater than ambient temperature are not uncommon in plants exposed to direct sunlight (Hopkins, 1992). A consequence of a higher leaf temperature is a greater vapour pressure difference between the leaf and the atmosphere, and an associated increased transpiration rate. Although vapour pressure difference at low relative water contents may not be reflective of true vapour pressure difference, it is indeed higher in high light plants (Figure 3.10), but transpiration, as measured within the IRGA cuvette, is not affected by this (Figure 3.12). Transpiration remains in the same range during drying for high and low light plants.

The quicker onset of drying in high light plants may be a feedback mechanism cued by a carbohydrate content (Rawson *et al.*, 1976). However, even if drying is controlled or instigated as a consequence of specific carbohydrate levels in conjunction with low water-availability, the mechanism by which plants dry more rapidly is not clear. High light plants do not transpire more rapidly than low light plants, and they do not facilitate drying by delayed stomatal closure; stomatal conductance does not differ significantly between high and low light plants during drying (Figure 3.13).

The lack of any apparent physiological process, such as stomatal conductance or transpiration rate, that can be related to the more rapid initiation of drying in high light leaves initiated further investigation of the phytotron environments in which the plants were dried. Although photoperiod, day and night temperatures and light intensity were controlled, the chambers

utilised airflow through the system to maintain temperature. The higher light intensity in the high light chamber resulted in a greater heat generation, and consequently a greater airflow to maintain day time temperatures at 25°C. The plants in the high light chamber thus experienced windier conditions (Chapter 2, section 1.1). Transpiration rates were not higher in the high light chamber, which is what the effect of a windier environment would have on the plants, if any. However, transpiration rates were measured while leaves were inside the constant environment of the leaf cuvette, where the external airflow rates would have no effect. It thus appears that the apparent effect of high light on rate of initiation of drying was, in this experiment, a consequence primarily of the higher airflow in the chamber.

The potential effect of light intensity on drying rate of *X. humilis* cannot be excluded as a factor over all, however. It has already been discussed that light intensity can affect leaf temperature and thus indirectly vapour pressure difference at the leaf surface, and this may still be a contributing factor to drying rate. *X. humilis* has previously been reported to dry to an air-dry state after nine days (Farrant *et al.*, 1999; Farrant *et al.*, 2002) and seven days (Dace *et al.*, 1998) without watering. This is a slower rate of drying than that occurring in either high or low light treatments in the present study. Such a rate difference may be again due to air-flow differences, but may also be contributed to by light level differences. No difference in soil-volume of pots existed between treatments, and as such the faster drying rate of high light plants is not due to any effect of different soil volumes.

Low levels of membrane damage, indicated by electrolyte leakage rates (Leopold *et al.*, 1981), had accrued in dry material in both treatments (Figure 3.3). Unlike the experiments of Farrant (2000) and Farrant *et al.* (1999) in which no increase in membrane damage occurred during drying, membrane damage (inferred from electrolyte leakage rates) in high light plants rose rapidly at the beginning of dehydration. This suggests that the hydrated tissue was experiencing a non-drying related stress that was immediately exacerbated when water-deficit stress was imposed, or possibly that lipid bilayers were merely being 'rearranged' (Hoekstra *et al.* 1999). It is well known that plants that have been 'hardened' to resist one stress are frequently found to be resistant to other stresses (Lichtenthaler, 1996; Hölmberg and Bülow, 1998), as although the stresses differ, the damage, and hence prevention and repair thereof, are similar. The decline in electrolyte leakage during drying (Figure 3.3) indicated that drying induced protection and repair mechanisms alleviated some of the original non-drying related damage before plants dried completely. Membranes in low light plants experienced an accrual of damage later on in the drying process. It is likely that such damage was associated with drying itself, and not another different stress. Both high and low light plants were unaffected by transient increases in membrane damage, as indicated by the low amount of damage in dry plants.

Lower quantum yield, as is seen in high light plants relative to low light plants (Figure 3.4), can indicate that light energy is being used in photorespiratory carbon and nitrogen recycling (Noctor *et al.*, 2002). This is of benefit when CO_2 is limited as is the case under water-deficit conditions, as it reduces

possible formation of hazardous compounds. The lower quantum yield in this case, however is also a function of the lower absolute quantum efficiency (Figure 3.5) of high light plants relative to low light plants in the stages before photosystems are broken down. Reduced absolute quantum efficiency is a result of photoinhibition (Lawlor and Cornic, 2002), or due to an increased epoxidation state of the xanthophyll cycle (Munné-Bosch and Alegre, 2000a). The latter explanation is likely to be applicable in this study, as significantly higher levels of non-photosynthetic fluorescence quenching occurred in high light plants (Figure 3.6B). Photosynthetic use of light energy is not lower in low light plants, indicating that low light levels are not limiting (Figure 3.6A), and are in fact a benefit to the plants, imposing less of a stress on them as they dry. Further evidence for the stress imposed by the higher light levels on the high light plants was the significantly greater accumulation of anthocyanins to limit light penetration of the leaves (Figure 3.15). That anthocyanins were upregulated in low light plants indicates that light is nevertheless hazardous to the plants at intermediate water contents, but it is probably much more so under higher irradiance.

High light plants also retained higher proportions of carotenoids relative to chlorophylls (Figure 3.14B), than low light plants did. Carotenoids are involved in protection against light-induced damage to photosystems (Demmig-Adams and Adams, 1996a), and the increase relative to chlorophyll concentration seen in high light plants further suggests that these plants experienced greater light stress.

The net assimilation rate declined with decreasing water contents (Figure 3.7), as did both maximal quantum efficiency (Figure 3.5) and stomatal conductance (Figure 3.13). Decline in the rate of net assimilation has been suggested to be due to breakdown of photosynthetic apparatus (Farrant, 2000). In this study, however, maximal quantum assimilation (indicative of functional status of PSII) and net assimilation decline independently of one another (Figure 3.8B). The decline in net assimilation was more closely correlated to decreasing stomatal conductance (Figure 3.8A).

In summary, plants of *X. humilis* subjected to drying under high and low light conditions varied little between treatments with respect to the rates of which, and levels to which, metabolic processes shut down. The water contents at which metabolic changes occurred were also no different between plants from different treatments. They varied instead in the degree they change metabolic factors associated with light stress. Mechanisms that protect against light stress were upregulated to a greater level in high light plants than in low light plants. The upregulation seemed to be sufficient to protect the plants as there was no excess membrane damage apparent in dry tissues of high light plants. In this experiment the different drying rates between light treatments may primarily be the result of the greater wind speed in the high light chamber.

Chapter 4

FIELD AND LABORATORY REHYDRATION

1. Introduction

During rehydration from the air-dry state desiccated plants are subjected to mechanical stresses associated with the rapid inflow of water (Iljin, 1957), as well as metabolic stresses associated with intermediate water contents (Pammenter and Berjak, 1999). 'True' desiccation-tolerant plants, such as many mosses and lichens, survive drying mainly through constitutive protection mechanisms and by repair of desiccation-induced damage during rehydration (Oliver and Bewley, 1997). 'Modified' desiccation-tolerant plants, such as *Xerophyta humilis*, are thought to rely more on the upregulation of protection mechanisms during stressful periods than on repair and constitutive protection (Farrant, 2000). They consequently require protection mechanisms to either be upregulated during rehydration or to be sufficiently instated prior to reaching an air-dry state. Poikilochlorophyllous resurrection plants dismantle their photosynthetic apparatus during drying, and thus have the further metabolic requirement during rehydration of resynthesis of pigments and reconstitution of organelles. It has been suggested by Sherwin and Farrant (1996) that recovery rate of desiccated resurrection plants is slower in poikilochlorophyllous species than homoiochlorophyllous species, due to the greater extent of reconstitution of subcellular organisation required.

Previous studies of physiological aspects of rehydrating *X. humilis* have been done by Dace *et al.*, (1998), Farrant *et al.*, (1999), and Farrant *et al.*, (2002),

in addition examinations of rehydration of other *Xerophyta spp* have also been made (eg. Hallam and Gaff, 1978; Tuba, *et al.*, 1993; Sherwin and Farrant, 1996). Farrant *et al.* (1999) examined rehydrating *X. humilis* to assess survival from very rapid drying rates, and Farrant *et al.* (2002) assessed survival of *X. humilis* dried in the absence of light. Rehydration of this species under natural field conditions has not previously been reported, possibly due logistic difficulties involved with accessing the natural habitat for this species.

X. humilis plants, dried naturally in the field and dried under high and low light laboratory conditions, were rehydrated by watering. A comparison between these treatments was done to assess if physiological aspects of recovery from desiccation differed under different environmental conditions.

2. Methods

Plants dried under low light and high light phytotron conditions, as well as naturally in the field, were rehydrated as described in Chapter 2 sections 1.1.2 and 1.2. Measurements were taken from just before watering and at regular intervals thereafter for four days in the laboratory studies and three days in the field study.

3. Results

3.1. Water contents

Rate of rehydration of tissues was no different among high light laboratory, low light laboratory and field-rehydrated plants, reaching maximum levels

within *ca.* 20 h (Figure 4.1A). Furthermore there was no difference in the maximum WC of the plants in the various environmental conditions, but sample variation was high. Even though the soil was watered to field capacity daily, high light plants remained at a constant WC thereafter, but low light plants declined to 2.7 (± 0.6) g. g^{-1} DM after 75 h of rehydration. WC of field-rehydrated plants also declined, falling below 2 g. g^{-1} DM after 45 h. Relative to WC measured on fully turgid leaves, (Figure 4.1B), both high and low light plants rehydrated to and remained at a RWC > 100%. Field-rehydrated plants rehydrated to 100% RWC, declining to a RWC of 70% in fully recovered plants.

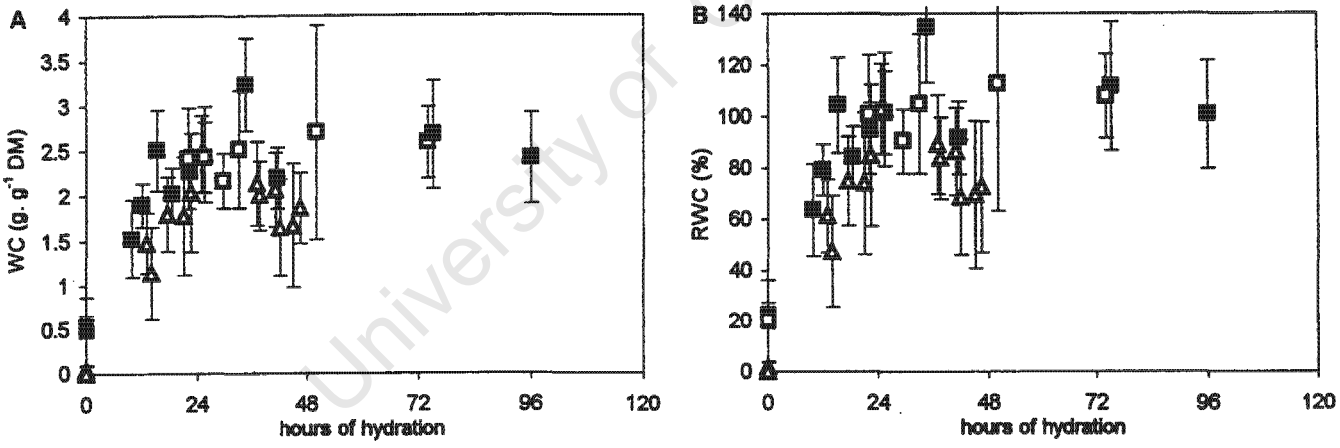

Figure 4.1. Change in water content, WC (A), and relative water content, RWC (B), of *Xerophyta humilis* during high light laboratory (*ca.*1200 µmol. m^{-2}. s^{-1}; □), low light laboratory (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) and field (Δ) rehydration (initated by watering). n>8.

3.2. Membrane integrity

Initial rates of electrolyte leakage, Figure 4.2, in high light plants were significantly higher than initial rates of electrolyte leakage in both low light and field-rehydrated plants ($n \geq 16$, $p \leq 0.013$), indicating lower membrane integrity

in high light plants. The electrolyte leakage rate in high light plants declined significantly (n = 16, P = 0.004) from 13 (± 4.9) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM to 2.7 (± 1.4) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM after 74 h of recovery, and reached levels similar to that of fully recovered field material. Electrolyte leakage rate in both low light and field-rehydrated plants did not change significantly from similar initial values of 9.6 (± 3) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM and 4.2 (2.4) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM respectively, although final values of 6.2 (± 3.6) μS. cm^{-1}. ml^{-1}. min^{-1}. g^{-1} DM for low light plants were significantly higher (n ≥ 16, P = 0.02) than high light and field-rehydrated levels.

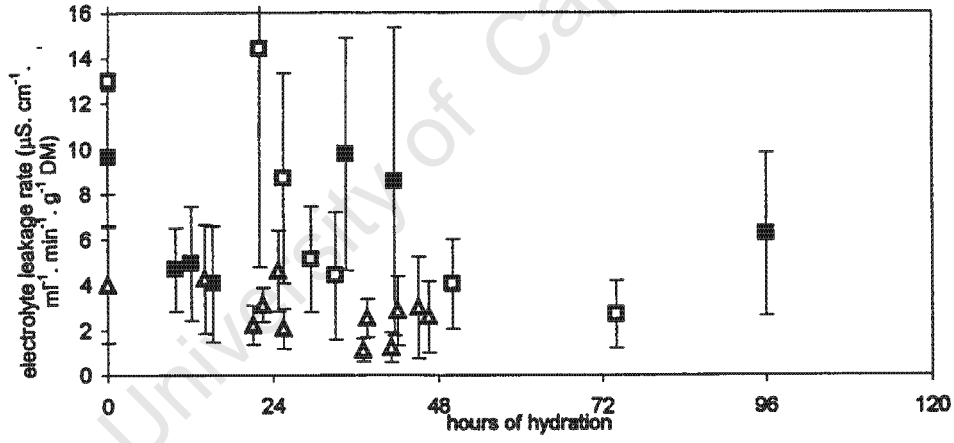

Figure 4.2. Rate of electrolyte leakage over time during rehydration (initiated by watering) of *Xerophyta humilis* under high light laboratory (*ca.*1200 μmol. m^{-2}. s^{-1}; □), low light laboratory (*ca.*320 μmol. m^{-2}. s^{-1}; ■) and field (Δ) conditions . n>8.

3.3. Chlorophyll fluorescence

Actual quantum yield of PSII (Φ_{PSII}) of high and low light plants recovered to maximum levels of about 0.66 after 33 and 38 h of rehydration respectively, whereas field-rehydrated plants had already recovered to maximum levels of

about 0.75 after 13 h of rehydration (Figure 4.3A). Thus recovery of efficiency of photochemistry (conveyed by Φ_{PSII} values) was most rapid in field-rehydrated plants, although field-rehydrated plants did not reach significantly higher levels of Φ_{PSII}. After maximum levels of Φ_{PSII} were attained, there were periodic declines in Φ_{PSII} in plants under all three conditions, returning to maximum levels after each decline. This regular cyclic pattern of periodic declines was most pronounced in field-rehydrated plants. Maximal quantum efficiency (Fv/Fm) for each treatment increased at a similar rate to the increases of Φ_{PSII} during rehydration. Thus field-rehydrated plants attained maximum Fv/Fm more quickly (13 h) than those at high and low light, which reached maximum levels of 0.7 (± 0.1) and 0.75 (± 0.08) after 25 and 38 h respectively (Figure 4.3B). Once Fv/Fm had attained maximum levels, between 0.7 and 0.76, these were maintained in all treatments.

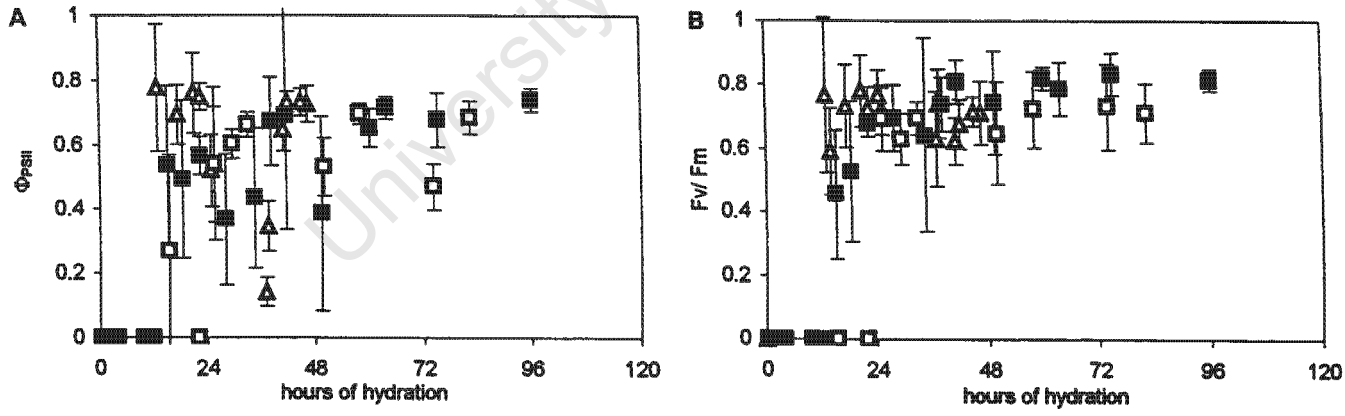

Figure 4.3. Change in chlorophyll fluorescence parameters, Φ_{PSII} (A) and Fv/Fm (B) during rehydration (initiated by watering) of *Xerophyta humilis* under high light laboratory (*ca.*1200 µmol. m^{-2}. s^{-1}; □), low light laboratory (*ca.*320 µmol. m^{-2}. s^{-1}; ■) and field (Δ) conditions. n>8.

Photosynthetic chlorophyll fluorescence quenching (qP), Figure 4.4A, recovered to maximum levels (*ca.* 1) in low light and field-rehydrated plants by 15 h of rehydration. At this time qP of high light plants was still much lower at 0.4 (± 0.59). The proportion of open reaction centres reached maximum levels (qP = 1.13 (± 0.15)) in high light plants after 30 h of rehydration. Although maximum levels of qP were recorded in fully recovered plants from all treatments, variation in mean qP occurred throughout the recovery period in all treatments, most notably in field-rehydrated plants. qP of field-rehydrated plants declined to 0.2 (± 0.05) at 37 h after initial watering, before recovering once more to *ca.* 1. Heat dissipation (non-photosynthetic chlorophyll fluorescence quenching, qN) increased more slowly than qP, in all treatments (Figure 4.4B). Maximum qN levels of *ca.* 0.45 – 0.5 were reached in low light and field-rehydrated plants after 25 h of rehydration, at which time qN in high light plants was only 0.06 (± 0.08). High light plants reached maximum levels by 50 h. Variation in the mean extent to which plants re-emitted light energy as heat occurred in all treatments, but as with qP this was most extensive in field-rehydrated plants. qN in field-rehydrated plants declined to 0.7 after 13 h and again after 38 h of recovery, increasing to a mean of at least 0.25 after each decline.

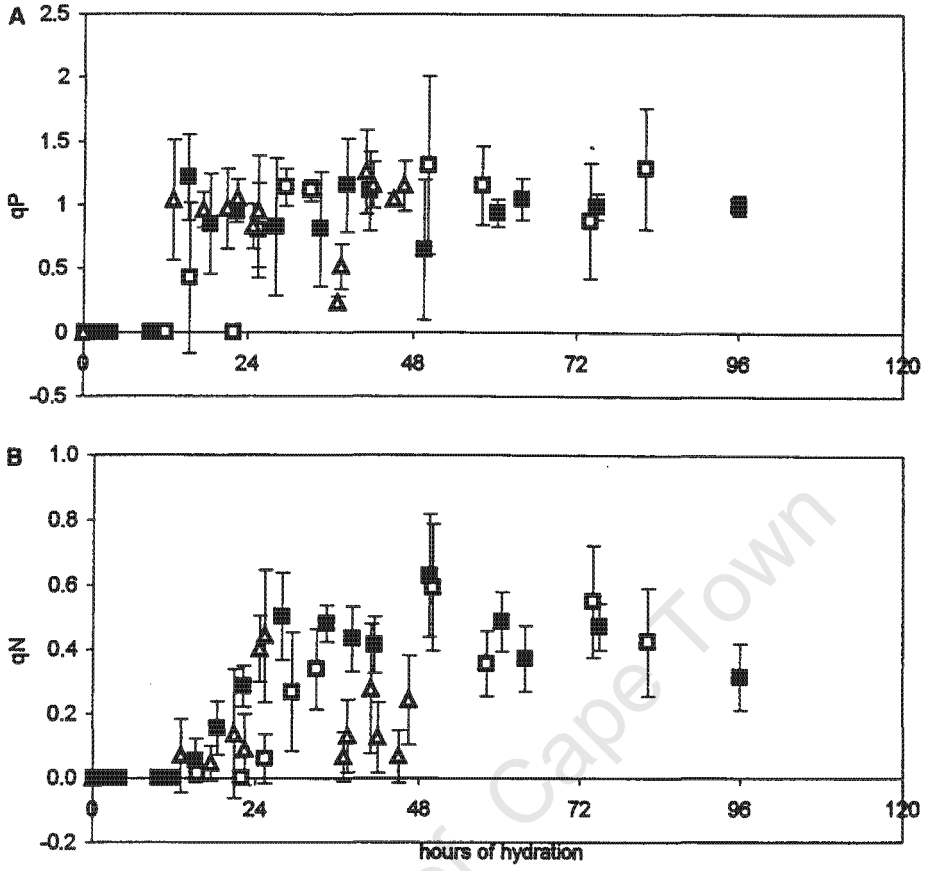

Figure 4.4. Changes in photosynthetic (qP) (A), and non-photosynthetic (qN) (B) chlorophyll fluorescence quenching of *Xerophyta humilis* during rehydration under high light laboratory (*ca.*1200 μmol. m^{-2}. s^{-1}; □), low light laboratory (*ca.*320 μmol. m^{-2}. s^{-1}; ■) and field (Δ) rehydration (initiated by watering). n>8.

3.4. Gas exchange

Gas exchange parameters were measured in high light and low light laboratory experiments only, and not during field rehydration due to technical problems.

Net photosynthetic CO_2 assimilation rates, A_{net} (Figure 4.5A), increased steadily during the rehydration period for both high light and low light plants, except for an anomalous decline below 0 μmol. m^{-2}. s^{-1} on d 4 of rehydration in

low light plants (between 60 h and 75 h of rehydration). A_{net} in low light plants returned to positive levels again on d 5, reaching a maximum value, in fully recovered plants, of 4.6 (± 2.2) μmol. m^{-2}. s^{-1}. Similar maximum photosynthetic assimilation rates were recorded in fully recovered high light plants. A_{net} was negative during the initial 20 – 30 h of rehydration for both treatments (Figure 4.5A) which suggests that dark respiration rates (R_D) were greater than CO_2 fixation rates even though plants were in the light. However, A_{net} was more negative during the initial period than can be accounted for by R_D (Figure 4.5B), and this might be attributable to possible increases in photorespiration during this period. Dark respiration resumed very soon (≤ 2 h) after initial watering (Figure 4.5B). There was much variation in dark respiration rates throughout the rehydration period in high and low light plants. However, there were increases in respiration in both treatments after 30 h of rehydration, but this increase was only significant in high light plants (n = 16, P = 0.035).

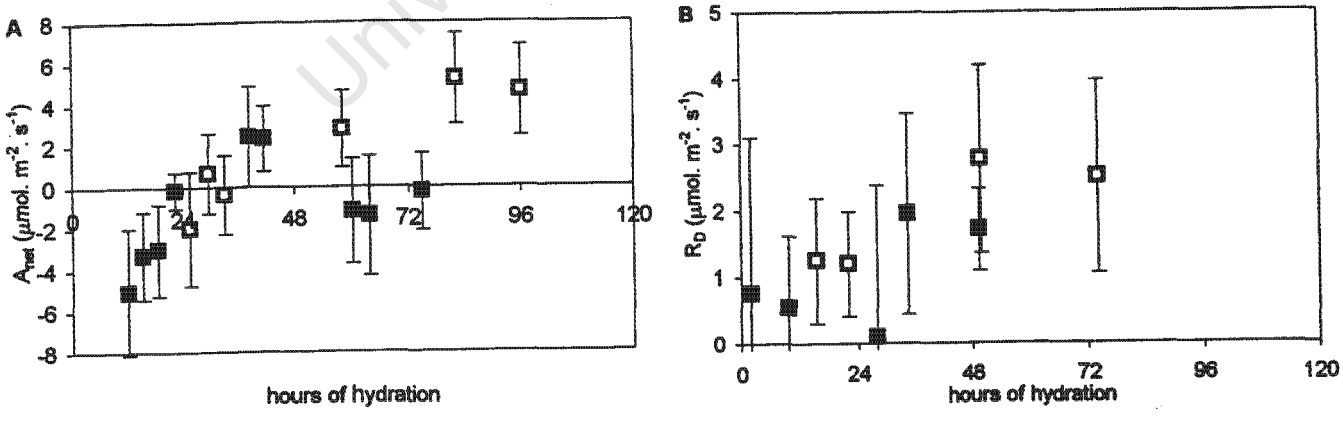

Figure 4.5. Net photosynthetic CO_2 assimilation rates, A_{net} (A), and dark respiration rates, R_D (B), during rehydration (initated by watering) of *Xerophyta humilis* under high light laboratory (*ca.*1200 μmol. m^{-2}. s^{-1}; □), low light laboratory (*ca.*320 μmol. m^{-2}. s^{-1}; ■) and field (Δ) conditions. n>8.

As occurred in A_{net}, stomatal conductance rates (g_s) increased steadily during the recovery period (Figure 4.6). Positive net assimilation rates in recovering plants were low when Fv/Fm was low, and high when Fv/Fm was high (Figure 4.7A). However, the two parameters changed independently of one another, as occurred during dehydration (Figure 3.8B). There hence appeared to be no dependant relationship. Increasing net assimilation rates were, however, correlated with both increasing stomatal conductance rates, and with increasing chlorophyll (a+b) concentration, (Figures 4.7B and C, respectively). The linear regression of net assimilation and g_s was significant in high light plants ($R^2 = 0.74$) but not in low light plants ($R^2 = 0.26$). Linear regressions of net assimilation and chlorophyll content were more significant, at $R^2 = 0.79$ and $R^2 = 0.94$ for high and low light plants, respectively (Anomalous d 4 net assimilation data were omitted for these curves).

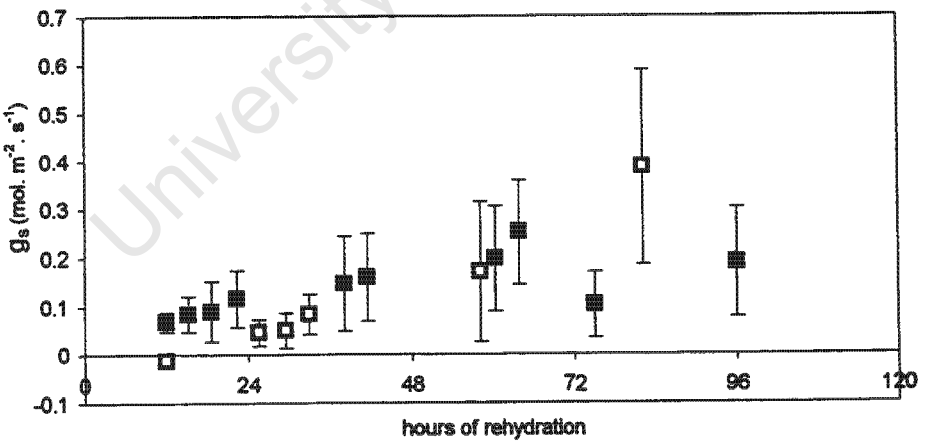

Figure 4.6. Change in stomatal conductance (g_s) during rehydration (initated by watering) of *Xerophyta humilis* under high light laboratory (*ca.*1200 µmol. m^{-2}. s^{-1}; □), low light laboratory (*ca.*320 µmol. m^{-2}. s^{-1}; ■) and field (Δ) conditions. n>8.

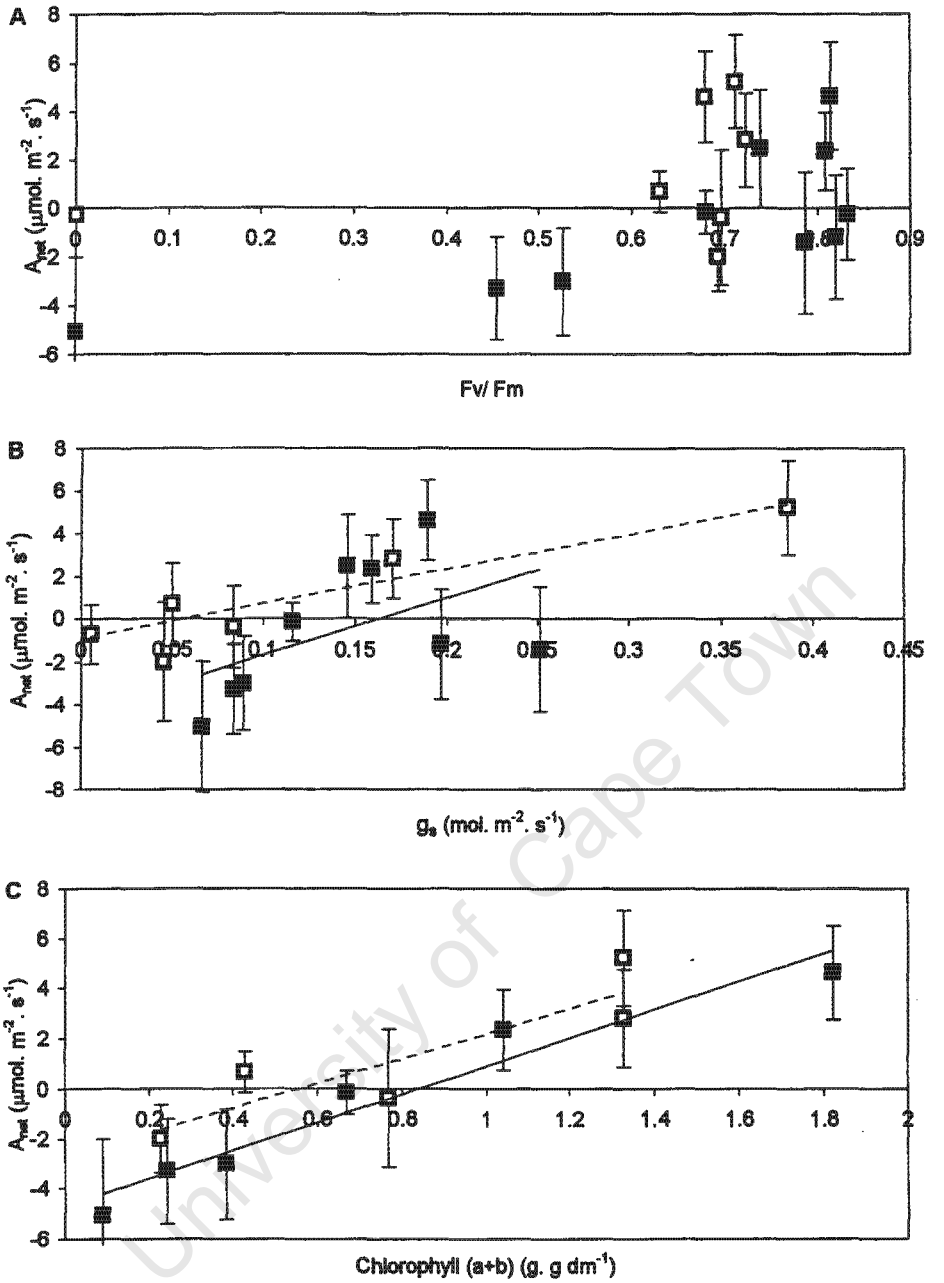

Figure 4.7. Change in net photosynthetic assimilation rates during rehydration (initiated by watering) of *Xerophyta humilis* relative to Fv/Fm (A), stomatal conductance, g_s (B), and chlorophyll (a+b) concentration (C). Linear regression lines through high light means (*ca.*1200 µmol. m^{-2}. s^{-1}; □) and low light means (*ca.*320 µmol. m^{-2}. s^{-1};■) are represented by (---) and (—) respectively. n>8.

3.5. Pigment concentrations

Chlorophyll concentrations (Figure 4.8A) increased consistently and at a similar rate in rehydrating high light and low light plants. Final concentrations

of 1.3 (± 0.6) and 2.23 (± 0.6) mg. g^{-1} DM were reached *ca.* 75 h after rehydration began. Chlorophyll concentrations increased more rapidly in field-rehydrated plants, and reached 2.1 (± 0.4) mg. g^{-1} DM after 25 h of rehydration. However, field-rehydrated plants did not maintain this chlorophyll concentration throughout the sampling period; chlorophyll concentration varied instead between a maximum value of 2.5 (± 0.85) mg. g^{-1} DM, and a minimum value of 0.9 (± 0.84) mg. g^{-1} DM.

Concentration of carotenoids (x+c), (Figure 4.8B) was *ca.* 0.3 mg. g^{-1} DM after 10 h of rehydration in all treatments. There was no significant change in carotenoid concentration during recovery in high and low light treatments. However, a gradual increase in the carotenoid concentration of high light plants meant that the final concentration of 0.57 (± 0.15) mg. g^{-1} DM in fully recovered high light leaves was significantly greater than the final concentrations of 0.21 (± 0.10) mg. g^{-1} DM in low light leaves ($n = 15$, $P = 0.04$). Carotenoid concentration in field-rehydrated plants increased significantly in fully recovered material to 0.45 (± 0.33) mg. g^{-1} DM ($n = 29$, $P < 0.00001$). Final carotenoid concentration in field-rehydrated plants was also significantly higher than final those in both high light ($n = 23$, $P = 0.03$) and low light ($n = 22$, $P < 0.00001$) plants.

The ratio of carotenoids (x+c) to chlorophylls (a+b) declined from 13.8 (± 3.5) in high light plants, 4.8 (± 2.7) in low light plants, and 31.8 (± 17.4) in field-rehydrated plants, to below 1 in fully recovered plants of all treatments (Figure 4.8C).

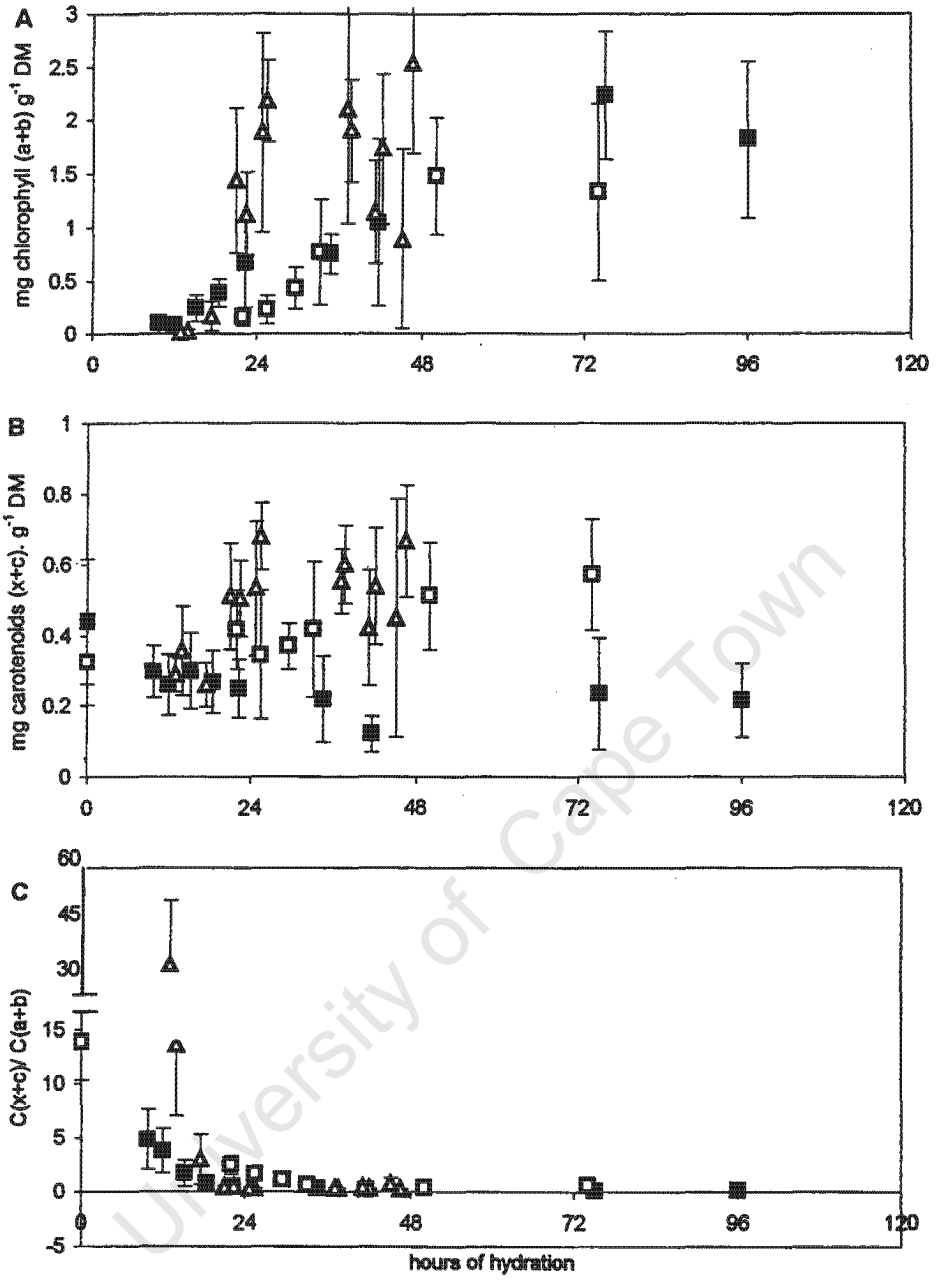

Figure 4.8. Change in concentration of chlorophylls (a+b) (A) and carotenoids (x+c) (B), and ratios of chlorophyll (a+b) to carotenoids (x+c) (C) in *Xerophyta humilis* during rehydration (initiated by watering) under high light (*ca.* 1200 μmol. m^{-2}. s^{-1}; □), low light (*ca.*320 μmol. m^{-2}. s^{-1};■) laboratory and field (Δ) conditions. n>8.

Anthocyanin concentrations at the start of rehydration were 51.7 (± 18.7) μmol. g^{-1} DM in low light plants, 165 (± 80.5) μmol. g^{-1} DM in high light plants, and significantly higher than both high light and low light plants (n = 31, P <

0.0001) at 443.3 (± 173) μmol. g^{-1} DM in field-rehydrated material (Figure 4.9). Final values were similar for low light and field-rehydrated plants, at *ca.* 45 μmol. g^{-1} DM, but were unexpectedly much lower (below 10 μmol. g^{-1} DM) in fully hydrated high light plants.

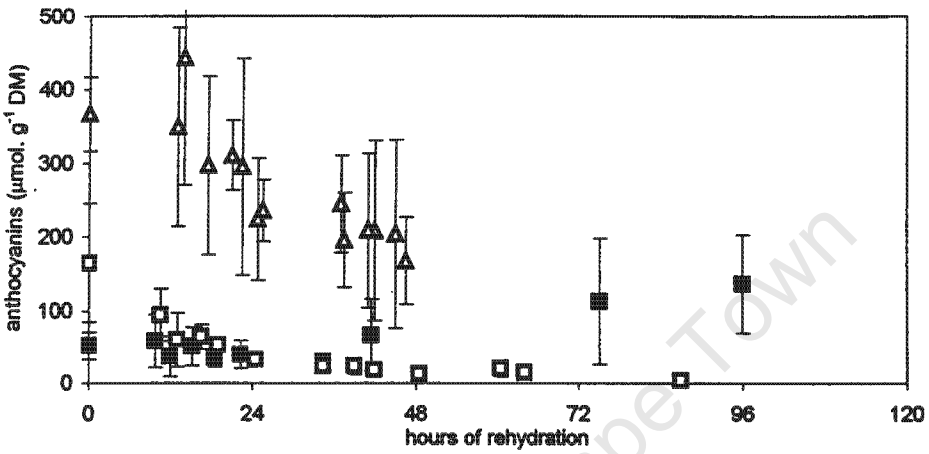

Figure 4.9. Change in concentration of anthocyanin during rehydration of *Xerophyta humilis* under high light (*ca.* 1200 μmol. m^{-2}. s^{-1}; □), low light (*ca.*320 μmol. m^{-2}. s^{-1};■) laboratory and field (Δ) conditions. n>8.

4. Discussion

Recovery from desiccation occurred in all treatments, indicating that although different conditions exposed the plants to different stresses during drying (Chapter 3), none of the treatments caused loss of viability during drying or rehydration. Rates of increasing WC (Figure 4.1A) and resumption of metabolic function such as maximal quantum efficiency (Figure 4.3B) were similar in high and low light laboratory treatments, as well as in the field-rehydration treatment. The rapid reconstitution of photosynthetic machinery is due to transcription of mRNA during dehydration, which is stored and then translated immediately during rehydration (Dace *et al.*, 1998). The increase of RWC in laboratory rehydrated plants to above 100% is theoretically impossible

(Figure 4.1B), however this result indicates that the absolute maximum level of WC is not a fixed parameter in *X. humilis*, and that it is possibly affected by environmental conditions.

Resumption of respiration occurred as early as 2 h after the initial application of water (Figure 4.5B). Although these data are for laboratory-rehydrated plants only, similar rehydration rates for field-rehydrated plants (Figure 4.1) indicate that metabolism also resumed soon after watering for these plants. Early recovery of respiration rates in rehydrating poikilochlorophyllous desiccation-tolerant plants is common (eg. Vander Willigen *et al.*, 2001; Tuba *et al.*, 1998), as these plants require energy for resynthesis of pigments and organelles. Compared with recovery of respiration, the recovery of net photosynthetic assimilation is relatively slower (Figure 4.5A), only reaching levels above the compensation point more than 20 h after initial watering, when the plants were already fully rehydrated (Figure 4.1). Recovery of photosynthesis thus appeared to occur only once the plants were turgid, in both high and low light conditions. The involvement of WC in the recovery of photosynthesis was further confirmed by the correlation of net assimilation to stomatal conductance levels (Figure 4.6B). Stomata opened progressively as WC increased (and water-deficit stress declined) over time (Figure 4.7). In this study the recovery of photosynthesis is also related to chlorophyll content (Figure 4.6C), which is resynthesised during the recovery of dehydrated poikilochlorophyllous desiccation tolerant plants (Hambler, 1961).

Light is required for the final stages of chlorophyll synthesis in angiosperms (von Wettstein *et al.*, 1995). *Xerophyta* spp. rehydrated under low light, such

as in experiments by Hallam and Gaff (1978) where light levels were 120 μmol. m^{-2}. s^{-1}, and Sherwin and Farrant (1996) where light was reduced by 30% shade cloth, resulted in the initiation of chlorophyll synthesis occurring in *X. villosa* and *X. viscosa* respectively, after 24 h. In experiments by Tuba *et al.* (1993a and 1993b) on *X. scabrida*, where leaves were rehydrated under higher light intensities of 1000 μmol. $m^{-2}.s^{-1}$ and 1200 μmol. m^{-2}. s^{-1} respectively, chlorophyll resynthesis began after 8 – 10 h. High light intensities (> 2000 μmol. m^{-2}. s^{-1}) in field experiments of this study thus possibly contributed to the rapid rates of chlorophyll resynthesis in *X. humilis* (Figure 4.8A). However, in most of the above mentioned experiments (Hallam and Gaff, 1978; Tuba *et al.*, 1993a, 1993b) detached leaves were rehydrated, and not whole plants. Leaves of *X. villosa, X. viscosa* and *X. scabrida*, at lengths of over 20 cm in mature plants, are much larger than those of *X. humilis*, which are seldom longer than 5 cm. Differences in chlorophyll-synthesis rates may therefore also be due to the larger size of the leaves.

High levels of anthocyanins in field-rehydrated plants (Figure 4.9) suggest that these plants experienced high levels of light-induced stress, against which protective mechanisms were upregulated. The extremely high levels of carotenoids (x+c) relative to chlorophylls (a+b) in field plants in the dry state and initial stages of rehydration, confirm this result as, like anthocyanins (Hendry, 1993), carotenoids function to protect plants from stressful levels of light (Demmig-Adams and Adams, 1996a). Light intensities in the high light treatment also imposed a light-stress on the high light plants, although not to the same extent as that in field-rehydrated plants. Levels of anthocyanins and relative

levels of carotenoids were significantly greater in high light plants than in low light plants, which experienced light intensities of only 30% of those in the high light treatment (Figure 4.8C, Figure 4.9). Interestingly, the plants exposed to the highest light stress levels (Figure 2.2; plants in the field) showed the lowest levels of damage in dry and hydrating leaves (Figure 4.2). It is possible that laboratory-rehydrated plants were experiencing additional stresses, such as stresses related to high air-flow rates, which caused (repairable) tissue damage not seen in field-rehydrated plants. As no membrane 'leakiness' was observed in field-rehydrated plants, it is unlikely that membrane reorganisation (rather than damage), as is suggested by Dace *et al.* (1998), was responsible for that seen in laboratory rehydrated plants.

Other than differences, between field and laboratory-rehydrated plants, of protective mechanisms, field-rehydrated plants showed greater variability of PSII quantum efficiency (Figure 4.3A) and photosynthetic and non-photosynthetic fluorescence quenching (Figure 4.4A and B). Chlorophyll concentrations varied widely during the same period, but it is unlikely that variation in the chlorophyll fluorescence parameters mentioned above were due to variations in chlorophyll content, as maximal quantum efficiency (Fv/Fm) remained constant (Figure 4.3B) during this period. Possible reasons for the periodic variation in certain physiological parameters are explored in the following chapter (Chapter 5).

In conclusion, it appeared that although there was no difference in survival among the treatments, laboratory-rehydrated plants experienced greater levels of damage than field-rehydrated plants. *X. humilis* was able to respond effectively to higher levels of light-stress, as seen in field-rehydrated plants. This suggests that

laboratory-rehydrated plants were experiencing stresses additional to those from excess light. Not only do high light levels impose stresses on these plants, high light intensity is also involved in chlorophyll resynthesis, thus playing an important role in recovery from dehydration. Additionally, the physiology of field rehydrated plants undergoes cyclic changes during rehydration.

Chapter 5

ACCLIMATION AND RHYTHMIC PHENOMENA

1. Introduction

Plants are adapted to survive in the environments in which they occur. Environments, however, are subject to change. Whether environmental change is long-term change, such as the change in temperature with the turning of the seasons, or short-term, such as that which occurs when a cloud moves across the sun, plants can respond with changes of their own. Changes in the physiology and biochemistry of plants, like environmental changes, occur over different time scales. Transient environmental changes, such as when a sunfleck reaches a forest understory, can result in transient changes in photosynthetic performance, for example (Hopkins, 1992; Lichtenthaler and Meihé, 1997). Longer term changes in the environment of a plant, such as when an old tree collapses and the previously shaded trees below are moved permanently into direct sunlight, can result in long-term adaptations, or acclimatisation, of the physiology of the plant. For example, pigment concentrations (Demmig-Adams and Adams, 1996b) and leaf morphology (Hopkins, 1992; Lichtenthaler, 1996) can become adapted to the new condition. Sun-adapted leaves have a chlorophyll a/b ratio of about 3.2 – 4 and shade-adapted leaves have a chlorophyll a/b ratio of about 2.5 – 2.9 (Lichtenthaler, 1987). In this chapter, plants of *Xerophyta humilis* that were used in the high light and low light studies reported in previous chapters (chapters 4 and 5) were assessed for acclimatisation to these different environments. An understanding of how these plants adapt to environmental change, if they adapt at all, can give

further insight into the effects of different ambient conditions of the physiology of *X. humilis*.

Some changes in an environment are unpredictable, and others occur at predictable periods throughout the life of a plant. The light of day-time and dark of night-time are examples of a predictable, cyclic change in the environment. Many aspects of plant physiology fluctuate synchronously with the changing light regime every 24 h, such as photosynthetic rate (Salisbury and Ross, 1992). In fact, such is the importance of cyclic phenomena that they have even been detected in the dormant seeds of some species, which exhibit cyclic variations of respiration rate (McClung, 2001). Physiological rhythms that occur during a day-night cycle can be either 'diurnal oscillations', which are exogenously controlled, or 'circadian rhythms' which, although they are entrained to a specific period by external factors, persist in the absence of such cues (McClung, 2001). In order to understand some of the variation that occurred during rehydration of field-based plants (Chapter 4), a brief examination was made of *X. humilis* physiology in the hydrated state in the laboratory. This was done to determine if the aforementioned variations during rehydration were rehydration-related, or perhaps due to natural daily variation in the physiology of the plants. The study of circadian rhythms and other rhythmic phenomena in plants is an extensive field in its own right. The assessment of rhythmic phenomena in *X. humilis* was by no means a comprehensive examination.

2. Methods

Hydrated plants, previously kept in a greenhouse, were moved into high and low light phytotrons. Experimental procedure is described in Chapter 2, section 1.1.3. Values for chlorophyll a and b concentrations at the end of the acclimatisation period were obtained from fully hydrated leaves, sampled at the beginning of the dehydration experiment.

3. Results

3.1. Acclimatisation

In order to determine whether any changes did occur, statistical tests (Students t-test) were performed on various initial vs. final data points for high and low light plants. The results from these tests are represented in Table 5.1.

Table 5.1. Statistical results of Student's t-test conducted between initial and final data points for the acclimation period of high light and low light *Xerophyta humilis* plants. "S" represents a statistically significant difference, "NS" represents no significant difference.

Student's t-test between initial and final samples						
Parameter	high light		significance	low light		significance
Fv/Fm	n = 16	p = 0.007	S	n = 13	p = 0.065	NS
Φ_{PSII}	n = 16	p < 0.0001	S	n = 13	p = 0.002	S
qN	n = 16	p = 0.0002	S	n = 13	p = 0.005	S
qP	n = 16	p = 0.25	NS	n = 13	p = 0.092	NS
A_{net}	n = 15	p = 0.035	S	n = 16	p = 0.015	S
R_d	n = 16	p = 0.103	NS	n = 16	p = 0.071	NS
g_s	n = 15	p < 0.0001	S	n = 16	p = 0.029	S

3.1.1. Chlorophyll fluorescence

Measurements of maximum quantum efficiency of PSII, Fv/Fm, on plants that had very recently been moved from the greenhouse into either of the phytotrons were high, around 0.8, indicative of healthy photosystems (Figure 5.1A). Plants placed under low light conditions maintained these levels, but high light plants became stressed (photoinhibition occurred), as indicated by a significant decline of Fv/Fm in high light plants to 0.69 (± 0.09). A significant decline of actual quantum yield (Φ_{PSII}) occurred in both high and low light plants. However, use of absorbed energy in photochemistry (Φ_{PSII}) in high light plants declined rapidly (within 1 d in the new environment) and dramatically more than low light plants to only 0.32 (± 0.063), indicative of reduced electron transport rates through PSII (Figure 5.1.B).

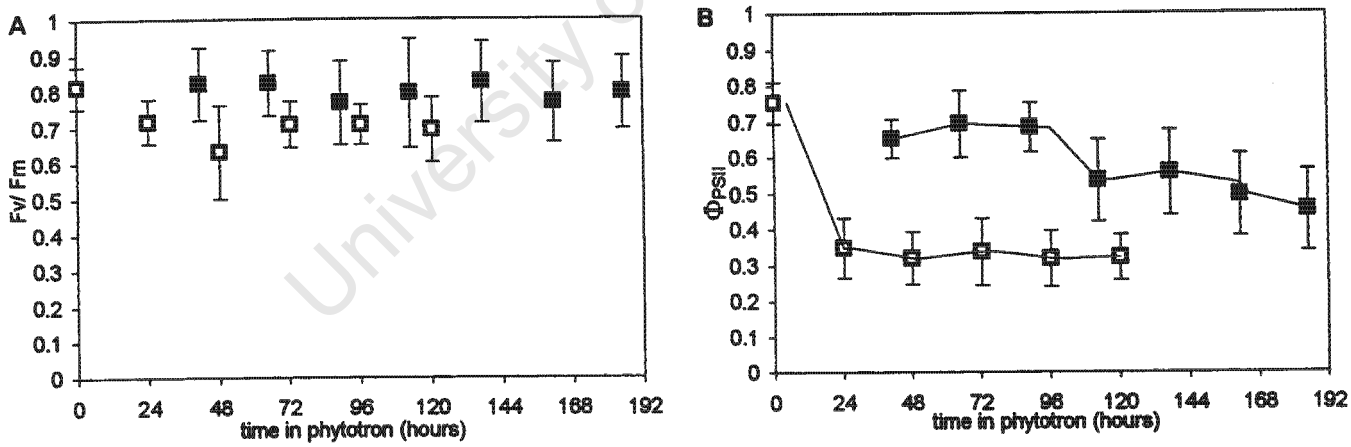

Figure 5.1. Maximal quantum efficiency, Fv/Fm (A) and actual quantum yield, Φ_{PSII}, (B) of hydrated *Xerophyta humilis* during a one-week acclimatisation period to high light (*ca.*1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) constant-environment laboratory conditions. n = 8.

Not surprisingly, given the reduced quantum yield of PSII and photoinhibition that occurred in high light plants (Figure 5.1), these plants experienced a

significant increase in non-photochemical chlorophyll fluorescence quenching (qN) of more than double initial values to 0.61 (± 0.12) (Figure 5.2A). Although re-emission of light energy as heat (qN) also increased in low light plants, these plants were not photoinhibited and the increase was only to 0.39 (± 0.05). Interestingly, despite evidence of photoinhibition in high light plants and a significant decline in linear electron transport (as indicated by decreased Φ_{PSII} values, Figure 5.1A) in both high light and low light plants, the proportion of open reaction centres, indicated by qP (Figure 5.2B) did not change, indicative of stressed photosystems.

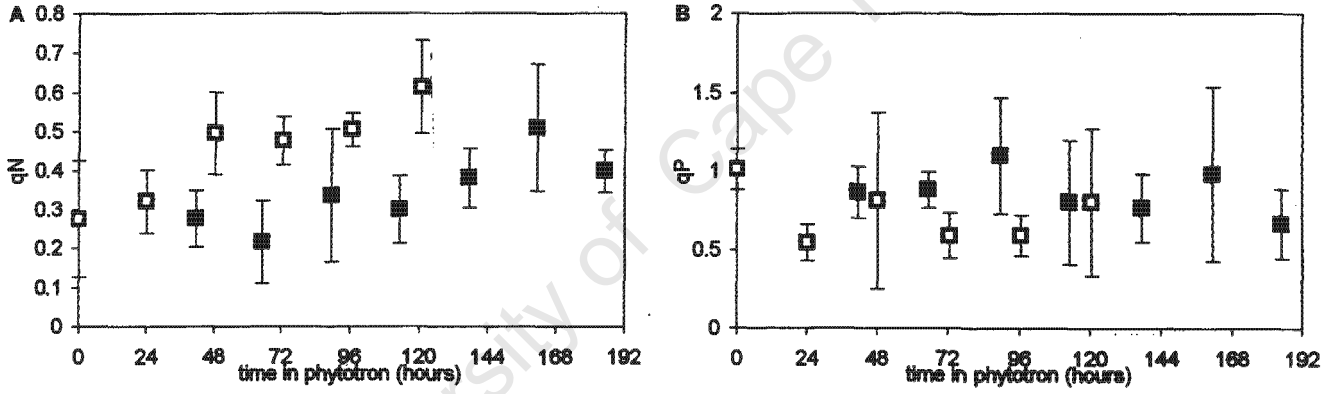

Figure 5.2. Non-photosynthetic (qN) (A), and photosynthetic (qP) (B), chlorophyll fluorescence quenching (A and B respectively) of *Xerophyta humilis* during a one-week acclimatisation period to high light (*ca.*1200 μmol. m^{-2}. s^{-1}; □) and low light (*ca.*320 μmol. m^{-2}. s^{-1}; ■) constant-environment laboratory conditions. n = 8.

3.1.2. Gas exchange

As might be expected from the decline in Φ_{PSII} (Figure 5.1B), rates of A_{net} (Figure 5.3A) also declined, relative to initial values, in high light plants. Photosynthetic rates above 15 μmol. m^{-2}. s^{-1} were recorded initially in high light plants, declining significantly to 10.86 (± 6.1) μmol. m^{-2}. s^{-1} after 130 h (*ca.* 5 d) in the phytotron.

Most of the decline occurred during the first 3 d. Initial A_{net} values in low light plants were very low (< 0 μmol. m^{-2}. s^{-1}), which may have been due to the high dark respiration rates in these plants (Figure 5.3B). A_{net} in low light plants increased to 4.6 (± 4) μmol. m^{-2}. s^{-1}, but this was still lower than the A_{net} that are possible for these plants, for example 16.2 (± 2) μmol. m^{-2}. s^{-1}, occurring initially in high light plants. This was not unexpected, as low light plants were also indicated to have reduced rates of electron transport (Figure 5.1B).

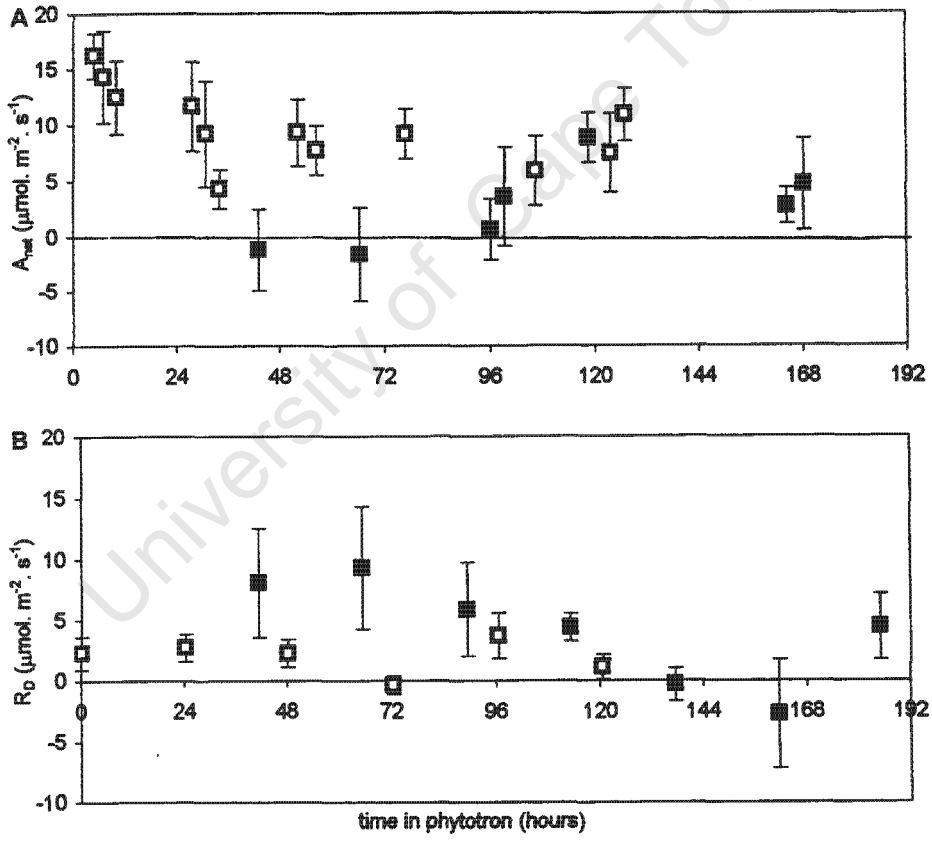

Figure 5.3. Rates of net photosynthetic CO_2 assimilation rates, A_{net} (A), and dark respiration rates, R_D (B), of *Xerophyta humilis* plants during a one-week acclimatisation period to high light (*ca*.1200 μmol. m^{-2}. s^{-1}; □) and low light (*ca*.320 μmol. m^{-2}. s^{-1}; ■) constant-environment laboratory conditions. n = 8.

Although stomatal conductance (g_s) varied within samples during the 'acclimatisation period', there was no significant change in either treatment over time (Figure 5.4).

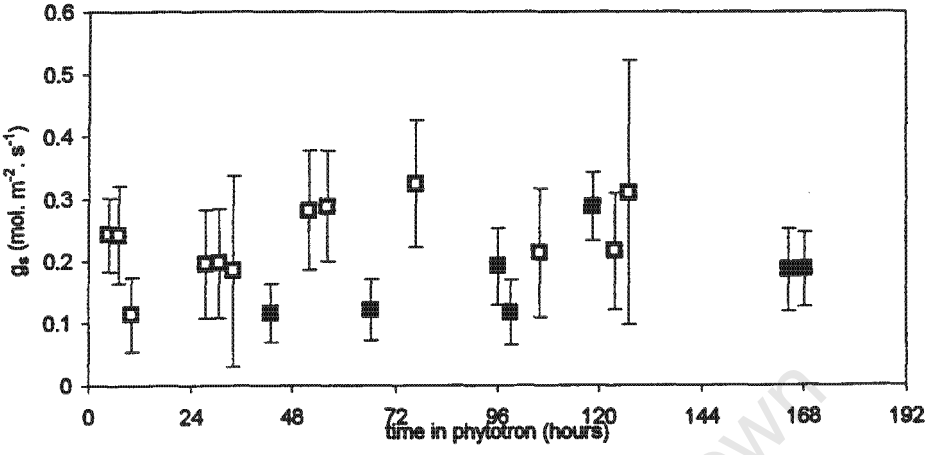

Figure 5.4. Rates of stomatal conductance (g_s) in plants of *Xerophyta humilis* during a one-week acclimatisation period to high light (*ca.*1200 μmol. m^{-2}. s^{-1}; □) and low light (*ca.*320 μmol. m^{-2}. s^{-1}; ■) constant-environment laboratory conditions. n = 8.

3.1.3. Light response curves

Predawn light-response curves, measured at intervals during the acclimatisation period on one leaf for each treatment (high and low light) are represented in Figure 5.5A-C (low light) and Figure 5.6A-D (high light). The same leaf was measured throughout the acclimatisation period, except in the low light treatment where the first light-response curve was constructed from a different leaf to the following two. The shape of the predawn light-response curves for the first morning in the phytotrons was similar for leaves under high and low light conditions, (Figure 5.5A and Figure 5.6A), with maximum net assimilation rates of *ca.* 10 μmol. m^{-2}. s^{-1} reached in both cases at a light intensity of *ca.* 500 μmol. m^{-2}. s^{-1}. Thereafter, maximum rates of net assimilation became increasingly lower, (except for the leaf measured on day 4 (Figure 5.5B) in the low light phytotron, which was a new leaf) for both high

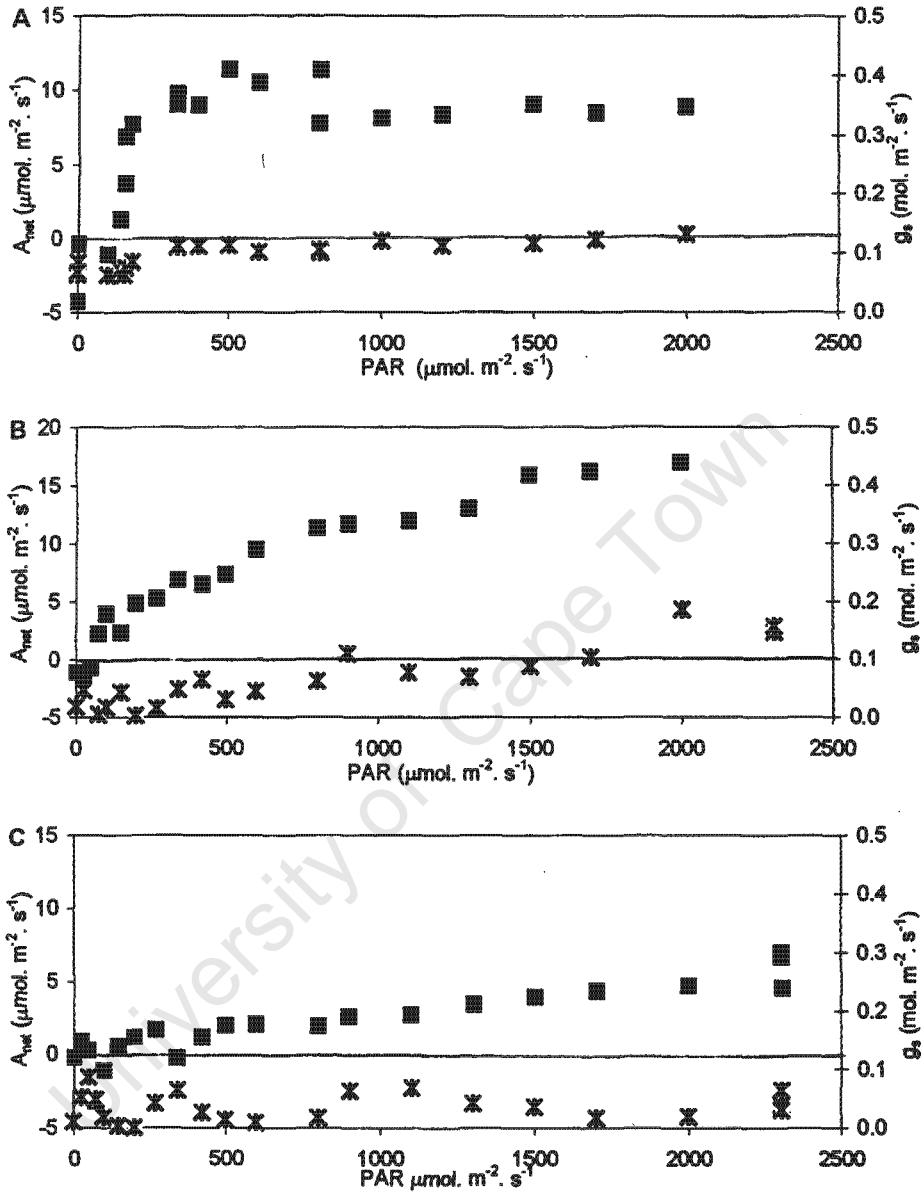

Figure 5.5. Predawn light response curves for a single leaf measured at intervals during the 'acclimatisation period' in the low light (*ca*.320 mmol. m^{-2}. s^{-1}) phytotron. Curves were constructed on d 1 (A), d 4 (B) and d 7 (C), where d 1 was the first full day the plants were in the phytotron, having been placed there the previous afternoon. (■) Represent net assimilation rates, and (Ж) represents g_s. n = 8

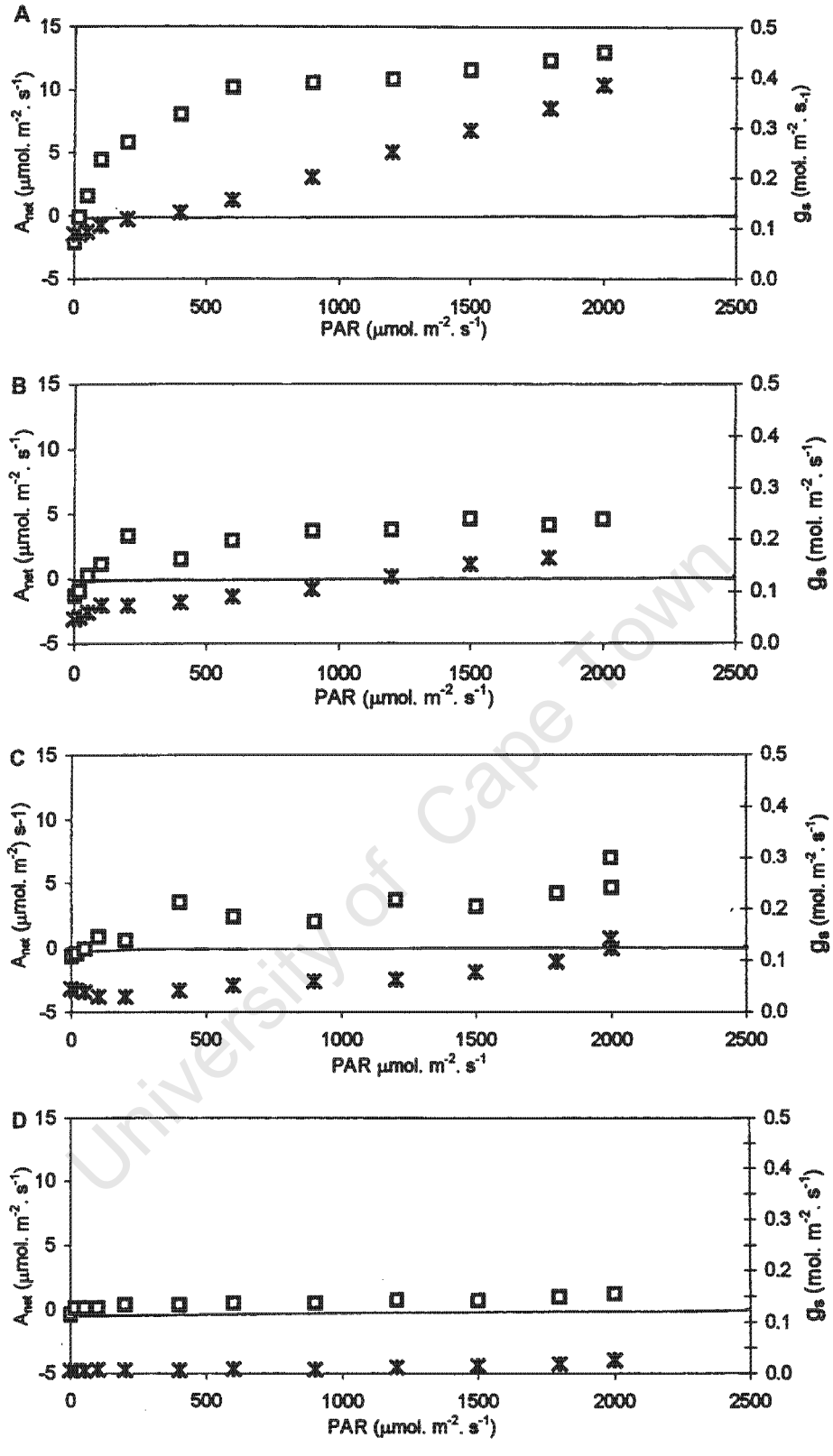

Figure 5.6. Predawn light response curves for a single leaf periodically during the 'acclimatisation period' in the high light (*ca.* 1200 µmol. m^{-2}. s^{-1}) phytotron. Curves were constructed on d 1 (A), d 3 (B), d 5 (C) and d 7 (D). (□) Represents net assimilation rates and (Ж) represents g_s. n = 8.

and low light treatments. The final light response curves for both high and low light plants reached very low net assimilation rates of less than 5 μmol. m^{-2}. s^{-1} in both treatments (Figure 5.5C and 5.6D), and in none of the curves was there any apparent difference, between high and low light plants, in the initial rate of increase of net assimilation (indicative of sun and shade adaption). The declines in maximum net assimilation rates are consistent with results presented in Figure 5.3A for high light plants. In all curves, stomata were slower to open than expected (even though the light response curves were measured using increasing light intensities), and stomatal conductance (g_s) was hence still increasing towards the final portion of the curve. Like net assimilation, maximum rates of g_s declined during the acclimatisation period.

3.1.4. Relative chlorophyll concentrations

The ratio of Chl a to Chl b (Chl a/b), as measured at the end of the acclimatisation period, of 3.05 (± 0.3) in high light plants was unexpectedly significantly lower (n = 18, p = 0.027) than that of low light plants (3.49, ± 0.5). However, Chl a/b ratios were in the normal range for sun-adapted plants for both high and low light plants (Figure 5.7.)

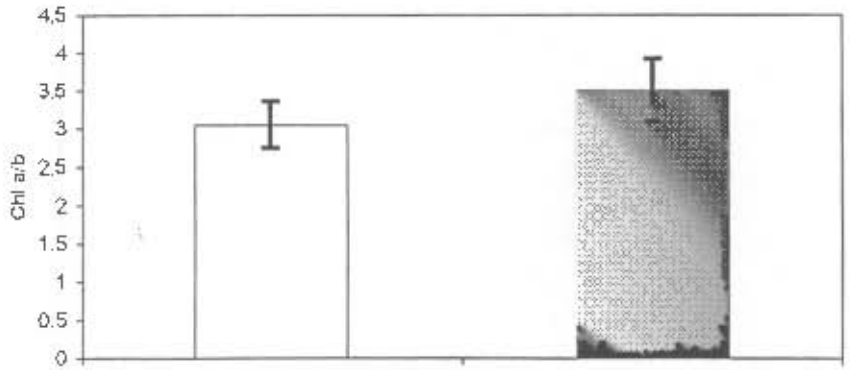

Figure 5.7. Ratio of Chl a to Chl b (Chl a/b) at the end of the 'acclimatisation period' in high light (*ca.*1200 μmol. m^{-2}. s^{-1}; open bar) and low light (*ca.* 320 μmol. m^{-2}. s^{-1}; closed bar) plants of *Xerophyta humilis*.

3.2. Rhythmic phenomena

Although the data from the chlorophyll fluorescence analysis performed on field-rehydrated plants have already been presented in Chapter 4, they are presented here again to represent more clearly the apparently rhythmic changes that occurred in some physiological parameters during the rehydration period (Figure 5.8 A-E).

During the rehydration period WC had reached maximum levels by the end of the first day (d 1) of recovery. During the rest of the recovery period, while WC remained at maximum levels, (Figure 5.8A) pigment concentrations (Figures 4.8, 4.9) and gas exchange parameters (laboratory plants; Figures 4.5, 4.6) returned to stable levels. Thus the rhythmic changes that occurred were not consequent of a varying WC, as might have been expected for rehydrating plants.

3.2.1 Field-rehydration rhythms

Fv/Fm, Φ_{PSII}, and qP had recovered to maximum levels during the night (*ca.* 13 h), subsequent to watering at dusk, (Figures 5.8 B, C, D, respectively). Although Fv/Fm did not decline again from maximum levels at any point thereafter, (suggesting that photoinhibition of PSII did not occur subsequent to recovery (Figure 5.8 B)) both Φ_{PSII} and qP did decline. Φ_{PSII} and qP were much lower in the morning of the second day, recovering to d 1 levels by midmorning. The lower quantum yield in the morning (Figure 5.8C) may in fact be consequent of the reduction of open reaction centres (Figure 5.8D). qN also showed rhythmic

change during the recovery period; values were low during d 1 but increased towards the end of d 1. qN values were at a low levels again on d 2.

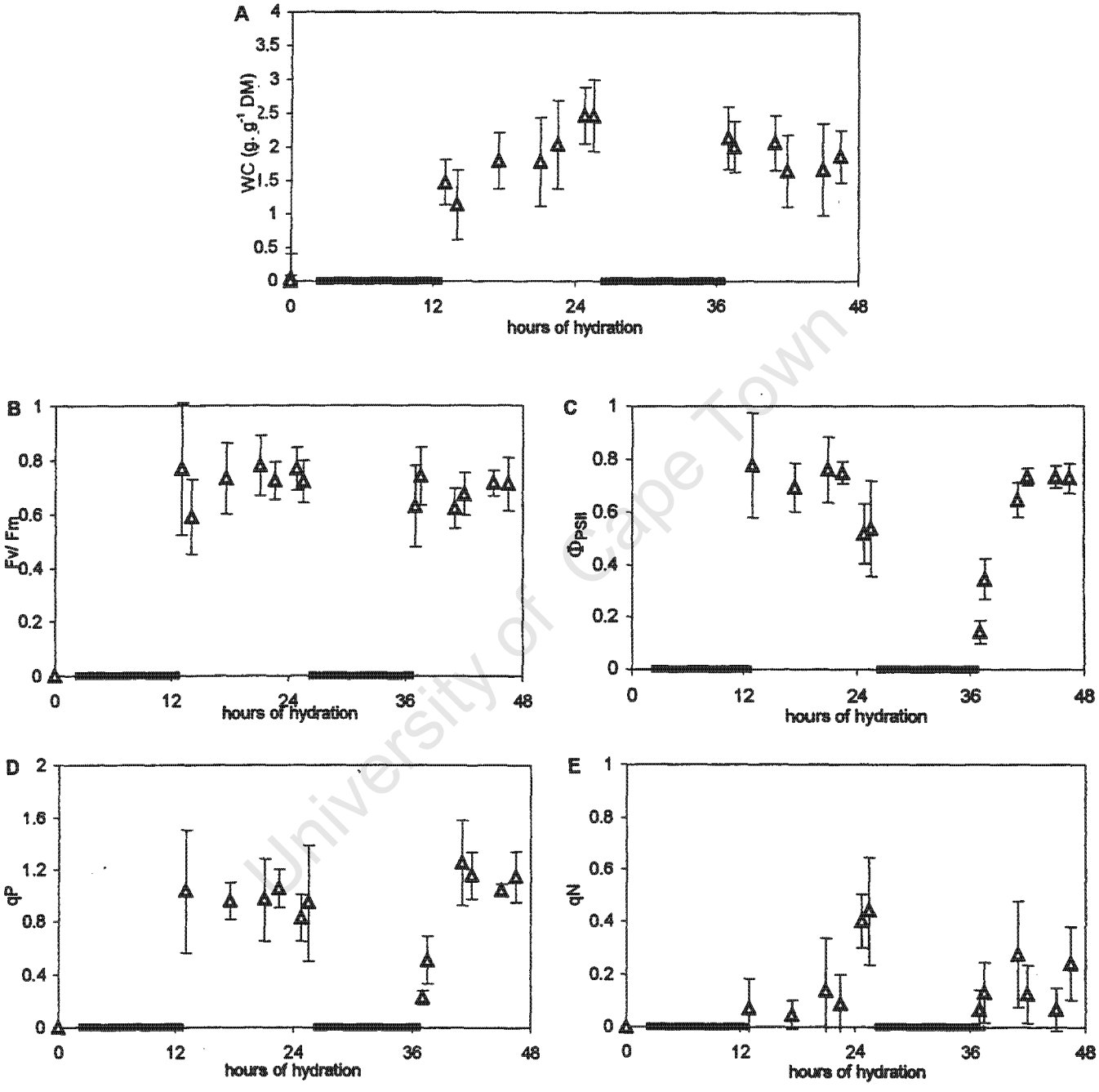

Figure 5.8. Changes in water content (WC), (A), and chlorophyll fluorescence parameters of *Xerophyta humilis* during rehydration (initiated by watering) under field conditions. (▬) represents the dark period. Fluorescence parameters represented are optimal quantum efficiency (Fv/Fm) (B), quantum yield (Φ_{PSII}) (C), photosynthetic (qP) and non-photosynthetic (qN) fluorescence quenching (D and E, respectively), n = 15.

3.2.2 Laboratory rhythms

As in the field-rehydration experiment, rhythmic variation of chlorophyll fluorescence and gas exchange parameters occurred during a 24 h period of plants under laboratory conditions. These plants had been maintained fully hydrated and had not just been rehydrated. As in field-rehydrating plants, Fv/Fm did not change at any point during the day in high light or low light plants, indicating that at no time did photoinhibition occur in either treatment (Figure 5.9A). However, Φ_{PSII} did vary (Figure 5.9B). Low predawn levels of Φ_{PSII} increased during the day in both treatments, but more so in low light plants, declining again at night, suggesting a decline in linear electron transport rates at night.

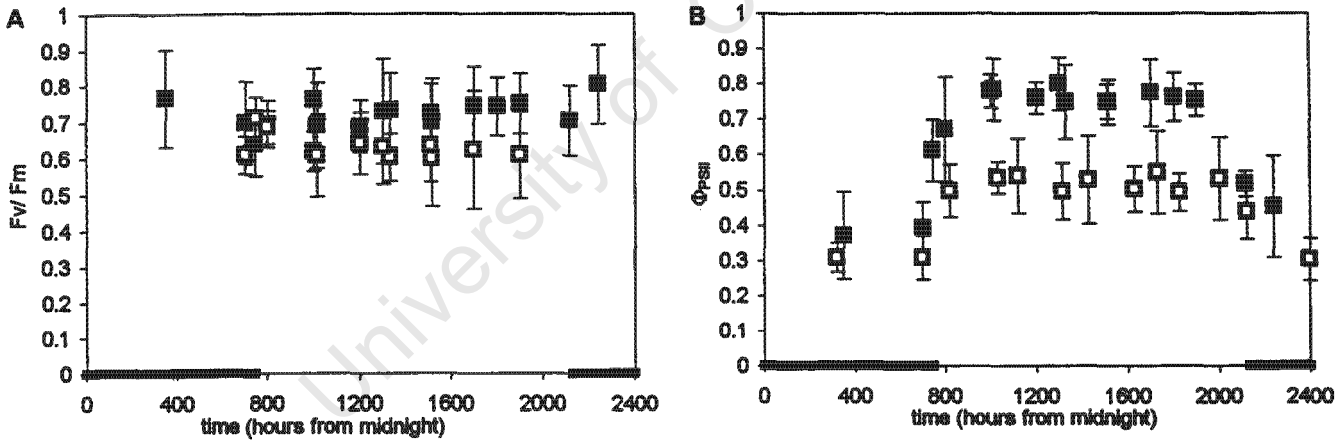

Figure 5.9. Daily variation of optimal quantum efficiency (Fv/Fm) (A) and quantum yield (Φ_{PSII}) (B) of fully hydrated *Xerophyta humilis* plants under high light (*ca.*1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1}; ■) laboratory conditions. (▬) represents the dark period. n = 15.

Variations that occurred in qP were no different between high and low light plants, but qP increased in both treatments during the day, declining again at night (Figure 5.10A). Similar to the variation seen in field-rehydrating plants,

qN for low light plants was lower during the day than at dawn and dusk (Figure 5.10B). qN of high light plants differed from field-rehydrated and low light plants as no daily variation was apparent. qN remained at high levels both day and night.

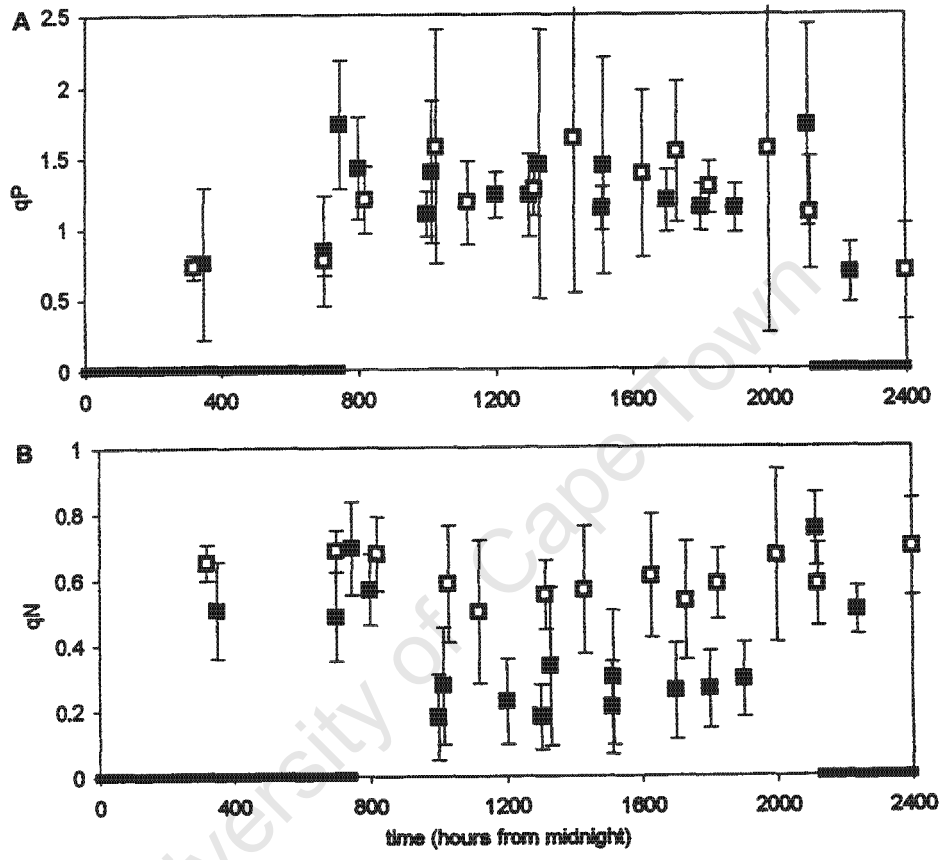

Figure 5.10. Daily variations in photosynthetic (qP) (A) and non-photosynthetic (qN) chlorophyll fluorescence quenching in *Xerophyta humilis* under high light (*ca.* 1200 µmol. m^{-2}. s^{-1};□) and low light (*ca.* 320 µmol. m^{-2}. s^{-1};■) in constant environment chambers. (▬) represents the dark period. n = 8.

As would be expected with plants exposed to light, and with increased rates of linear electron transport (Figure 5.9B), rates of net CO_2 assimilation (Figure 5.11) increased during the day in both high and low light plants. Net assimilation rates reached maximum levels after *ca.* 3 h of light exposure during the day and began to decline again after only *ca.* 7 h of light. Plants were thus not

photosynthesising maximally during the last 7 h of the light period. Although Φ_{PSII} increased to higher levels in low light plants, maximum net assimilation rates were similar in high and low light plants.

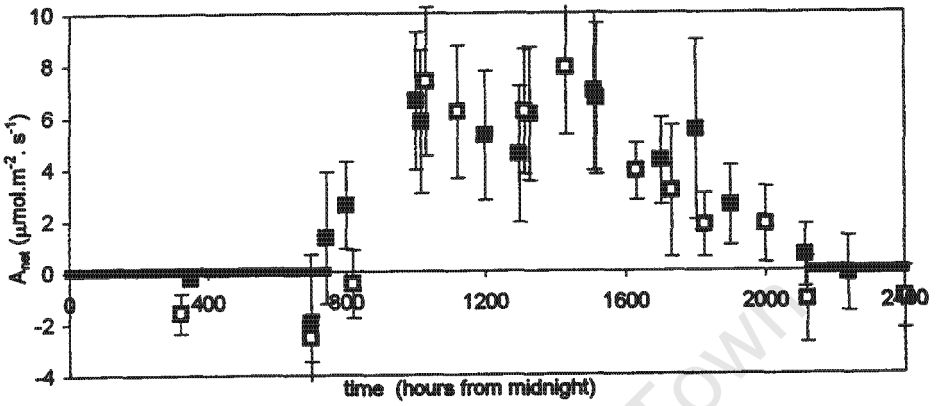

Figure 5.11. Daily variation in rate of net photosynthetic CO_2 assimilation (A_{net}) of *Xerophyta humilis* under high light (*ca.* 1200 µmol. m^{-2}. s^{-1}; □) and low light (*ca.* 320 µmol. m^{-2}. s^{-1};■) constant environment conditions. (▬) represents the dark period, n = 8.

Stomatal conductance rates (g_s) (Figure 5.12A) showed similar daily variations to net photosynthetic CO_2 assimilation rates, increasing during the first part of the day, and declining to minimum levels thereafter. Variation of g_s was similar in high and low light plants, and began to decline about an hour prior to the decline observed in net assimilation. As expected from the daily variation that occurred in g_s, transpiration rates (E) increased during the first half of the day and declined after reaching maximum levels after *ca.* 5 - 6 h of light (Figure 5.12B), when stomata began to close. This is a common phenomena which occurs as water usually becomes limiting by midday.

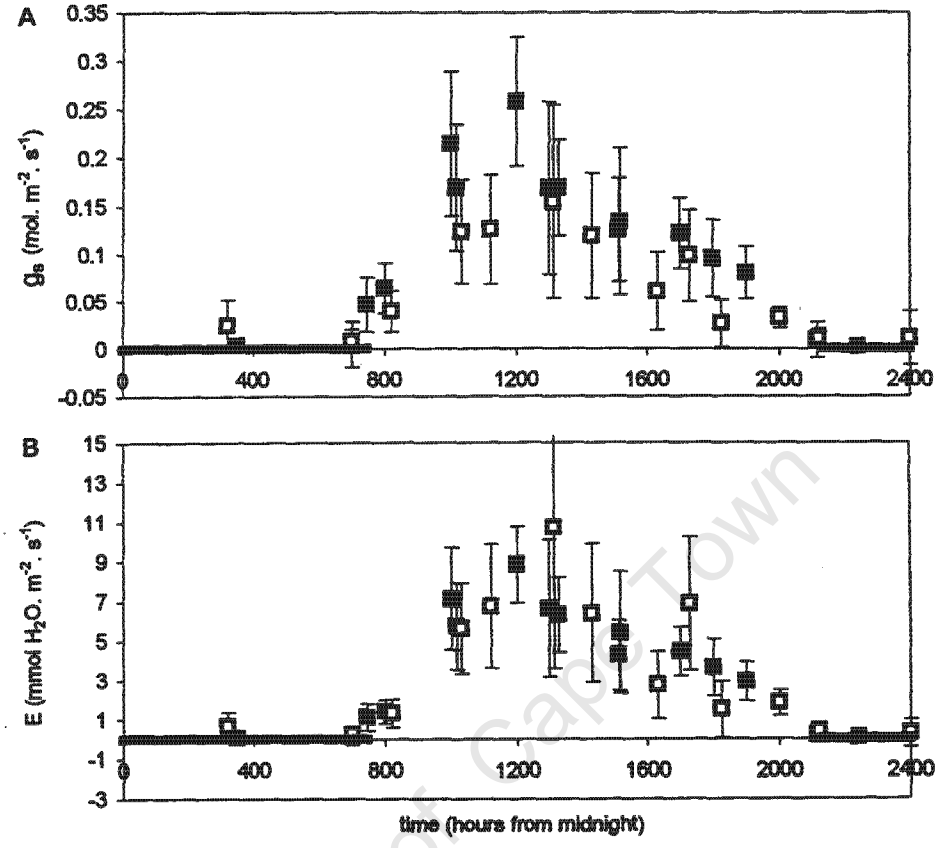

Figure 5.12. Daily variation in stomatal conductance rates (g_s) (A) and transpiration rates (E) (B) of *Xerophyta humilis* under high light (*ca.* 1200 µmol. m^{-2}. s^{-1};□) and low light (*ca.* 320 µmol. m^{-2}. s^{-1};■) constant- environment conditions. (▬) represents the dark period.

4. Discussion

4.1. Acclimatisation

Although plants showed some changes in physiology when moved into the new environments of high and low light phytotrons, changes did not appear to occur in physical parameters such as the maximal quantum efficiency of PSII (Fv/Fm, Figure 5.1A), or certain metabolic rates such as respiration rates (Figure 5.3B). Although there was a significant difference in Chl a/b ratios between high and

low light plants after 8 d of acclimatisation (Figure 5.7), high light plants had a *lower* ratio than low light plants, which is unusual as this would be expected of shade adapted species (Lichtenthaler, 1987). This indicates that plants had not altered chlorophyll ratios into those common of sun and shade leaves after 8 d of acclimatisation, but lack of initial values for Chl a/b ratios means that such a suggestion is speculative. Instead changes appeared to be more transient in nature, such as a decline in rates of net assimilation (Figure 5.3A) (most likely due to factors other than changes in pigment concentrations) and increased non-photochemical chlorophyll fluorescence quenching (Figure 5.2A). No categorical statement can be made about the physical nature of the changes of *X. humilis* when placed in the phytotrons, as no examination of cellular ultrastructure or specific leaf area were conducted. Also, in order to verify the transience or permanence of a physiological change, measurements of plants *removed* from the chambers and placed back in the greenhouse is necessary. However, what can be deduced from these results is that physiologically, the plants were largely unaffected by the 'new environments' except that high light plants experienced higher levels of light stress. This is indicated by both decreased maximal photochemical efficiency, Fv/Fm – Figure 5.1A, (Maxwell and Johnson, 2000) and increased non-photochemical fluorescence quenching; Figure 5.2A (Demmig-Adams and Adams, 1992). A decrease of Fv/Fm is a consequence of photoinhibition (due to stressed or damaged photosystems) and increased qN occurs when light energy is greater that which than can be utilised, and is also an indicator of light-induced stress.

4.2. Rhythmic phenomena

The cyclic variation that was apparent in some aspects of field-rehydrated *X. humilis* physiology (Chapter 4) is clearly correlated to the daily photoperiod (Figure 5.8). Examination of fully hydrated plants of *X. humilis* under laboratory conditions revealed the occurrence of rhythmic changes in quantum yield of PSII (Figure 5.9B), in chlorophyll-fluorescence quenching (Figure 5.10) as well as in exchange of CO_2 (Figure 5.11) and water vapour (Figure 5.12). Thus one can conclude that the daily variations in field-rehydrated plants were at least in part a consequence of naturally occurring rhythmic phenomena, and not a consequence of rehydration.

Whether the rhythmic oscillations presented in this study are true circadian rhythms, controlled endogenously and entrained to the day-night photoperiod, or whether they are merely direct responses to environmental change cannot be ascertained from these results. True circadian rhythms have been detected in gas exchange and stomatal aperture (McClung, 2001), but daily stomatal closure and reduction in net assimilation rate can also be directly due to environmental stress (Zhang and Gao, 1999). However, regardless of whether they were circadian rhythms or diurnal oscillations, the significance of the rhythmic changes occurring in *X. humilis* plants on a daily basis is important to note.

Daily variations in xylem pressure in the resurrection plant *Myrothamnus flabellifolius* (Schneider *et al.* 1999), and daily variations in concentrations of carbohydrates in the resurrection plant *Craterostigma plantagineum* (Norwood

et al., 2000) have been detected in hydrated plants. Daily variations in surface reflectance properties and some photochemical parameters (such as Fv/Fm) have also been examined in the desiccation-tolerant moss, *Tortula ruralis* (Hamerlynck *et al.*, 2000). In the latter example, plants were also undergoing concurrent daily dehydration and rehydration cycles, with which the surface reflectance and photochemistry varied. Rhythmic variations during the recovery of a poikilochlorophyllous desiccation-tolerant plant subsequent to initiation of rehydration have not previously been reported, but are of relevance to understanding results of any rehydration experiment.

Imbibition in certain seeds is reported to act as an entraining signal for circadian rhythms (Salisbury and Ross, 1992). The possibility for rehydration in resurrection plants to act as an entraining signal can not be excluded. Perhaps, as in seeds, hydration of tissues 'sets', or in the case of vegetative tissues, 'resets' the circadian clock. Thus the time of day when rehydration is initiated is also an important variable to consider when designing experiments, and the rhythms themselves require consideration when determining sampling intervals.

Chapter 6

OVERVIEW AND FUTURE STUDIES

A reductionist approach to the study of biological systems, where underlying processes are examined in order to understand the functioning of the whole, is common (Brown, 1994), and successful (Smith, 1982). However, as pointed out by both Brown (1994) and Smith (1982), the reductionist approach is severely limited if information so gathered is not applied to the system as a whole.

The aims of the present study were to investigate if the physiology of rehydration differed between field and laboratory conditions, and to investigate how differing light levels might affect the physiology of desiccation tolerance (dehydration and rehydration) under otherwise identical laboratory conditions. The motivation behind these aims was to gain some understanding of the *relevance* of information collected about *Xerophyta humilis* during experimentation under laboratory conditions, that are often very different to those found in nature. How applicable to the 'wild' plant system is the information that is gathered from plants examined and maintained under laboratory conditions? Is our current understanding of some aspects of the physiology of desiccation tolerance in *X. humilis* applicable to the system in which this plant is naturally found?

In the present study plants dried under controlled ambient conditions that were set to differ only with respect to light intensity, were found to differ in the

rate of onset of drying (once drying had begun changes in water content occurred at the same rate in high light and low light plants). Upon closer examination, it appeared that the earlier onset of drying in plants under high light conditions was probably due to a greater speed of airflow across the plants, and not in fact due to any light-related causes. *X. humilis* is a diminutive species existing within the boundary layer on the soil surface, frequently in depressions, and as such is unlikely to experience high rates of airflow under natural conditions. Consequently, although the higher light intensities approximated natural levels more closely than low light intensities, conditions in the high light phytotron, as a whole, were not representative of the natural environment.

Examining the protection and repair mechanisms against the hazards of dehydrating and rehydrating is of importance to understanding the ways in which resurrection plants tolerate desiccation of their tissues. Although the higher light levels in the high light treatment were not involved in initiating the more rapid onset of drying, the higher light levels imposed a greater stress on PSII in high light plants, as indicated by the upregulation of protective pigments to a greater extent in these plants compared with low light plants. It is thus apparent that in attempting to understand the protection and repair mechanisms that are utilised by plants in the field, the use of high light intensities in experimentation (to emulate those naturally experienced) is necessary. It is also important that stresses not experienced in the field, such as increased wind velocities, are excluded. Failure to account for non-drying related stresses will lead to an incorrect understanding of protection and

repair mechanisms that are upregulated during desiccation, as well as any damage observed. High light intensities are stressful under water limited conditions, and although *X. humilis* can survive drying in the dark, (Farrant *et al.*, 2002) light is necessary for the resynthesis of chlorophyll during rehydration of poikilochlorophyllous plants, and is thus necessary for the complete recovery from dehydration. The light intensity under which plants are rehydrated, however, from data presented in this study does not appear to play an important role in any aspect of rehydration physiology.

The ways in which different experimental conditions can affect the physiology of dehydrating and rehydrating desiccation-tolerant seeds and vegetative tissues has been explored by Pammenter *et al.* (2002). As discussed by these authors, different experimental conditions can result in large differences in the observed responses to desiccation. The results of the present study support this observation.

It is apparent from this study that not only the light intensity, but also the light regime to which the plants are exposed, is of importance. Although the rhythmic diurnal variations that were very apparent during recovery of field-rehydrated plants were not as distinct in laboratory rehydrated plants, rhythmic variations were nevertheless clearly present in laboratory plants maintained at field capacity. Daily variation in factors such as the net photosynthetic rate and the performance of the photosynthetic machinery are important factors to consider when interpreting experimental results. As *X. humilis* dehydrates and rehydrates over periods of a few days, the 'window

period' during which metabolic changes occur, and hence the window period of interest to most modified desiccation-tolerant plant researchers, is brief. Consequently, distinct metabolic changes occur over periods of hours, as do the natural daily cycles. Thus a clear understanding of rhythmic phenomena is important to avoid misinterpretation of physiological changes. Changes in physiology are likely to be mirrored by biochemical changes and changes in gene regulation, and understanding rhythmic phenomena is also likely to be relevant to studies of such factors.

Not only are rhythmic phenomena important to consider when interpreting experimental results, such phenomena are also important to consider when designing experiments. The time of day during which samples are taken, and if samples are taken at different times during the day will effect the results obtained. The time of day that drying is initiated, or rehydration begun, may also affect aspects of the physiology of dehydration and rehydration.

The natural variability of biological systems is often exacerbated by experimental error, or the inaccuracies of the instrumentation used. Even highly sophisticated, accurately calibrated instruments are prone to unavoidable error, such as the overestimation of respiration rates that can occur in IRGA measurements (Pons and Welschen, 2002). However, although much experimental error is unavoidable, a holistic understanding of the physiology of *X. humilis* requires a closer simulation of field conditions in laboratory studies, and an awareness of the natural daily rhythms that occur in the physiology of this plant.

Future study

There is much scope for further study into many aspects of the physiology (as well as the ecology and biochemistry) of resurrection plants as a whole, and *X. humilis* specifically.

Additional examination of stresses experienced by *X. humilis* under phytotron conditions that are not experienced in the field, and how these stresses interact with the 'natural' stresses involved with desiccation, would augment the results obtained in the present study.

In this study dry plants were watered in the late afternoon in all experiments, as rainfall commonly occurs in the afternoon in their natural habitat. Thus rehydration occurred predominantly during the following night and the stressful period of intermediate water contents was consequently not concurrent with light stress, which may have contributed to the fact that different light intensities had negligible effect on the rehydration physiology measured for these plants. Initiating rehydration during the morning or at midday would give insight into the effects of different light intensities during rehydration.

The occurrence and nature of rhythmic phenomena in the physiology of *X. humilis*, and how these interact with the metabolic changes that occur during dehydration and rehydration is an exciting and important topic for further research. Moreover, should such rhythmic phenomena prove to be true

circadian rhythms, there would be opportunity for investigations into entraining factors as well as periodicity of the cycles.

Different wavelengths of light are known to affect plant physiology. Brief periods of exposure to red light, even at very low levels, is known to be an entrainment signal (Hopkins, 1992), and as a red light is utilised by the modulated fluorometer, taking measurements of chlorophyll fluorescence at night may affect the plants' circadian rhythms. There is much scope for further study into the effects that both dark-adaption of leaves during the day, and the provision of light, both red and white, during fluorescence measurements at night can have on *X. humilis*.

An holistic and applicable understanding of the physiology of *X. humilis* is more than just an end in itself, but can contribute to the understanding of genetic, metabolic and biochemical parameters as well as the ecology of this plant.

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