SPATIOTEMPORAL DYNAMICS OF HIGH-TEMPERATURE TOLERANCE IN AUSTRALIAN ARID-ZONE PLANTS

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DOCTOR OF PHILOSOPHY IN SCIENCE

CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree, nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This research is supported by an Australian Government Research Training Program Scholarship.

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Date: 26 September 2017

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Figure 2.1 (For corresponding, published figure, see Fig. S2.1)**. Step 1.** Leaves were sampled from branches collected from the north-facing outer canopy of a minimum of five plants per species. **Step 2.** For each species, six batches of ten leaves were randomly chosen from the sampling pool and treated to one of six temperature treatments. **Step 3.** Control measurements of maximum quantum yield of PSII ($PS_{FV/FM}$)

and effective quantum yield ($PS_{\Delta F/FM'}$) were measured prior to heat stress. F_V/F_M was measured two hours (2 hr) after stress treatment and after a further recovery period of ca. 16 hours ($D2_{FV/FM,}$ indicating day two of measurements) at 46, 48, 50, 52 to 54 °C and a control temperature of 28 °C. $\Delta F/F_M'$ was measured immediately following stress treatment (0 hr), 1.5 hours after and on day two following dark-adapted measurements and an additional 15 minutes under control conditions in order to light-adapt samples ($D2_{\Delta F/FM'}$). For each treatment temperature, dark measurements were used to quantify the damage metric, D_{PSII}, and light measurements were used to quantify the recovery metric, $R_{\Phi PSII}$. For all data points $n = 10 \pm$ SE. The alignment of dark- and light-adapted measurements, F_V/F_M and $\Delta F/F_M'$ respectively, with time and treatment temperature indicated with arrows. Graphs inset show the photochemical quantum yield for leaves in the dark- and light-adapted state in response to heat stress treatments, as demonstrated in *Acacia papyrocarpa* during summer. Dashed lines are for ease of reading patterns and not representative of continuous time.

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species exhibiting minimal changes in their T_{50} between winter and spring but a substantial jump from spring to summer; **Steady Increase,** species exhibiting a steady increase in T_{50} values from winter to summer, with no marked step increase from winter to spring or spring to summer; **No Response,** species showing little change in T_{50} seasonally. Error bars are an estimation of within-species variation in T_{50} interpolated from 95% confidence intervals (see Methods and Results). For full species names see Methods and Table 4.1.

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Supporting information: tables

Table S3.1 Pearson correlations (*r* values) of T_{50} with the minimum, maximum, range and mean of three climate-based parameters: mean annual rainfall, mm; solar radiation, $MJ.m^{-2}.day$; mean annual aridity index. Results shown are for all 42 species and individually for W_{high} ($n = 10$), W_{var} ($n = 14$), and W_{low} ($n = 18$) species. $P > 0.05$ in all cases except where indicated in bold ($P < 0.05$).

Table S3.2 Species rank according to their warming tolerance (WT), calculated as the difference between a species' physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on the highest value across each species' Australia-wide distribution using four different thermal indices. Here, T_{hab} values were based on the highest annual maximum mean temperature (WT_{amm}).

Table S3.3 Species rank according to their warming tolerance (WT), calculated as the difference between a species' physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on the highest value across each species' Australia-wide distribution using four different thermal indices. Here, T_{hab} values were based the highest annual mean temperature.

Table S3.4 Species rank according to their warming tolerance (WT), calculated as the difference between a species' physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on the highest value across each species' Australia-wide distribution using four different thermal indices. Here, T_{hab} values were based on the highest warmest maximum period (WT_{wmp}).

Table S3.5 Species rank according to their warming tolerance (WT), calculated as the difference between a species' physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on the highest value across each species' Australia-wide distribution using four different thermal indices. Here, T_{hab} values were based on the highest warmest quarter (WT_{wq}).

Table S4.1. Pearson correlations between species' thermal damage thresholds and the daily minimum, daily maximum, daily cumulative minimum, daily cumulative maximum and daily range temperatures obtained one to ten days prior to physiological measurements; here termed 'priming temperature'. Analyses revealed few significant relationships between T_{50} and priming temperature for the days preceding our experiment in winter and spring. For summer measurements, statistically significant correlations were consistent across priming temperature metrics by the third day prior to T_{50} measurements (indicated by dashed line); with the exception of daily temperature range, which remained nonsignificant irrespective of day or season. Results suggest ambient temperature has a stronger influence on T_{50} during summer than typically cooler seasons. From these analyses, we chose to incorporate daily ambient temperature recordings from day three as a proxy for priming temperature in all generalised linear models. Statistically significant relationships appear in bold. $\alpha = 0.05$.

Table S5.1 Seasonal minimum, maximum and mean (± SE) values for six leaf morphological and structural traits: leaf thickness, leaf mass per area, leaf water content, effective leaf width, and visible and near infrared reflectance. Values are for all species within each season (total) and for species grouped by microhabitat: high water, W_{high}; low water, W_{low}; variable water availability, W_{var}.

ABSTRACT

Many aspects of the Earth's climate are predicted with high certainty to undergo substantial and rapid changes in the near future, potentially resulting in a plethora of new high stress conditions to which plants must respond to survive. Living in extreme environments, desert plants are expected to be among the most vulnerable. Due to the thermal dependence of photosynthesis, changes in temperature are particularly important for plants. Extreme high-temperature events are becoming more frequent and intense and projected to increase in many regions. General expectations are that species' vulnerability to increased temperatures varies with latitude, but less is known about how local-scale habitat variation influences thermal tolerance. Variation in the ability to plastically adjust thermal tolerance will undoubtedly influence the distribution of different species and affect community composition. Yet, the extent of variation in thermal acclimatisation in plant species is poorly understood. The overall objective of my PhD research was to provide insight into leaf-level thermal responses of plants under extreme high temperatures in light of a warming climate. Through a series of linked experiments, my research demonstrates how dynamic and varied the heat stress response can be, including cross-species variation of critical thermal limits, heat stress recovery, acclimatisation patterns within and among species over time, and spatial differences relating to native microhabitat. I developed a novel protocol for measuring biologically relevant, species-specific thermal damage thresholds (Chapter 2), which I subsequently used to demonstrate seasonal and spatial effects on species' thermal responses (Chapters 3 and 4). The latter findings emphasise that a deeper understanding of plant thermal responses requires insight into their capacity to shift their thermal response over time and space. I then showed that species' innate physiological thermal tolerance aligns in multi-trait space with two alternative leaf-level morphological pathways of thermal protection (Chapter 5). This raises the possibility that other thermal protective processes, e.g., heat shock protein production and increased membrane stability, may also sit along these axes. Lastly, I demonstrated intracanopy variation in leaf-level physiological response, which expands our mechanistic understanding of plant-environment interactions and could benefit models predicting the cost to species of a warming climate (Chapter 6). By revealing these and other key thermal response

patterns, this thesis offers a meaningful contribution to the field of plant ecophysiology, and provides information that is crucial for our understanding and management of desert– and potentially many other – ecosystems.

DECLARATION OF CONTRIBUTION TO EACH PUBLICATION

The following publications have resulted directly from the research presented within this thesis:

1) Curtis EM, Knight CA, Petrou K, Leigh A. 2014. A comparative analysis of photosynthetic recovery from thermal stress: a desert plant case study. *Oecologia,* 175: 1051-1061.
   Associate Professor Andy Leigh and I designed the thermal tolerance work; I also collected and analysed the data and led the writing, with all authors contributing to the text. Dr Katherina Petrou provided valuable insight into chlorophyll a fluorescence protocols. Professor Charles Knight advised on the initial experimental setup.
2) Curtis EM, Gollan JR, Murray BR, Leigh A. 2016. Native microhabitats better predict tolerance to warming than latitudinal macro-climatic variables in arid-zone plants. *Journal of biogeography*, 43.6 (2016): 1156-1165.
   Associate Professor Andy Leigh and I designed the thermal tolerance work; I also collected and analysed the data and led the writing, with all authors contributing to the text. Dr Brad Murray contributed to analyses and interpretations, and Dr John Gollan contributed the ideas on warming tolerance.

The following chapters are in review or prepared for imminent submission for publication:

1) Chapter 4: Curtis EM, Murray BR, Leigh A. (In review) Temporal dynamics of upper thermal damage thresholds: variation among desert plant species with contrasting microhabitat associations. Submitted to *Global Change Biology*.
   Associate Professor Andy Leigh and I planned and designed the thermal tolerance work; I also performed experiments, conducted fieldwork, collected and analysed the data; Dr. Brad Murray provided analytical advice; I led the writing, with all authors contributing to the text.
2) Chapter 5*:* Curtis EM, Knight CA, Leigh A. Extending the global leaf economics spectrum to include metrics of plant thermal tolerance: Two new axes identified from a suite of desert plant species. Ready for submission to the *Journal of Vegetation Science.*

Associate Professor Andy Leigh and I generated hypotheses and designed the thermal tolerance work; I also collected and analysed the data; Professor Charles Knight provided advice on the initial experimental setup and contributed intellectual input; I led the writing, with all authors contributing to the text.

3) Chapter 6: Curtis EM, Knight CA, Leigh A. Intracanopy adjustment of leaf-level thermal tolerance is associated with microclimatic variation across the canopy of *Acacia papyrocarpa* Benth. Ready for submission to *Oecologia.*

   Associate Professor Andy Leigh and I generated hypotheses and designed the thermal tolerance work; I also collected and analysed the data; Professor Charles Knight provided advice and contributed intellectual input; I led the writing, with all authors contributing to the text.

Signature of Student:

Ms. Ellen Curtis

Date: 26 September 2017

Chapter 1

Introduction

1.1 PLANT STRESS PHYSIOLOGY: OUTLINE

The focus of my PhD thesis is high-temperature stress in terrestrial plants. The concept of plant stress is inherently complex, crossing disciplines, temporal and spatial scales. In this Introduction, I first briefly define stress, especially heat stress in relation to plant physiology, providing rationale for my focus on temperature as a stressor. Expanding the initial simplified definition, I introduce the dynamic nature of stress and explain the 'press-pulse' disturbance concept. The importance of plants as primary producers and the potential impact temperature can have on their photosynthetic machinery follows. I then highlight the need to understand species' acclimatisation potential. I contrast this protective mode of physiological tolerance via acclimatisation with protection via thermoregulation and the leaf traits involved in protecting against thermal damage. I then take a step back to consider plant thermal tolerance in the context of climate change and highlight why desert systems are of particular importance in this regard. I finish this chapter by outlining the specific questions I address in the remaining chapters of my thesis.

1.2 WHAT IS STRESS?

The answer to this question will vary depending on the context to which it is applied. At a basic level, 'stress' can be thought of as a state of tension, indicating the effects of a 'load' on an organism imposed by external stimuli. These stimuli are termed stressors and trigger a response leading to a change at one or all functional levels of an organism (Larcher, 2003; Schulze *et al.*, 2005; Mohr *et al.*, 2012). For plants, stress is generally defined as an external factor, either biotic (biological) or abiotic (environmental), that *negatively* influences plant growth, productivity, reproductive capacity or survival (Rhodes & Nadolska-Orczyk, 2001; Gaspar *et al.*, 2002). Stress can therefore be defined as limitations on an organism imposed by factors that induce a physiological change, which often compromises performance, including metabolism, growth or development (Lichtenthaler, 1996; Nilsen & Orcutt, 1996)

The natural environment is composed of a complex set of abiotic and biotic stressors, of which temperature is a key limiting factor for most living organisms. This limitation arises from the fundamental dependence on temperature for many key biological processes, including growth and reproduction, which in plants, largely includes the processes of respiration and photosynthesis (Körner, 2007; O'Sullivan *et al.*, 2013; Teskey *et al.*, 2015). Across a range of temperatures, the functional rate of these and other processes will generally increase until optimum rates are reached, but either side of this optimal range, maximum rates decline and functional performance is inhibited (Fig. 1.1). Thermal optima are species-specific and often reflect the *in situ* growth environment, but to a certain extent can change over time to suit novel conditions (Schulze *et al.*, 2005). Often the plastic response requires time and is thus preceded by a lag, where changes in the environment and phenotype become uncoupled. The length of uncoupling is determined by a given species' capacity to respond to changes in its environment (Tattersall *et al.*, 2012). By interrupting the state of biochemical mechanisms associated with functional processes – which are bound by universal biological limits – extreme changes in temperature can exert severe stress and thereby influence an organism's performance and survival. This makes temperature one of the

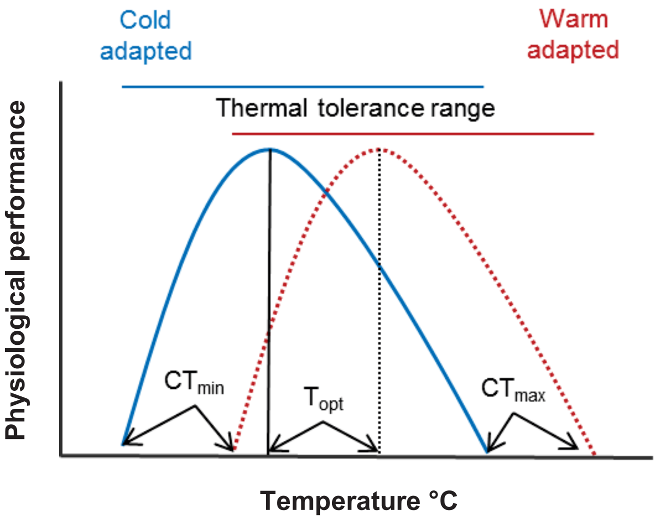

Figure 1.1 Theoretical thermal performance curves for cold- (solid blue curve) and warm-adapted (dashed red curve) conditions. Physiological performance is maintained within limits of a species' thermal tolerance range (horizontal lines) that is bound by a lower and upper critical thermal limit: CT_{min} and CT_{max}, respectively, beyond which performance drops to zero. Peak performance occurs at the thermal optima, T_{opt} (vertical lines). A species' thermal tolerance range and T_{opt} can shift to an extent over time to match novel growth conditions.

most important environmental drivers influencing the distributions of species (Tattersall *et al.*, 2012; Körner *et al.*, 2016). The stresses imposed by temperature on plants in particular have been classified into three types, depending on the nature of the stressor: 1) high-temperature stress, 2) low temperature stress above freezing and 3) low temperature stress below freezing (Iba, 2002). In plants, both extreme low and high temperatures can profoundly influence the rates of biochemical reactions, alter the structure and fluidity of cell membranes, detrimentally effect enzyme function and destroy proteins (for review see, Fig. 1 in Sung *et al.*, 2003). Here, I focus on high-temperature stress, with low temperature stresses outside the scope of the present study (the interested reader is referred to comprehensive reviews on these topics by others: Guy, 1990; Hughes & Dunn, 1996; Uemura & Steponkus, 1999; Pearce, 2001; Thomashow, 2001; Körner, 2012; Körner, 2016; Körner *et al.*, 2016).

The above relatively simple definition of stress does not account for the nuanced nature of plant stress physiology. Below, I detail the dynamic nature of stress, followed by an introduction to the thermal sensitivity of the photosynthetic apparatus of terrestrial plants.

1.3 STRESS DYNAMICS

It is important to recognise that stress is dynamic and ranges along a spectrum of mild to extreme. Consequently, the stress response of a plant is dynamic also. Heat stress can be considered *mild* if an impacted system, for example plant metabolism, deviates little from the physiological norm and if it returns to homeostasis or prestress base-line levels rapidly and completely with no long-term damage. This contrasts functional impairment or structural damage due to *moderate* levels of heat stress, which, although reparable require an indeterminate time for recovery before the system can return to a prestressed state. Temperatures that range between 30 – 40 °C (Atwell, 1999; Sharkey, 2005) are typically considered as moderate; 40 °C is also considered to be the upper thermal optimum for photosynthesis (Larcher, 2003). Temperatures that exceed 40 °C are considered *extreme*. For plant tissue exposed to extreme temperatures, thermal damage may be irreversible or even lethal (Sharkey & Schrader, 2006). High temperatures that elicit extreme heat stress therefore may be defined as those that exceed an individual's tolerance threshold or capacity to acclimatise (see p. 13 below, '1.6 Thermal acclimatisation') (Gutschick & BassiriRad, 2003). These above three categories are not discrete but rather they form a spectrum along which an individual's perceived stress level is dynamic. For example, absolute temperatures required to inflict an extreme stress response may depend on a species' location, thermal history and resource availability and thus can vary with time and spatial scale.

The stress response is dose-dependent. That is, a species' response will vary depending on the magnitude, either intensity or duration, and frequency, including timing, of a stress event. Leaf death may result from a single extreme event if its magnitude is large, causing irreversible damage. Eventual leaf death may also result if thermal damage is accrued over time from recurrent, typically non-lethal, low intensity events. The latter scenario may occur in situations where normally reversible damage is not possible due to insufficient recovery time between stress events. Stress frequency and magnitude are central to the 'press-pulse' disturbance concept that is commonly applied in riverine studies examining the effects of altered flow regimes or changed toxin concentrations (Parsons & Surgeoner, 1991a; Parsons & Surgeoner, 1991b; Lake, 2000; Balmonte *et al.*, 2016). To determine whether an event is a pulse, press, or ramp disturbance, changes over time in the strength or magnitude of a stress are observed (Lake, 2000); these three disturbances are thus defined by the temporal pattern of intensity and duration. Pulses are short-term and sharply delineated disturbances; for example, flood events, after which a system is returned to its previous state. By contrast, presses may arise sharply, eventually reaching a constant level that is maintained for an extended period; for example, sedimentation after a landslide. Ramp disturbances may in fact be thought of as a class of press disturbance (Hadwen *et al.*, 2012) that arise when the strength of a disturbance increases steadily over time and sometimes space. Ramp disturbances may continue to increase steadily or, after an extended period of time, reach an asymptote; for example, the worsening conditions of drought (Fig. 1.2) (Lake, 2000). Lake's classification highlights the importance of defining the temporal scale at which an event or stress acts upon a particular system of interest. In practice, whether or not a disturbance is characterised as a pulse, press or ramp is context driven and depends on how long the physical effects of a disturbance lasts and the system that is being investigated. For example, Collins *et al.* (2017) recently applied the press-pulse theory to understand desert grass community structure and dynamics. In their study, comparatively short-term acute stress events, including wildfire and inter-annual

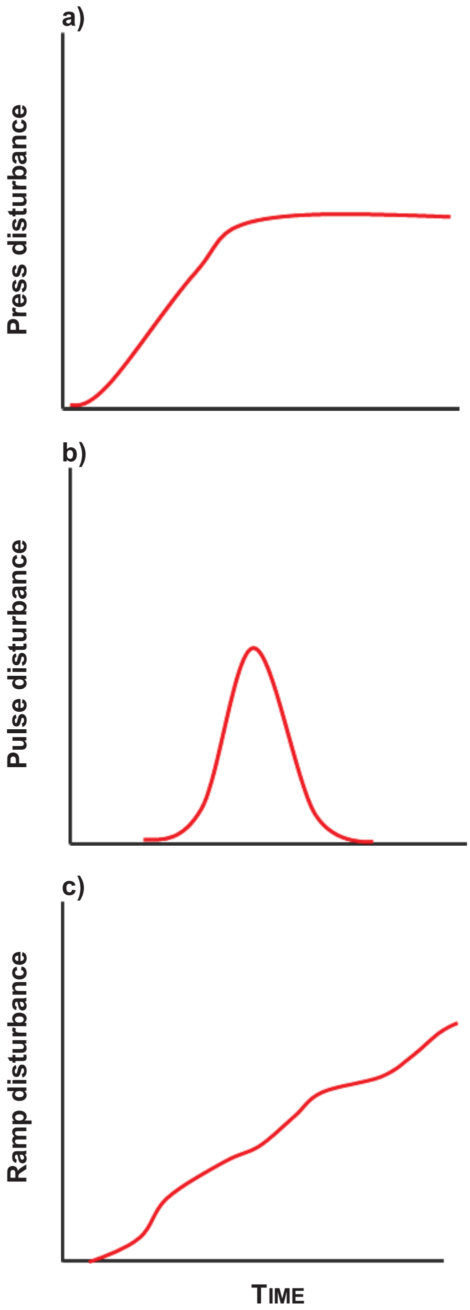

Figure 1.2 Press, pulse and ramp disturbances can be distinguished by temporal trends in the strength of the disturbing force. Press disturbances may arise sharply and eventually reach a constant, chronic level **a)**. Pulse disturbances are short-term and sharply delineated, acute disturbances **b)**. Ramp disturbances can arise where a stressor increases in strength steadily over time **c)** (modified from Lake, 2000).

precipitation variability, were defined as ecological pulses. By contrast, climate change variables, the effects of which are more subtle, persistent and in some cases accelerating over time, were termed ecological presses. Examples given of ecological presses included changing precipitation regimes, increasing atmospheric nitrogen deposition, and rising mean daytime and nighttime temperatures (Collins *et al.*, 2017). While the term 'press disturbance' can refer to long term events that may span decades (Grosse *et al.*, 2011), shorter perturbations, relevant to plant functioning, including photosynthesis, are less well understood and are the context of the present study. The processes of photosynthesis and energy partitioning will now be outlined, followed briefly by the effect of temperature on the photosynthetic machinery of plants.

1.4 PHOTOSYNTHESIS

Photosynthesis is the process used by vascular plants to harness and convert solar energy (light) into chemical energy. This energy is then used to fuel metabolism and growth of primary producers and these in turn fuel consumers. Photosynthetic organisms, including land plants, utilise light for photosynthesis in the visible range of the electromagnetic spectrum: wavelengths from approximately 400 to 700 nm (Fig. 1.3). The predominant photosynthesising organs of a plant are its leaves; however, other chlorophyll containing tissues can also photosynthesise. For the purpose of the present study I will refer to all relevant photosynthesising leaf-like structures as leaves. Photosynthesis takes place within membrane-bound organelles called chloroplasts, which for plants are located in the spongy and palisade mesophyll cells of leaves (Fig. 1.4). Here, integrated into an elaborately folded network of thylakoid membranes, is an array of protein complexes that together make up the photosynthetic electron transport system (Knox & Ladiges, 2006; Freeman, 2008). Photosynthesis consists of two sets of linked reactions (Fig. 1.5): 1) photochemical, light dependent reactions, the rate of which is independent of temperature and 2) non- photochemical, light independent reactions, which do not require light to function and are dependent on temperature (Bonner & Varner, 2012). During light dependent reactions, harvesting of light energy and its conversion to chemical energy occurs. Specifically, energy absorbed by pigment molecules in the light harvesting complexes is transferred among neighbouring pigment molecules until reaching photosystem II (PSII), the site of photochemistry and the start of the electron transport chain. Products arising from the light-dependent reactions include energy rich adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) molecules. These molecules store energy temporarily for subsequent use in the light-independent reactions, which consist of carbon fixation and the production of sugars from carbon dioxide (CO_2).

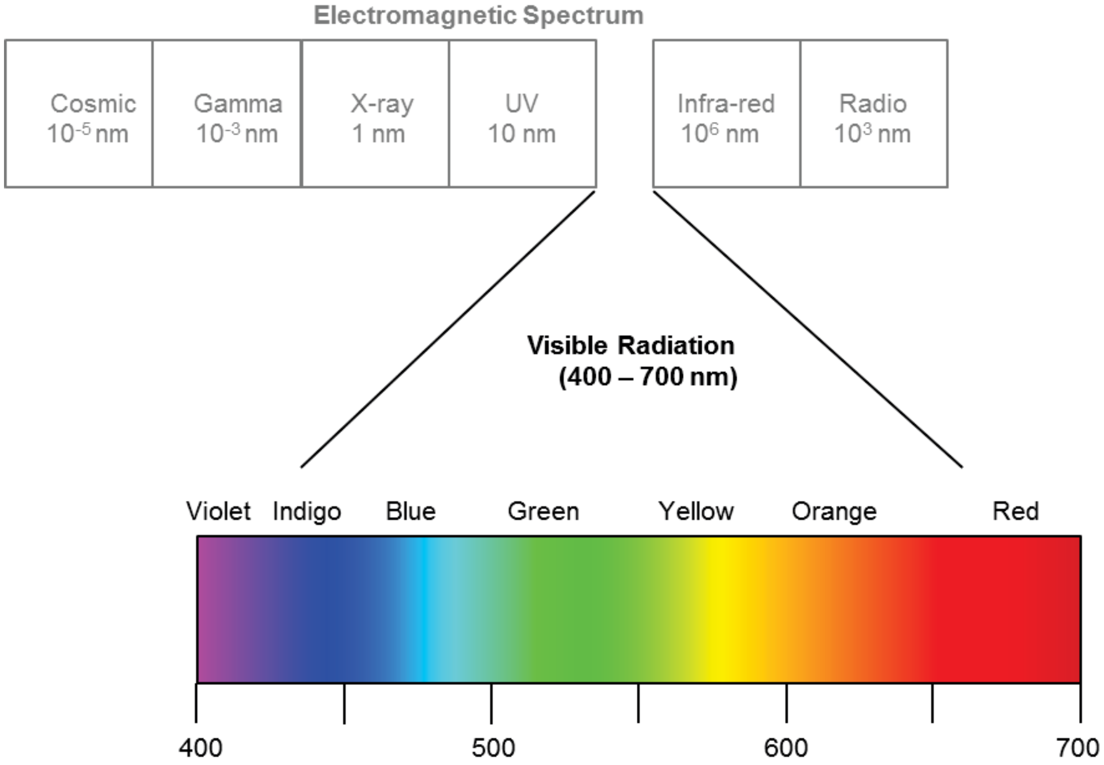

Figure 1.3 The electromagnetic spectrum is the wavelengths of energy ranging from cosmic radiation to radio waves. The solar spectrum is generally subdivided into three components, commonly referred to as short-wave radiation and includes ultraviolet radiation (UV: 300 to 400 nm, up to 4 – 7% of solar radiation), photosynthetically active or visible radiation (PAR: 400 – 700 nm, 21 – 46%) and near infrared radiation (NIR: 700 – 1100 nm, 50 – 70%) (Lambers *et al.*, 1998; Jones & Rotenberg, 2001). Visible wavelengths represent the portion of light that is used by plants during photosynthesis; it is also responsible for the colours that we see (modified from Knox & Ladiges, 2006).

Together with photochemistry, the light energy absorbed by chlorophyll molecules within a leaf can travel one of three pathways: it can be 1) used in the process of photochemistry, 2) dissipated as heat or 3) re-emitted as light, measured as chlorophyll fluorescence (Maxwell & Johnson, 2000; Govindjee, 2004). During fluorescence, light energy escapes from the pigment bed and is re-emitted or fluoresced at longer wavelengths between 690 and 730 nm (Lichtenthaler, 1996; Maxwell & Johnson, 2000). The process of partitioning energy into these three pathways is regulated to maximise photosynthetic rates under a given set of conditions (Pedros *et al.*, 2008; Ruban *et al.*, 2011). There is wide agreement that at room temperature, 90% of chlorophyll *a* fluorescence is emitted by PSII (Strasser *et al.*, 2004). In addition to the intrinsic temperature dependence of the reactions of photosynthesis, moderate and extreme high

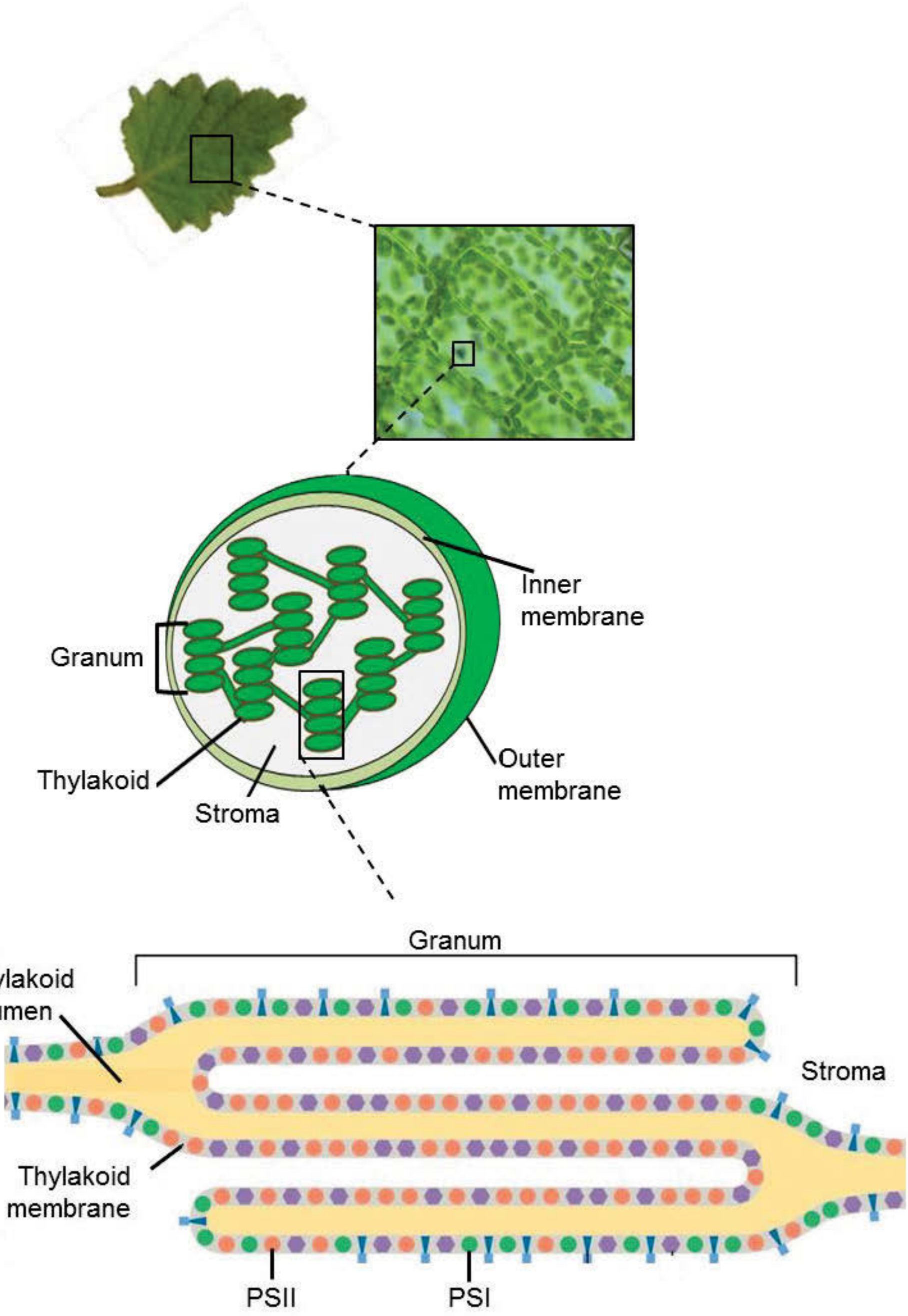

Figure 1.4 Photosynthesis takes place within highly structured, membrane-rich organelles located within the chloroplasts of leaves. The elaborately folded, internal membranes within chloroplasts are called thylakoids, which stack to form grana. Photosystem I and II (PSI and PSII, respectively) of the photosynthetic electron transport system are located within these membranes (modified from Freeman, 2008).

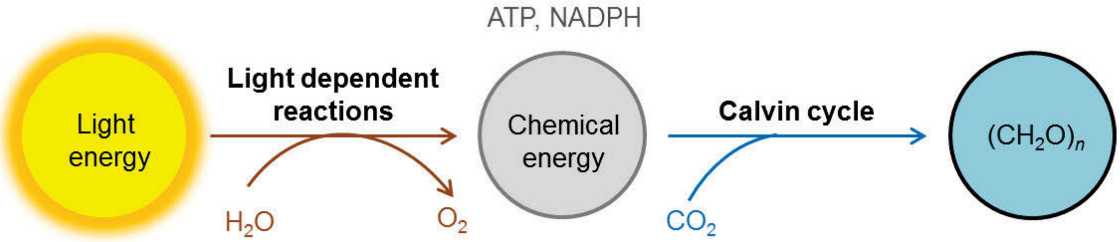

Figure 1.5 Photosynthesis consists of two reactions. During the light dependent reactions light energy is turned into chemical energy in the form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH). During this process, molecular oxygen (O_2) forms from the splitting of water molecules (H_2O). The energy rich molecules, ATP and NADPH, produced during the light-independent reactions, are used in the Calvin cycle, to reduce carbon dioxide (CO_2) to carbohydrates ($(CH_2O)_n$) (modified from Freeman, 2008).

temperatures can drastically affect the integrity of the system; for example, by limiting photosynthetic repair processes and inducing structural damage (Berry & Bjorkman, 1980; Pastenes & Horton, 1996; Eaton-Rye *et al.*, 2011). Measuring changes in the yield of chlorophyll *a* fluorescence provides insight into changes in the relative functional state of photosynthesis (Maxwell & Johnson, 2000; Strasser *et al.*, 2004). Because damage to the photosynthetic machinery often occurs long before visual cues are evident, chlorophyll *a* fluorescence can act as an early means of detecting the physiological effects of stress on plants.

1.5 PLANT THERMAL OPTIMA AND HEAT STRESS

It is because of the temperature-dependent nature of photosynthesis that plants have an optimum thermal range at which growth is maximised. The optimum temperature for photosynthesis is species-specific and varies with their growth conditions. Generally xeric-adapted desert or arid-zone species have high thermal optima ranging between 30 to 40 °C (see, Larcher, 2003 Table 2.11). This contrasts alpine species, for example, which have substantially lower thermal optima ranging between 15 to 25 °C (Larcher, 2003). Within these ranges, the rate of photosynthesis responds rapidly and reversibly, and the functional integrity of the photosynthetic apparatus remains intact (Berry & Bjorkman, 1980). Temperatures above a species' optimal range induce stress.

Individual components of the photosynthetic apparatus respond to moderate and high heat stress differently. For plants, moderately stressful temperatures are described as being sufficiently high to alter the physical state of thylakoid membranes, deactivate Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo, a key enzyme involved in carbon fixation) and stimulate the production of toxic reactive oxygen species (ROS), which can impair the recovery processes of PSII (for reviews see, Sharkey, 2005; Murata *et al.*, 2007). Whereas PSII is not *directly* affected by moderately stressful temperatures, it is particularly responsive to heat stress at higher temperatures (Berry & Bjorkman, 1980; Havaux, 1993; Georgieva & Yordanov, 1994; Havaux, 1994), with damage often incurred at temperatures above 45 °C (Pessarakli, 1999; Sharkey, 2005). If high temperatures exceed species' upper critical thermal thresholds, the photosynthetic apparatus, in particular PSII, can be severely and irreversibly damaged. At higher temperatures, damage to PSII has been associated with injury to a number of its constituents including the disruption of the oxygen evolving complex; degradation of PSII reaction centre subunits, especially the D1 protein; dissociation of the light-harvesting complex from the PSII core complex; and altered membrane properties, including increased membrane leakiness (Yamane *et al.*, 1998; Atwell, 1999; Sharkey, 2005; Allakhverdiev *et al.*, 2008). Thermal damage to PSII can be measured by fluorometry as reductions in F_V/F_M: the maximum quantum yield of PSII in the dark-adapted, unquenched state (Baker & Rosenqvist, 2004; for review see, Buonasera *et al.*, 2011). Specifically, F_V/F_M is the proportion of variable to maximum fluorescence of dark-adapted leaves, with F_V calculated as the difference between dark-adapted maximum and minimum fluorescence (F_0). More generally, F_V/F_M is indicative of photosystem health, with similar values between plants suggesting a similar proportion of healthy, functional PSII reaction centres (DeEll & Toivonen, 2003). Healthy, non-stressed plants tend to have an F_V/F_M close to 0.8 (DeEll & Toivonen, 2003). High-temperature stress causes F_M to decline and F_0 to increase, which result in a decrease in the F_V/F_M ratio (Krause & Weis, 1984; see Fig. 2b in Knight & Ackerly, 2003). A shift in these fluorescence parameters for leaves exposed to high-temperature indicates a change in the functional state of PSII and its components, including the light-harvesting and oxygen evolving complexes. For example, heat stress can cause the separation of the light-harvesting complex from the PSII core complex and the inactivation of oxygen evolution, which lead to increasing F_0 and decreasing F_M, respectively (Yamane *et al.*, 1997). The temperature causing a 50% decline in F_V/F_M of PSII from prestress levels corresponds to the onset of irreparable thermal damage, T_{50} (Knight & Ackerly, 2003;

Curtis *et al.*, 2014) (Fig. 1.6), and is a useful tool for quantifying high-temperature tolerance among species. The use of T_{50} for determining plant photosynthetic thermal tolerance is a relatively new development. Past studies typically used the temperature-dependent rise in F_0, the F-T curve, which, in contrast to the method for obtaining T_{50}, is obtained by slowly ramping up the temperature of a leaf and simultaneously recording changes in F_0 over time (e.g., Schreiber & Berry, 1977; Berry & Bjorkman, 1980; Bilger *et al.*, 1984; Seemann *et al.*, 1984; Havaux, 1993; Knight & Ackerly, 2002; Morgan-Kiss *et al.*, 2002; O'Sullivan *et al.*, 2013). The F-T curve is typified by an initial slow-rise in F_0 at moderate temperatures, which is followed by a sharp increase at higher temperatures (Knight & Ackerly, 2002). The increase in F_0 with temperature is associated with the disruption of electron transport due to increased membrane fluidity and dissociation of membrane-bound proteins involved in photosynthesis (Schreiber & Berry, 1977; Krause & Weis, 1984). Various points along the F-T curve have been characterised, including T_{crit} or T_c (Schreiber & Berry, 1977; Bilger *et al.*, 1984) and T_{S20} (Knight & Ackerly, 2002); these metrics provide important species-specific information of the temperature representing the break-point between reparable and irreparable thermal damage (see Fig. 1a in Knight & Ackerly, 2002). Traditional metrics

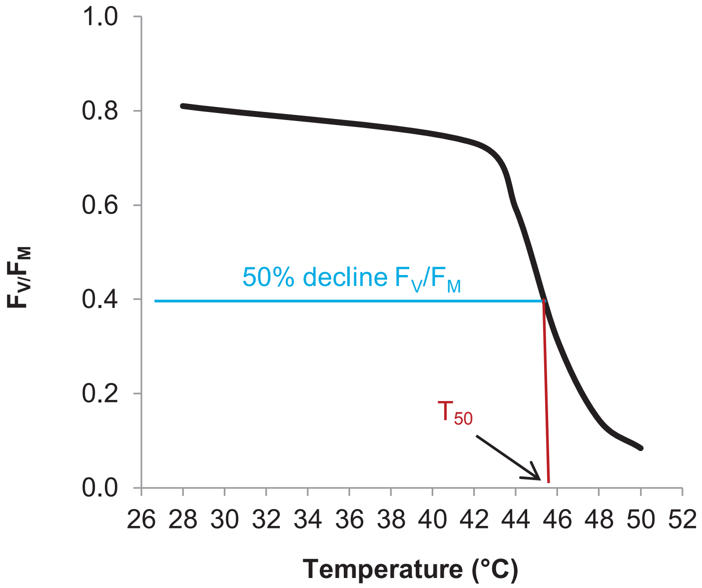

Figure 1.6 Chlorophyll *a* fluorescence can be measured for quantifying a species' thermal damage threshold, T_{50}. Specifically, the temperature causing a 50% decline in F_V/F_M of PSII from pre-stress levels corresponds to the onset of irreparable thermal damage, T_{50}, where F_V/F_M is the maximum quantum yield of PSII.

derived from the F-T curve are strongly and positively correlated with one another (Schreiber & Berry, 1977; Downton & Berry, 1982; Knight & Ackerly, 2003). Importantly, this break-point is strongly correlated with the thermal damage metric applied throughout this thesis, T_{50} (Knight & Ackerly, 2002; Knight & Ackerly, 2003). The T_{50} thermal tolerance threshold was used here as it enables relatively rapid sampling of multiple leaf replicates and species in a given experimental campaign (see, Curtis et al. 2014). Notably, thermal damage measured using the temperature-dependent decline in F_V/F_M, is significantly reduced if heat stress occurs in the presence of light (Buchner *et al.*, 2015). The protective capacity of light during heat stress is thought to be due to its influence on recovery and other processes, including the repair and almost immediate synthesis of the D1 protein, which becomes suppressed under dark conditions (Yamauchi *et al.*, 2011; Marutani *et al.*, 2012). **In the present study, I employ chlorophyll *a* fluorescence to measure species' T_{50}. In the process of refining this methodology, I developed a new protocol for monitoring thermal damage and damage recovery post heat stress (Curtis *et al.*, 2014). The methodological approach that I developed is outlined in Chapter 2 and was subsequently applied to questions I addressed in the remaining data chapters.**

To avoid heat stress as temperatures changes through time, many species have the capacity to acclimatise through plastic alterations of plant functional traits (Arnone III & Körner, 1997; Franks *et al.*, 2014). This means that species' thermal sensitivities are dynamic, and that the information provided from single, static measures of species' thresholds is valuable only in the context of comparisons within a given time period. Given its importance for understanding thermal tolerance variation among species, I now turn to discuss acclimatisation in plants in response to temperature changes.

1.6 THERMAL ACCLIMATISATION

The climate is in a state of rapid change (see p. 19 below, '1.8 The big picture'), resulting in new, potentially high stress, conditions to which plants must respond to survive. A plant species may show distributional changes and maintain growth only in localities where conditions are favourable. Alternatively, a species may stay and adapt by evolutionary selection and/or plastically acclimatise to the new conditions. For many species, the ability to disperse or evolve at a pace equal to the rate of environmental change will be limited, being strongly reliant on their ability to make intra-generational adjustments. A high phenotypic plasticity is expected to be an important attribute influencing a species’ ability to cope with novel environmental conditions (Gratani, 2014). Variation in plasticity will undoubtedly influence the abundance and distribution of species and affect community composition. Therefore, measuring species’ potential to physiologically acclimatise to novel temperatures is an important means of evaluating their vulnerability to heat stress.

Experimentally manipulating a single environmental variable and subsequently determining an organism’s performance generally is referred to as ‘acclimation’ (Way & Yamori, 2014). This contrasts with the process of individuals gradually adjusting to natural climatic changes in their environment, which can be considered ‘acclimatisation’. That is, the former is the influence on a trait or set of traits of a specific abiotic or biotic parameter, which can be measured by an increase in performance. The latter is the change in a trait that may occur in response to multiple environmental variables, e.g., seasonal changes in temperature, rainfall and light, and does not necessarily equate to increased performance. Thermal acclimatisation requires exposure to a non-lethal temperature stress event, triggering a change in the way genetic information is expressed over a short time period, such as days to weeks (Franks *et al.*, 2014; van Zanten *et al.*, 2014). This process is termed thermal priming and signals a change in the heat response pathways of plants, including upregulation of heat shock protein expression and changes in cell membrane fluidity (Penfield, 2008; McClung & Davis, 2010). Individuals within and among species can exhibit unique thermal acclimatory adjustments specific to growth location, season and even their developmental stage (Mooney & West, 1964; Smillie & Nott, 1979; Knight & Ackerly, 2002; Knight & Ackerly, 2003). When transferred to novel environments, warm- and cool-origin species can adjust physiologically, increasing or decreasing thermal tolerance to suit their new temperature regime (Knight & Ackerly, 2003). Seasonal shifts have also been recorded, with temperature tolerance higher in summer compared to winter (Lerner, 1999). Acclimatisation can result in greater fitness under growth conditions (often termed 'adaptive plasticity', e.g., Schmitt *et al.*, 1995; and 'acclimation', e.g., Atkin *et al.*, 2006; Way & Yamori, 2014) and is expected to be important for the persistence of plants within a warming climate (Angilletta, 2009); yet few studies explicitly test the hypothesis that a change in a given trait is adaptive

(Schmitt *et al.*, 1995). Similarly, variation among species' capacity to make these seasonal adjustments is not well understood. Testing the adaptive nature of acclimation is beyond the scope of the current research. **To address the latter gap in knowledge, I designed an experiment to measure inter- and intra-specific variation in T_{50} over three seasons: winter, spring and summer. Numerous species spanning plant families and functional types were compared, including a number of phylogenetically independent paired species contrasts. Due to the potential for local adaptation to influence outcomes, I selected paired contrasts based on differences in species' preferred native microhabitat. This work is outlined in Chapter 4 of this thesis.**

1.7 TEMPERATURE REGULATION

Although plants can acclimatise, their ability to do so is limited both by individual genetic constraints and the universal thresholds of biological function. Lacking an infinite capacity to increase their physiological temperature tolerance, plants require alternative means of protecting their leaves. An important way that plants can reduce heat stress is to thermoregulate. To explain this process, I will first outline the ways that leaves gain and lose heat.

1.7.1 Leaf energy budgets

Leaf temperature is the result of the balance between incoming energy and energy loss (Gates, 1965; Ehleringer, 2000; Jones & Rotenberg, 2001; Gates, 2012). When energy coming in is equal to energy leaving, leaf temperature remains unchanged and is said to be at 'steady state', which is rarely the case under natural conditions. When energy input exceeds output, leaf temperatures increase and this occurs mainly via absorbed radiation (Fig. 1.7). To reduce the proportion of absorbed incident light, leaves possess a range of traits, including optical properties, e.g., reflectance, varied morphology and orientation. Contrasting energy gains, the predominant pathways through which energy loss occurs from a leaf are reradiation of previously absorbed radiation, sensible heat exchange processes (i.e., convection and conduction) and latent heat exchange (i.e., transpiration) (Jones & Rotenberg, 2001). Plant and environmental attributes including plant water status, air temperature and humidity and wind speed, can strongly influence sensible and latent heat exchange processes.

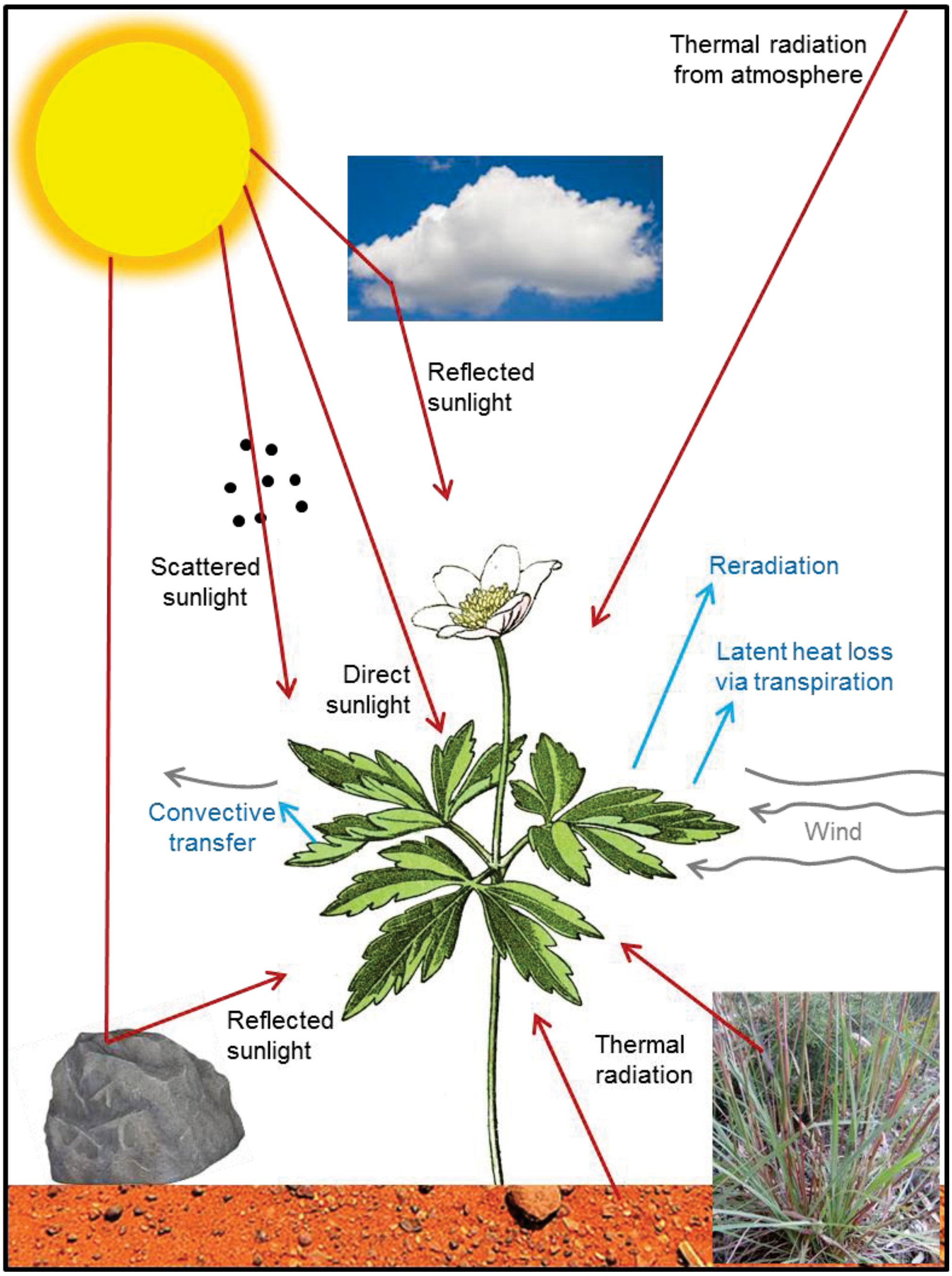

Figure 1.7 Pathways through which energy is transferred to and from plant leaves. Leaf temperature is the result of the balance between incoming energy and energy loss. Absorbed radiation, including solar radiation and the emission of thermal or infrared radiation from the surroundings, i.e., soil and other vegetation, is the main process by which energy is gained (red arrows). Contrasting energy gains, the predominant pathways through which energy loss occurs (blue arrows) from a leaf are: reradiation or the emission of previously absorbed radiation, sensible heat exchange processes, e.g., convection and latent heat exchange via transpiration (adapted from Gates, 1965).

1.7.2 Sensible heat exchange (convection and conduction)

Sensible heat exchange describes the processes of convection and conduction, by which heat is transferred between an object, e.g., a leaf, and its surroundings (Jones & Rotenberg, 2001). Conduction is the process of heat transfer through a medium or between substances that are in direct contact with one another and form a thermal gradient. This contrasts the process of convection, which describes the transfer of heat from or to an object via the physical movement of a surrounding fluid. The fluid motion of wind through the layer of air adjacent to a leaf – the boundary layer (Nobel, 2012) – exemplifies convective heat exchange. For plants, convection is a particularly important process influencing leaf temperature, and can occur as free or forced convection (Jones & Rotenberg, 2001; Vogel, 2009). Although the influence of free convection on leaf temperature can be important during wind stilling, it is less efficient at transferring heat than forced convection, which dominates in most field conditions.

Forced convection occurs when an outside source causes fluid to move over the surface of an object. In a natural terrestrial setting, the predominant source of forced convection is wind. Wind speeds of 0.5 m s^{-1} are at the threshold of human perception. For plants, even gentle wind speeds just above this speed are sufficient to disturb the leaf boundary layer and increase the rate that heat is transferred away from the surface of a leaf; however, when air movement drops, leaf temperature may increase rapidly (Leigh *et al.*, 2012). The latter scenario is more likely if the thermal time constant of a leaf, i.e., the time required for a leaf to change temperature, is short and/or if a protracted lull in wind speed is coupled with high radiation.

1.7.3 Latent heat exchange (transpiration)

Contrasting sensible heat exchange processes, latent heat exchange describes the transference of energy via an associated phase change of a substance, e.g., the change of liquid water to a vapour. Latent heat from leaves occurs via the process of transpiration, where liquid water at the mesophyll cell surfaces is converted to a gas via evaporation and exits through stomata. Due to water having a high heat of vaporisation, the change from liquid to gas consumes a large amount of energy and, together with the evaporated water, exits a leaf and cools the plant (Atwell, 1999).

Although transpiration can lower the temperature of a leaf substantially (e.g., by as much as 15 °C, Gates, 1965), it requires that stomata remain open. Numerous environmental cues can affect whether stomates open or close, including light, CO_2 concentrations within the leaf, water status of the plant, soil water availability, air-leaf vapour pressure deficit (VPD) and temperature (Gates, 2012). For example, for a well-watered plant in a warm environment, light stimulates stomata to open in the morning, and as temperature increases during the day, both photosynthesis and transpiration rates will steadily rise until maximum rates are reached (Farquhar & Sharkey, 1982). Irrespective of leaf cooling via latent heat exchange, the temperature of a leaf may continue to rise above its physiological optimum and slow photosynthesis. The reduced rate of photosynthesis is associated with an increase in the concentration of CO_2 within the leaf. The latter corresponds with lower internal pH, triggering osmotic adjustments in the guard cells of stomates, which close to conserve water: transpiration ceases, leaf temperatures increase and photosynthesis stops (Gates, 1965; Farquhar & Sharkey, 1982). Plants in a hot, dry environment such as a desert are faced with the combination of high temperatures, high VPD and low available water. To prevent desiccation under such conditions, plants often limit stomatal conductance, triggering leaf temperatures to rise rapidly (Macinnis-Ng & Eamus, 2009; Teskey *et al.*, 2015). The microclimatic conditions governing heat convection and latent heat loss may vary spatially, not only among plants, but also within a single plant (Chapter 6); however, studies have yet to determine whether leaf thermal damage thresholds are influenced by these finer scale environmental changes within a canopy. **I devised an experiment to investigate how microclimatic variation across the canopy of a dominant tree species of Australia's southern arid-zone may result in localised variation in leaf thermal tolerance.**

1.7.4 Structural and spectral properties influencing leaf thermal dynamics

Whereas physiological tolerance works on leaves that reach high temperatures, innate structural and spectral properties can prevent them from reaching excessively high temperatures in the first place. Differences in the width, size, thickness and shape of a leaf can influence its temperature. For example, smaller, narrower and more deeply lobed leaves have reduced boundary layers, which enable greater convective heat exchange and can subsequently maintain average leaf temperatures closer to ambient than larger or less-lobed leaves (Schuepp, 1993; Nobel, 2012; Leigh *et al.*, 2017). In extremely hot environments such as deserts, however, tracking ambient temperatures may not always be an advantage. To reduce the rate at which their temperature fluctuates in very hot conditions, plants can increase the thickness of their leaves. A thick leaf has a greater thermal mass than a thin leaf, which increases its thermal time constant and slows its rate of heating (Ball *et al.*, 1988; Gates, 2012). A slower rate of heating would be of particular importance for sun exposed leaves during a lull in wind speed and experiencing water stress, where latent heat loss is often minimal. With regards to leaf thermal dynamics, the benefit of a thick leaf is irrespective of whether or not leaves are thick and sclerophyllous or thick and succulent (Leigh *et al.*, 2012), corresponding to high leaf mass per area (LMA) and high leaf water content, respectively.

The structural properties of a leaf also can affect its temperature via various spectral properties that influence the passage of light into, out of and through a leaf (Woolley, 1971; Vogelmann, 1993; Vogelman *et al.*, 1996). For example, by increasing the reflectiveness of leaves, special epidermal coatings, including waxes, cutin, hairs and salt crystals, can reduce their thermal loading (Mooney *et al.*, 1977; Ehleringer, 1981; Skelton *et al.*, 2012). Although common in high irradiance environments, not all leaves are highly reflective and even those that are can maintain high average temperatures if they are large (Leigh *et al.*, 2012). **Given that both physiological mechanisms and a range of structural traits protect leaves from heat stress, a key question I address in the present study is whether species trade off a higher physiological thermal tolerance with various structural and spectral properties that influence their leaf thermal dynamics.**

1.8 THE BIG PICTURE

1.8.1 Climate change

Earth's climate is a naturally variable system that is being constantly influenced by natural variation in solar orbital patterns, continental movements and seismic activity (Matthews *et al.*, 2004). The current rate of climate change, however, cannot be explained by natural environmental variation alone. Distinct anomalies in the historical climate records have been linked with 20th century industrialisation, leading to elevated levels of greenhouse gases from fossil fuel use (Etheridge *et al.*, 1996) and large-scale land use changes (Chase *et al.*, 2000). Predictions of future climate change include a reduction in rainfall and increase in the frequency and magnitude of temperature extremes, conditions that are expected to co-occur in many areas globally (Field *et al.*, 2014). Now recall that transpiration through stomata is an important means by which plants can reduce heat stress (see section, '1.7.3 Latent heat exchange (transpiration)' above). An increase in atmospheric greenhouse gases, namely CO_2, may reduce stomatal conductance (Wong *et al.*, 1978; Wong *et al.*, 1979), elevating thermal stress by limiting latent heat loss (Siebke *et al.*, 2002; Barker *et al.*, 2005; but see the review by, Wang *et al.*, 2012). Stomatal closure also occurs under conditions of low soil water and/or high atmospheric evaporative demand (Atwell, 1999; Sperry, 2000). In many regions, including across Australia (Roderick *et al.*, 2009a; Roderick *et al.*, 2009b), a steady decrease in atmospheric evaporative demand has been observed for the last 30 to 50 years (Peterson *et al.*, 1995; Gifford, 2005), suggesting there may be more soil water available to support transpirational cooling. These latter observations regarding evaporative demand are complicated, however, by the potential effects of an array of climate, soil and plant factors (McVicar *et al.*, 2012a; McVicar *et al.*, 2012b). Further, the decline in evaporative demand has been associated with instrumentational features (Slaymaker *et al.*, 2009) and thus does not necessarily translate to plant evapotranspiration rates (Roderick *et al.*, 2009b).

Increased coupling of high-temperature extremes and water stress may have drastic consequences for plant growth and survival and community dynamics. This is especially pertinent for vegetation communities throughout desert and arid regions, where warming is expected to be fastest (Stahlschmidt *et al.*, 2011; Field *et al.*, 2014) and where species already are living at the upper thermal limits of what many biological processes can withstand. The arid region of Australia is one such area where the frequency and intensity of extreme events, including heatwaves and drought, are predicted to increase (Australian Bureau of Meteorology and CSIRO, 2016; CSIRO and Bureau of Meteorology, 2016), yet the amount and rate of climatic change will likely vary across the known geographic range of a species (Diamond *et al.*, 2012). To establish which arid-zone species may suffer detrimentally with more frequent and extreme temperatures, we need to understand their current physiological limits. **Given**

this insight, my research examines the spatiotemporal dynamics of high-temperature heat stress for Australian arid-zone plants.

1.8.2 Arid regions of Australia

Arid and semi-arid regions, where rainfall is very low and potential evaporation very high, cover up to 40% of the earth's land surface (Field *et al.*, 2014; Fig. 1.8). Australia is the driest inhabited continent in the world, with up to 70% of the land surface being arid or semi-arid (collectively known as 'desert' – ABS, 2006; Fig. 1.9). The Australian arid-zone is defined as receiving an average annual rainfall of approximately ≤ 250 mm in the south and ≤ 350 mm in the north (Eamus *et al.*, 2006). By contrast, the semi-arid-zone receives an average annual rainfall of 250 to 500 mm in the south and 350 to 750 mm in the north (Linacre & Geerts, 2002). Together these vast regions form a coherent biome (Byrne *et al.*, 2008; Davis *et al.*, 2013), with its own distinct and diverse floral assemblage (ABS, 2006). Australia's desert flora is a unique group to study, not only because species here must tolerate extremely nutrient poor soils, but also they are distinct from many northern hemisphere desert plants by being predominantly evergreen (Beadle, 1954). The rich and diverse range of floral assemblages found across

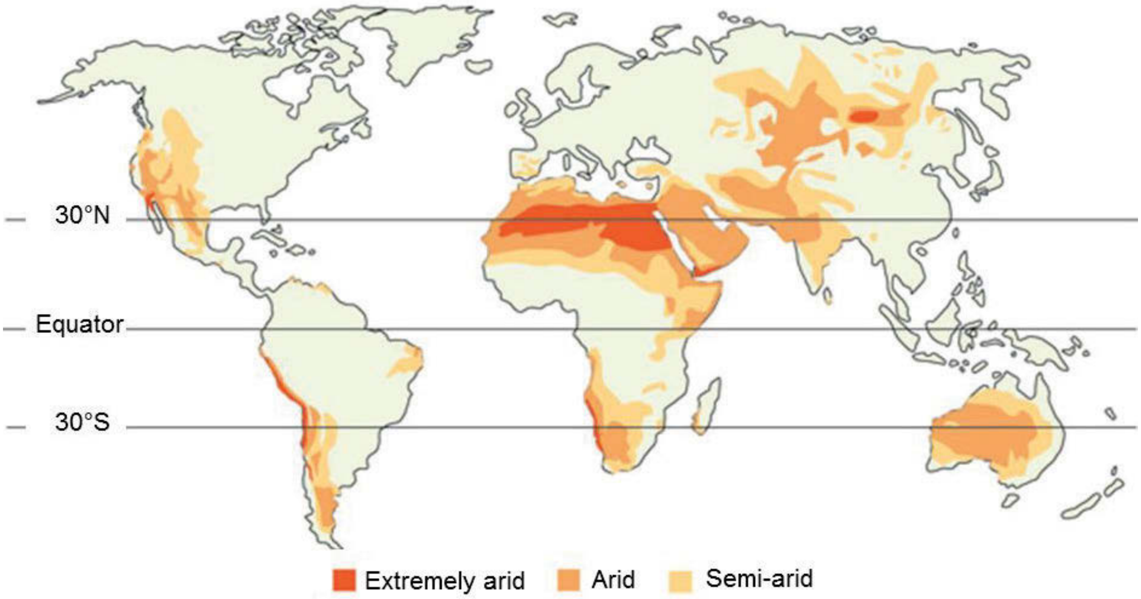

Figure 1.8 Approximately 40% of the world's terrestrial land surface is occupied by deserts which includes extremely or hyper-arid, arid and semi-arid regions. These regions generally have high daytime temperatures, receive little rainfall and have a high potential evaporation (© 2011 Nature Education, All rights reserved).

Australia's desert region range from vast spinifex-dominated (*Triodia* spp.) grasslands, which are confined largely to the arid interior of the continent, *Acacia*-dominated low woodlands or shrublands in the southern district of the Australian arid-zone, *Eucalyptus* woodlands that establish growth along ephemeral waterways and Chenopod shrublands that are found mainly throughout the southern arid-zone and are dominated by hardy, succulent species including *Atriplex* (saltbush) and *Mairena* (bluebush) (ABS, 2006). Both gymnosperm and angiosperm species are present. Compared with angiosperms, the species richness of gymnosperms is limited; however, Cyprus Pine (*Callitris* sp.) is common throughout some regions. Within the larger grouping of angiosperms, the most common plant families include Myrtaceae (e.g., *Eucalyptus* spp.), Asteraceae (daisies), Mimosaceae (e.g., *Acacia* sp. or wattle), Poaceace (grasses), Fabaceae (peas or legumes) and Amaranthaceae (formerly Chenopodiaceae; saltbush, bluebush,

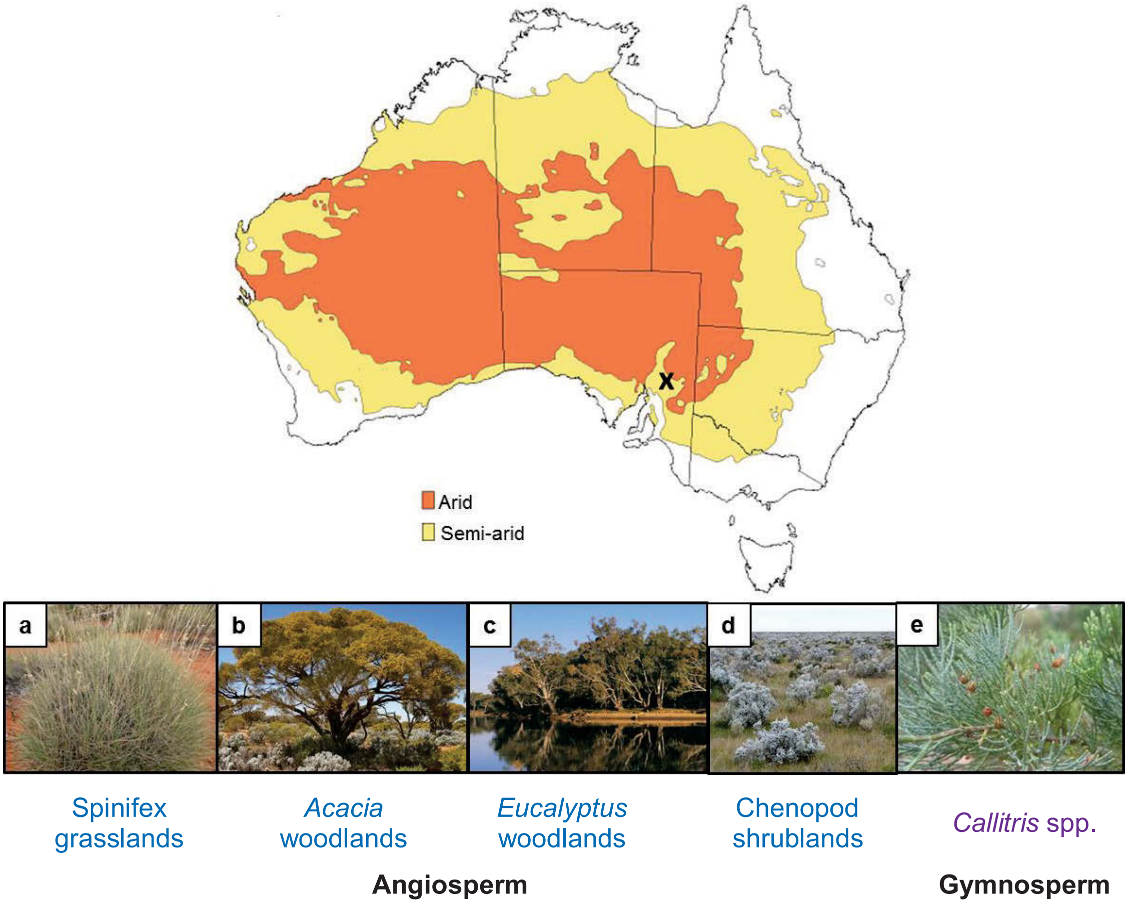

Figure 1.9 Arid and semiarid regions extend across 70% of the Australian continent making it the largest desert region in the southern hemisphere. Panels a – e: examples of the rich and diverse range of floral assemblages found across Australia's desert region. Bold 'x' marks the approximate location of the Australian Arid Lands Botanic Garden study site in Port Augusta, South Australia. Source: http://www.bom.gov.au/.

cottonbush). At the level of genus, *Acacia* dominates throughout arid Australia, but species of *Eremophila* and *Eucalyptus* also are common throughout arid Australia. Within these and other groups are several life history strategies, ranging from long-lived perennials, which must cope with climatic stresses all year round, to ephemerals or annuals, which effectively avoid certain stressful periods by dying back to rootstock or completing their life cycle within a few weeks or months. **I sampled among these and other genera and families to incorporate a diverse range of plant functional types and leaf morphologies. For this study, all species experienced similar conditions within a naturally arid environment at the Australian Arid Lands Botanic Garden in Port Augusta (Fig. 1.9) located in the southern arid region of South Australia.** From the information I have outlined thus far, a series of key questions have emerged which form the basis of this thesis.

1.9 RESEARCH OBJECTIVE AND THESIS OUTLINE

The overall objective of the work described in this thesis is to provide insight into leaf-level thermal responses of plants to extreme high temperatures in light of a warming climate. To do so, I addressed seven questions:

1) **To what extent do plant thermal damage thresholds vary among species?**
2) **Is a species' capacity to recover from short term heat stress related to its critical thermal damage threshold?**

I address these first two questions in Chapter 2, where I detail a novel methodological approach for measuring thermal damage and damage recovery post heat-stress in 41 species of arid-zone plants. A reformatted version of this chapter has been published as a peer-reviewed paper in the scientific journal *Oecologia* (Curtis *et al.*, 2014). Subsequently, the procedure outlined here for determining species' thermal damage thresholds was applied in all remaining data chapters.

3) **Is a species' thermal damage threshold more strongly related to macro-scale, broad climatic indicators or to its preferred native microhabitat based on typical water availability?**

Chapter 3 addresses the question of scale, specifically with regards to environmental conditions that are commonly used to infer species' thermal vulnerability; it examines the influence of broad-scale climatic conditions and fine-scale habitat variation**.** A reformatted version of this chapter has been published in the *Journal of biogeography* (Curtis *et al.*, 2016).

4) **Do seasonal thermal acclimatisation trajectories vary among species of arid-zone plants; what implications does this have for understanding species-specific thermal tolerances?**

In Chapter 4, I examine differences in the temporal variation of thermal damage thresholds among species with contrasting preferred native microhabitats. This chapter expands on the findings in Chapter 3 by considering aspects of temporal acclimatisation and thermal priming. This chapter is about to be submitted to the journal of *Global Change Biology* (by end of June 2017).

5) **Do morphology and spectral properties that influence leaf thermal dynamics trade-off or covary with the physiological index of thermal tolerance, T_{50}, among arid-zone plants?**

I investigate this question in Chapter 5, which uses a series of multi-trait analyses to examine the relationship among physiological and morphological properties that influence leaf thermal dynamics. Linking findings of previous chapters, I discuss the importance of season, native microhabitat and phylogenetic relatedness for multi-trait patterns. This chapter is in preparation for submission to the *Journal of Vegetation Science* (by end of June 2017).

6) **Do local-scale microclimatic differences equate to varied thermal tolerances among leaves from contrasting canopy positions?**

Chapter 6 explores the relationship between localised canopy microclimatic conditions and T_{50} variation for the widespread Australian southern arid species, *Acacia papyrocarpa* Benth. Aspects including ambient air temperature, air vapour pressure deficit and relative humidity, as well as wind speed and leaf thermal time constants are considered. This chapter is in preparation for submission for publication by September 2017.

7) **How do changes in the frequency and magnitude of repeated heat stress events influence species' thermal response?**

The final chapter of my thesis synthesises my key findings, highlighting new insights that this work has contributed to our understanding of the spatiotemporal dynamics of high-temperature tolerance in Australian arid-zone plants. I addressed question 7 during my PhD with a 2-week experiment, for which I conducted preliminary analysis. Although these findings did not end up as a data chapter due to time constraints, the final chapter includes reference to these preliminary findings (included as an appendix) in light of question 7. I discuss potential applications for this work, along with the rest of my research, and identify future areas for investigation.

Because each data chapter has been either published or prepared for imminent submission for publication, the reader will notice some level of repetition, particularly among the Introduction sections of each data chapter. I hope that the reader will appreciate my decision to include each of these publications/manuscripts in their entirety to maintain flow and context.

Chapter 2

A comparative analysis of photosynthetic recovery from thermal stress: a desert plant case study

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Keywords: High-temperature; heat stress; thermal threshold; chlorophyll fluorescence; arid-zone.

2.1 ABSTRACT

Our understanding of the effects of heat stress on plant photosynthesis has progressed rapidly in recent years through the use of chlorophyll *a* fluorescence techniques. Methods frequently involve treatment of leaves for several hours in dark conditions to estimate declines in maximum quantum yield of PSII (F_V/F_M), rarely accounting for recovery of effective quantum yield ($\Delta F/F_M'$) after thermally-induced damage occurs. Exposure to high-temperature extremes, however, can occur over minutes, rather than hours, and recent studies suggest that light influences damage recovery. Also, current focus on agriculturally important crops may lead to assumptions about average stress responses and a poor understanding about the variation among species' thermal tolerance. We present a chlorophyll *a* fluorescence protocol incorporating subsaturating light to address whether species' thermal tolerance thresholds (T_{50}) are related to the ability to recover from short-term heat stress in 41 Australian desert species. We found that damage incurred by 15 minute thermal stress events was most strongly negatively correlated with the capacity of species to recover after a stress event of 50°C in summer. Phylogenetically independent contrasts analyses revealed that basal divergences partially explain this relationship. Although T_{50} and recovery capacity were positively correlated, the relationship was weaker for species with high T_{50} values (>51°C). Results highlight that, even within a single desert biome, species vary widely in their physiological response to high-temperature stress and recovery metrics provide more comprehensive information than damage metrics alone.

2.2 INTRODUCTION

High-temperature events – heat waves – are increasing in both frequency and magnitude worldwide (Carter *et al.*, 2007; CSIRO and AGBoM, 2007). The rate of climate change also is predicted to surpass the rate of many plant species' ability to migrate or evolve (Jump & Peñuelas, 2005). Being immobile, plants potentially are more vulnerable than organisms that are able to migrate. Plant community compositional changes will depend on both species-specific threshold parameters and species' responses to the type and rate of change (Walther, 2003). Therefore, to better predict which plant species are more vulnerable to changing climatic conditions, it is important to understand how they currently cope with high-temperature stress. This includes understanding both current tolerance and the potential for species to recover after an episode of heat stress. Often, heat stress studies are confined to agriculturally significant crops and generally investigate only a small number of species (Harding *et al.*, 1990; Derocher *et al.*, 1991; Pessarakli, 1994; Tsonev *et al.*, 1999; Claussen, 2005; Singh *et al.*, 2007). Although useful within a given context, information only on one or a few species does not provide the full range of responses and may even misrepresent a perceived norm. Cross-species comparisons make it possible to investigate broad patterns relating to the functional significance of plant thermal tolerance and their capacity to recover. Further, crop species, whilst agriculturally important, generally represent a limited set of climatic conditions, whereas other biomes also warrant our attention. For example, desert plant species may be particularly vulnerable to increased heat stress as they already live on the upper threshold of what many biological tissues and processes can withstand. Given the large area of terrestrial ecosystems occupied by desert or semi-desert worldwide, this is a significant gap in our understanding of ecosystem response to future temperature extremes.

Chlorophyll fluorometry can provide robust estimates of thermal stability whilst also enabling rapid measurements of a large number of field-grown replicates (Seemann *et al.*, 1984; Willits & Peet, 2001; Knight & Ackerly, 2002; Knight & Ackerly, 2003). Photosystem II (PSII), in the thylakoid membrane of chloroplasts, generally is considered to be the site that is most sensitive to heat stress (Georgieva & Yordanov, 1994). Fluorescence methods used to evaluate thermal damage to photosynthetic tissue traditionally measure the maximum quantum yield of PSII or $F_{V/}F_M$. $F_{V/}F_M$ is measured on dark-adapted tissue to estimate the maximum portion of absorbed quanta used in reaction centres, therefore providing an indication of the capacity of PSII to accept light (Havaux *et al.*, 1991; Baker & Rosenqvist, 2004). Due in part to its relatively rapid measurement time, $F_{V/}F_M$ is well-established as a reliable way of quantifying the health of plant photosystems after a period of stress (Berry & Bjorkman, 1980). Similar values of $F_{V/}F_M$ between plants suggests that they have an equivalent proportion of healthy functional PSII centres (DeEll & Toivonen, 2003). However, estimating the health of reaction centres provides information on only the first step in the photochemical pathway, which does not necessarily correlate with the efficiency of photosynthetic electron transport after light is absorbed (Demmig-Adams & Adams, 1996). Photosynthetic efficiency ($\Delta F/F_M'$) is the realised operating efficiency of PSII under actinic light (drives photosynthesis); where $\Delta F = F_M' - F$ or the difference in the maximum (F_M') and minimum (F) fluorescence yield, determined in the light-adapted state (Schreiber *et al.*, 1995), and can vary in response to other environmental conditions, including temperature stress (Baker, 2008). $\Delta F/F_M'$ is an estimate of the effective or actual portion of absorbed quanta that is used in photosynthesis (Genty *et al.*, 1989). Although rarely considered, the extent to which photosynthetic efficiency recovers following heat stress, could provide a more complete understanding of plant thermal tolerance (Tsonev *et al.*, 1999).

It is known that low, subsaturating levels of light during heat stress are important for recovery processes in heat stressed plants (Marutani *et al.*, 2012). Inhibition of photosynthetic oxygen evolution and photochemical energy storage has been shown to occur when heat stress is induced under dark conditions, but not in the light (Havaux et al., 1991). Sub-saturating, low to moderate levels of light can effectively protect against thermally induced inactivation by facilitating recovery processes and may be critical in mitigating thermal damage (Marutani *et al.*, 2012; Buchner *et al.*, 2013). Protective mechanisms associated with exposure to light are not clear, but it is thought that light may help mitigate heat-induced disassembly of the water-splitting system of PSII (Havaux & Tardy, 1997). Light-induced transthylakoid-acidification also could act to stabilise thylakoid membranes, which would enable PSII reaction centres to be maintained in their normal configuration (Weis, 1982; Havaux *et al.*, 1991). As heat stress is likely to occur during daylight hours, plant thermal tolerance experiments that include light potentially are more ecologically relevant than those conducted on dark-adapted leaves only.

The duration of heat stress treatments applied also can vary considerably among studies: from one or more hours to days. Although high ambient temperatures can indeed endure for hours, the temperatures experienced by leaves do not always couple with ambient temperatures for protracted periods due to convective air currents (Roden & Pearcy, 1993). Transient lulls in air movement, however, may cause leaf temperatures to rise dramatically in minutes (Vogel, 2005), especially when alternative mechanisms of cooling, e.g., transpiration, are limited (Leigh *et al.*, 2012). Indeed leaf temperatures are known to exceed air temperatures by, e.g., 10°C in a matter of minutes, even seconds (Wise *et al.*, 2004; Vogel, 2005). In the Australian desert, air temperatures often exceed 45°C on summer afternoons. Under such conditions, with a lull in air movement for a period of several minutes, leaf temperatures of between 49 °C to > 52 °C have been recorded, sustained for around ten minutes before falling close to ambient when wind speed increases once more (A. Leigh unpublished data). Importantly, even short durations of heat stress can suppress photosynthesis and lead to oxidative stress (Vallélian-Bindschedler *et al.*, 1998). The potential for leaves to recover after enduring short episodes of heat stress of a matter of minutes is considerably understudied.

In the present study we asked: is the thermal tolerance threshold of desert plant species correlated with their capacity to recover from a short term heat stress event? In addressing this question, we advanced existing protocols for evaluating thermal tolerance based on maximum quantum yield (health of PSII) and the longer-term recovery of effective quantum yield (photosynthetic efficiency) using chlorophyll fluorescence. Our technique involved applying subsaturating light to leaves, both during short periods of heat stress and for a phase of recovery of photochemistry, and monitoring their response throughout treatments. To investigate relationships between species' thermal tolerance threshold, damage and recovery from damage we measured a range of Australian desert plants during the height of summer in a common desert environment.

2.3 METHODS

2.3.1 Study area and species sampling

The study was addressed using plants growing at the Australian Arid Lands Botanical Garden, located in Port Augusta, within the southern arid region of South Australia. The site spans 250 ha, which encompasses naturally occurring chenopod plains and Western Myall (*Acacia papyrocarpa*) woodlands. All species sampled experience similar climatic and soil conditions representative of the adjacent desert environment. Port Augusta has a mean annual rainfall of ~ 250 mm and a mean maximum summer temperature of ~ 31.3 °C, with maximum temperatures reaching > 45 °C in summer (AGBoM, 2013a; Curtis et al., unpublished data). Forty-one southern arid region shrub and tree species were sampled and, to reduce phylogenetic bias, species were selected across twenty-one plant families. Species also were selected to encompass a range of growth forms and leaf morphology. To reduce variation in the data, due to potential seasonal effects, all measurements took place over a six week period during high summer 2013 (late January to early March). By minimising the effect of regional climatic and seasonal variation, physiological differences among species could be more reliably attributed to adaptive variation in heat stress response, rather than localised acclimatisation.

Leaves were sampled from branches collected from the north-facing outer canopy of a minimum of five plants per species (in eight cases, 3 – 4 plants were sampled as this was the number available). To prevent photoinhibition, branches were collected predawn and placed into a dark plastic bag with a moistened sponge to prevent desiccation. All fully expanded, healthy leaves of similar age were sampled from each branch. Leaves were pooled together and kept in a sealed container with moist paper towel at room temperature (25 – 28 °C) under ambient light conditions (~ 15 µmol photons m^{-2} s^{-1}) until randomly allocated to a treatment temperature. 'Leaves' included both true leaves and photosynthetic plant organs that function as leaves when true leaves are absent, i.e., cladodes and phyllodes.

2.3.2 Chlorophyll *a* fluorescence: thermal tolerance threshold and recovery

Chlorophyll *a* fluorometry was used to determine thermal tolerance, damage and recovery from heat stress. A thermal tolerance threshold (T_{50}) was calculated using the temperature dependent decline in maximum quantum yield of PSII (F_V/F_M) to determine the temperature at which it dropped by 50% (Knight & Ackerly, 2003). Specifically, T_{50}

was calculated by fitting a straight line through the temperature points bracketing the 50% decline in F_V/F_M (using [y-b]/m = x). F_V/F_M measurements recorded on leaves after being allowed to recover until the following day (see below) were used in order to capture long-term or irreversible damage caused by the application of heat stress. F_V and F_M are the variable and maximum fluorescence of dark-adapted leaves, respectively, with F_V calculated as the difference between dark-adapted maximum and minimum fluorescence (F_0). The T_{50} thermal tolerance threshold was used due to the ability of this method to enable relatively rapid sampling of multiple replicates in a given experimental campaign. The T_{50} metric has been shown to strongly correspond with the temperature-dependent increase in minimum fluorescence (T_{S20} or T_{crit}) among numerous species, representing the onset of irreversible damage to PSII (Schreiber & Berry, 1977; Downton & Berry, 1982; Knight & Ackerly, 2003). In the present study, to complement measurements of T_{50}, damage to PSII in response to heat stress and the potential to recover from stress were also estimated via metrics incorporating the subsequent increase in photosynthetic function, using dark-adapted ($F_{V/}F_M$) and light-adapted tissue ($\Delta F/F_M'$), respectively.

The experimental process was initiated within approx. 6 hr of leaves being collected. Fluorescence measurements were made using a pulse modulated fluorometer (MINI-PAM) with fibre optics and leaf-clip attachment (Heinz Walz, Effeltrich, Germany). For each species, six batches of ten leaves were chosen from the sampling pool and each batch was placed into a zip-lock plastic bag on moistened paper towel and sealed, removing air from within. Each batch of leaves was treated to one of six temperature treatments (five treatment temperatures and one control temperature) using temperature controlled water baths. Treatment temperatures ranged from 46 °C to 54 °C (except for one species, *Triodia irritans*, which required an additional final heat treatment of 56 °C in order to adequately extrapolate T_{50}), varying by 2 °C increments, plus a control of 28 °C. Set temperatures of each treatment and control bath were maintained constant (± 0.2 °C) for the duration of the treatments using thermoelectric controllers (Cynebar – Precision Electrical Heating, Brisbane, Australia). Treatment temperatures of each bath were verified using calibrated k-type thermocouples and monitored for the duration of the experiment using a DT85 data logger (Thermo Fisher Scientific, Melbourne, Australia). In each bath, an aquarium pump (Aqua Nova 300 L/H) was used to circulate the water, preventing thermal stratification. To stimulate recovery throughout the treatment process, leaves were exposed to a subsaturating light level of ca. 280 μmol photons m^{-2} s^{-1} from a red/blue dominant 15 W LED grow light (white, 660 nm, 630 nm, 710 nm and 460 nm bulbs, www.livingapartments.com. au) suspended above each water bath. Light levels were determined under the surface of the water at a level corresponding to the placement of samples using a 4π underwater quantum probe (Li – 250A light meter, LICOR) and represent average light levels obtained from several measurements made under the illuminated field. All samples were placed beneath the water at a depth of 6 cm, with lights suspended 11 cm above the surface of the water. Although photoinhibition is likely to occur during periods of heat stress under field conditions, for cross-species comparisons to be made, controlled photoinhibitory light levels would require a light source far more powerful than was possible in our field laboratory. As our goal was to subject leaves to short-term heat stress representing a transient lull in air movement, we chose a treatment time of 15 minutes, based on field-recorded wind lulls of < 2.0 m $s^{-1}$for at least 10 minutes (A. Leigh, unpublished data). To ensure that leaves of different species experienced stress temperatures for equivalent periods, a trial was conducted to determine the rate at which leaves reached treatment temperature. Using six replicate leaves of 12 species representing the range of leaf morphologies of the greater sampling set (e.g., species ranging in leaf thickness, succulence and surface coatings: waxes and hairs), K-type fine-wire thermocouples were taped, without interfering with the thermocouple junction, to leaves, which were sealed in treatment bags and submerged in the water bath. Leaf temperatures were monitored every 5 seconds using a DT85 data logger (Thermo Fisher Scientific, Melbourne, Australia). These trials found that, regardless of morphological variations among species, leaves rapidly reached the set temperature of the water baths (on average 47.01 seconds, SE ± 5.68) equating to an average difference of ~ 5% among species.

Fluorescence was measured at intervals suitable for capturing variation in recovery over time. These intervals were chosen after a series of trials measuring $\Delta F/F_M'$ (on leaves exposed to actinic light, 280 μmol photons m^{-2} s^{-1}) on a sub-set of species immediately after heat stress and then every fifteen minutes for three hours. F_V/F_M (measured on dark-adapted leaves) was then monitored at 3.5, 4.5, 6.5, 8.5 hours and the following morning (additional ca. 16 hrs. later). These preliminary results identified within-species variation in response with time post heat stress, which greatly reduced after leaves were left to recover until the next day. This observation agrees with those of others who have shown decreased variability in fluorescence data after a period of 24 hours (Marias *et al.*, 2016). A number of tests confirmed that measuring severed leaves the day after collection was a robust approach. First, in all instances, the quantum yield of leaves under control conditions remained stable over the duration of the testing period (Fig. S2.1a), indicating that observed responses to temperature stress treatments could be attributed to heat stress. Second, readings taken up to 24 hours after sample collection, showed F_V/F_M and $\Delta F/F_M'$ of control leaves to be the same as pre-stress measurements taken on the day of collection (see Fig. S2.1a, b, which is representative of the control response for all species measured). Third, a trial comparing leaves measured 2 – 3 hours post-collection (10 runs of 10 leaves for each temperature treatment and control = 600 leaves) with those measured 4 – 6 hours post-collection (another 600 leaves), suggest that temporal variations in experimental starting times did not influence F_V/F_M and $\Delta F/F_{M'}$ post heat stress (all treatment temperature comparisons $P > 0.05$).

Following these preliminary experiments, the resolved protocol for applying heat stress was as follows (Fig. 2.1): Pre-stress F_V/F_M was measured after a 30 minute period of dark-adaptation. Samples then were placed for 15 minutes under control conditions (28 °C, 280 μmol photons m^{-2} s^{-1}), after which pre-stress measurements of $\Delta F/F_M'$ were recorded. $\Delta F/F_M'$ was again measured on leaves immediately after 15-minute treatments in one of the five temperature baths (dark-adaptation was not possible at this point so F_V/F_M was not measured immediately following heat stress). Following 90 minutes of recovery under control conditions, $\Delta F/F_M'$ was measured and samples were then dark-adapted for 30 minutes before F_V/F_M was measured at two hours post heat stress. Final measurements of F_V/F_M and $\Delta F/F_M'$ were recorded on day two after leaves had recovered in the dark for a further ca. 16 hours followed by 15 minutes at control conditions, respectively.

The difference between pre- and post-stress levels of photosystem health was used to determine damage incurred by different treatment temperatures (D_{PSII}) (Fig. 2.1, Fig. S2.1c):

$$D_{PSII} = 1 - (D2_{FV/FM}) / (PS_{FV/FM}) \qquad (eq.2\ 1)$$

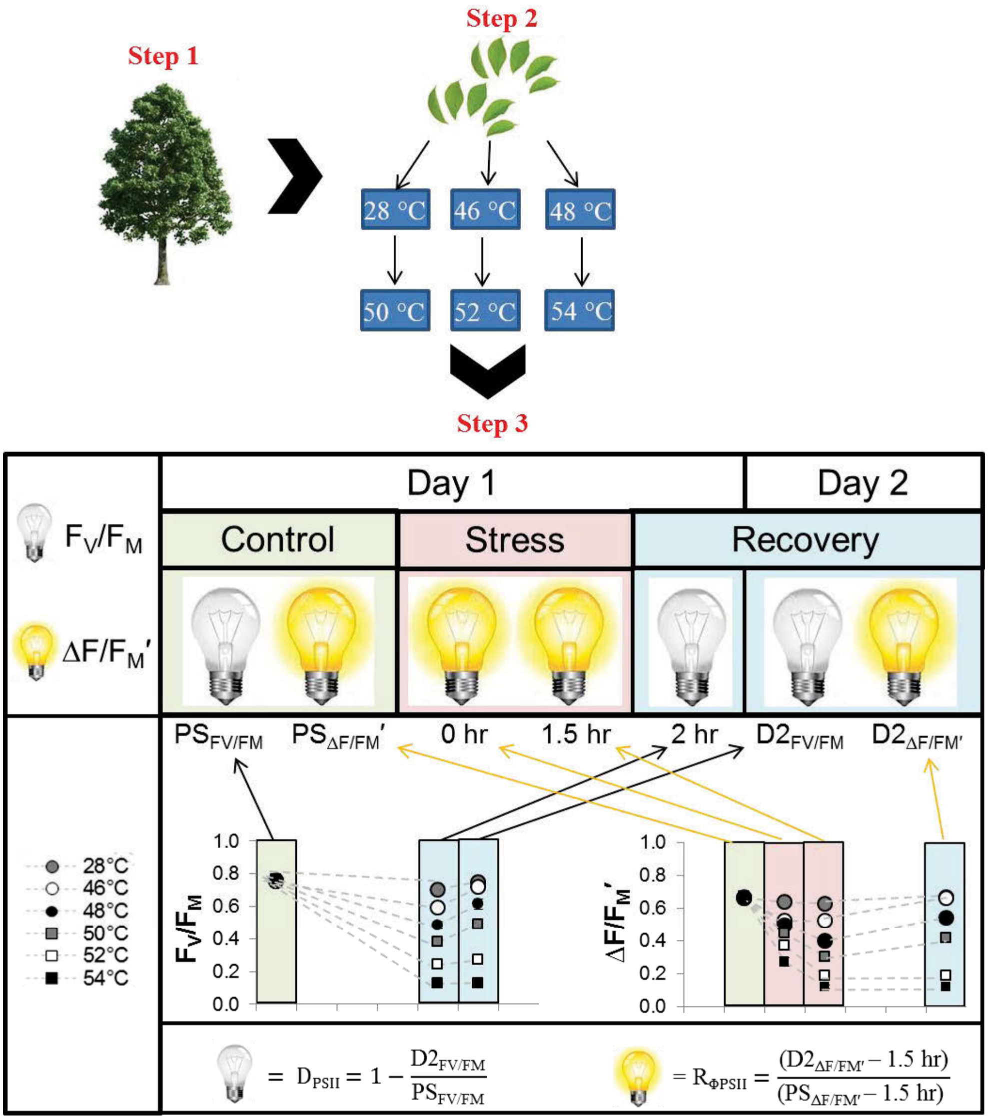

Figure 2.1 (For corresponding, published figure, see Fig. S2.1)**. Step 1.** Leaves were sampled from branches collected from the north-facing outer canopy of a minimum of five plants per species. **Step 2.** For each species, six batches of ten leaves were randomly chosen from the sampling pool and treated to one of six temperature treatments. **Step 3.** Control measurements of maximum quantum yield of PSII ($PS_{FV/FM}$) and effective quantum yield ($PS_{\Delta F/FM'}$) were measured prior to heat stress. F_V/F_M was measured two hours (2 hr) after stress treatment and after a further recovery period of ca. 16 hours ($D2_{FV/FM,}$ indicating day two of measurements) at 46, 48, 50, 52 to 54 °C and a control temperature of 28 °C. $\Delta F/F_M'$ was measured immediately following stress treatment (0 hr), 1.5 hours after and on day two following dark-adapted measurements and an additional 15 minutes under control conditions in order to light-adapt samples ($D2_{\Delta F/FM'}$). For each treatment temperature, dark measurements were used to quantify the damage metric, D_{PSII}, and light measurements were used to quantify the recovery metric, $R_{\Phi PSII}$. For all data points $n = 10 \pm$ SE. The alignment of dark- and light-adapted measurements, F_V/F_M and $\Delta F/F_M'$ respectively, with time and treatment temperature indicated with arrows. Graphs inset show the photochemical quantum yield for leaves in the dark- and light-adapted state in response to heat stress treatments, as demonstrated in *Acacia papyrocarpa* during summer. Dashed lines are for ease of reading patterns and not representative of continuous time.

where D_{PSII} is the inverse of maximum quantum yield of PSII measured on day two ($D2_{FV/FM}$) as a proportion of the pre-stress level ($PS_{FV/FM}$). D_{PSII} therefore quantifies long-term heat-induced photoinhibition relative to pre-stress conditions. Values of D_{PSII} close to 1.0 indicate a higher degree of irreversible damage for a given recovery time.

Recovery from heat stress was quantified by considering the proportion of initial loss of photosynthetic efficiency that was recovered the next day ($R_{\Phi PSII}$) (Fig. 2.1, Fig. S2.1c):

$$R_{\Phi PSII}= (D2_{\Delta F/FM'} - 1.5\ hr)/ (PS_{\Delta F/FM} - 1.5\ hr) \qquad (eq.2\ 2)$$

where $D2_{\Delta F/FM'}$ is the photosynthetic efficiency of PSII measured on day 2, 1.5 hr indicates the photosynthetic efficiency of PSII measured 90 minutes after heat stress and $PS_{\Delta F/FM}$ is photosynthetic efficiency of PSII measured prior to heat stress. Higher values of $R_{\Phi PSII}$ correspond to an increase in $\Delta F/F_M'$ on day two following stress. $R_{\Phi PSII}$ therefore indicates the level of functional recovery from heat stress at a given temperature.

2.3.3 Statistical analyses

Data were analysed using the software IBM SPSS® (version 19). After considering data for assumptions of normality and heterogeneity of variances, Pearson product-moment correlation coefficient (*r*) analyses were used to assess the relationships between species' thermal tolerance thresholds (T_{50}), the ability for leaves to recover from heat stress and damage ($R_{\Phi PSII}$ and D_{PSII}, respectively). A Student's t-test was used to assess differences in recovery between leaf samples in the light- vs dark-adapted state. Further, we performed Phylogenetically Independent Contrasts following the method of Felsenstein (1985) and Garland *et al.* (1992) using the 'ape' package of the R statistical programming language. We obtained the phylogeny of our species set using Phylomatic (Webb *et al.*, 2008). We randomly resolved the two polytomies in our phylogeny for the purpose of the independent contrast test, and generated branch lengths using the method of Grafen (1989) again using the 'ape' package of R.

2.4 RESULTS AND DISCUSSION

2.4.1 Thermal tolerance (T_{50})

Although our method did not allow investigation of whole plants, leaves being detached from the plant did not cause a decline in the yield at control temperatures for any species (e.g., Fig. S2.1a, b, which is representative of control treatment responses for all species, data not shown). By working with detached leaves, we were able to measure a minimum of 60 replicate leaves (10 leaves for each temperature treatment) for 41 species and obtained remarkably low variation for each sampling point within treatments. To obtain this level of consistency and high replication on whole plants *in situ* under field conditions would be extremely challenging. We found that thermal tolerance (T_{50}) determined via traditional measurements of maximum quantum yield (F_V/F_M) was strongly correlated with thermal tolerance determined using effective quantum yield ($\Delta F/F_M'$) (r = 0.908, n = 41; $P < 0.000$). In other words, maximum potential efficiency of PSII and functional efficiency both decreased at similar rates at a given temperature. Accordingly, any reference to T_{50} hereafter relates to thermal tolerance based on F_V/F_M.

During this hot summer period, T_{50} among the 41 species ranged from 47.9 to 54.3 °C and averaged 51.1 °C (Table 2.1). As our study controlled for spatial and seasonal variation that might influence tolerance thresholds through acclimatisation, these results highlight that even within this single desert biome there is considerable variation in species' innate thermal tolerance, as has been suggested for smaller numbers of species (Downton *et al.*, 1984; Knight & Ackerly, 2003). A difference of almost 6 °C between the lowest and highest thermal tolerance threshold may not appear substantial. However, it is known that considerable increases in damage can be incurred with only small escalations in ambient temperature and effects can be cumulative (Pessarakli, 1999). From a physiological perspective, very minor temperature variations can mean the difference between damage that can be repaired and the accumulation of irreparable damage (Yamane *et al.*, 1998; Sharkey & Schrader, 2006). Our findings demonstrate that even transient exposure to high temperatures can lead to a slowing of recovery from photoinhibition, reflecting irreversible damage to PSII over a given period of recovery. Interestingly, observed longer-term effects are incurred with increases of ≤ 2 °C (see Fig. 2.1).

Table 2.1 Thermal tolerance thresholds (T_{50}) in degrees Celsius measured for 41 Australian southern arid plant species *in situ* during summer.

Species	**Family**	**T_{50}**
Cassinia laevis	Asteraceae	50.7
Cratystylis conocephala	Asteraceae	52.7
Casuarina pauper	Casuarinaceae	51.6
Atriplex nummularia	Chenopodiaceae	50.2
Maireana pyramidata	Chenopodiaceae	52.6
Atriplex vesicaria	Chenopodiaceae	52.5
Maireana sedifolia	Chenopodiaceae	53.2
Callitris glaucophylla	Cupressaceae	52.4
Bauhinia gilva	Fabaceae - Caesalpinioideae	49.4
Senna pleurocarpa var. *pleurocarpa*	Fabaceae - Caesalpinioideae	50.5
Bossiaea walkeri	Fabaceae - Faboideae	52.1
Acacia aneura	Fabaceae - Mimosoideae	52.3
Acacia ligulata	Fabaceae - Mimosoideae	51.1
Acacia papyrocarpa	Fabaceae - Mimosoideae	49.6
Gyrostemon ramulosus	Gyrostemonaceae	49.6
Amyema miraculosa subsp. *miraculosa*	Loranthaceae	48.9
Amyema quandang subsp. *quandang*	Loranthaceae	48.3
Brachychiton gregorii	Malvaceae	52.0
Lasiopetalum behrii	Malvaceae	52.2
Commersonia magniflora	Malvaceae	51.8
Marsilea drummondii (terrestrial form)	Marsileaceae	48.7
Eucalyptus camaldulensis subsp. *camaldulensis*	Myrtaceae	50.0
Melaleuca uncinata	Myrtaceae	51.3
Callistemon teretifolius	Myrtaceae	51.8
Eucalyptus pimpiniana	Myrtaceae	50.7
Nitraria billardierei	Nitrariaceae	50.8
Jasminum didymum	Oleaceae	52.8
Cymbopogon obtectus	Poaceae	50.9
Triodia irritans	Poaceae	54.3
Grevillea stenobotrya	Proteaceae	51.8
Hakea francisiana	Proteaceae	48.9
Geijera parviflora	Rutaceae	50.7
Exocarpos aphyllus	Santalaceae	50.6
Santalum acuminatum	Santalaceae	51.7
Santalum lanceolatum	Santalaceae	53.1
Dodonaea viscosa subsp. *angustissima*	Sapindaceae	49.8
Eremophila bignoniiflora	Scrophulariaceae	47.9
Eremophila longifolia	Scrophulariaceae	50.6
Solanum orbiculatum subsp. *orbiculatum*	Solanaceae	51.6
Pimelea microcephala	Thymeleaceae	48.2
Xanthorrhoea thorntonii	Xanthorrhoeaceae	53.5

T_{50}, Thermal tolerance threshold: the temperature at which F_V/F_M [maximum quantum yield of photosystem II (PSII) declined to 50% of the maximum prestress levels]

2.4.2 Damage and recovery from heat stress

While field-based measurements allow thermal tolerance measurements under natural solar radiation (Buchner *et al.*, 2013), varying light conditions (depending on time of day, cloud cover and aspect) and high light can lead to photoinhbition, removing the ability to determine effects of heat stress alone. The constant, subsaturating levels of light across all treatments in the current study enabled direct comparisons to be made among species grown – but not measured in – their native environment. In agreement with other studies (Havaux *et al.*, 1991; Marutani *et al.*, 2012; Buchner *et al.*, 2013), we found that recovery of photosynthetic function improved (F_V/F_M more than doubled) when low levels of light were applied during treatments compared to measurements on dark-adapted tissue ($t_{(38)} = -3.988$, $P = 0.01$).

Like T_{50}, recovery from heat stress also showed considerable variation among species and in response to treatment temperature. As might be expected, recovery was greater in species that were less affected by a particular heat treatment. Our two response variables, the stress-induced damage to PSII (D_{PSII}) and the proportion of initial loss of photosynthetic efficiency that was recovered the day after stress ($R_{\Phi PSII}$) were significantly correlated (Table 2.2). That is, the recovery of photosynthetic efficiency of PSII generally was greater in species maintaining higher maximum quantum yield of PSII post heat stress (Table 2.2). This relationship was strongest for leaves treated at 50 °C, where the greatest range in species' responses was evident (Fig. 2.2, Table 2.2). For both 48 °C and 52 °C treatments, the relationships were weaker and the strength of the relationship declined further at 46 °C and 54 °C treatments (Fig. 2.2, Table 2.2). For treatment temperatures below 50 °C, plants showed minimal photoinhibition (low D_{PSII}) with almost complete recovery of PSII function (high $R_{\phi PSII}$). The four negative data points for 46 °C treatments sitting well below the horizontal axis (see Fig. 2.2), represent a calculation artefact for four species with high damage thresholds (*Triodia irritans*, $T_{50} = 54.3$ °C; *Maireana pyramidata*, $T_{50} = 52.6$ °C; *Cratystylis conocephala*, $T_{50} = 52.7$ °C; *Jasminum didymium*, $T_{50} = 52.8$ °C). For these species, $D2_{\Delta F/FM'}$ was marginally lower (≤ 6% deviation) than $\Delta F/F_M'$ at 1.5 hours (refer to Fig. 2.1), having the effect of simulating incomplete recovery (Fig. 2.2). However, in terms of biological relevance, there was no appreciable difference in $\Delta F/F_M'$ measured at these two time points, which was confirmed by the low D_{PSII} values, indicating little to no inhibition of the photosynthetic apparatus (Fig. 2.2). By contrast, at extremely high treatment temperatures (52 °C to 54 °C), negative and near zero values for most species indicate the likely collapse of photosynthetic machinery and that recovery processes were heavily compromised. In contrast to stress treatment temperatures that were too cool (46 °C and 48 °C) or too hot (52 °C and 54 °C), the intermediate stress treatment temperature of 50 °C enabled the variation in stress response to clearly be determined across these desert species in summer. When stressed at 50 °C, one can observe the full spectrum of species' responses, ranging from sustained heat-induced photoinhibition (high D_{PSII}, low $R_{\phi PSII}$) to near-complete recovery and high levels of PSII function (low D_{PSII}, high $R_{\phi PSII}$). Sustained depression of quantum yield at higher temperatures is indicative of species suffering chronic heat stress and suggests irreparable damage to the chloroplast structure and function. Heat-induced decline in F_V/F_M is known to correspond to inhibition of both Calvin cycle and electron-transport processes, with sustained suppression of F_V/F_M suggesting irreversible damage to the photosynthetic apparatus (Law & Crafts-Brandner, 1999; Haldimann & Feller, 2004). Importantly, the identification of an ideal experimental treatment temperature has clear implications for decisions regarding the selection of temperatures for heat stress trials. Heating leaves to

Table 2.2 Pearsons correlation (*r*) relationships between damage and recovery after five heat stress treatment temperatures for 41 Australian arid-zone species measured during summer. Damage to PS II following heat stress (D_{PSII}) was calculated as the difference between pre- and post-stress levels of maximum quantum yield of PSII ($F_{V/}F_M$). Recovery of photosynthetic efficiency ($R_{\Phi PSII}$) was calculated as the proportion of the initial loss of photosynthetic functional efficiency ($\Delta F/F_M'$) that was recovered the day after heat stress. Heat stress was applied for 15 minutes at five treatment temperatures (46, 48, 50, 52 and 54 °C). Levels of significance are indicated as:$* = P < 0.05$. $** = P < 0.01$. $*** = P < 0.001$. $n = 41$. Values in bold-type are complimentary to correlative relationships depicted in Fig. 2.2.

°C	$R_{\Phi PSII}$				
	46	48	50	52	54
D_{PSII}					
46	**-0.255**	-0.471^{**}	-0.510^{**}	-0.199	-0.093
48	-0.033	$\mathbf{-0.619}^{***}$	-0.778^{***}	-0.222	-0.108
50	0.061	-0.664^{***}	$\mathbf{-0.862}^{***}$	-0.350^{*}	-0.108
52	0.250	-0.524^{***}	-0.643^{***}	$\mathbf{-0.504}^{**}$	-0.042
54	0.189	-0.439^{**}	-0.516^{**}	-0.503^{**}	**-0.085**

temperatures that represent climate average maxima might intuitively seem the most appropriate method for investigating plant thermal tolerance. However, especially when not transpiring, the leaves of plants can reach temperatures from as little as 2 °C and up to 15 °C above ambient, depending on leaf morphology, spectral properties, orientation, local wind speed, canopy position and distance from ground (Ball *et al.*, 1988; Roden, 2003; Nobel, 2012). The 50 °C treatment in the present study was higher than the climate average maxima (~ 47 °C) but nevertheless is a temperature quite possible for leaves to achieve in summer desert conditions, even if only briefly (Leigh et al. unpublished).

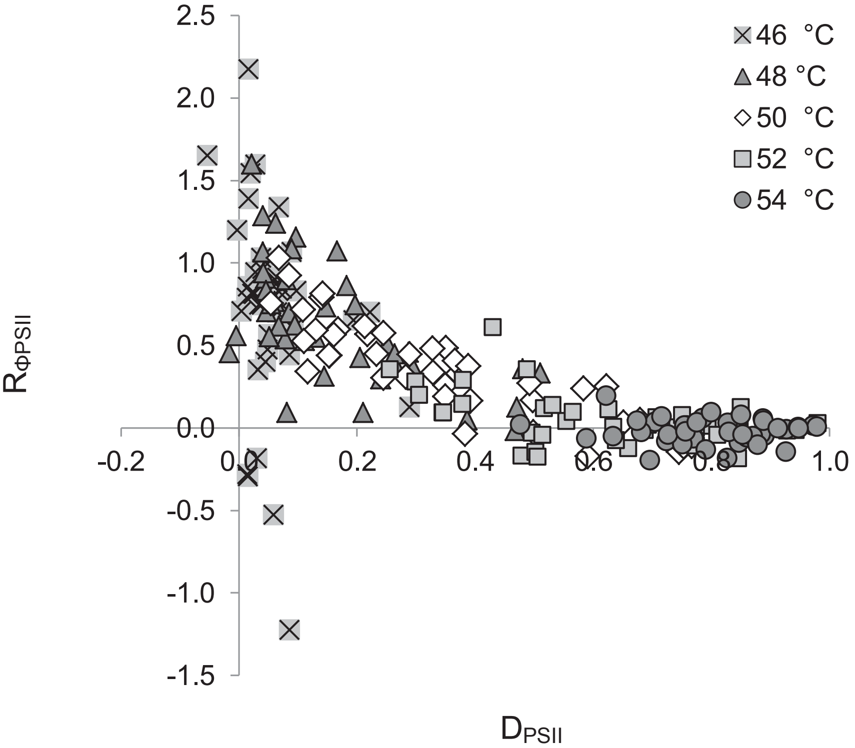

Figure 2.2 Correlative relationships between recovery and damage measures at five stress treatment temperatures of leaves of 41 Australian arid-zone species during summer. Heat stress was applied for 15 min at the five treatment temperatures (46, 48, 50, 52 and 54 °C). Details of the recovery and health method are as for Table 2. For each series, *n* = 41. Higher D_{PSII} values (difference between pre- and post-stress levels of photosystem health) indicate more long-term damage: F_V/F_M suppressed overnight. Higher $R_{\Phi PSII}$ (quantification of recovery from heat stress by considering the proportion of initial loss of photosynthetic efficiency that was recovered the next day) is indicative of species having a greater capacity for recovery.

2.4.3 Relationships between thermal tolerance and recovery

Species with a higher thermal tolerance threshold exhibited less long-term damage and higher rates of recovery than species with lower thermal tolerance thresholds (Fig. 2.3). These relationships again were dependent on treatment temperature, being strongest for the 50 °C treatment (Table 2.3, Fig. 2.3). Although these correlations were expected and significant, damage and recovery responses varied in the nature of their relationship with T_{50}. In response to a 50 °C stress event, the level of damage to PSII could be readily predicted for species with thermal tolerance thresholds lower than 51 °C. For higher T_{50} values, the relationship was weaker, indicating that very high thermal tolerance thresholds do not necessarily translate to an equivalent level of damage to or protection of PSII (Fig. 2.3a). For example, although T_{50} values for *Triodia irritans* and *Commersonia magniflora* differed by 2.5 °C (Table 2.1) both species sustained only ~ 10% damage to PSII (low D_{PSII}) (Fig. 2.3a). Recovery of photosynthetic efficiency ($R_{\Phi PSII}$) again was not tightly correlated with species' thermal tolerance threshold but this was true for any T_{50} value, i.e. not only for those above 50 °C (Fig. 2.3b). The variation in $R_{\Phi PSII}$ among species could reflect differences among species' D1 protein turnover rates (degradation and de novo synthesis of the D1 protein) and subsequent recovery of the photochemical efficiency of PSII (Aro *et al.*, 1994). These responses suggest that, at stress temperatures of 50 °C and above, a higher thermal tolerance threshold does not precisely equate to enhanced regulation of electron transport or reversibility of photoinhibition. The weaker relationship between T_{50} and $R_{\Phi PSII}$ suggests that looking only at damage to PSII does not necessarily provide a complete assessment of a species' ability to recover and that $R_{\Phi PSII}$ may be a useful metric to provide a more complete understanding of species' responses to high-temperature stress.

2.4.4 Effect of phylogenetic independent contrasts: thermal tolerance and recovery

Leaves in the 50 °C heat stress treatment showed a strong and significant ($P < 0.001$) relationship between T_{50} and both $R_{\Phi PSII}$ and D_{PSII} (correlation coefficients of 0.715 and -0.906, respectively). Independent contrasts analysis weakened both correlations, yet the

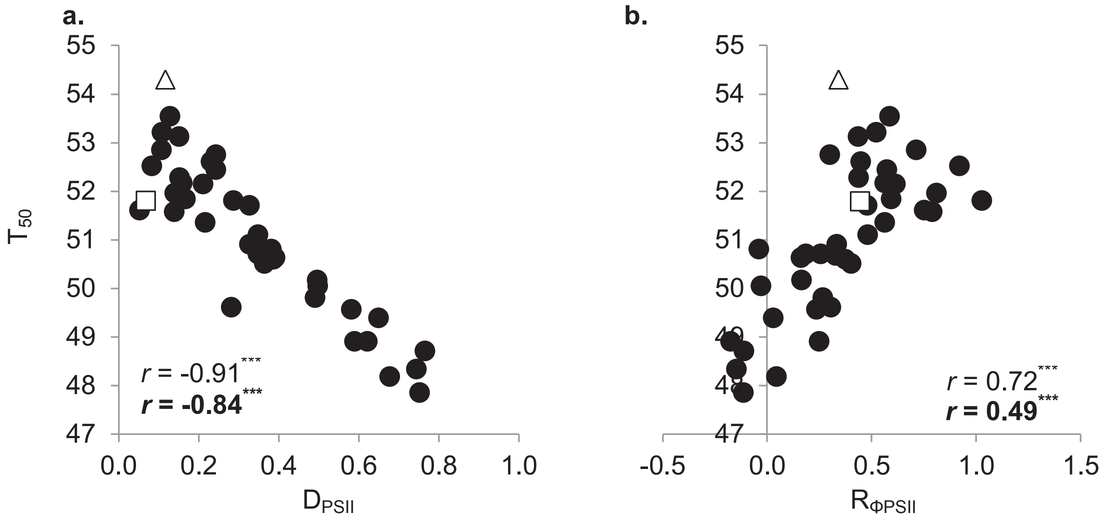

Figure 2.3 Bivariate relationships between the thermal tolerance threshold (T_{50}) with thermal damage (D_{PSII}) **a)** and recovery of photosynthetic functional efficiency ($R_{\Phi PSII}$) **b)** in leaves of 41 Australian arid-zone species measured during summer. Damage and recovery are presented for a 15 minute heat stress at a 50 °C treatment. The points for *Triodia irritans* (open triangle) and *Commersonia magniflora* (open square) are indicated separately and discussed in text. Correlations resolved using independent contrast analysis are indicated in bold. A significance level of $P < 0.001$ is indicated as ***.

Table 2.3 Relationships between damage and recovery after stress with thermal tolerance thresholds during summer, measured at five stress treatment temperatures, for 41 Australian arid zone species using Pearson correlations (r). Details of how damage and recovery were derived are as for Table 1. Species' thermal tolerance thresholds (T_{50}) were defined as the temperature at which $F_{V/}F_M$ declined to 50% of the maximum prestress levels. Levels of significance are indicated as: $* = p < 0.05$. $** = p < 0.01$. $*** = p < 0.001$. $n = 41$.

°C	**D_{PSII}**	**$R_{\Phi PSII}$**
46	-0.409^{**}	-0.270
48	-0.809^{***}	0.584^{***}
50	-0.906^{***}	0.715^{***}
52	-0.903^{***}	0.415^{**}
54	-0.809^{***}	0.126

relationships were still highly significant using this test ($R_{\Phi PSII}$: $r = 0.49$ and D_{PSII}: $r = -0.84$, $P < 0.001$). This indicates that closely related species (Supporting information, Fig. S2.2) were more likely to be similar in their thermal tolerance thresholds, damage and recovery capacity and that basal divergence is at least partially responsible for the strong relationships between T_{50} and $R_{\Phi PSII}$ and D_{PSII}. This minor level of phylogenetic signalling, however, did not completely obscure the strong relationship between thermal tolerance with $R_{\Phi PSII}$ and D_{PSII}, indicating correlated evolution for these traits.

2.4.5 Conclusion

Heat-induced impairment of plant photosynthetic machinery is a well-documented and easily quantified phenomenon. We present a chlorophyll fluorescence protocol incorporating subsaturating light to measure plant thermal tolerance thresholds (T_{50}), thermal damage and recovery capacity in 41 Australian desert species in summer. Measurements of damage response and recovery on individual leaves sampled from plants grown under natural conditions enabled a large number of species to be compared in a relatively short timeframe. We provided leaves with light throughout stress treatments to ensure that results were more ecologically relevant than if conducted on dark-adapted tissue. Providing light to samples during heat stress and recovery improved T_{50} and increased their capacity to recover photosynthetic function. Our method is robust across a wide range of species and demonstrates the validity of examining thermal tolerance not only with respect to maximum potential efficiency of PSII but also in terms of leaves' ability to recover photosynthetic efficiency.

Even within this single desert biome, among the 41 species we measured, thermal tolerance varied by almost 6 °C, a considerable range, given the difference only 1 °C to 2 °C can make to plants' ability to recover from heat stress or incur permanent damage. To our knowledge, recovery from high-temperature stress for such a large number of species has not previously been shown. Thermal tolerance thresholds based on dark- and light-adapted leaf tissues were strongly correlated with one another, especially when comparing responses to a heat stress treatment of 50 °C. Relationships between T_{50} and damage and recovery from heat stress were highly significant at the stress temperature of 50 °C, indicating a critical heat stress temperature greater than the average maximum temperature for the growth environment of these study species. We suggest that research on the variation among species' responses to heat stress should trial a range of treatment temperatures close to the average climate maxima to determine the appropriate stress treatment temperature to use. Although independent contrasts revealed an effect of phylogeny, illustrating a degree of functional similarity among related species, cross-species analysis of the relationship between thermal tolerance, damage and species’ capacity to recover remained robust, suggesting that these traits exhibit a degree of correlated evolution. This study has shown that photosystem health and recovery from heat damage was greater with higher $T_{50,}$ but this relationship was not consistently robust, particularly with respect to recovery, suggesting that a high thermal threshold does not necessarily confer a greater benefit in terms of species’ capacity to avoid long-term damage. The methodology described in this study highlights the importance for understanding how species, with differing T_{50}, vary in their capacity to recover photosynthetic function following heat stress. It provides a new way to help elucidate the potential risk that plant species face with increasing global temperature extremes.

Chapter 3

Native microhabitats better predict tolerance to warming than latitudinal macro-climatic variables in arid-zone plants

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Keywords. Climate change; desert plants; arid-zone; high-temperature stress; species' distributions; thermal damage threshold (T_{50}); warming tolerance (WT).

3.1 ABSTRACT

Aim. Understanding species' ability to withstand heat stress is paramount for predicting their response to increasing temperatures and decreasing rainfall. Arid systems are subject to climatic extremes, where plants, being immobile, live on the frontline of climate change. Our aim was to investigate whether: 1) warming tolerance (WT = the difference between a species' physiological thermal damage threshold (T_{50}) and the maximum temperature within its distribution (T_{hab})) for desert plants is higher at high latitudes, as has been shown for terrestrial ectotherms, and 2) if T_{50} of desert plants better correspond with broad climatic indicators or species' native microhabitats?

Location. The Australian Arid Lands Botanic Garden, Port Augusta, South Australia.

Methods. Using chlorophyll fluorescence techniques, we measured T_{50} for 42 Australian arid plant species native to different microhabitats based on water availability. WT was calculated (T_{50}-T_{hab}) and each metric was compared against microhabitat and broad-scale climatic variables for each species.

Results. Whereas T_{50} was unrelated to macro-scale climate or latitude, WT increased for species whose distributions extend into higher latitudes, a pattern hitherto not shown for terrestrial plants. We also found that species adapted to higher water availability in their native microhabitat had significantly lower T_{50} and WT than species from drier microhabitats.

Main conclusions. 1) WT increased with latitude, but the strength of this relationship was related to the way WT is quantified, with T_{hab} and latitude being linked. 2) Species' T_{50} thresholds did not correlate with latitude, but both T_{50} and WT strongly related to their microhabitats. Specifically, water availability is important, such that even within a desert biome, species associated with 'wetter' microhabitats, may be particularly vulnerable to heat stress. Thus, we show that local-scale patterns better capture plant physiological responses to temperature than broad-scale distributions.

3.2 INTRODUCTION

Extreme high-temperature events are increasing in both frequency and magnitude worldwide (IPCC, 2014). Identifying the vulnerability of plant species to increasing temperatures is important, as local extinctions can have consequences for other organisms relying on plants for food or habitat and for carbon cycling and productivity (Walther, 2003). Plant vulnerability to climate change is uncertain and some ecosystems are particularly under-researched; for example, desert and semi-desert (arid) systems. Collectively, these environments comprise approximately one-third of the land surface area globally (Prentice *et al.*, 2001). Recent studies have shown that some semi-arid regions contribute far more to carbon cycling than previously thought (Cleverly *et al.*, 2013), highlighting the importance of understanding these systems. Plants in these regions must withstand biologically stressful and highly variable conditions, especially with regard to temperature extremes and drought (Noy-Meir, 1973; Wahid *et al.*, 2007). Examining the effects of heat stress on desert vegetation will provide a means of understanding the effects of a changing climate on plants living at the upper edge of what many species can tolerate (O'Sullivan *et al.*, 2017).

Predictive models characterising the bioclimatic envelope (or climate niche) of a species are often used to forecast vulnerability and potential distributions under future climate change scenarios (Pearson & Dawson, 2003; Beaumont *et al.*, 2005). While providing useful ecological insight and strong predictive potential, these models, largely focus on linking coarse-scale spatial and climatic data to species' distribution records (Hampe, 2004; Thuiller *et al.*, 2005; Elith & Leathwick, 2009). Importantly, such models generally do not take into account species' unique physiological limits under particular environmental conditions or the scale at which species interact with their surroundings (Ashcroft *et al.*, 2014). Consequently, predictions are constrained by the exclusion of important mechanistic links between species' functional traits and their native microhabitat (Kearney & Porter, 2009). Incorporating such links into future models is key to more accurate forecasting of species survival and persistence within a given location.

A popular measure for ranking species' vulnerability to a warmer world is warming tolerance (WT): the difference between a measure of a species' physiological thermal limit (heat stress damage threshold) and a thermal index of its habitat (Deutsch *et al.*, 2008). An increasing positive value indicates species less vulnerable to effects of climate warming, whereas values close to zero suggest species are more vulnerable (Hoffmann *et al.*, 2013). The use of WT allows multiple species to be ranked by their relative vulnerability to a warming climate. Studies on terrestrial ectotherms (including reptiles, amphibians and insects) have consistently found WT to be greater for species at higher latitudes relative to those nearer the equator (Fig. 3.1) (Deutsch *et al.*, 2008; Huey *et al.*, 2009; Diamond *et al.*, 2012; Hoffmann *et al.*, 2013). Species at lower latitudes are considered to be most vulnerable because they already are living at the thermal limits of what many organisms can withstand. Importantly, however, higher latitudes are expected to experience larger increases in average temperature and species at these latitudes therefore may be at an increased risk of thermal stress (IPCC, 2007; Deutsch *et al.*, 2008; O'Sullivan *et al.*, 2017). Being immobile, plants, especially long-lived perennials with limited dispersal, are potentially more vulnerable than organisms that are able to migrate. Warming tolerance, however, has not yet been recorded for plants. Thus, an aim of the current study was to determine whether the well-documented latitudinal trend in WT exhibited for animals (Fig. 3.1) can be generalised to plants.

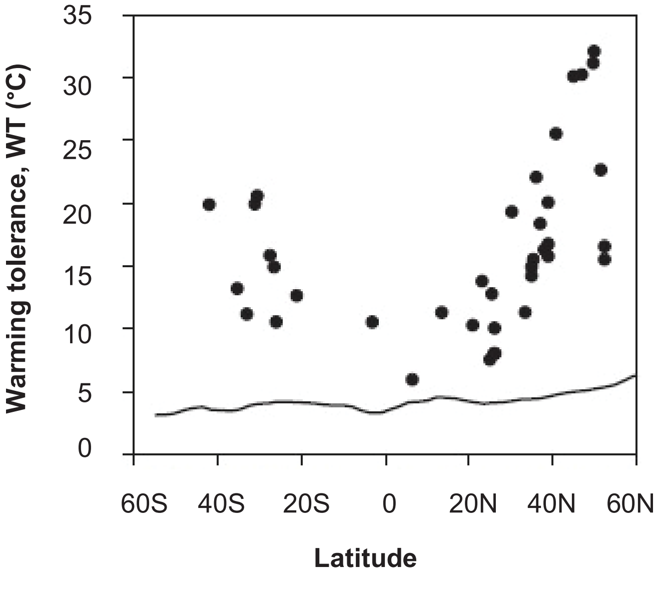

Figure 3.1 Estimates of the impact of warming on insects by comparing the relationship between warming tolerance (WT, based on the annual mean temperature) and latitude with the projected magnitude of warming expected by 2100 (black line) (adapted from Deutsch *et al.*, 2008, Copyright (2008) National Academy of Sciences, U.S.A.)).

In contrast to these broad-scale relationships, recent studies have highlighted the importance of understanding local landscape heterogeneity in the provision of refugia for making more informed predictions of species' vulnerability under climate change (Suggitt *et al.*, 2011; Ashcroft *et al.*, 2014). A multidisciplinary approach, integrating species' physiology and ecology, together with their known distributions and environmental data at multiple scales, is necessary to improve predictions of community and species-level responses (Cooke *et al.*, 2013). This approach can provide valuable insight for identifying species or functional types that will be most at risk from future temperature regimes (Tsonev *et al.*, 1999; Curtis *et al.*, 2014).

With respect to assessing physiological vulnerability, the availability of water in plant microhabitats is likely to be an important factor influencing the way they experience high temperatures. Although arid biomes are dry on average, water availability can vary at local scales; for example, an ephemeral river bed adjacent to a well-drained hill slope or dune (Morton *et al.*, 2011; Free *et al.*, 2013). Under conditions of water limitation, plants often restrict transpiration to reduce water loss (Barradas *et al.*, 1994; Hamerlynck *et al.*, 2000), but the resulting drop in latent heat loss can cause leaf temperatures to rise considerably above ambient (Ball *et al.*, 1988; Nobel, 2012), particularly under hot, still conditions (Leigh *et al.*, 2012). Also, multiple stresses can have confounding effects (Suzuki *et al.*, 2014), such that a drought-stressed plant may be more severely damaged by heat stress than a well-watered plant. Accordingly, even within a given bioclimatic envelope, differences in microhabitat type, based on access to water, may influence species' ability to cope with heat stress. Such differences at the microhabitat scale could well be irrespective of any macro-scale latitudinal trends relating to species' distribution.

In this study, we investigated whether – and at what scale – species-specific thermal threshold parameters might best be used to predict plant vulnerability under future climate change. Specifically we asked: 1) is warming tolerance (WT) for desert plants higher at high latitudes, as has been shown for terrestrial ectotherms? and 2) do the physiological thermal damage thresholds of desert plants correspond most strongly with macro-scale, broad climatic indicators or species' native microhabitat? We investigated these questions for Australian southern arid species grown under natural conditions in a common environment, but differing with respect to the water availability characterising the microhabitat in which they normally grow.

3.3 MATERIALS & METHODS

3.3.1 Site description

All species in this study grew in a common arid environment at the Australian Arid Lands Botanical Garden (AALBG) in Port Augusta, South Australia (32°27'56.3"S 137°44'40.7"E). Mean annual rainfall is approximately 250 mm and mean maximum summer temperature is approximately 31.3 °C, with maximum temperatures exceeding 45 °C in summer (AGBoM). All measurements took place during summer 2013 (late January to early March).

3.3.2 Measuring species-mean values of T_{50}

We measured T_{50} for 42 Australian arid shrub, tree and one herb species, following the Flora of New South Wales (accessed online via PlantNET, The Royal Botanic Gardens and Domain Trust, 2013) and the Flora of South Australia (accessed online via eFLORA SA, DEWNR South Australia, 2013). Species were selected from 21 plant families to encompass a range of growth forms (Table 3.1). One of the study species, the herbaceous fern *Marsilea drummondii*, was selected in both its aquatic and terrestrial form as the two different microhabitats of this plant were of interest. Unless otherwise stated, analyses presented only include measurements from the terrestrial form of *M. drummondii*.

To determine species-mean values of T_{50} for each species, maximum quantum yield of PSII of dark-adapted tissue (F_V/F_M) was measured with chlorophyll *a* fluorometry using a pulse modulated fluorometer (Heinz Walz, Effeltrich, Germany), following the protocol of Curtis *et al.* (2014). Values of F_V/F_M are useful for assessing the health of plant photosynthetic reaction centres under numerous stressors, including temperature, by providing an indication of the capacity of PSII to accept light (Baker & Rosenqvist, 2004). Our method of quantifying T_{50} utilises the temperature-dependent decline in F_V/F_M to determine the temperature at which it drops to 50% of prestress levels, a point corresponding to the onset of irreparable thermal damage. Briefly, leaves of each species were subjected to five temperature treatments (46, 48, 50, 52 and 54 °C) and one control (28 °C) using temperature controlled water baths, accurate to ± 0.2 °C. Leaves were exposed to a subsaturating light level of ca. 280 µmol photons m^{-2} s^{-1}

Table 3.1 List of the 42 Australian desert plant species used in this study, arranged from lowest to highest thermal damage thresholds (T_{50}, °C). Growth form is given in parentheses: g, grass; h, herb; p, hemi-parasite; s, shrub; t, tree. T_{50} was calculated as the temperature at which maximum quantum yield (F_V/F_M) declines to 50% of the maximum prestress F_V/F_M measurement. Native microhabitats were defined as the environments that species naturally tend to occupy and that differ broadly on the availability of water: W_{low}, relatively low water availability, W_{var}, availability of water is variable; W_{high}, relatively high water availability. Warming tolerance (WT) was defined as the difference between a species' physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on maximum values across each species' Australia-wide distribution using four different thermal indices: annual maximum mean temperature (amm); annual mean temperature (am); warmest maximum period (wmp); warmest quarter (wq).

Species	**Family**	**$^{\#}T_{50}$ (°C)**	**Microhabitat**	**WT_{amm}**	**WT_{am}**	**WT_{wp}**	**WT_{wq}**
Marsilea drummondii (aquatic form) (h)	Marsileaceae	45.1	W_{high}	10	15.6	3.9	12.4
Eremophila bignoniiflora (t)	Scrophulariaceae	47.9	W_{high}	12.5	19.5	6.7	15.2
Pimelea microcephala (s)	Thymeleaceae	48.2	W_{var}	13.4	20.2	7.2	15.5
Amyema quandang subsp *quandang* (p)	Loranthaceae	48.3	W_{high}	15.5	21.6	8.2	16.9
Marsilea drummondii (terrestrial form) (h)	Marsileaceae	48.7	W_{high}	13.6	19.2	7.5	16
Amyema miraculosa subsp *miraculosa* (p)	Loranthaceae	48.9	W_{high}	22.1	28.2	11.8	21.5
Hakea francisiana (s)	Proteaceae	48.9	W_{var}	19	25.8	9.4	18.3
Bauhinia gilva (s-t)	Fabaceae - Caesalpinioideae	49.4	W_{high}	15	26.8	8.4	16.6
Acacia papyrocarpa (t)	Fabaceae - Mimosoideae	49.6	W_{var}	19.9	26.5	9.7	18.6
Gyrostemon ramulosus (t)	Gyrostemonaceae	49.6	W_{low}	16.2	23.1	8.4	17.1
Dodonaea viscosa subsp *angustissima* (s)	Sapindaceae	49.8	W_{low}	15	21.8	8.9	17.1
Eucalyptus camaldulensis subsp *camaldulensis* (t)	Myrtaceae	50.0	W_{high}	20.5	26.6	10.9	18.9
Atriplex nummularia (s)	Chenopodiaceae	50.2	W_{high}	18.6	25.5	10.2	18.7

Table 3.1 cont.

Senna pleurocarpa var *pleurocarpa* (s)	Fabaceae - Caesalpinioideae	50.5	W_{low}	17.3	24.3	9.6	18.2
Exocarpos aphyllus (s)	Santalaceae	50.6	W_{low}	19.5	24.2	10.6	19.2
Eremophila longifolia (t)	Scrophulariaceae	50.6	W_{var}	15.3	22.6	9	17.8
Cassinia laevis (s)	Asteraceae	50.7	W_{low}	21.6	27.8	12.8	21.1
Eucalyptus pimpiniana (t)	Myrtaceae	50.7	W_{low}	23.6	30.7	14.4	24.1
Geijera parviflora (t)	Rutaceae	50.7	W_{low}	19.8	26.7	12	20
Nitraria billardierei (s)	Nitrariaceae	50.8	W_{var}	20.6	27.3	10.3	19.5
Cymbopogon obtectus (g)	Poaceae	50.9	W_{high}	16.7	23	9.5	18.3
Acacia ligulata (t)	Fabaceae - Mimosoideae	51.1	W_{low}	16.3	23.1	9.9	18.5
Melaleuca uncinata (s)	Myrtaceae	51.3	W_{high}	18.9	25.2	11.4	19.1
Casuarina pauper (t)	Casuarinaceae	51.6	W_{low}	22.1	28.7	11.8	20.7
Solanum orbiculatum subsp *orbiculatum* (s)	Solanaceae	51.6	W_{low}	21.1	27.6	11.1	20.3
Santalum acuminatum (t)	Santalaceae	51.7	W_{var}	18.5	25.4	10.9	19.2
Commersonia magniflora (s)	Malvaceae	51.8	W_{var}	22	29	13.7	22
Callistemon teretifolius (s)	Myrtaceae	51.8	W_{low}	25.3	31.2	15.4	24.4

Table 3.1 cont.

Grevillea stenobotrya (s)	Proteaceae	51.8	W_{var}	17.3	24	10.3	19.2
Brachychiton gregorii (t)	Malvaceae	52.0	W_{var}	21.1	27.9	11.9	20.5
Bossiaea walkeri (s)	Fabaceae - Faboideae	52.1	W_{low}	24.5	31.1	14	23.2
Sida ammophila (h-s)	Malvaceae	52.2	W_{high}	17.97	24.79	11.59	19.69
Lasiopetalum behrii (s)	Malvaceae	52.2	W_{low}	27.9	34.3	18.4	26.7
Acacia aneura (t)	Fabaceae - Mimosoideae	52.3	W_{low}	25.5	31.6	15.2	24.9
Callitris glaucophylla (t)	Cupressaceae	52.4	W_{low}	20.8	25.5	12.7	20.9
Atriplex vesicaria (s)	Chenopodiaceae	52.5	W_{var}	19.5	25.5	11.7	20.1
Maireana pyramidata (s)	Chenopodiaceae	52.6	W_{var}	20.1	27.3	11.1	20.1
Cratystylis conocephala (s)	Asteraceae	52.7	W_{low}	25.5	32.5	16.4	25.9
Jasminum didymum (s)	Oleaceae	52.8	W_{var}	17.5	23.2	11.3	20.1
Santalum lanceolatum (t)	Santalaceae	53.1	W_{var}	17.7	24.3	11.4	20.3
Maireana sedifolia (s)	Chenopodiaceae	53.2	W_{var}	24.5	30.9	14	23.4
Xanthorrhoea thorntonii (g)	Xanthorrhoeaceae	53.5	W_{low}	24.1	30.7	15	23.7
Triodia irritans (g)	Poaceae	54.3	W_{low}	23.4	30.5	15.1	23.3

[#] thermal threshold measurements for all species (with the exception of *Marsilea drummondii*, aquatic form) are taken from Curtis (2014) throughout the process. Applying heat stress at different treatment temperatures was performed on replicate batches of 10 leaves for each species in the following sequence. Prestress F_V/F_M was measured after a 30 minute period of dark-adaptation. Leaves then were placed for 15 minutes under control conditions (28 °C, 280 μmol photons m^{-2} s^{-1}), allowing them to reach steady state under the treatment light conditions prior to stress. Each batch of replicate leaves then was transferred to one of the five temperature baths for 15 minutes. Following 90 minutes of recovery under control conditions (28 °C, 280 μmol photons m^{-2} s^{-1}), samples were dark-adapted for 30 minutes before F_V/F_M was again measured. Final measurements of F_V/F_M were recorded after leaves had recovered overnight in the dark. Previous studies have shown a strong positive correlation across species between T_{50} measured this way and other fluorescence methods (e.g. T_{S20}), which measure whole leaves that remain attached to the plant (Knight & Ackerly, 2003). Our method of assessing T_{50} allows for relatively rapid measurement of a large number of species and appropriate data replication, while controlling for external conditions including light levels.

3.3.3 Bivariate relationships among WT, T_{50} and latitude across species

In this study, warming tolerance for a given species was defined as: WT = T_{50} - T_{hab}, where T_{hab} is a thermal index of a species' habitat (Deutsch *et al.*, 2008; Diamond *et al.*, 2012). To estimate T_{hab}, studies have used various temperature indices, including the long-term mean annual temperature and mean temperature during the warmest quarter of the year (e.g., Deutsch *et al.*, 2008; Huey *et al.*, 2009; Diamond *et al.*, 2012). We calculated several values of WT for each plant species by selecting four T_{hab} temperature variables. These included maximum values for annual mean, annual maximum mean, warmest maximum period and warmest quarter using occurrence records from across the entire Australia-wide distribution of each species. Climate data were sourced from the online data portal Atlas of Living Australia (ALA) database (http://www.ala.org.au/, sourced May, 2013). Species occurrence records suspected of being erroneous are recognised by the ALA and were excluded from our analyses. Retrieved data were plotted and the maximum value for each climatic variable was extracted. Temperature variables defining T_{hab} were generated using distributional data and the software package ANUCLIM 6, which is used for obtaining climate data from climate surfaces built using the ANUSPLIN package (Xu & Hutchinson, 2011).

Data for Australia-wide latitudinal distributions were also obtained from ALA records (sourced May, 2013). Minimum, maximum, mean and range in latitude were quantified for the distributional envelope of each species. Preliminary analysis indicated that WT varied predictably with latitude across species when either the most northerly latitudinal distributions or latitudinal range (the most northerly minus the most southerly distribution) were used. We chose to examine the WT-latitude relationship based on most northerly latitudinal distributions as this depicted the relationship most strongly.

We note here that T_{50} values are not fixed for a given species and can depend upon factors such as the length of the applied heat treatment and plant health. Values of WT, incorporating metrics such as T_{50}, are therefore a coarse measurement, which does not represent a species' absolute critical thermal limit with regards to climate warming. That is, WT is not an indication of the absolute amount of climate warming that we could expect species to tolerate before substantial declines are observed. Rather, WT should be thought of as a relative way to rank species potential vulnerability across a large spatial scale.

3.3.4 Relating T_{50} and WT to macro-scale climate and native microhabitat

We wanted to determine if macro-scale climatic indicators or species' native microhabitats most strongly corresponded to their resistance to heat stress (T_{50} and WT). To address this question, information on the climatic variables of species' Australia-wide distribution and their affinity for a local microhabitat were collated. For broad climate variables, the temperature indices outlined above were used, as well as water availability (mean annual rainfall, mm), solar radiation (annual mean, $MJ.m^{-2}.day$) and site aridity (mean annual aridity index) (http://www.ala. org.au/, sourced May, 2013). Microhabitat determination was based on the availability of water to each species within its native environment. Because transpirational cooling is often reduced under conditions of drought stress, from a plant's perspective, apparent temperature is thus likely to be greater in areas with less water. Using this criterion, water availability served as a proxy indicator of potential temperature stress plants may experience in their native microhabitats. To identify each species' affinity for a microhabitat, a range of literature was reviewed (Jessop *et al.*, 1986; Cunningham *et al.*, 1992; Department of Parks and Wildlife: Western Australian Herbarium, 2013; DEWNR South Australia, 2013; The Royal Botanic Gardens and Domain Trust, 2013). Species were found to fall into three microhabitats with respect to water access: W_{high}, W_{low} and W_{var} (Table 3.1). W_{high} (n = 10) incorporated microhabitats where water is relatively available and included hemi-parasitic species that have ready access to their hosts' xylem (Ehleringer, 1985; Goldstein *et al.*, 1989), species restricted to the banks of seasonally flooded rivers, road-side depressions or wadis, and ephemeral species that tend to respond rapidly to wet weather events. Species naturally found where water is less often available and/or where water drains away readily were classified as W_{low} (n = 18). Microhabitats fitting this description included sand dunes and exposed rocky hill slopes. The remaining species were categorised as W_{var} (n = 14) and can be found in areas fitting the water availability of both W_{high} and W_{low} species. It is important to note that these native microhabitat details were irrespective of the conditions in which the sample plants used for measurements were grown and therefore represent the innate preference for a given set of microhabitat conditions.

3.3.5 Statistical analyses

Pearson product-moment correlation coefficient analyses were used to quantify bivariate relationships among the variables WT, T_{50} and latitude across species. Pearson correlations were also used to quantify relationships between broad-scale climate variables and T_{50}. One-way ANOVA was used to determine differences in T_{50} and WT among the three native microhabitats (as a fixed factor). Data were tested for assumptions of normality and heterogeneity of variances using Kolmogov-Smirnof and Levene's tests, respectively. Data were analysed using the statistical software IBM SPSS® (v19).

3.4 RESULTS

3.4.1 Native microhabitats and T_{50}

Species-mean values of T_{50} ranged from 45.1 °C (*Marsilea drummondii*, aquatic form) to 54.3 °C (*Triodia irritans*) and varied significantly with respect to native microhabitat (Table 3.1, Fig. 3.2a). We found that W_{high} species had significantly lower T_{50} values on average than either W_{low} or W_{var} species ($F_{(2,38)} = 7.643$, $p = 0.002$). Thus, we show that our study species can be ranked in terms of their vulnerability to future climate change with respect to their native microhabitats. Interestingly, T_{50} did not covary significantly

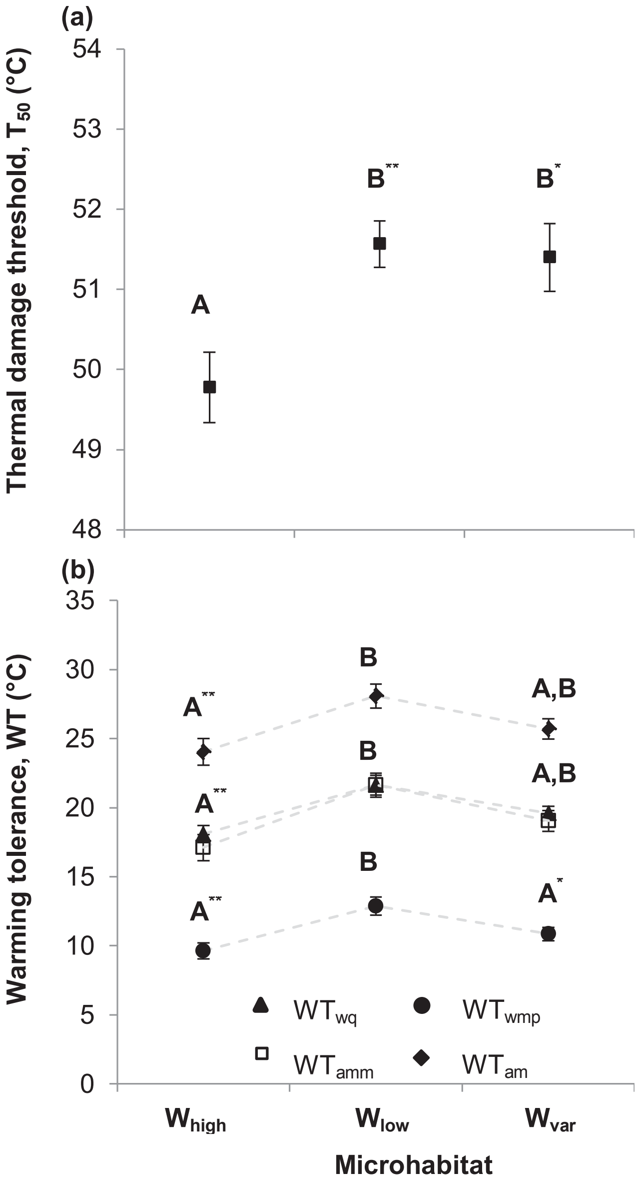

Figure 3.2 Species variation as a function of microhabitat type: W_{high}, high water availability; W_{low}, low water availability; W_{var}, variable water availability. T_{50}, mean summer thermal damage threshold **a)**, WT, mean warming tolerance **b)**. Filled diamonds, WT highest annual mean temperature; filled triangles, WT highest warmest quarter; open squares, WT highest mean annual maximum temperature; filled circles, WT highest warmest maximum period. Dashed lines are for ease of reading patterns. Points with letters different from one another are significantly different pairwise comparisons ($*P < 0.05$, $**P < 0.01$). (Note that the letters above the middle points apply to both sets of data points that overlap: solid triangles and open squares.)

with any of the climate variables both across and within microhabitats, although for W_{high} species, there was a strong, if non-significant, increase in T_{50} with precipitation (see Table S3.1).

3.4.2 Warming tolerance, T_{50} and latitude

We found that T_{50} increased significantly with increasing WT (Table 3.2). This is not surprising, given that T_{50} is used to calculate WT and a relationship should be expected. Nevertheless, it is interesting that the strength of this relationship was dependent on the specific climate variable used to calculate T_{hab} for each species, with the strongest relationship occurring when the warmest maximum period was used (Table 3.2). We found that T_{50} and latitude were not strongly correlated with one another (Table 3.2, Fig. 3.3a), with species-mean values of T_{50} stable across latitude and little variation apparent. By contrast, T_{hab} increased from higher to lower latitudes (Fig. 3.3a). When WT was calculated by subtracting T_{hab} from T_{50} (the largest and smallest differences indicated by arrows, Fig. 3.3a), it emerged that the WT-latitude relationship was primarily generated by the inherent relationship between T_{hab} and latitude.

Table 3.2 Pearson correlations (*n* = 42) between warming tolerance (WT) and i) species' thermal damage thresholds (T_{50}) and ii) their mean maximum latitudinal Australia-wide distributions. WT is calculated as the difference between the maximum recorded values of any relevant long-term mean climatic temperature variable across a given species' distribution minus its thermal damage threshold (see Table 3.1 legend). Here, WT was calculated using four different thermal indices: annual maximum mean temperature (amm); annual mean temperature (am); warmest maximum period (wmp); warmest quarter (wq). The strongest relationship for each bivariate combination is shown in bold (***$P < .001$).

	WT_{amm}	**WT_{am}**	**WT_{wmp}**	**WT_{wq}**	**T_{50}**
T_{50}	0.622^{***}	0.595^{***}	**0.735^{***}**	0.710^{***}	-
Max. latitude	-0.745^{***}	**-0.794^{***}**	-0.621^{***}	-0.634^{***}	-0.247

As has been found in previous studies for animals (Fig. 3.1), we found that WT increased with distance from the equator, regardless of the measurement of T_{hab} used. That is, WT was greatest among species with distributions that extend to higher latitudes (more negative values: southward bearing - Fig. 3.3b; Table 3.2). Of the four measurements of WT used in this study, its relationship with latitude was the strongest

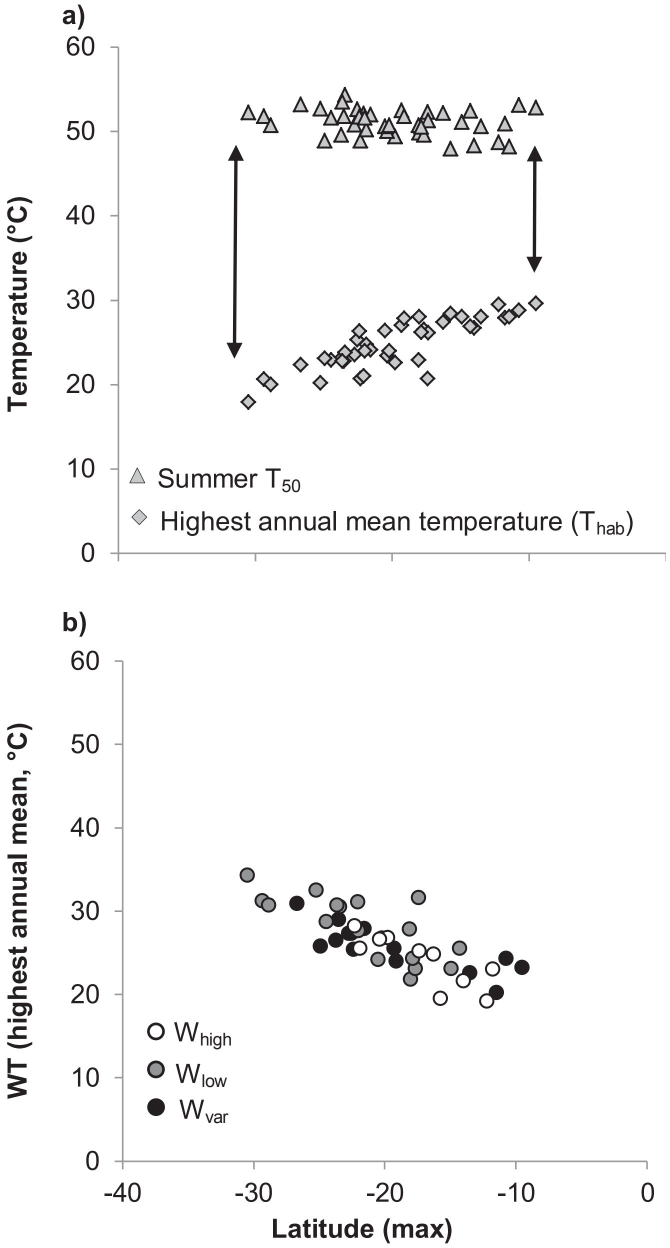

Figure 3.3 Pearson's correlation (r) relationship between latitude and: species' thermal damage threshold (T_{50}) and the highest annual mean temperature across their Australia-wide distributions (T_{hab}) **a)**; warming tolerance (WT, based on the highest annual mean) **b)**. Latitude was defined as the most northerly distribution in Australia for each of the 42 species investigated (see Table 3.2). More negative latitudinal values indicate that species' distributions extend further south. Arrows on panel a are referred to in text in the Results. For **panel b**, microhabitat preference (see Fig. 3.2) is indicated for each species: W_{high} (open circles), W_{low} (black circles), W_{var} (grey circles).

when T_{hab} was based on the highest annual mean temperature for a given species' distribution (WT_{am}, Table 3.2, Fig. 3.3b). There was considerable overlap among microhabitat groupings along the WT-latitude spectrum (Fig. 3.3b). In spite of this overlap, average warming tolerance was significantly different among native microhabitat groups: W_{low} species had a significantly higher mean WT than W_{high} species, with W_{var} species WT being intermediate between these two (Fig. 3.2b).

Depending on the measure of T_{hab} used to calculate warming tolerance, the range of WT within each microhabitat grouping was as much as 15.2 °C (Fig. 3.2b). Regardless of this variation, the general pattern among microhabitats of lower warming tolerance in W_{high} species compared with W_{low} and W_{var} species remained unchanged (Fig. 3.2b, WT highest annual mean temperature: $F_{(2,38)} = 4.999$, $p = 0.012$; WT highest warmest quarter: $F_{(2,38)} = 7.379$, $p = 0.002$; WT highest mean annual maximum temperature $F_{(2,38)} = 6.261$, $p = 0.004$; WT highest warmest maximum period $F_{(2,38)} = 7.501$, $p = 0.002$). The order that species were positioned in the relationship did vary among measures of WT; however, only marginally so (see Tables S3.2–S3.5).

3.5 DISCUSSION

3.5.1 Species' distributions and T_{50}

The average summer thermal damage thresholds of these Australian desert plant species can vary by > 5 °C, with maximum thresholds exceeding 54 °C for some species (Curtis *et al.*, 2014). In the current study, we set out to understand if this variation corresponded more closely with macro-scale, broad climatic indicators or to differences in the microhabitats where species are known to occur naturally. Broad-scale climatic variables change predictably with latitude and often this pattern is used to help explain species' distributions (Walther, 2003; Thomas, 2010). One might therefore infer that species' physiological thresholds for heat stress would vary with climatic variables or latitude. We found no clear relationship between species' upper physiological thermal damage thresholds (T_{50}) and macro-scale climate variables or latitude, both across and within microhabitat groups. These results indicate that the climatic conditions defining a species geographic range are not the best predictors of its physiological heat stress threshold. Instead, we found that species' native microhabitat clearly influenced T_{50}. In particular, species adapted to conditions of higher water availability had significantly lower thermal damage thresholds than the other species examined. As all plants were grown under common climatic conditions, these findings suggest a genotypic effect on plant tolerance to high temperatures.

Maintaining a higher level of protection against thermal damage implies a metabolic cost for plants, as they need firstly to protect against structural and functional disruption of cell membranes and proteins, and secondly to repair damage (Pierce *et al.*, 2005). Such processes may be particularly costly for desert plants, which typically live in resource-poor environments. If a situation arose in which the ongoing likelihood of heat stress was reduced, unnecessarily sustaining a high thermal damage threshold would not be economical. The lower thermal damage thresholds we observed for W_{high} species therefore might be explained in terms of resource conservation. For example, if the cooling benefits of transpiration afforded by relatively better access to water enabled a lower leaf temperature, then costly high thermal thresholds would be less necessary. The three lowest T_{50} values of all species measured were for *Marsilea drummondii* (W_{high})*, Eremophila bignoniiflora* (W_{high}) and *Pimelea microcephala* ssp. *microcephala* (W_{var}). Although *P. microcephala* is a W_{var} species, resource trade-offs may yet explain its low T_{50}. Under stressed conditions *P. microcephala* plants are known to drop their leaves (B. Haase, pers. comm., 2013; E. Curtis, pers. obs., 2013). The leaves of this species are especially thin, with low leaf mass per area (unpublished data), and therefore may represent a lower production cost than would be required to maintain a long-lived leaf with a higher thermal damage threshold. By contrast, the highest T_{50} values of all species measured were found in *Santalum lanceolatum* and *Maireana sedifolia*, both W_{var} species capable of occupying very dry sites, and *Xanthorrhoea thorntonii* and *Triodia irritans*, both extremely xerophytic, W_{low} species. With relatively lower access to water, these species may invest resources in higher physiological tolerance and trade off this cost by having slower growth rates or longer-lived leaves, e.g., *Xanthorrhoea* spp. are known to have slow leaf growth rate in warmer temperatures (Lamont *et al.*, 2004).

In addition to some unique adaptive abilities suggested above, species also may have potential to acclimatise via short-term physiological adjustments to changing environmental conditions (Downton *et al.*, 1984; O'Sullivan *et al.*, 2017). In the present study, evidence was seen for spatial acclimatisation as a response to changed water availability in the two *M. drummondii* forms (i.e., aquatic and terrestrial forms). Although the two forms were the same species, and growing within close proximity to one another, T_{50} was > 3 °C lower for the individuals growing in a permanent pool of water compared with those situated on drier sites (Table 3.1). As both climate and water availability are dynamic through time, the effect of temporal variation with respect to acclimatisation must be accounted for to obtain a more holistic understanding of species' physiological responses to heat stress, something we investigate in an upcoming paper.

3.5.2 WT: species' distribution and T_{50}

Unlike T_{50}, which is a plant-specific, raw measure of a species' physiological heat stress threshold, WT integrates this measure with the realised temperature conditions to estimate vulnerability of that species to increased climate warming. We were interested in whether plants mirrored the clear latitudinal trend demonstrated for numerous animal taxa, where species with distributions extending into lower latitudes consistently show lower WT than species at higher latitudes (Fig. 3.1). Our results confirmed this pattern in desert plants, a pattern that has not, to our knowledge, previously been shown for terrestrial vegetation (but see a recent study by O'Sullivan et al. 2017, which using a related metric - the thermal safety margin - show a similar pattern for species across biomes). Nevertheless, T_{50} and WT differed in their relationships with broad-scale indicators, causing us to question how the WT-latitude relationship should be interpreted.

To help understand and interpret these WT findings, it is necessary to unpack the method used for obtaining them. Warming tolerance comprises two metrics, the damage threshold, T_{50}, and a measure of a species' thermal environment, T_{hab}. Of these two metrics, T_{50} held no relationship with latitude, whereas T_{hab} did (Fig. 3.3a), something also seen for animal taxa (Addo-Bediako *et al.*, 2000; Sunday *et al.*, 2011; Diamond *et al.*, 2012). Generally speaking, habitats at higher latitudes experience lower temperatures than those closer to the equator (Jones *et al.*, 1999; Sunday *et al.*, 2011). It is this inherent relationship between latitude and temperature that drives the observed pattern between WT and latitude: high latitude species have a larger difference between T_{hab} and T_{50} than low latitude species (see arrows, Fig. 3.3a).

Because of its dependence on T_{50}, WT also differed with microhabitat, being highest for species adapted to low water access, and this variation was irrespective of latitude (Fig. 3.3b). That WT varied at this micro-scale has implications for interpreting broad-scale ecological conclusions about the relative vulnerability of species to climate change based on calculations of WT and related metrics (e.g., Deutsch *et al.*, 2008; O'Sullivan *et al.*, 2017). From the present study, we might conclude that W_{high} species at lower latitudes are comparatively more vulnerable, particularly if, as suggested, climate change brings localised reductions in rainfall (Hennessy *et al.*, 2007). Such changes may conceivably reduce the availability of specialised habitats, on which W_{high} species rely. On the other hand, higher latitudes are expected to experience larger increases in temperature (Diamond *et al.*, 2012), potentially offsetting the presumed benefits of a higher WT towards the poles.

3.5.3 Concluding summary

The search for widespread, repeatable patterns to explain species' distribution and performance with predicted increases in temperature has become a frontrunner of ecological research. Our findings for desert plants agree with those for various animal taxa that warming tolerance is greater at higher latitudes. Far from providing a clear-cut picture of species' future vulnerability, however, we suggest interpreting such broad patterns with caution. Rather than macroclimatic measures of temperature, it is with the thermal characteristics of microhabitats with which most plants interact at a physiological level. In support of other authors (Biederman & Whisenant, 2011; Ashcroft & Gollan, 2013), our results demonstrate the importance of small-scale differences in the landscape for explaining species' ability to cope with high temperature. They also could help shed light on the within-site variability in species' thermal limits observed across biomes (O'Sullivan *et al.*, 2017). Therefore, we place much greater importance on our other key finding: that warming tolerance was highest for plant species adapted to microhabitats with lower access to water.

Desert plants, already living in extreme environments, may be especially vulnerable to changes in climate, particularly where increases in temperature are coupled with more variable rainfall (IPCC, 2014). Under such a scenario, species more reliant on the availability of 'wetter' microhabitats may therefore be particularly vulnerable to heat stress under conditions of reduced water availability (Suzuki *et al.*, 2014). Ignoring fine-scale thermal attributes (or features that influence heat stress) of a species' microhabitat may therefore lead to their persistence with global warming either being under or overestimated (Pincebourde & Casas, 2014). Predictive distribution models would be improved by incorporating local-scale variation in water availability, including soil types and local topography, which influence water holding capacity and run-off, respectively. Such an approach would refine predictive outcomes for individual species with respect to not only survival from heat stress, but also to growth, productivity, reproduction and recruitment.

Chapter 4

Temporal dynamics of upper thermal damage thresholds: variation among desert plant species with contrasting microhabitat associations

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4.1 ABSTRACT

Measurements of plant thermal thresholds generally are based on a single snapshot in time and space, potentially misrepresenting a species' vulnerability to high-temperature extremes. Our goal was to determine whether species vary in their ability to adjust their thermal threshold seasonally and if so, whether this variation related to native microhabitat. We measured T_{50} seasonally (winter, spring, summer) for 47 Australian desert species. To address the question of evolutionary divergence patterns between pairs of species contrasted on native microhabitat (more mesic- *vs* xeric-adapted species), phylogenetically-informed analyses of changes in T_{50} in relation to season were performed on a subset. To account for the influence of ambient temperature on individual T_{50}, species-specific priming temperatures (PT) were determined. We found that thermal responses varied significantly with season, increasing by 5 °C from winter to summer. Irrespective of phylogeny, mesic-adapted species had significantly lower T_{50} than more xeric-adapted species, particularly in summer. The potential for a species to acclimatise from winter to summer did not necessarily equate to a high thermal damage threshold during the hottest time of year. Finally, we found that the temporal trajectory for changing thermal tolerance varied among species. Using a novel framework for classifying the temporal dynamics of species' T_{50}, we identified that the rate and timing of thermal adjustments throughout the year fell into one of five groups: Avoid, Early Jump, Late Jump, Steady Increase, and No Response. This finding has clear implications for how different groups of species might experience a sudden early heatwave. Also, the dramatic seasonal shifts in thresholds hold importance for models seeking to understand plant distributional changes with climate warming. To advance the predictive accuracy and application of mechanistic models, we suggest a sampling approach spanning critical seasonal periods and incorporating key microhabitat types representative of higher *versus* lower environmental stress.

4.2 INTRODUCTION

Climate extremes, including high-temperature events, are increasing in both magnitude and frequency (Hennessy *et al.*, 2007; IPCC, 2014), posing a very real threat to the ongoing survival of many species. Organisms most at risk from extreme high-temperature events are those with a limited potential for acclimatisation and reduced ability to move to more favourable environments (Deutsch *et al.*, 2008). Plants are of particular concern as their ability to disperse to regions with more accommodating conditions may not keep pace with the rate of climate change (Jump & Peñuelas, 2005). Among plants, warm-origin species, covering two thirds of the terrestrial planet and already operating near their upper thermal limit, may be at an even greater risk from increased warming. This is because the capacity of these species to physiologically adjust to higher temperatures may be constrained by the universal limits to physiological function above a certain threshold (Way & Oren, 2010; Drake *et al.*, 2015).

The photosynthetic machinery of plants is very heat sensitive (Havaux *et al.*, 1991; Georgieva & Yordanov, 1994), with even short durations of exposure to high-temperature able to illicit a stress response in leaves (Vallélian-Bindschedler *et al.*, 1998; Buchner & Neuner, 2003; Curtis *et al.*, 2014). Photosystem II (PSII) is recognised as one of the most thermally labile components of photosynthesis, especially in response to high temperatures, e.g., above 45 °C (Schrader *et al.*, 2004). Variation among species in the thermal sensitivity of PSII can thus be used to gauge the relative tolerance of species to high temperatures. A species' thermal tolerance can be defined by its measured physiological thermal damage threshold, defined here as T_{50}: the temperature causing a 50% decline in maximum quantum yield of PSII and corresponding to the onset of irreparable thermal damage (Knight & Ackerly, 2003; Curtis *et al.*, 2014). The T_{50} index is useful for comparison among species as it is highly repeatable and allows for numerous measurements in a relatively short timeframe. Mechanistic models incorporating detailed physiological measures such as T_{50} are becoming increasingly recognised as a means of understanding species' interactions with, and distribution in, the environment (Elith *et al.*, 2010; Kearney *et al.*, 2010). Yet, single threshold measurements that represent just a static snapshot in time are in most cases the only available measures to use in such models. The accuracy and application of such snapshots are limited because thermal thresholds are dynamic in response to changing environmental conditions (Buchner & Neuner, 2003; Valladares *et al.*, 2014; Curtis *et al.*, 2016; O'Sullivan *et al.*, 2017).

Variation in responses among species may represent either genetic constraints (i.e., species are adapted to a particular suite of conditions) or plastic modification to suit varying environmental conditions (i.e., within an organism's lifetime; Givnish, 2002; Nicotra *et al.*, 2010). To avoid heat stress as temperatures change through time, many species have the capacity to acclimatise through plastic alterations in plant functional traits (Arnone III & Körner, 1997; Franks et al., 2014). Thermal acclimatisation requires exposure to a non-lethal temperature stress event, triggering a change in the way genetic information is expressed over a short time period, such as days to weeks (Franks *et al.*, 2014; van Zanten *et al.*, 2014). This thermal priming signals a change in the heat response pathways of plants, including upregulation of heat shock protein expression and changes in membrane fluidity (Penfield, 2008; McClung & Davis, 2010). Priming temperature (PT) therefore has a role in preparing plants for future high-stress conditions by signalling mechanisms that stimulate an increase in their physiological thermal tolerance.

The ability of plant species to plastically respond and adjust their physiology enables them to cope with considerable temperature variation. For example, when transplanted to contrasting growth temperatures, many warm- and cool-origin species show an associated downward or upward shift in their thermal tolerance, respectively (Knight & Ackerly, 2003). Seasonal shifts also have been recorded, with temperature tolerance higher in summer compared to winter (Lerner, 1999; see Table S6 in O'Sullivan *et al.*, 2017). This acclimatisation often results in greater fitness under growth conditions (Atkin *et al.*, 2006) and is expected to be important for plant species' persistence in a warming climate (Angilletta, 2009). Variation among species in the capacity to make these seasonal adjustments is not well documented; thus, there remains a need for studies to provide better temporal resolution for more species.

Not only does ambient temperature vary, but also the *realised* heat stress experienced by plants can be indirectly influenced by other environmental factors. For plants, variation in local water availability is likely to influence their realised heat stress. This is because an adequate supply of water provides one of the most effective means of mitigating heat stress via latent heat loss, which occurs as evaporative cooling through stomata during transpiration. This process, however, is not assured where the availability of water is highly variable in space or time. Therefore, irrespective of broad-scale average climatic conditions, local-scale environmental variation creates distinct microhabitats, each with their own unique conditions of stress (Buchner & Neuner, 2003; Austin & Van Niel, 2011; Curtis *et al.*, 2016; McLaughlin *et al.*, 2017). Variability in water availability is typical of desert systems, creating spatially segregated microhabitats. Plants in hot, dry microhabitats often restrict transpiration to conserve water (Hamerlynck *et al.*, 2000). Lack of transpirational cooling in hot conditions can result in leaf temperatures rapidly increasing, from 2 °C to 15 °C above ambient (Ball *et al.*, 1988; Nobel, 2012; Leigh *et al.*, 2017). Within a given biome, therefore, microhabitats with low water availability (e.g., well drained slopes) and those with higher water availability (e.g., ephemeral river beds or depressions) likely provide different selection pressures on species for thermal tolerance.

The overall aims of our study were first to quantify interspecific, seasonal variation in T_{50} for 47 desert plant species and second, to determine whether seasonal adjustments vary as a function of microhabitat. We examined variation in T_{50} across seasons and among native microhabitats differing in water availability. We also investigated the extent to which priming temperature influenced or masked these broader drivers. Specifically, we asked the following questions: 1) is there a consistent response across species to increase T_{50} from winter to summer? 2) is the magnitude of change in T_{50} influenced by species' native microhabitat? 3) is there evidence for repeated evolutionary divergence in T_{50} between pairs of species contrasted for microhabitat affiliation? In addressing these questions, we present a novel framework for classifying the temporal dynamics of species' upper thermal damage thresholds.

4.3 METHODS

4.3.1 Study area and sampling period

The study was conducted at the Australian Arid Lands Botanic Garden (AALBG), located in Port Augusta, within the southern arid region of South Australia (32°27′56.3″S, 137°44′ 40.7″E). Here, plants native to Australia's southern arid region typically grow on red sandy loam soils in a common arid environment, exposed to natural irradiance and temperature (Supporting information, Fig. S4.1). The site receives mean annual rainfall of < 250 mm, with mean monthly rainfall often below 20 mm (AGBoM 2016). Mean minimum and maximum temperatures are 12.8 °C to 26.3 °C in autumn, 5.4 °C to 18.6 °C in winter, 12.0 °C to 27.3 °C in spring and 18.5 °C to 33.3 °C in summer (AGBoM 2016). Temperatures of > 45 °C are common throughout summer and can sometimes occur in spring during aseasonal heatwave events (AGBoM, 2013a; unpublished data). Plants within the garden were drip irrigated to provide sufficient water to prevent stress under the hot arid conditions, i.e. approximately 2 hours on an 8 L/hr cycle, once per week in winter and spring, and twice in summer. Therefore, for all species, water availability was more reliable than if they were grown in the field, but not typical of a temperate garden setting. Most measurements took place throughout 2013: summer, late January to early March; winter, late June to early August; spring, late September to early November. Due to an absence of suitable growth from which to sample foliage, summer measurements for *Sida ammophila* were made the following season in 2014. During the sampling period, above average temperatures were experienced across the entire Australian continent and several climate records were exceeded, at the time reflecting Australia's warmest period on record (AGBoM, 2013b).

4.3.2 Species selection and sampling protocol

To address the question of seasonal variation, a range of arid-zone shrub and tree species, spanning multiple plant families, were sampled seasonally to assess intra- and inter-specific variation of thermal responses, namely temporal adjustments in T_{50} among seasons. Specifically, our aim was to sample species throughout the year and capture their thermal response during seasons representing annual climate extremes and also intermediate weather conditions. Winter, spring and summer measurements captured species thermal response to extreme low, intermediate and extreme high temperature, respectively and enabled the acclimatisation potential of various species to be quantified. For this study, T_{50} was measured for a total of 23 species during winter, 22 species in spring and 42 species in summer: a combined total of 47 species from 23 families. Thermal damage thresholds for 41 of the 42 species measured during summer have been presented elsewhere (Curtis et al. 2014), but here are combined with winter and spring measurements to provide novel insight into the seasonal differences among these and additional arid-zone species. Next, to investigate the influence of microhabitat associations on T_{50}, among-season, T_{50} adjustments were considered for species when grouped into one of three categories on the basis of typical water availability in their native microhabitats, following the protocol described in Curtis *et al.,* (2016). The three microhabitat categories were, from most mesic to most xeric: (1) high water availability (W_{high}), e.g., species restricted to the banks of seasonally flooded rivers and road-side depressions, and ephemeral species that tend to grow only during favourable periods; (2) low water availability (W_{low}), e.g., species growing on well-drained soils and rocky outcrops; and (3) variable water availability (W_{var}), species able to grow in both the above microhabitats. In this context, 'mesic' is a relative term within a desert context, referring to desert species with comparatively more reliable access to water. Note that the above descriptions of microhabitats pertain to the field conditions in which each species is naturally found, whereas the growth conditions used in the present study were similar among species within a given season. Although T_{50} is known to significantly vary among the described microhabitat categories in summer (see Curtis *et al*., 2016), an objective of the present study was to establish if this pattern is consistent among seasons. Among-microhabitat comparisons, therefore, were also conducted within each season. Lastly, phylogenetically-informed analyses of changes in T_{50} in relation to season were used to address the question of evolutionary divergence patterns in this threshold between pairs of species contrasted on native microhabitat. For these phylogenetic analyses, 22 of the 47 species were sampled in all three seasons and formed 11 phylogenetically-independent contrasts (PICs). Contrasts were for species with relatively high- and low-available water in their native microhabitats (referred to hereafter as mesic- and xeric-adapted species, respectively). We selected both congeneric and confamilial pairs to establish the largest set of PICs possible, enabling the data to be more effectively generalised (Westoby, 2007). Final contrasts were between congener pairs in *Acacia*, *Atriplex*, *Eremophila*, *Eucalyptus* and *Solanum* and between confamilial pairs in Asteraceae, Fabaceae, Lamiaceae, Poaceae, Malvaceae and Myrtaceae. Possible pairings, where the first of each potential scenario represents a more mesic-adapted species, were as follows: 1) W_{high} with $W_{low,}$ 2) W_{high} with W_{var} and 3) W_{var} with W_{low}. Of the 22 species selected for PIC analyses, four of the W_{high} species could not be measured during summer as they either exhibit an ephemeral life history (*Solanum chenopodinum*, Solanaceae) or drop their leaves during unfavourable conditions typical of summer, i.e., were facultatively deciduous (*Olearia ferresii*, Asteraceae, and *Prostanthera striatiflora* and *Plectranthus intraterraneous*, Lamiaceae).

4.3.3 Leaf collection and T_{50} measurements

Leaves were collected and T_{50} measured following the protocol of Curtis *et al*. (2014). Briefly, all fully expanded, healthy leaves of similar age were sampled from branches collected from the north-facing outer canopy from a minimum of five (on eight occasions, three to four) plants per species. The same individual plants were used in each sampling season for all species except *Sida ammophila*, the individuals of which had unreliable seasonal regrowth, most notably in summer. A sub-sample of ten leaves from the larger pool was randomly selected for each temperature and control treatment and used to measure species' T_{50}. T_{50} was calculated using chlorophyll *a* fluorescence as the temperature-dependent decline in average F_V/F_M to determine the temperature at which it dropped by 50%. F_V/F_M is an index of health of the photosynthetic reaction centres within leaves; it is measured on dark-adapted tissue to estimate the maximum portion of quanta absorbed by reaction centres, providing an indication of the capacity of PSII to accept light (Baker & Rosenqvist, 2004). Fluorescence measurements were made using a pulse modulated fluorometer (HeinzWalz, Effeltrich, Germany). Seasonally, leaves of each species were treated with one of six temperature treatments using temperature controlled water baths, accurate to ± 0.2 °C. Of the six temperature treatments, one was a control treatment (28 °C) and the other five were heat stress treatments increasing by 2 °C increments: 42 °C – 50 °C (winter), 44 °C – 52 °C (spring) and 46 °C – 54 °C (summer). Leaves were exposed to a subsaturating light level of ca. 280 μmol photons m^{-2} s^{-1} throughout the treatment process (for details see Curtis *et al.*, 2014). Two species, *Olearia ferresii* and *Triodia irritans*, required additional low (i.e., down to 36 °C) and high (i.e., up to 56 °C) treatment temperatures to adequately extrapolate T_{50} in winter and summer, respectively.

4.3.4 Classifying seasonal changes of T_{50}

In developing a framework for classifying the temporal dynamics of species' T_{50}, our aim was to identify distinct patterns of seasonal change among the 11 phylogenetic contrasts. To ensure objective classification, we established a criterion for determining that T_{50} for a given species differed substantially from that of another in a given season by generating an error term for each species' T_{50}. We generated this quantitative error term using an interpolative approach similar to that used to determine T_{50} values (Fig. 4.1a), but this time based on the 95% confidence intervals (CI) around F_V/F_M (Fig. 4.2b). First, for each species we determined values corresponding to upper and lower confidence limits around the mean F_V/F_M of each treatment and control temperature. These values define the range of a CI. Next, linear interpolation was used to determine the temperatures at which the upper and lower limits dropped to 50% of prestress (control) conditions (here upper_{50} and lower_{50}) (Fig. 4.1b). The difference between species' upper_{50} and average T_{50}, and lower_{50} and average T_{50} were then determined. The mean of these two differences was then applied as the error term around species' average T_{50} in each sampling season (Fig. 4.1c). These estimated CIs were subsequently used as the cut-off criterion for classifying seasonal changes of T_{50} among paired species. Seasonal adjustments of T_{50} were considered substantial if the CI from one season to the next showed no overlap. Species classifications were the same if their determined acclimatisation patterns followed similar trajectories.

4.3.5 Acclimatisation and priming temperature

Experimentally manipulating a single environmental variable and subsequently determining an organism's performance generally is referred to as 'acclimation' (Way & Yamori, 2014). This contrasts with the process of individuals gradually adjusting to natural climatic changes in their environment, which can be considered 'acclimatisation'. Along with evaluating acclimatisation across seasons, our aim was also to define the potential for species to adjust their threshold from the coolest to warmest period of the year. We use the term 'acclimatisation potential' (AP) to describe the maximum shift in T_{50}, which is the difference in T_{50} between winter to summer: AP = (summer T_{50} – winter T_{50}). This definition necessarily excluded ephemeral or facultatively deciduous species, which were not present during the warmer summer months. Therefore, AP data were available for a total of 18 species, which were measured both in winter and summer.

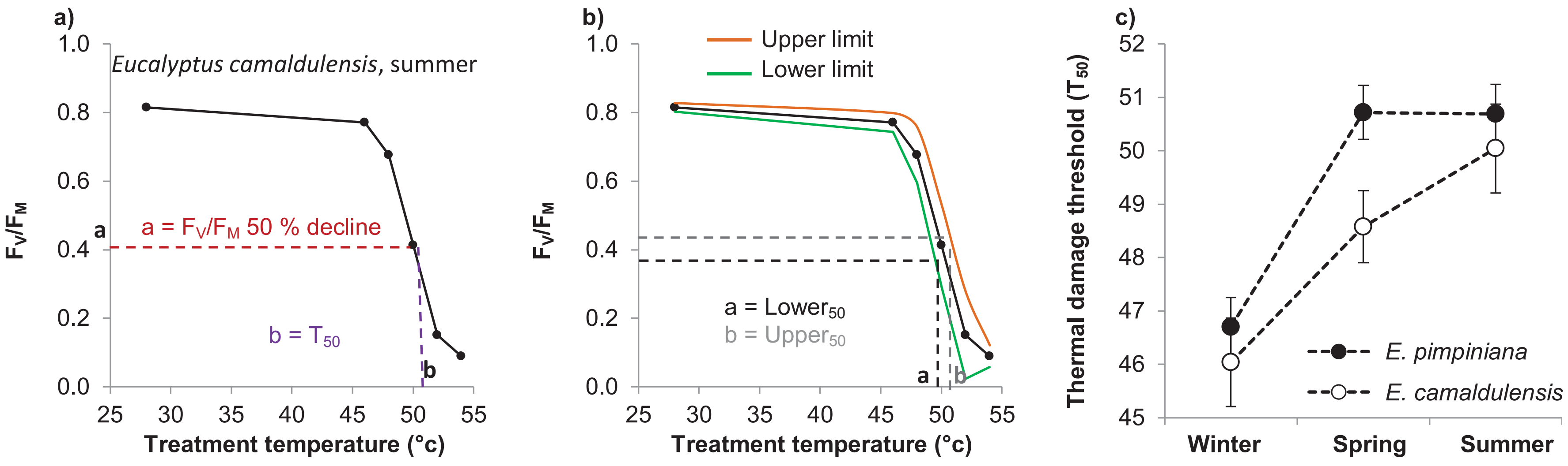

Figure 4.1 Using linear interpolation, a species' thermal damage threshold (T_{50}) is defined as the temperature-dependent decline of F_V/F_M chlorophyll fluorescence from prestress values **a)**. Here, we employed a similar approach to estimate within-species variation of T_{50} from the 95% confidence interval (CI) around the sample mean of F_V/F_M at each treatment and control temperature (for each data point $n = 10$). First, for each species we determined values corresponding to upper and lower confidence limits around the mean F_V/F_M of each treatment and control temperature. These values define the range of a CI. Next, linear interpolation was used to determine the temperatures at which the upper and lower limits dropped to 50% of prestress (control) conditions (here $upper_{50}$ and $lower_{50}$) **b)**. The difference between species' $upper_{50}$ and average T_{50}, and $lower_{50}$ and average T_{50} were then determined and their mean applied as the error term around species average T_{50} seasonally (winter, spring, summer) **c)**. In this way, the interpretation of seasonal patterns of change in individual species' T_{50} could be kept consistent. In the example shown, the summer T_{50} (see **panel a**) for *Eucalyptus camaldulensis* ssp. *camaldulensis* was interpolated as 50.0 °C ± 0.83, where 0.83 is the mean difference between T_{50} and temperatures corresponding to $lower_{50}$ (49.3 °C) and $upper_{50}$ (51.0 °C), respectively, equating to the CI around T_{50} for this species (see **panel b**). **Panel c** compares seasonal differences in T_{50} between paired species contrasted on typical water availability in their native microhabitats: *E. camaldulensis* (high-water) and *E. pimpiniana* (low-water). In this panel, lines are for ease of reading patterns and do not represent continuous time. With estimated CI being applied, we can see that the error bars for T_{50} for each species do not overlap in spring, whereas clear separation of species T_{50} is not present in either winter or summer. Further, both species exhibit an Early Jump strategy (see Results and **Fig. 4.2**), defined for a given species as no overlap of their CI between winter and spring, but with overlap in spring and summer.

To evaluate acclimatisation among our species, our aim was to compare T_{50} among three seasons; however, we recognise that adjustments are likely to occur within a season. To reduce the effect of temporal environmental variation on species' physiological thermal responses, the sampling period within each season was restricted to the shortest timeframe possible. Nevertheless, thermal damage threshold measurements took a period of weeks, with only one or two species able to be measured per day. There existed the possibility, therefore, that differences in ambient temperature of the days preceding measurements influenced each species' thermal damage threshold (Mittler *et al.*, 2012). That is, any observed interspecific variation in T_{50} could have been additionally influenced by thermal priming. Here priming temperature (PT) was quantified as the daily minimum and maximum temperatures recorded three days preceding sampling a given species' T_{50}, or PT_{min} and PT_{max} respectively (climate data sourced from AGBoM (2017a)). Alternative thermal metrics, including the daily minimum and maximum temperature, daily thermal range and cumulative daily minimum and maximum temperature across multiple days preceding T_{50}, also were considered as candidates for priming. Each were excluded due to the following reasons. First, analyses were conducted to establish the extent to which T_{50} was correlated with each of the retrieved ambient temperature records or thermal metrics. The latter revealed that ambient temperature records corresponding to three days prior to T_{50} measurements were significantly and at times more strongly correlated with T_{50} than daily thermal data from other days, particularly during summer (Table S4.1). The latter analyses identified that damage thresholds were most strongly associated with the minimum daily temperature and cumulative minimum daily temperatures of the three days prior to measurements. Similarly, T_{50} was closely related to both the maximum daily temperature three days prior and the cumulative maximum daily temperatures of the three days prior to measurements. Third, T_{50} was not significantly correlated with the daily thermal range. Final analyses therefore included only PT_{min} and PT_{max} from three days prior to T_{50} measurements, and can be considered a snapshot of species' short-term thermal history.

4.3.6 Data analyses

Statistical analyses were performed using the statistical software package IBM SPSS$^{®}$ (v19). To investigate whether T_{50} differed among levels of the explanatory variables season and microhabitat, we used generalised linear models (GzLM) with a Gaussian distribution and identity link function. Priming temperature was included in models as a covariate. We included the two priming temperature metrics, PT_{min} and PT_{max}, in separate models. Initially, models consisted of the full factorial design, including all main effects and interaction terms. Models were reduced by eliminating all nonsignificant interaction terms until only significant interactions remained (Engqvist, 2005). The goodness-of-fit for each model was assessed using Akaike's information criteria corrected for small sample sizes (AICc, with low AICc indicating a better model fit) (Garson, 2013b). For both the PT_{min} and PT_{max} models, the most parsimonious reduced models consisted of all main effects and the season × priming temperature interaction. Fischer's Least Significant Difference (LSD) post-hoc tests were used to identify significant pairwise comparisons within factors, based on estimated marginal means from the GzLM (Streiner & Norman, 2011; Armstrong, 2014). To aid interpretation of significant interaction terms between factor and covariates in the GzLM (i.e., season x PT_{min} and season x PT_{max}), one-way analysis of covariance (ANCOVA) was used, as estimated marginal means of covariates were automatically excluded from selection by the analytical program. The ANCOVA model was structured identically to the GzLM with the exception that individual seasons were modelled separately (winter, spring, summer). For phylogenetically-independent contrasts, we first used paired *t*-tests to determine if PT differed between paired species, which would potentially influence differences in T_{50} between the two groups. Next, a paired *t*-test was used to assess differences in T_{50} between the two groups. To understand the influence of a season on AP, Pearson's correlations (*r*) were computed to test the linear association between AP and T_{50} seasonally (winter, spring, summer).

4.4 RESULTS

4.4.1 Influence of season, priming temperature and microhabitat on T_{50}

Species' T_{50} values varied considerably within and among seasons, with values as low as 37.9 °C (*Olearia ferresii*) in winter and as high as 54.3 °C (*Triodia irritans*) in summer. Average T_{50} values across species increased by 5 °C, from 46 °C (SE = ± 0.45, *n* = 23) in winter to 51 °C (SE = ± 0.24, *n* = 42) in summer (Fig. 4.2). Average T_{50} in spring was 47.8 °C (SE = ± 0.39, *n* = 22). Average T_{50} values also varied significantly

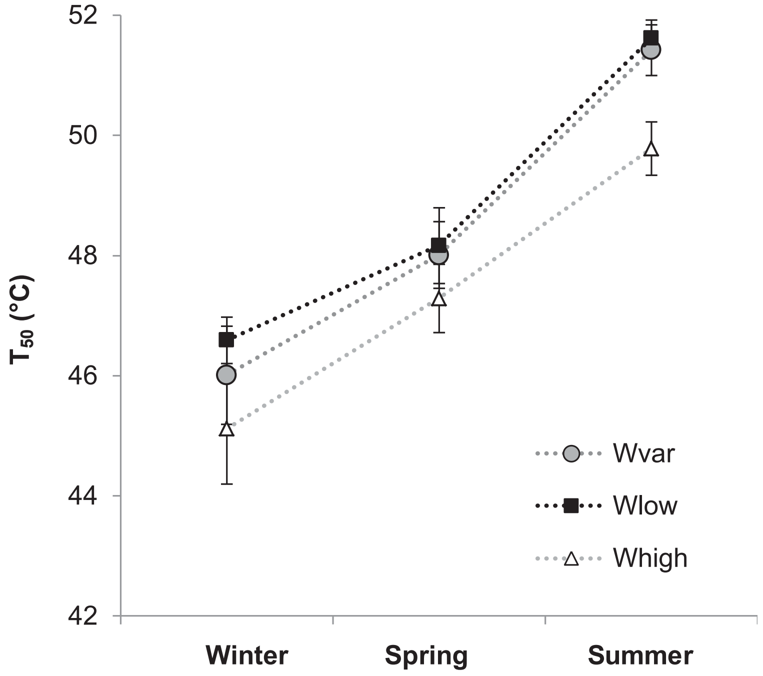

Figure 4.2 Mean (± SE) thermal damage thresholds (T_{50}) for species from each microhabitat across seasons: winter (*n* = 23), spring (*n* = 22) and summer (*n* = 42). Native microhabitat was defined by three levels of water availability, variable (W_{var}), low (W_{low}), and high (W_{high}). Dashed lines are shown for ease of reading patterns and do not indicate continuous time.

among microhabitats, being lower for W_{high} species compared with W_{var} and W_{low} species (Fig. 4.2). After accounting for the effect of priming temperature, the variation in T_{50} among season and native microhabitat group was statistically significant (Table 4.1). Specifically, for both models (model 1 = PT_{min}; model 2 = PT_{max}), the main effect of priming temperature was nonsignificant and the main effect of microhabitat was statistically significant (Table 4.1). The average T_{50} of W_{low} and W_{var} species did not significantly differ from one another, but both were significantly higher than the average T_{50} of W_{high} species (Table 4.2). The main effect of season was nonsignificant for model 1 (PT_{min}), but it was significant in model 2 (PT_{max}) (Table 4.1). The average T_{50} values in winter and spring were significantly lower than in summer, but T_{50} in winter and spring did not significantly differ (Table 4.2). For both models there was a significant interaction between PT and season: suggesting that the influence of PT_{min} and PT_{max} on T_{50} differed among season (Table 4.1). Analysis of covariance tests within each season, confirmed a strong significant main effect of both PT_{min} and PT_{max} on T_{50} in summer, but no significant effect of either PT_{min} or PT_{max} in winter and spring (Table 4.3, Fig. 4.3a, b).

Table 4.1 Comparison of models predicting the influence of season, microhabitat and priming temperature (PT) on the thermal damage threshold (T_{50}). Each model incorporates a different priming temperature metric as a covariate: PT_{min} (model 1) and PT_{max} (model 2), respectively based on the daily minimum and maximum temperature recorded three days preceding collection of species' T_{50}. Results are for the most parsimonious models, assuming Gaussian distributions with identity link functions. Omnibus tests confirmed that each fitted model was significantly different from its null model Significant differences appear in bold ($\alpha = 0.05$).

Model parameters	Model 1: PT_{min}			Model 2: PT_{max}		
	df	Wald χ^2	*P*	df	Wald χ^2	*P*
Main Effects						
Season	2	5.011	0.082	2	7.991	**0.018**
Microhabitat	2	8.812	**0.012**	2	13.376	**0.001**
PT	1	0.081	0.776	1	1.031	0.310
Interactions						
Season x PT	2	13.648	**0.001**	2	9.675	**0.008**

Table 4.2 Pairwise comparisons of the estimated marginal means for significant model main effects (see Table 4.1). Model compares the effects of season and microhabitat on species' thermal damage thresholds (T_{50}), while accounting for effects of minimum (PT_{min}, model 1) or maximum (PT_{max}, model 2) priming temperatures. Dashes indicate where the main effect was nonsignificant in the overall model. Significant differences appear in bold ($\alpha = 0.05$).

	Model 1: PT_{min}				**Model 2: PT_{max}**			
Season	Mean difference (contrasts)	SE	df	*P*-value	Mean difference (contrasts)	SE	df	*P* -value
winter *vs* spring	-	-	-	-	-0.792	1.232	1	0.520
winter *vs* summer	-	-	-	-	-2.977	1.244	1	**0.017**
spring *vs* summer	-	-	-	-	-2.185	0.406	1	**< 0.001**
Microhabitat								
W_{var} *vs* W_{low}	-0.116	0.379	1	0.753	-0.141	0.353	1	0.689
W_{var} vs W_{high}	1.054	0.452	1	0.020	1.260	0.429	1	**0.003**
W_{low} vs W_{high}	1.170	0.404	1	0.004	1.402	0.395	1	**<0.001**

Table 4.3 Analysis of covariance investigating the influence of priming temperature on species' thermal damage threshold (T_{50}) within season (winter, spring, summer) (results complement Table 4.2). For each season, separate models incorporating the two different priming temperature metrics as a covariate were conducted: PT_{min} (model 1) and PT_{max} (model 2). Significant differences appear in bold ($\alpha = 0.05$).

Season	Model 1: PT_{min}	Model 2: PT_{max}
Winter	$F_{(1,19)} = 1.108$ (*P* > 0.306)	$F_{(1,19)} = 0.606$ (*P* > 0.446)
Spring	$F_{(1,18)} = 1.079$ (*P* > 0.313)	$F_{(1,18)} = 2.276$ (*P* > 0.149)
Summer	$F_{(1,38)} = 9.177$ **(*P* = 0.004)**	$F_{(1,38)} = 10.332$ **(*P* = 0.003)**

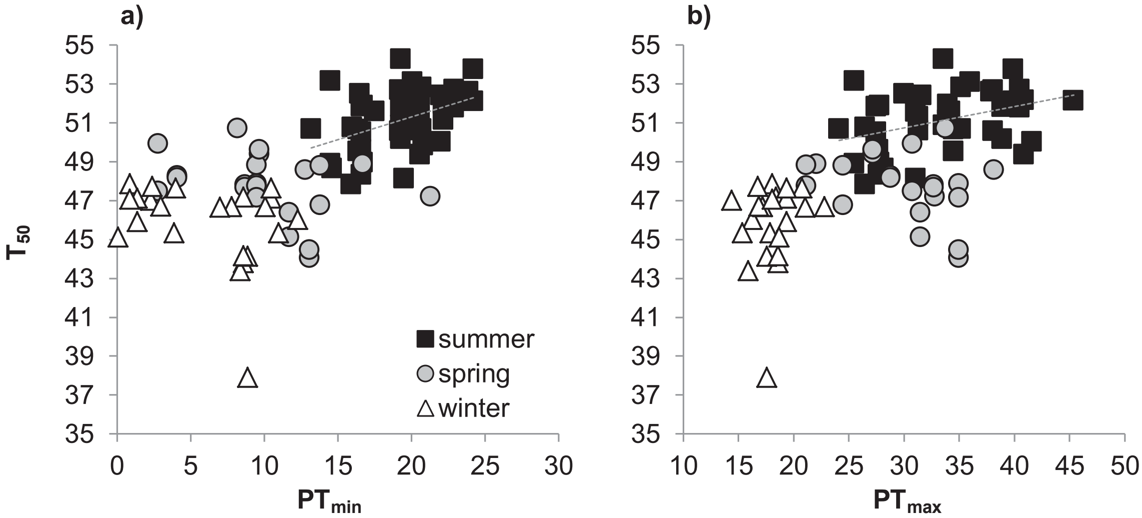

Figure 4.3 Relationships between T_{50} and PT_{min} **a)** and PT_{max} **b)** seasonally (winter, spring, summer). For corresponding ANCOVA results, see Table 4.3.

4.4.2 Acclimatisation

For species assessed both in winter and summer, acclimatisation potential ranged from 0.7 °C (*Eremophila bignoniiflora*) to 8.3 °C (*Atriplex vesicaria*) and averaged 4.5 °C (SE = ± 0.4, *n* = 18) (Fig. 4.4). Being the difference between winter and summer T_{50} values, acclimatisation potential for a given species may increase not only via a high summer T_{50}, but potentially also low winter T_{50}, providing the latter was followed by a comparatively large jump to a moderate summer T_{50}. We found AP to be significantly and positively correlated with T_{50} in summer (high AP with high summer T_{50}: $r = 0.734$, $n = 18$, $P = 0.001$) and negatively correlated with T_{50} in winter (high AP with low winter T_{50}: $r = -0.612$, $n = 18$, $P = 0.007$). Although non-significant, average AP tended to increase from mesic- to more xeric-adapted species: $W_{high} < W_{var} < W_{low}$ ($W_{high,}$ 3.9 °C ± 0.7, $n = 7$; W_{var}, 4.6 °C ± 1.9, $n = 3$; W_{low}, 5.1 °C ± 0.5, $n = 8$).

Table 4.4 Paired *t*-tests used to assess differences in seasonal thermal damage thresholds (T_{50}) between phylogenetically independent contrasts ($n = 11$ pairs). Species are contrasted on low- *versus* high-water availability in their native microhabitat. As priming temperature was found to influence variation in T_{50} (see Tables 4.1, 4.3), *t*-tests also were performed on PT_{min} and PT_{max}. Significant differences appear in bold ($\alpha = 0.05$).

		Low-water		High-water					
Variable	Season	Mean	SE	Mean	SE	*n*	*t*	df	*P*-value
T_{50}	Winter	46.47	0.40	45.45	0.83	11	1.88	10	$P = 0.090$
	Spring	48.05	0.52	47.52	0.50	11	1.45	10	$P = 0.178$
	Summer	51.93	0.46	50.20	0.47	8	3.78	7	$\mathbf{P = 0.008}$
PT_{min}	Winter	5.01	1.18	4.16	1.46	11	0.94	10	$P = 0.371$
	Spring	7.03	1.37	10.16	1.82	11	-1.57	10	$P = 0.148$
	Summer	17.99	0.85	17.05	2.58	8	0.32	7	$P = 0.760$
PT_{max}	Winter	18.33	0.53	18.30	0.62	11	0.05	10	$P = 0.962$
	Spring	30.20	1.55	29.83	1.48	11	0.17	10	$P = 0.865$
	Summer	33.90	2.16	35.74	2.46	8	-0.47	7	$P = 0.653$

For the analysis of PICs, comparing mesic- with xeric-adapted species, results were consistent with the analysis across species. That is, despite potential effects of PT, T_{50} for xeric-adapted species were significantly higher than their mesic-adapted counterparts in summer, with no significant differences in T_{50} between paired species in either winter or spring (Table 4.4). Using our classification framework, we identified that species could be grouped into one of five discernible ways of changing their T_{50} from winter to spring and spring to summer (Fig. 4.4, Table 4.5). In clear contrast to other species in this data set, which maintain growth throughout the year, species in the first group have an ephemeral life history and/or are facultatively deciduous, thus exhibiting a strategy of heat stress avoidance during summer (Fig. 4.4, Table 4.5, Group 1: Avoid). The four Avoider species, however, did show a significant increase in T_{50} from winter to spring. The second pattern of change was one of a step increase in

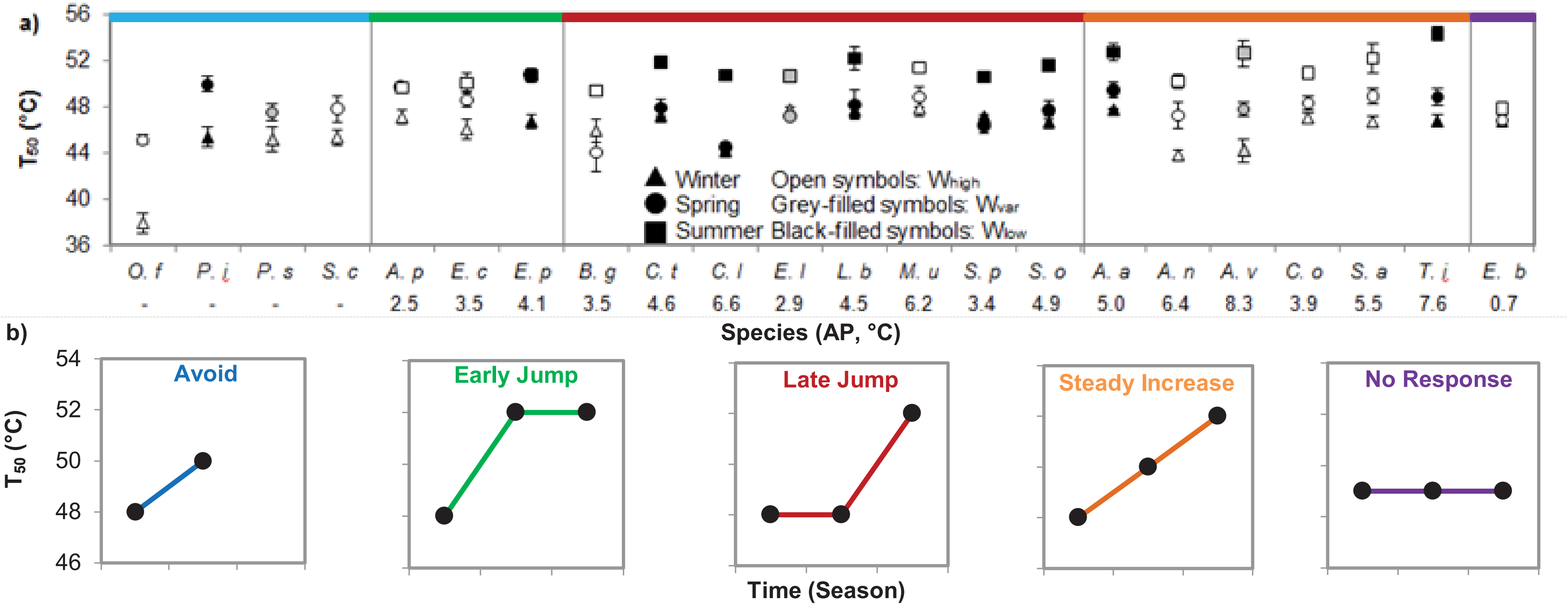

Figure 4.4 Thermal damage thresholds (T_{50}) measured seasonally for 22 Australian southern arid-zone species varying in their native microhabitat: variable water, W_{var}; low water, W_{low}; high water, W_{high} **a)**. Acclimatisation potentials (AP = winter T_{50} – summer T_{50}) are listed below each species name; AP is not shown for ephemeral or facultatively deciduous species (dashed lines), the leaves of which were not present in summer. Species are arranged into groups reflecting differences in their thermal response with season (groups are colour-coded to match panel b). Theoretical representations of these groupings are shown in **panel b)**: **Avoid,** species with an ephemeral life history and/or exhibiting facultative deciduousness during less favourable conditions; **Early Jump,** species exhibiting a step increase in T_{50} between winter and spring, with minimal change between spring and summer; **Late Jump,** species exhibiting minimal changes in their T_{50} between winter and spring but a substantial jump from spring to summer; **Steady Increase,** species exhibiting a steady increase in T_{50} values from winter to summer, with no marked step increase from winter to spring or spring to summer; **No Response,** species showing little change in T_{50} seasonally. Error bars are an estimation of within-species variation in T_{50} interpolated from 95% confidence intervals (see Methods and Results). For full species names see Methods and Table 4.1.

Table 4.5 List of species belonging to each of five acclimatisation groups reflecting different trajectories of seasonal changes in T_{50}. Details in text Methods. Shading for ease of reading.

Group 1: **Avoid**	**Group 2:** **Early Jump**	**Group 3:** **Late Jump**	**Group 4:** **Steady Increase**	**Group 5:** **No Response**
Olearia ferresii	*Eucalyptus pimpiniana*	*Eremophila longifolia*	*Cymbopogon obtextus*	*Eremophila bignoniifolia*
Plectranthus intraterraneous	*Acacia papyrocarpa*	*Senna pleurocarpa* var. *pleurocarpa*	*Sida ammophila*	
Prostanthera striatiflora	*Eucalyptus camaldulensis* ssp. *camaludulensis*	*Melaleuca uncinata*	*Atriplex nummularia*	
Solanum chenopodinum		*Bauhinia gilva*	*Triodia irritans*	
		Callistemon teretifolius	*Atriplex vesicaria*	
		Solanum orbiculatum ssp. *orbiculatum*	*Acacia aneura*	
		Lasiopetalum behrii		
		Cassinia laevis		

thermal thresholds between winter and spring and only minimal change occurring between spring and summer (Fig. 4.4, Table 4.5, Group 2: Early Jump). In contrast to the Early Jump group, the third group of species exhibited minimal changes in their T_{50} between winter and spring but a substantial jump from spring to summer (Fig. 4.4, Table 4.5, Group 3: Late Jump). The fourth group consisted of species exhibiting a steady increase in T_{50} values from winter to summer, with no marked step increase from winter to spring or spring to summer (Fig. 4.4, Table 4.5, Group 4: Steady Increase). The final group consisted of a single species (*Eremophila bignoniiflora*), which exhibited no substantial change in T_{50} through the year (Fig. 4.4, Table 4.5, Group 5: No Response).

4.5 DISCUSSION

4.5.1 Predictors of T_{50}: microhabitat, season, and priming temperature

Generational exposure of plant species to a unique set of environmental conditions allows the evolution of novel plant genotypes that function more efficiently under the particular regimes of their native habitats (Berry & Bjorkman, 1980). Indeed, species from hotter environments, such as deserts, tend to have a higher intrinsic thermal damage threshold compared to species from more mesic coastal regions (Knight & Ackerly, 2003). These biome-based differences can represent adaptation in species that have evolved under spatially separate and climatically contrasting biomes. Previously, we have shown that physiological thresholds may also correspond to environmental variation within the landscape at even finer spatial scales than that of a biome, specifically with respect to water availability (Curtis *et al.*, 2016). In the current study, we have demonstrated for numerous desert species that this pattern is seasonally dynamic: with differences in thermal thresholds among microhabitat groups (W_{var}, W_{low}, W_{high}) most pronounced during summer (Fig. 4.2). The influence of different microhabitats on thermal damage thresholds occurred even when phylogenetic relatedness was explicitly considered. Exposure to similar selective pressures could explain the convergence of similar thermal tolerance among distantly related species that have evolved in comparable desert microhabitats (Cavender-Bares *et al.*, 2009). For instance, limited available water in their native environments may expose xeric-adapted species more frequently to extended periods of water stress, during which time they are prevented from taking advantage of the substantial cooling benefits of transpiration. Notably, plant physiological responses corresponding to the onset of water stress also protect against exposure to high temperatures (Valladares & Pearcy, 1997; Ghouil *et al.*, 2003). We suggest that, over generations, xeric-adapted species have become better adapted to cope with conditions of high thermal stress than mesic-adapted species, which may grow only metres away, but with more assured water access. The co-occurrence of water stress and high-temperature heat stress are most typical of summer, which may explain the clear differences among microhabitats observed at this time of the year. Species compositional changes have potentially also influenced the patterns we found. Ephemeral species with their naturally lower damage thresholds are expected to be dormant during summer when heat stress is exacerbated by reductions in available water across the landscape.

Superimposing the gradual and cyclical change in species critical thermal limits, associated with seasonal change are rapid and short-term fluctuations. The thermal tolerance of plant photosynthetic apparatus can increase concomitantly with an increase in daily growth temperatures (Seemann *et al.*, 1984; Buchner & Neuner, 2003) and that adjustment can happen rapidly (Kee & Nobel, 1986; Havaux, 1993). In the present study the association between T_{50} and PT was particularly strong during summer. The magnitude and/or intensity of priming events are likely greater during summer than other times of the year, meaning species will endure comparatively greater levels of thermal stress; similarly, leaf temperatures would be more frequently prone to nearing their upper thermal thresholds at this than other times of the year. Few studies, however, differentiate between effects of daily minimum *versus* maximum ambient growth temperatures on species' responses (Prasad *et al.*, 2008; Cheesman & Winter, 2013). An increase in daily minimum temperatures can have significant effects on plant photosynthesis and whole-plant growth. For example, warm overnight temperatures may lead to carbon loss through increased respiration or the production of reactive oxygen species (ROS) (Larkindale *et al.*, 2005b; Prasad *et al.*, 2008; Peng *et al.*, 2013). While ROS can lead to increased cell damage when in high concentrations, at lower concentrations they are thought to be involved in several protective pathways (Quan *et al.*, 2008). Notably, their production is linked to the development of stress tolerance in plants, making them an important part of the heat stress response (Neill *et al.*, 2002; Gechev *et al.*, 2006; Driedonks *et al.*, 2015). Among our desert species, T_{50} was as strongly associated with minimum priming temperatures as it was with maximum priming temperatures. Note that there was no relationship between T_{50} and the temperature range of the previous days (maximum minus minimum PT), suggesting both low and high-temperature extremes are important forces driving species' thermal acclimatory responses. Potentially, the relationship observed here between T_{50} and PT is indicative of upregulation of the production of ROS or other similarly protective regulatory processes, stimulated by more intense summer priming events. Given the complexity of the plant heat stress response (Larkindale *et al.*, 2005a) and the paucity of research into priming conditions, further work would benefit our understanding of the mechanisms driving the thermal responses seen here for a diverse range of arid species.

4.5.2 Species' heat stress vulnerability and the importance of seasonal thermal response patterns

Along with evidence suggesting intergenerational adaptation to their thermal environments, species in the current study showed signs of short-term seasonal acclimatisation. The seasonal increase in T_{50} from winter to summer parallels temporal increases in the average climatic temperatures experienced by these plants, a pattern reported elsewhere (Monson & Williams, 1982; Downton *et al.*, 1984). That the ability to acclimatise is itself a genetically determined trait suggests that species' responses observed in this study are an example of their genetic predisposition to plastically respond to variable environmental conditions (Givnish, 2002; West-Eberhard, 2003; Nicotra *et al.*, 2010). Interestingly, species with high acclimatisation potential were not necessarily the least vulnerable to high-temperature extremes and vice versa (Fig. 4.4). For instance, *Atriplex vesicaria* achieved the highest AP overall (8.3 °C), which was driven not only by a high summer T_{50}, but also by a below average winter T_{50} of 44.2 °C. By contrast, the high AP of *Triodia irritans* largely was a function of this species exceptionally high summer T_{50} of 54.3 °C, the highest T_{50} recorded for any of our species. Another comparison is *Senna pleurocarpa* var. *pleurocarpa* and *Cassinia laevis,* both Late jump W_{low} species that each attained a summer T_{50} value of ~ 50.5 °C. The photosynthetic apparatus of these two species therefore might be described as being equally tolerant to high summer temperatures; however, due to a very low winter T_{50}, *C. laevis* had an AP that was almost twice that of *S. pleurocarpa*. In spite of showing a greater plastic temporal response to thermal change, *C. laevis* potentially is more vulnerable than *S. pleurocarpa* to aseasonal extreme temperature events in cooler months, when its T_{50} would be comparatively lower.

Perhaps more informative than AP in understanding variation in species' vulnerability to high-temperature extremes is what happens to their physiology in milder seasons. Consider a sudden, extreme thermal event occurring in spring. A recent example from our study area showed the daily maximum temperature exceeding the long-term monthly mean maximum by > 12 °C, i.e., 39.5 °C in October, relative to an average 27.1 °C (AGBoM, 2015). Under such extreme conditions, especially if coupled with low water supply, plant leaves and the photosynthetic apparatus within, can reach damagingly high temperatures (Skelton *et al.*, 2012). In our study, the different trajectories of altering thresholds during spring ranged from almost no to large increases in T_{50} between winter and spring or spring and summer (Fig. 4.4b). Differences in acclimatisation patterns suggest that the above spring heatwave scenario may be particularly damaging to certain groups of species. Late Jump and No Response species may have insufficient time to acclimatise, resulting in tissue damage or death. Avoiders, with their restricted or absent summer growth, may fail to flower or set seed before dying off. Steady Increasers, on the other hand, would have a better chance at survival and Early Jumpers may be unaffected.

Maintaining the physiological processes associated with damage prevention and repair can be costly (Leroi *et al.*, 1994; Hoffmann, 1995; Loeschcke & Hoffmann, 2002). Given that T_{50} corresponds to the prevention of damage to PSII, one might expect that an increase in T_{50} also would be accompanied by a decrease in other processes such as growth. The various acclimatisation responses of species described above may therefore indicate unique strategies corresponding to the way that species allocate available resources. Returning to *C. laevis,* with its comparably large AP but low winter T_{50}, it may be utilising a more resource-conservative strategy than species maintaining a higher thermal damage threshold in cooler seasons, irrespective of the likelihood of heat stress. Although potentially risky, by drawing resources away from protective processes when a higher thermal tolerance is not likely to be necessary, species like *C. laevis* may be able to allocate more towards growth. By contrast, sustaining a higher T_{50} across seasons would require more resources to be allocated into thermal damage prevention, potentially slowing growth rates in exchange for more reliably protected foliage (Mitra & Bhatia, 2008) and higher survival rates, particularly in summer.

4.5.3 Conclusion

Understanding species' responses to changing temperature extremes will inform more targeted management strategies (Williams *et al.*, 2008). Results of our study for desert plants reveal that, despite inherent variation in thermal responses based on microhabitat affiliation, species showed clear and distinct patterns of change in T_{50} over the course of the year. Importantly, season and microhabitat remained strong predictors of species' thermal thresholds despite the short-term influence of ambient (priming) temperatures, the effect of which was significant in summer. We first found that thermal damage thresholds increased by 5 °C from winter to summer. Second, irrespective of gradual changes across season, species' propensity for a high thermal tolerance reflected selection for conditions of compounding water and temperature stress in their native microhabitat that was independent of phylogenetic relatedness. Third, the potential for a species to acclimatise from winter to summer (AP) did not necessarily equate to a high thermal damage threshold during the hottest time of year. Fourth, possibly reflecting different resource use strategies, the temporal trajectory for changing thermal tolerance varied among species, with the rate and timing of thermal adjustments throughout the year falling into one of five groups: Avoid, Early Jump, Late Jump, Steady Increase, and No Response. Such variation in seasonal acclimatisation dynamics among species suggests that some groups will be more vulnerable than others to thermal damage during early heatwaves occurring, e.g., in spring. These findings emphasise that not only are species' thermal responses highly dynamic, but also that static measurements provide limited insight about species' vulnerability to temperature extremes. Our findings have strong implications for predictive models aiming to understand species compositional changes and distributional shifts with climate warming. To advance the predictive accuracy and application of mechanistic models, we suggest a sampling approach spanning critical seasonal periods and incorporating key microhabitat types representative of higher *versus* lower environmental stress.

Chapter 5

Extending the global leaf economics spectrum to include metrics of plant thermal tolerance: Two new axes identified from a suite of desert plant species

This chapter is in preparation for submission to the *Journal of Vegetation Science*

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Keywords: Thermal damage thresholds; LMA, reflectance; leaf morphology; leaf thickness; leaf shape; high-temperature stress; plant strategies; arid-zone plants; desert plants; principal components analysis.

5.1 ABSTRACT

Aims: Physiological measures of plant thermal tolerance can be used to indicate species' vulnerability to high-temperature stress. Some morphological and spectral properties of leaves also confer greater thermal protection, e.g., leaf thickness and spectral properties, but are rarely considered in this context. We investigated relationships among structural traits that influence leaf thermal dynamics and a physiological measure of thermal protection, T_{50}. Objectives were to: 1) identify the major axes of trait variation among a cross-section of Australian arid-zone plant species and 2) determine if species' preferred native microhabitat with respect to water availability could predict their positions in multi-trait space.

Location: The Australian Arid Lands Botanic Garden, Port Augusta, South Australia.

Methods: Leaf metrics were quantified seasonally (winter, spring, summer) for 47 species, with principal component analysis (PCA) used to determine their positions in multi-trait space. Scores were extracted along two principal components for each season. We investigated whether variation in PCA scores were associated with species' native microhabitat.

Results: Plant structural leaf traits covaried with physiological thermal tolerance, but the strength and configuration of relationships varied seasonally. Microhabitat preference successfully predicted species' placement along PC1. Xeric-adapted species had higher LMA and T_{50} and lower leaf water contents than their mesic counterparts. PC2 was consistently driven by variation in visible reflectance and somewhat by T_{50}, but was independent of microhabitat.

Conclusions: Our findings suggest the presence of two key thermal protection strategies among arid-zone plant species. The strong association of LMA on PC1 suggests a strategy relating to protecting long lived leaves; whereas thermal protection described by PC2 was independent of LMA and the leaf economics spectrum. Whether species employ a heat stress resistance or avoidance strategy will influence community composition and can affect broader ecosystem processes. Identification of trait-based thermal protection strategies offers important insight into predicting which species might be most resilient under a changing climate.

5.2 INTRODUCTION

The breadth of structural variation displayed by leaves and its potential adaptive significance for plants has received great interest both from ecologist and physiologist alike (Vogel, 1970; Ackerly & Reich, 1999; Reich *et al.*, 2003; Wright *et al.*, 2004; Vogel, 2009; Nicotra *et al.*, 2010; Leigh *et al.*, 2017). Being the primary photosynthetic organs of plants, the functional significance of leaf trait variation is often discussed from an economic perspective of carbon gain. Emphasis has been placed on global correlations within a small group of leaf functional traits, such as leaf nitrogen content and specific leaf area (the inverse of leaf mass per area, LMA). The 'leaf economics spectrum' (LES) describes universal plant resource use strategies characterised by a suite of closely coordinated leaf traits that form a continuum of variation, along which plants range from quick to slow returns on investment of nutrients and dry mass in leaves (Wright *et al.*, 2004). In this light, LMA is considered a particularly important indicator of plant strategies (Westoby *et al.*, 2002; Diaz *et al.*, 2004; Westoby & Wright, 2006; Flores *et al.*, 2014). Notwithstanding the importance of the LES in developing our understanding of plant community dynamics globally, leaf structural traits such as LMA can also contribute to increased plant tolerance under numerous abiotic and biotic stress conditions (Poorter *et al.*, 2009), yet are rarely considered in this way.

In desert environments, plants face frequent high temperatures and a range of leaf traits can minimise heat stress. Aside from high ambient temperatures, water is a key variable affecting how plants experience heat stress. This is because adequate soil moisture allows plants to cool via latent heat loss through stomata, but when water is scarce, transpirational cooling becomes impractical. In response, plants will often close their stomata to restrict water loss, reducing the cooling benefit of transpiration (Barradas *et al.*, 1994; Hamerlynck *et al.*, 2000). In deserts, high temperatures often are accompanied by drought, so plants in these environments need alternative means of regulating leaf temperature. Reflective hairs, waxes and salt crystals, for example, can reduce the amount of solar radiation reaching a leaf, helping maintain temperatures well below what they might otherwise reach (Mooney *et al.*, 1977; Ehleringer, 1981; Skelton *et al.*, 2012). Another important attribute that can influence leaf temperature is leaf size, where thinner boundary layers of smaller, narrower leaves increase convective heat loss compared to larger forms (Smith, 1978; Gates, 2012; Nobel, 2012). Leaves can also regulate their temperature by increasing the time it takes them to heat in response to rapid temperature increases, e.g. during a lull in wind speed. A slower heating response time is achieved by having a larger thermal mass, accomplished through greater leaf thickness, either by a higher LMA, leaf water content (succulence) or a combination of both (Leigh et al., 2012). Many of these leaf traits have been shown to vary with environmental conditions. For example, xerophytes tend to have higher LMA than mesophytic species that are adapted to environments with a more reliable source of water (Abrams *et al.*, 1994; Lewis *et al.*, 2011; Park *et al.*, 2016). LMA not only varies among broad groups of species but also within a single species or individual as environmental conditions change over time and space (Ellsworth & Reich, 1993; Shipley, 1995; Poorter *et al.*, 2009; Hulshof *et al.*, 2013; Coble *et al.*, 2016). Also, to optimise radiation loads under changing conditions of light and heat, many plants exhibit seasonal variation of leaf reflectance, which coincides with leaf structural changes over time (Mooney *et al.*, 1977; Ehleringer & Björkman, 1978; Skelton *et al.*, 2012).

In addition to regulating their temperature via structural means, plants make physiological adjustments to protect against heat stress and regulate their heat tolerance limits. Physiological protective mechanisms span cellular and metabolic responses, including changes to membrane structures and increased production of thermally stable molecules such as heat shock proteins, which serve to increase tolerance to heat stress by protecting photosynthetic tissue (Knight & Ackerly, 2001; Wahid *et al.*, 2007; Bita & Gerats, 2013). An easily measured estimate of the physiological thermal damage threshold of a plant is T_{50}: the temperature-dependent decline in the maximum quantum yield of photosystem II (F_V/F_M) (Curtis *et al.*, 2014). A higher T_{50} corresponds with greater upregulation of photoprotective mechanisms (Knight & Ackerly, 2002; Knight & Ackerly, 2003), so T_{50} provides a useful physiological index of thermal protection, which can be used to compare plant thermal tolerance across species, time and space (Curtis *et al.*, 2016). Plant physiological thermal responses and critical limits are indeed dynamic and respond to changes in the local environment at various spatial and temporal scales (Havaux, 1992; Knight & Ackerly, 2003). For example, in a recent study by Curtis *et al.* (2016), among a range of desert plants grown under similar environmental conditions, species native to drier sites had higher thermal thresholds than species normally found in wetter sites. Physiological thermal tolerance is also temporally dynamic, with short-term acclimatisation coinciding with seasonal shifts in environmental conditions (Thesis Chapter 4).

It has been shown that heat-shock induced increase in HSP production is positively correlated with LMA (Knight & Ackerly, 2001). These authors suggest that HSPs and their production may be correlated with a suite of structural and physiological traits associated with optimal carbon gain with respect to the opportunity cost of stress. That is, some species may utilise traits that maximise growth during favourable periods at the expense of losing the opportunity for carbon gain during stressful periods, whereas others may exploit marginal growing conditions by investing in effective stress response pathways (Knight & Ackerly, 2001). We postulate here that structural and physiological leaf traits conferring protection against thermal stress may group together in one of two ways. First, physiological thermal tolerance may covary with structural leaf traits, creating a suite of thermally protective traits. This first option, however, dictates that while some species would be well protected, others would be poorly protected against heat stress. Alternatively, various traits may trade off with one another, suggesting multiple thermal protection strategies. In the current study we focused on relationships among key morphological traits that influence leaf thermal dynamics and the physiological index of thermal tolerance, T_{50}. The objectives were: 1) to identify the major axes of trait variation among a cross-section of Australian arid-zone plant species and 2) to determine if species' preferred native microhabitat associations could predict their positions in multi-trait space. Microhabitat was broadly defined as relative water availability typical of the preferred native environment for a given species: high, low, and variable (W_{high}, W_{low}, W_{var}, respectively (Curtis *et al.*, 2016). To accommodate any seasonal variation in traits, we investigated relationships in three seasons, winter, spring and summer. Also, because many functional traits are highly conserved and lead to strong phylogenetic signatures (Freckleton *et al.*, 2002; Webb *et al.*, 2002; Westoby, 2007; Li *et al.*, 2017), we also examined relationships for a subset of phylogenetically independent species contrasts (PICs).

5.3 METHODS

5.3.1 Study area

All species in this study were growing in the same desert environment at the Australian Arid Lands Botanic Garden (AALBG) in Port Augusta, within the Southern Arid Region, South Australia (32°27'56.3"S 137°44'40.7"E). Mean annual rainfall is approximately 250 mm and mean maximum summer temperature is approximately 31.3 °C, with maximum temperatures at times exceeding 45 °C (AGBoM). Measurements took place seasonally in 2013: winter (late June to early August), spring (late September to early November) and summer (late January to early March). Due to an absence of suitable growth from which to sample foliage from, summer measurements for *Sida ammophila* were made the following season in 2014.

5.3.2 Species selection and trait measurements

Six morphological leaf traits were measured for a total of 47 plant species encompassing 23 plant families. Plants were sampled if suitable foliage was present on site and measurements were repeated seasonally during winter (23 spp.), spring (22 spp.) and summer (42 spp.). Previously, thermal damage thresholds (T_{50}) for these species were shown to vary temporally with season (Thesis chapter 4) and as a function of their native microhabitat (Curtis *et al.*, 2016). For preferred native microhabitat association, species were assigned to one of three microhabitat types based on typical water access from most mesic to most xeric: W_{high}, W_{var} and W_{low} species. Note that in this context, 'mesic' is a relative term with a desert context, referring to desert species with comparatively more reliable access to water. W_{high} species associate with native microhabitats where water is relatively available including banks of seasonally inundated rivers, and include ephemeral species, which respond rapidly to wet weather events. Species establishing growth where water is less often available and/or where water drains away readily, including crests of sand dunes and rocky slopes, are classified as W_{low} species. Lastly, W_{var} species are able to grow in areas fitting the water availability of both W_{high} and W_{low} species. Catergorisation of species into these microhabitat groups was based on distribution records from multiple sources (Curtis *et al.*, 2016).

To determine $T_{50,}$ chlorophyll *a* fluorescence was used to measure the temperature at which F_V/F_M drops to 50% of prestress levels using a pulse modulated fluorometer (HeinzWalz, Effeltrich, Germany). Briefly, to measure each species' T_{50}, ten leaves were treated with one of six temperature treatments using temperature controlled water baths, accurate to ± 0.2 °C (60 leaves per species). Of the six temperature treatments, one was a control treatment (28 °C) and the other five were heat stress treatments increasing by 2 °C increments: 42 °C – 50 °C (winter), 44 °C – 52 °C (spring) and 46 °C – 54 °C (summer). These temperature treatment ranges encompassed the temperatures that bracketed the lowest and highest T_{50} for all species within a given season. Leaves were exposed to a subsaturating light level of ca. 280 µmol photons m^{-2} s^{-1} throughout the treatment process (for details see Curtis *et al.*, 2014; Thesis Chapter 4).

Morphological leaf trait data were measured synchronous with T_{50} measurements. Leaf traits were: thickness (mm), LMA (g m^{-2}) and water content (%); effective leaf width (mm), which is an indication of leaf size (Cornelissen *et al.*, 2003); reflectance of visible and near-infrared radiation (%). For all trait measurements and species, samples were randomly selected from a larger pool of leaves, earlier removed from the branches of a minimum of three plants. For each leaf trait, a minimum of ten leaves were sampled and the mean obtained. This process was repeated in winter, spring and summer. Leaf thickness was measured multiple times on each leaf using digital callipers (accurate to 0.01 mm) placed away from major veins (LPG200 0 – 25mm/1" pocket digital gauge, Sciencetech Instruments). Leaf area and effective leaf width, hereafter leaf width, were obtained from scanned images using the graphic software program ImageJ (version 1.50a, United States National Institute of Health, Bethesda, MD, USA). Leaf width was measured as the diameter of the largest circle that can be placed within the leaf margin (Leigh *et al.*, 2017). The scanned leaves were oven-dried for a minimum of 48 h at 60 °C and LMA was calculated as a ratio using leaf dry mass and one-sided leaf area. Leaf water content was calculated as a percentage: (fresh weight – dry weight) / fresh weight). All fresh and dry weights were measured using a precision analytical balance sensitive to 0.001 g (Mettler Toledo, city).

Reflectance was measured in 0.5 nm increments between 400 and 1100 nm as a percentage of incoming light using a SpectraWiz fibre optic spectroradiometer probe and configured SL1 Tungsten Halogen light source (StellarNet Inc., Tampa, FL, USA). Prior to each leaf measurement the instrument was referenced to dark and light standards. Again, reflectance measurements were made on the upper surface of a minimum of ten leaves for each species, which were taken from the same pool as those used for T_{50}. Visible and near infrared reflectance were quantified as the average reflectance across wavelengths spanning 400 – 700 nm and 750 – 1000 nm, respectively.

5.3.3 Data analysis

All data analyses were carried out using the statistical software IBM SPSS® (v23). Principal component analysis (PCA) is a variable reduction technique that reduces multidimensional data to a smaller number of artificial variables, called principle components or axes, with minimal loss of information (Shang, 2014). PCA is useful when data from numerous variables have been obtained for many observations or subjects (Smith, 2002) and for identifying patterns of similarities among various groups of variables (Smith, 2002; Field, 2009). Here we used PCA for the detection of potential leaf trait syndromes relating to thermal protection among a cross-section of Australian southern arid-zone plants. To assess short-term intra-annual temporal variability of multi-trait patterns among these species, individual PCAs were conducted for trait data measured in each of the three seasons. Note that one species, *Sida ammophila*, was excluded from summer analyses due to missing morphological data ($\therefore n = 41$). PCA proceeded as follows. The ratio of the number of species sampled to number of variables was used as an indication of sample size adequacy (de Winter *et al.*, 2009) and here ranged from ~ 3:1 to ~ 6:1 seasonally (winter, 23/7; spring, 22/7; summer, 41/7, respectively). To ensure PCA produced stable solutions, the following conditions were also met: extracted components were checked for simple structure (i.e., that they had minimal to no complex variables), and few variables achieved low communalities (< 0.3 – 0.4, (Stevens, 2012; Leech *et al.*, 2014)). We also placed high importance on the interpretability of components retained in light of whether it would be biologically reasonable to expect loaded variables to co-occur (Lawless & Heymann, 2010; Garson, 2013a). Because data represented variables with different measurement scales and units, prior to running PCA all leaf trait data were standardised by *z*–transformation, i.e., data were centred to zero mean with a unit variance of 1 (Jongman *et al.*, 1995; Abdi & Williams, 2010). Next, the Bartlett's test of sphericity within each season was conducted, which found that variables were adequately correlated ($P < 0.05$), indicating data were suitable for PCA (Stevens, 2012). To determine the number of principal components to retain for the three seasonal datasets, we utilised an online parallel analysis engine (Patil *et al.*, 2007; Patil *et al.*, 2008). Compared with more commonly used methods, parallel analysis is considered one of the more robust and accurate factor-extraction approaches (Matsunaga, 2010). For all three seasons, results of these analyses indicated that two principal components should be extracted. Next, to maximise component interpretability, we sought to achieve matrix solutions yielding simple structure, where variables load highly on a single component with little to no cross-loading, by submitting the initial un-rotated matrix solutions to an oblique rotation, in this case direct oblimin (Fabrigar *et al.*, 1999; Osborne, 2015). The resulting pattern matrices, which list the variable loadings on each retained axis (Osborne & Costello, 2009), were used to interpret components from the three seasonal datasets.

For a given principal component, variables with high positive loading (> 0.6) indicate a strong correlation with the component and explain a large proportion of the variation among species for that axis. Traits with strong negative loadings (> -0.6) also explain a large proportion of the variation among species for that axis, but in the opposite direction to positively loaded traits (Quinn & Keough, 2002). Here, a moderate loading magnitude of ± 0.4 was applied as the minimum cut-off criterion for which a variable was deemed to contribute meaningfully to a component (Quinn & Keough, 2002). Complex variables were defined as those cross-loading on more than one component (Beavers *et al.*, 2013) at values near or above ± 0.4. As complex variables can be difficult to interpret, the following additional criteria were applied to simplify their allocation to a component. Firstly, to determine which component loaded most clearly on a complex variable, we considered the difference between its primary (i.e., the component loading most highly on the variable) and secondary loadings. Complex variables exhibiting sufficiently large loading discrepancies (e.g., 0.3 – 0.4) were considered to contribute in a meaningful way to the primary component (Matsunaga, 2010). Where more than one component loaded similarly on a particular variable, we considered if the result made sense conceptually. That is, whether the cross-loading occurred because the offending variable was genuinely applicable to both components in a biological context, reflecting two independent patterns of variation (Acton *et al.*, 2009).

Component scores were extracted for two principal components (PC1, PC2) in each of the three seasons, creating six composite dependent variables for use in subsequent analyses. To investigate if preferred native microhabitat functioned as a good predictor of species' placement along the extracted principal axes, analysis of variance (ANOVA) was carried out within each season. Where data violated the Levene's assumption of homogeneity, Welch's ANOVA was used, with post-hoc comparisons based on the Games-Howel test, suitable for unequal sample sizes (Field, 2009). To determine if phylogeny was influencing patterns of significance between microhabitat preference, we also ran the analyses with a subset of phylogenetically independent species contrasts (PICs) of congeneric and confamilial pairs. Contrasts were for relatively more mesic- *vs* xeric-adapted species. Possible pairings, where the first of each potential scenario represents a more mesic-adapted species were as follows: 1) W_{high} with $W_{low,}$ 2) W_{high} with W_{var} and 3) W_{var} with W_{low}. Seasonal differences in PCA scores between PICs were analysed using paired *t*-tests (Thesis Chapter 4).

5.4 RESULTS

5.4.1 Species' characteristics in multivariate trait space

Standardised PCA with complementary parallel analysis produced two principal components per season (Table 5.1, Fig. 5.1a – c, Supporting information Fig. S5.1). Collectively, these two principal components (PC1 and PC2) explained 57.7%, 65.7% and 52.2% of the total variability of the original data in winter, spring and summer, respectively (Table 5.1). The first axis explained 34.4% (winter), 41.9% (spring), and 20.0% (summer), while the second axis explained 23.3%, 23.8%, and 22.2% of the variance, respectively. In winter, strong loadings along PC1 represented species characterised by low per cent leaf water content and high leaf mass per area, $T_{50,}$ and NIR (Table 5.1). Winter PC2 loadings represented species characterised by wide, thin, low LMA leaves that were highly reflective of visible wavelengths (VIS) (Table 5.1). Given its much larger loading onto PC1 than PC2 in winter (contrast PC1, 0.738; PC2, -0.418), the primary loading for LMA was ascribed to PC1. For spring, PCA patterns were similar to those observed for winter. Namely, PC1 was negatively associated with per cent leaf water content and positively associated with LMA, NIR and T_{50}, but spring PC1 differed by also loading highly on leaf thickness (Table 5.1). Spring PC2 was positively associated with VIS and leaf width and cross-loaded on T_{50} (Table 5.1). The primary-secondary difference in this instance was < 0.3, meaning the allocation of T_{50} to either component was less clear. Although the difference fell short of being ≥ 0.3, the higher primary loading of 0.561 suggests T_{50} is slightly

Table 5.1 Variable component loadings along PC1 and PC2 for winter, spring, and summer PCA. Moderate to high loadings in bold, complex variables appear in italics if 1) they cross-load at or near the cut-off criterion of ± 0.4 and 2) their primary-secondary difference is small (< 0.3), making clear placement to either component difficult. Initial eigenvalues, variation explained by each principal component, and communalities listed. See Methods for detailed descriptions of selection criteria and leaf traits. Descriptive statistics for structural leaf traits are provided in Supporting information, Table S5.2.

	PC1 variable component loadings			PC2 variable component loadings			Communalities		
Traits	winter	spring	summer	winter	spring	summer	winter	spring	summer
Leaf mass per area (LMA), g/m	**.738**	**.921**	**.873**	**-.418**	-.113	-.052	.740	.856	.751
Effective leaf width (LW), mm	-.254	-.264	**-.593**	**.766**	**.792**	.088	.666	.688	.344
Leaf thickness (LT), mm	-.006	**.862**	**.625**	**-.546**	.034	.135	.298	.746	.433
Near infrared reflectance (NIR), %	**.665**	**.623**	.079	.059	.105	**.784**	.444	.402	.638
Thermal damage threshold (T_{50}), °C	**.682**	***.561***	*.342*	.118	***.447***	***.441***	.474	.525	.355
Visible reflectance (VIS), %	.339	.155	-.257	**.772**	**.840**	**.913**	.692	.735	.833
Leaf water content (WC), %	**-.851**	**-.740**	**-.550**	-.002	.334	.017	.723	.649	.300
Initial Eigenvalues	2.407	2.935	2.098	1.629	1.665	1.556			
Variation explained (%)	34.384	41.931	29.972	23.275	23.791	22.228			

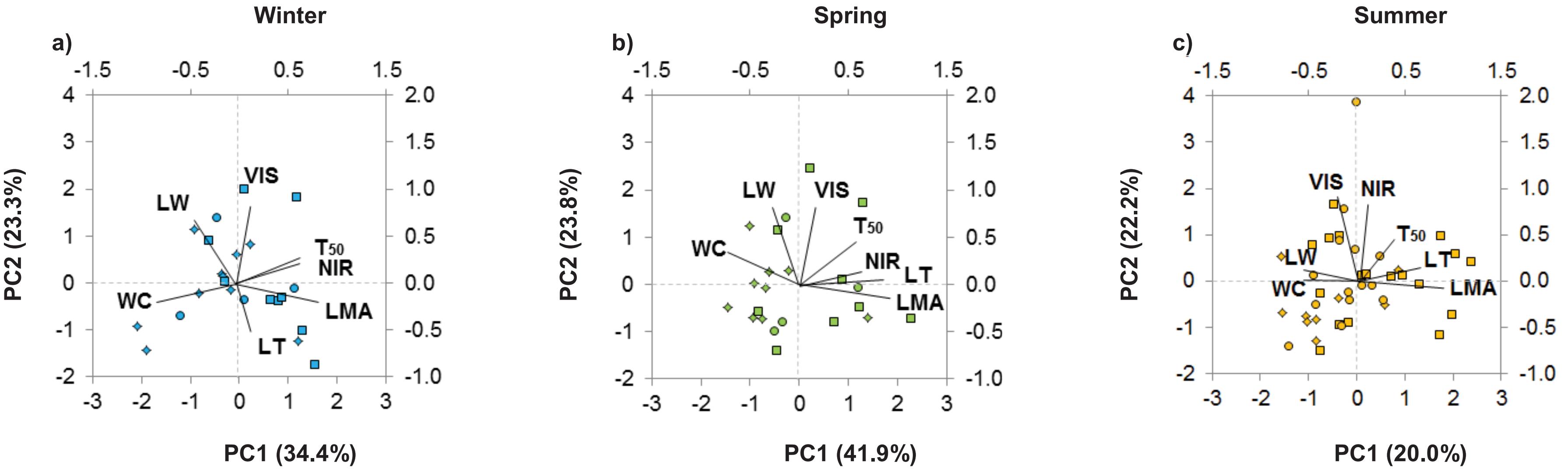

Figure 5.1 Seasonal projections of plant species grouped by preferred native microhabitat on the plane defined by principal component axes (PC) 1 and 2. Diamond symbol, W_{high}; round symbols, W_{var}; Square symbols, W_{low} **(a – c)**. Solid lines indicate direction and weighing of vectors representing the seven traits considered: Leaf thickness, LT; leaf mass per area, LMA; near infrared reflectance, NIR; thermal damage threshold, T_{50}; visible reflectance, VIS; effective leaf width, LW; water content, WC. Per cent variance explained by each axis indicated.

more closely correlated with variables of PC1 than those of PC2. Nevertheless, the small difference (~ 0.1) in component loadings on T_{50} suggests that both axes explain the variance observed for T_{50} (Beavers *et al.*, 2013). For summer, PCA yielded some similarities as well as considerable differences to winter and spring. Similar to winter and spring, the summer PC1 was negatively associated with per cent leaf water content and positively associated with LMA, while also sharing a loading for thick leaves with the spring PC1. Contrasting the cooler seasons, however, summer PC1 was negatively associated with leaf width and had a weak positive loading on T_{50} (Table 5.1). Spectral properties again dominated the second axis, with the summer PC2 being strongly and positively associated with high reflectance in both VIS and NIR, and loading moderately on T_{50} (Table 5.1).

5.4. 2 Relating trait syndromes to species' preferred native microhabitat

To determine if species grouped by microhabitat preference showed significant differences in their trait syndromes, season-based PCA component scores were subjected to individual analysis of variance. Along the first principal axis, the three microhabitat groups emerged to form a spectrum of variation moving progressively from xeric species at the positive end to more mesic species at the negative end: W_{low} to W_{var} to W_{high} (Fig. 5.2a – c). The difference between W_{low} and W_{high} species was statistically significant, a result that was consistent for PC1 in each season (Table 5.2). In winter, W_{high} species were characterised by leaves with relatively low LMA, T_{50}, and NIR, but high leaf water content, contrasting W_{low} species, which had the opposite leaf characteristics (Fig. 5.2a). Similar leaf traits characterised W_{high} species along the spring PC1, but thicker leaves also distinguished W_{low} species from W_{high} species (Fig. 5.2b). Along PC1 in summer, W_{high} species had wider, thinner leaves, with comparatively higher leaf water contents and lower LMA relative to W_{low} species (Fig. 5.2c). Similar to winter and spring PC1, W_{high} species on the summer PC1 have lower T_{50} than species positioned at the positive end of the spectrum. In contrast to PC1, seasonal results for PC2 did not clearly distinguish among native microhabitat (Table 5.2; Fig. 5.2d – f).

Results from phylogenetically independent contrasts mirror these observations from the overall dataset. Specifically, the average component scores of the more xeric PIC

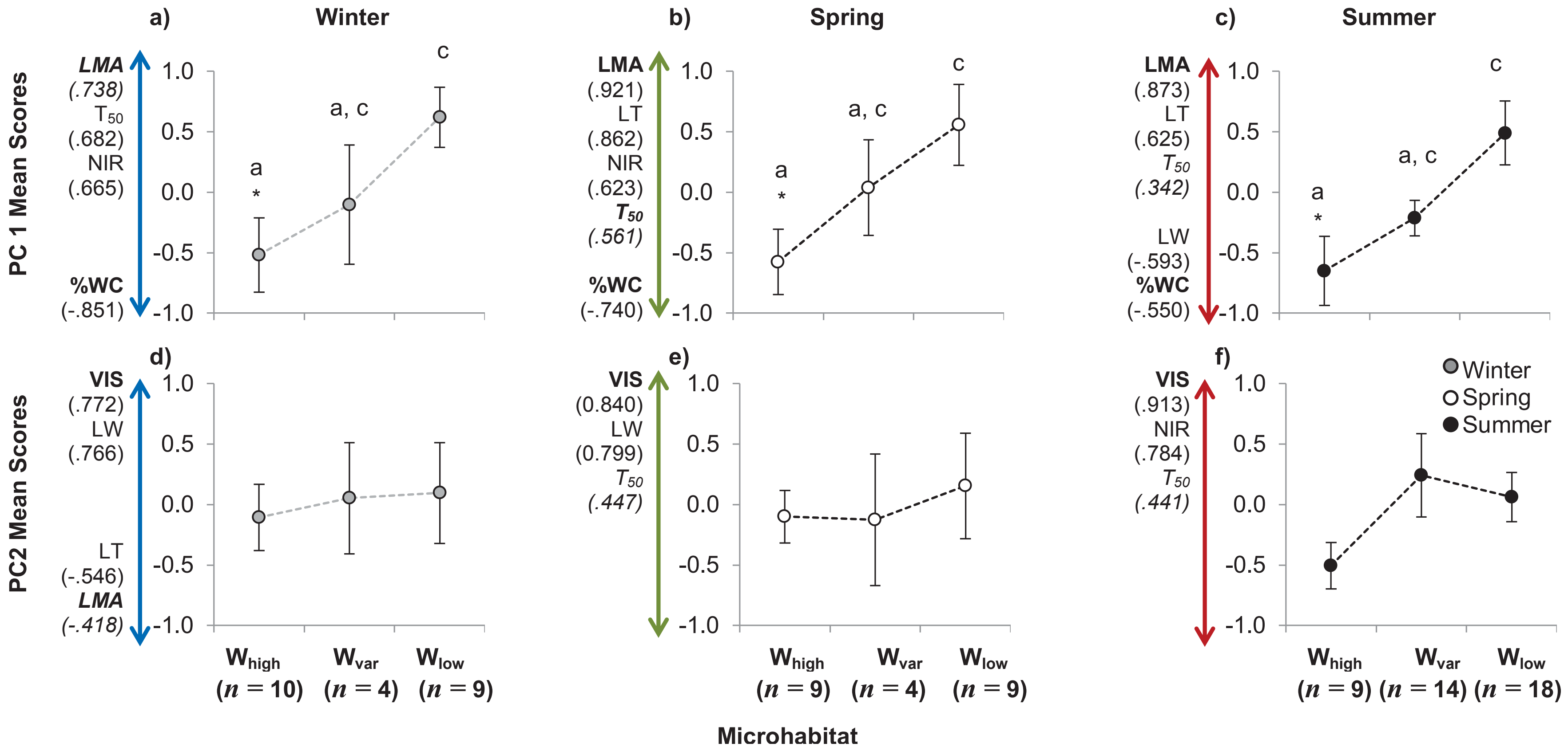

Figure 5.2 Mean seasonal (winter = 23 spp., spring = 22 spp., summer = 41 spp.) score distributions along the first **(a – c)** and second **(d – f)** principal components (PC1, PC2). Species grouped by preferred native microhabitat based on water availability W_{high}, W_{var}, W_{low}. Variables loading moderately to highly (≥ ± 0.4) on each axis are presented to the left of each graph (see Table 5.1 for description of variables). Variables in bold consistently load across all seasons for a given PC axis. Variables in italics cross-load, having moderate loadings on both PC axes within a given season (≥ ± 0.4). Data points with different letters above differed significantly at * $P < 0.05$. Component loadings between ± 0.4 and ± 0.6 are moderate in strength, with values above and below considered weak and strong, respectively (see Methods).

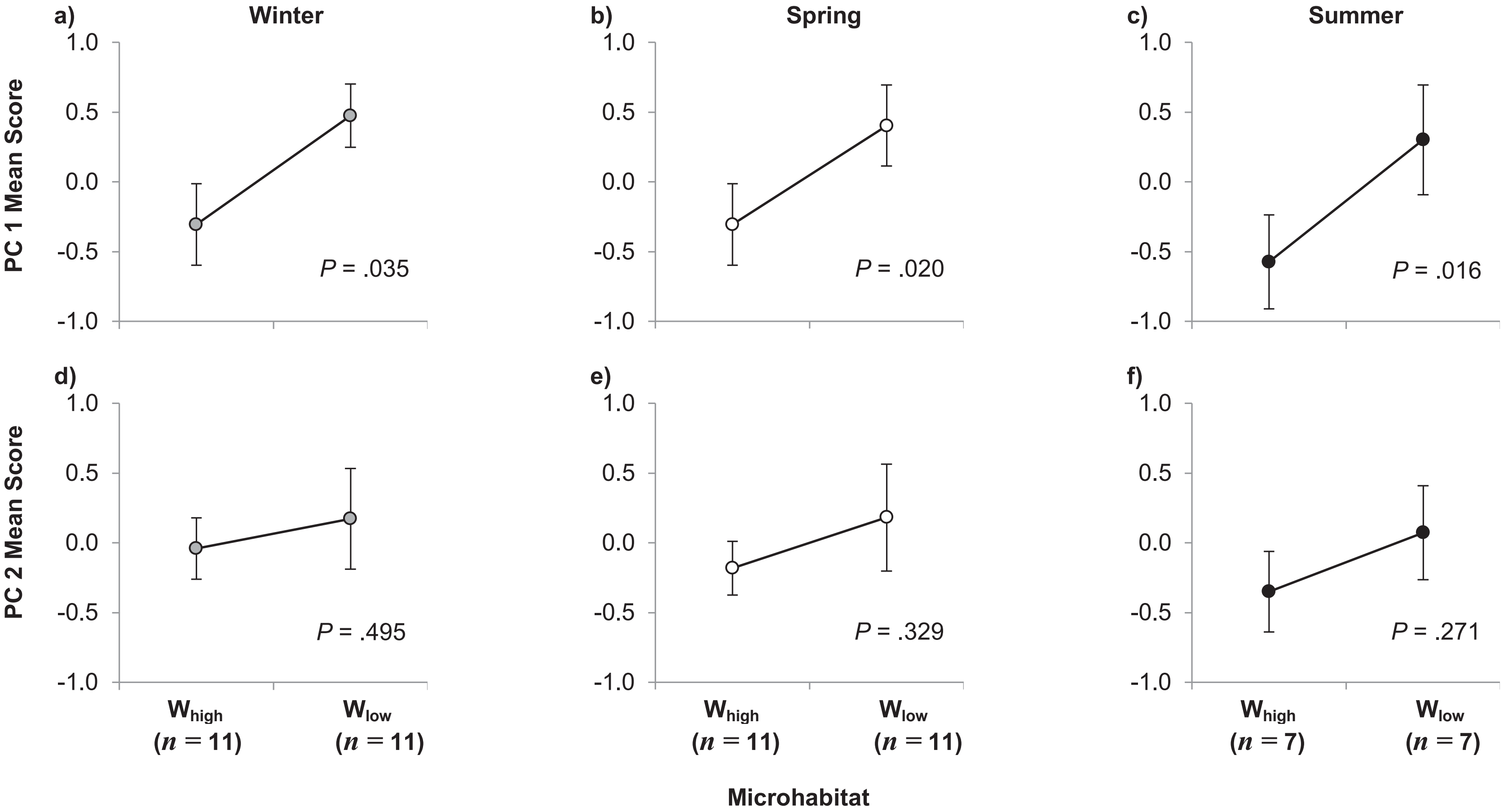

Figure 5.3 Mean seasonal score distributions along the first **(a – c)** and second **(d – f)** principal components (PC1, PC2) for phylogenetically independent species contrasts. Species contrasted on preferred native microhabitat based on water availability, being mesic-adapted or xeric-adapted species, respectively. Results of paired *t*-test provided inset ($\alpha = .05$). Refer to Fig. 5.1. for list of variables loading highly on each axis.

Table 5.2. Seasonal ANOVA for differences among three microhabitats based on species' native water availabilities: W_{high}, W_{var}, W_{low} (see Methods). Results are for traditional F-tests with the exception of summer PC1, which was based on Welch's F-test ($\alpha = 0.05$). Results correspond with Fig. 5.2.

Season	PC1	PC2
Winter	$F_{(2,20)} = 3.914$ (***P* = .037**)	$F_{(2,20)} = 0.096$ (*P* = .909)
Spring	$F_{(2,19)} = 3.612$ (***P* = .047**)	$F_{(2,19)} = 0.171$ (*P* = .844)
Summer	$F_{(2,19.76)} = 4.396$ (***P* = .026**)	$F_{(2,38)} = 1.648$ (*P* = .206)

members were statistically and significantly higher than their mesic counterparts along PC1, but differences were not statistically significant along PC2 (Fig. 5.3). For brevity, and because the outcome for all species *versus* PICs were similar, both are discussed in the same light below.

5.5 DISCUSSION

Extensive variation within a given trait or group of traits can suggest the presence of multiple ecological strategies among species for coping in an environment (Westoby & Wright, 2006; Stahl *et al.*, 2013). In terms of abiotic stress, it is generally accepted that plants cope in one of two ways: they endure or evade (Osmond *et al.*, 1987). In understanding plant-environment interactions it is therefore valuable to recognise the various strategies utilised and the key traits associated with these strategies (Lavorel & Garnier, 2002). Ultimately, the strategies plants employ can influence species' persistence, community composition, and critically can affect broader ecosystem processes (Pierce *et al.*, 2005; Grime, 2006; Aitken *et al.*, 2008). Among the desert plants in the current study, we found two clear axes of trait variation, with many key traits characterising each axis being consistent among season (Fig. 5.1, 5.4, Fig. S5.1). Notably, the first principal axis consistently linked physiological thermal tolerance with the leaf economics spectrum (LES) via leaf mass per area (LMA). Traits aligned with PC2 were seasonally more variable, but suggest that spectral reflectance properties play a key, independent role in leaf thermal protection processes for these desert species. We suggest that these two components represent different thermal protection strategies, the importance of which we highlight here.

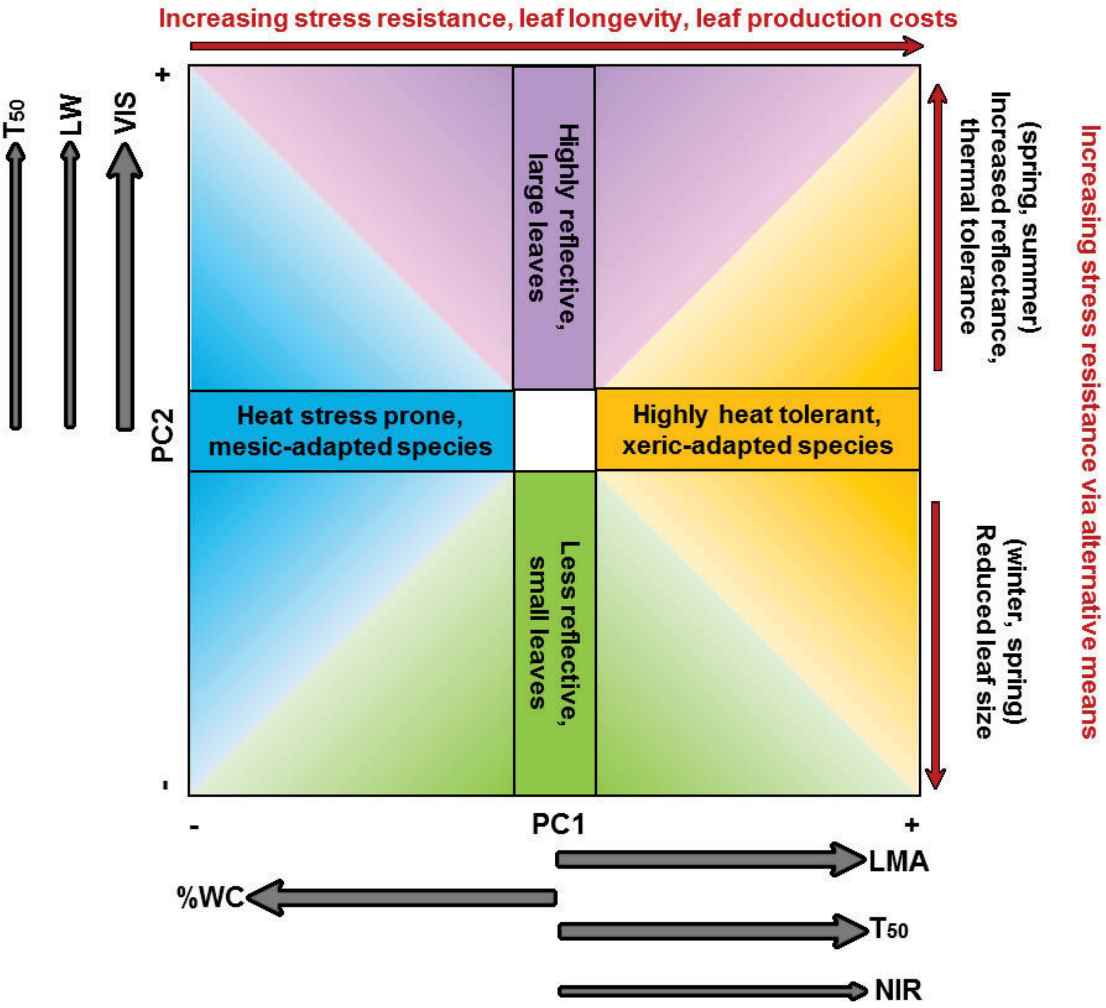

Figure 5.4 Thermal protection strategies among arid-zone plant species fell along two principal component (PC) axes. Microhabitat preference successfully predicted species' placement along PC1. Xeric-adapted species had higher LMA and T_{50} and lower leaf water contents than their mesic counterparts. PC2 was consistently driven by variation in visible reflectance, and somewhat by leaf size (winter, spring) and T_{50} (spring, summer) but was independent of microhabitat. The strong association of LMA on PC1 suggests a strategy relating to protecting long lived leaves; whereas thermal protection described by PC2 is independent of LMA and the leaf economics spectrum. Solid black arrows indicate the direction and strength of leaf traits loading highly on each axis: 1) For a given principal component, variables with high positive loading indicate a strong correlation with the component and explain a large proportion of the variation among species for that axis. Traits with strong negative loadings also explain a large proportion of the variation among species for that axis, but in the opposite direction to positively loaded traits. 2) Greater arrow thickness indicates a comparatively higher loaded variable. 3) Variables depicted further away from the axis have loadings that become progressively weaker as indicated by the reduced arrow thickness. LMA, leaf mass per area; %WC, per cent water content; T_{50}, leaf thermal damage threshold; NIR, near infrared reflectance; VIS, visible reflectance; LW, effective leaf width. For seasonal results from the present study see, Fig. 5.1.

5.5.1 Heat stress resistance strategies among desert plants

5.5.1.1 PC1: Co-occurrence of leaf traits conferring increased structural and physiological thermal protection places species along a heat stress resistance axis, with links to the LES.

Considering the consortium of leaf traits driving PC1, we suggest that this component represents a spectrum of stress resistance. We propose that, independent of phylogeny, the three native microhabitat groups along that spectrum identify contrasting plant functional types (Pla *et al.*, 2012), ranging from highly heat tolerant, xeric-adapted species at the positive end to more heat stress prone, mesic-adapted species at the negative end (Fig. 5.2, Fig. 5.4). At the positive end of the spectrum, xeric-adapted species invest in traits that increase resistance to structural damage and physiological damage from heat stress. By contrast, mesic-adapted species at the opposite end of the spectrum appear less resistant to damage. Due to the strong and consistent association on this spectrum of LMA, a central trait of the leaf economics spectrum, the present results provide a quantitative link between structural and physiological thermal protection traits and plant resource use and acquisition strategies (Michaletz *et al.*, 2015; Michaletz *et al.*, 2016).

Together with leaf thickness, LMA is typically discussed in light of its role in enhancing leaf structural strength and increasing leaf resistance to physical damage, including from herbivory (Witkowski & Lamont, 1991; Castro *et al.*, 2000; Wright & Cannon, 2001; Groom & Lamont, 2015). Apart from its association with the leaf economics spectrum, LMA also is an important trait influencing leaf thermal dynamics through buffering against rapid fluctuations in leaf temperature (Leigh *et al.*, 2012; Michaletz *et al.*, 2015). Rarely is this trait explored with respect to physiological thermal protection. The positive relationship between LMA and thermal tolerance in the current study contrasts with the results of some that have shown thermal tolerance to be independent of LMA (e.g., woody savanna species, Zhang *et al.*, 2012), but is in agreement with the results of others (e.g., desert and coastal species, Knight & Ackerly, 2003). These apparently contradictory results likely represent differences in context, with respect to both environment and functional strategies of species. Zhang *et al.* (2012) investigated deciduous woody species, where leaf ages were comparably short, whereas the current study and that of Knight and Ackerly (2003) comprise desert and coastal species, including leaf ages of several years. Here, we propose that for xeric-adapted species at the high LMA-T_{50} end of PC1 (Fig. 5.2a – c), thick, tough leaves may play a multifaceted role: protecting leaves against not only structural, but also thermal damage. To protect against the effects of heat stress, xeric-adapted species thus appear to have evolved an arsenal of defences for reducing heat stress: investing both in structural and physiological traits conferring higher levels of protection. The involvement of LMA on this axis also suggests that species typically investing more in protection against thermal damage have long-lived leaves compared with other species (Wright *et al.*, 2004). This makes sense as well-protected leaves would likely be more costly to make and physiologically protect (Sharkey & Yeh, 2001; Al-Whaibi, 2011), requiring longer turn-over times for debt recovery than a less protected leaf. In an arid environment, well-protected leaves may be particularly important for a long-lived species, which must also often contend with nutrient-poor soils (Beadle, 1954). A well-protected leaf would be an unnecessary investment for comparatively mesic-adapted species situated at the low LMA-T_{50} end of the spectrum, which we suggest utilise a live-fast-die-young strategy for evading stressful conditions, and rely on favourable conditions for growth.

5.5.1.2 PC2: Spectral reflectance – an alternative strategy for heat stress resistance among desert plants

The first noteworthy association along PC2 is the increase in visible reflectance with an increase in leaf width for species at the positive end of the spectrum in winter and spring (Table 5.1). Although summer might be expected to be most stressful for plants in terms of high temperatures, for large-leafed species, a bright sunny day during the cooler months can still present conditions for heat stress, exacerbated by numerous factors including proximity to the ground, water stress or greater albedo from lack of plant cover. We suggest PC2 represents a secondary heat stress resistance strategy that is independent of PC1 and the leaf economics spectrum. Unlike PC1, species along the PC2 axis did not separate into microhabitat groups. Regardless of where they are situated in the landscape, desert plants all tend to be subjected to high levels of radiation and therefore have a high chance of photoinhibition. High temperatures exacerbate the adverse effects of photoinhibition by interfering with repair processes, potentially leading to photodamage (Powles, 1984; Murata *et al.*, 2007). For large-leafed species with higher leaf temperatures, greater spectral reflectance would be a strategic advantage. Species situated at the low-end of the winter and spring PC2 may utilise an alternate strategy, benefiting from smaller leaves that track ambient temperatures more closely than large leaves. Transitioning from winter to summer, at the positive end of PC2, visible reflectance remains strong, but leaf size was replaced by near infrared reflectance and T_{50} as leading variables. A closer look at the data suggests that this replacement was driven by a number of factors. First was an increase in the number of species sampled in summer, the larger dataset capturing greater interspecific variation for each leaf trait. Second was the absence of ephemeral and facultatively deciduous species in summer, some of which had large leaves. The third, and apparently strongest driver of this change in leaf size in our data, was a marked reduction in leaf size in many species from winter and spring to summer, while spectral reflectance significantly increased over the course of the year (Fig. S5.2). For long-lived desert species, increasing reflectance in the near infrared likely represents a greater need to reduce these thermal wavelengths in summer. On the other hand, the greater prominence of T_{50} suggests that mediating high temperatures via morphological means alone is not enough to protect leaves from incurring high-temperature damage at this time of year. Indeed, the effectiveness of structural leaf traits for maintaining leaf temperatures below critical limits varies with environmental conditions (Ehleringer & Björkman, 1978; O'Sullivan *et al.*, 2017). Especially considering that all the species we sampled are likely to at some point experience considerable heat stress, the benefit of investing in higher physiological thermal tolerance under such conditions may therefore likely exceed its cost.

5.5.2 T_{50}: a complex variable

For spring and summer, both PC1 and PC2 loaded onto T_{50}. The physiological stress response is ubiquitous among all organisms (Kristensen *et al.*, 2002). The highly conserved nature of the heat stress response, for example through the production of HSPs, suggests that it is a fundamental and essential process (Vierling, 1991). As well as being important for thermal tolerance, HSPs also play a pivotal role in cell maintenance and function under normal growth conditions (Wang *et al.*, 2004; Asea *et al.*, 2016). The cross-loading observed for T_{50} may reflect these distant origins of stress response, such that all species are expected to exhibit a degree of physiological stress resistance. What is most interesting here is the clear clustering of T_{50} with certain leaf-level morphological traits along two different axes, suggesting two unique thermal tolerance strategies among this desert flora (Fig. 5.4). That is, species complement innate physiological thermal tolerance with two alternative leaf-level morphological pathways of thermal protection. The first strategy couples physiological protection with traits that can increase structural strength, but which also have a role in buffering leaves against reaching high temperatures. The second strategy couples spectral traits with physiological protection during warmer periods of the year. Both axes describe a spectrum of thermal protection or resistance, which, especially in summer, places less protected species at one end and highly protected species at the other.

5.5.3 Summary and ecological implications

That plant species appear to utilise numerous independent heat stress resistance strategies makes intuitive sense when you consider fine-scale landscape variability (McLaughlin *et al.*, 2017). Although average ambient temperature in a given desert region may be comparable, deserts are a complex network of ecologically distinct microhabitats permitting a diversity of vegetation communities with varying functional responses and strategies (Noy-Meir, 1973; Ward et al., 1993; Tongway and Ludwig, 1994; Tewksbury and Lloyd, 2001; Schwinning and Ehleringer, 2001; von Willert, 1992). Yet accounts of desert flora, and in particular their leaf attributes, remain fairly consistent, with depictions of small, thick, tough or succulent leaves overwhelmingly common (Fonseca et al., 2000; Niinemets, 2001; Turner, 1994; Givnish, 1987; e.g., Smith, 1978). Certainly, many desert plants do exhibit such leaf traits, but as shown here and elsewhere, they are by no means characteristic of all arid-zone species (Table 2; see also Curtis et al 2012). Indeed, trait variation can be even greater within communities than among them (Poorter *et al.*, 2009; Freschet *et al.*, 2011). Our results are consistent with studies showing that, even within the same environment, trait variation among species is considerable, reflecting the repertoire of strategies plants engage to manage diverse growth conditions (Diaz *et al.*, 2004). That these results for all species were echoed in our subset of PICs suggests that the ability to use these strategies is independent of any phylogenetic links with trait variation. That is, these strategies are not phylogenetically constrained; rather species from a variety of lineages at various phylogenetic 'depths' can employ these defence strategies, suggesting that these abilities are deeply rooted. Our findings expand the well-established notion that the diversity of plant traits among species can be represented by a continuous distribution of traits characterised by contrasting states of resource use, capture, and availability (the LES: Reich *et al.*, 1997; Westoby *et al.*, 2002; Wright *et al.*, 2004) to include leaf level traits conferring increased thermal tolerance. The above finding is in contrast with results from a recent study, which investigated high-temperature tolerance of plant species across biomes (O'Sullivan et al. 2017). The authors concluded that species' thermal environment was a predictor of their thermal limits, while leaf traits such as SLA, leaf nitrogen and phosphorous could not improve our ability to predict variability in high-temperature tolerance. The apparent dichotomy in findings could be due to a number of logistical and statistical design differences between the current study and that of O'Sullivan et al (2017). First, sampling of all Australian southern hemisphere species in that study was spread across the southern hemisphere seasons of winter, autumn, spring and summer (see Table 1 in O'Sullivan et al 2017). The extensive range of sampling times may have confused any potential relationship between species' high-temperature tolerance and leaf structural/chemical composition traits, which may vary seasonally, as highlighted in this thesis. Second, species composition varies between studies, with the most notable difference being the inclusion of both evergreen and deciduous species in the study of O'Sullivan et al (2017), while I compare evergreen species only. These design and logistical differences may have contributed to the contrary findings of these two studies. For the purpose of predicting species' thermal response, it therefore is important to consider if the given trait data compare species within a single season, functional type or even biome.

A critical prerequisite for making accurate model predictions of ecosystem responses to environmental change are comprehensive studies that describe functional trait diversity and their relationships across species, time and space; yet we lack this level of detail for most ecosystems (Violle *et al.*, 2014). The clear quantifiable differences in trait spectra for 47 species across time and space presented in the current study thus have the potential to refine models aiming to understand processes shaping community dynamics. In particular, the suggestion of an axis of trait variation that is centred on easily measured leaf traits inferring differences in resource use and stress resistance, could serve as a tool for understanding responses of species – and groups of species – to climate warming. Importantly, these findings also stress the influence of measurement timing on species' thermal responses and trait expression, which must be considered when attempting to interpret or generalise results across species. Finally, we suggest that species that require a more reliable source of water for growth, and with innately lower physiological thermal tolerance limits will be most at risk from changing climatic conditions.

Chapter 6

Intracanopy adjustment of leaf-level thermal tolerance is associated with microclimatic variation across the canopy of *Acacia papyrocarpa* Benth.

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Keywords: Canopy microclimate; desert plants; thermal damage thresholds; aspect; height from ground; plasticity.

6.1 ABSTRACT

Aims: This study investigated whether microclimatic variation across the canopy of a dominant tree species of Australia's southern arid-zone may result in localised variation in leaf thermotolerance. We asked: 1) to what extent does the microclimate vary within the canopy of *Acacia papyrocarpa* Benth? and 2) do these microclimatic differences equate to varied thermal tolerances among canopy positions of height and/ or aspect?

Location: The Australian Arid Lands Botanic Garden, Port Augusta, South Australia.

Methods: We measured four microclimatic variables that influence canopy temperature at four canopy positions contrasting in height and aspect: upper north, upper south, lower north, and lower south. Microclimatic variables were air temperature, vapour pressure deficit (VPD), relative humidity and wind speed. Principal component analysis was used to generate a composite climatic stress variable (C_{STRESS}) from temperature, VPD, and humidity variables. To provide an indication of how closely leaf temperatures might track ambient temperature, we also predicted the thermal time constant (τ) for each canopy position. The leaf-level thermal damage threshold, $T_{50,}$ was measured for leaves at the same four canopy positions. ANOVA was used to compare microclimatic differences among canopy positions. Generalised linear models were used to investigate the influence of height and aspect on $T_{50,}$ while accounting for C_{STRESS} and predicted τ as potential drivers of T_{50} variation.

Results: Temperature and VPD were higher and humidity was lower in north-facing, lower-canopy positions than elsewhere in the canopy, resulting in significantly higher C_{STRESS} at lower-canopy than upper-canopy positions. Differences in wind speed with height resulted in significantly longer predicted τ for leaves positioned at lower, north-facing positions. The combinations of height with C_{STRESS}, and aspect with predicted τ, were significant drivers of localised T_{50} variation. Namely, T_{50} was highest for leaves in the more environmentally stressful lower and north-facing canopy positions.

Conclusions: Intracanopy T_{50} variation was driven by differences in microclimatic conditions across the canopy of *A. papyrocarpa*. These findings suggest that this species optimises resources to protect against thermal damage at a whole plant level. If such variation were found to be consistent across a range of dominant species, this information could help to refine models of carbon flux dynamics at an ecosystem-level.

6.2 INTRODUCTION

Temperature is one of the most influential climate variables driving the physiological responses of plants (Hikosaka *et al.*, 2006; Teskey *et al.*, 2015). Outside of their optimum thermal range, plants experience thermal stress, which can impair growth, survival and reproductive output (Pearcy *et al.*, 1987; Bauerle *et al.*, 2007; Laisk & Nedbal, 2009). In many regions, an increase in the frequency and intensity of maximum and minimum temperatures are expected under climate change (IPCC, 2014). Conditions eliciting heat stress in plants are therefore likely to occur more frequently and will be longer lasting than current episodes of stress (Teskey *et al.*, 2015). The photosynthetic machinery within the leaves of plants, in particular, photosystem II (PSII), is especially sensitive to thermal change (Havaux *et al.*, 1991; Georgieva & Yordanov, 1994; Schrader *et al.*, 2004). Measures of a plant's physiological thermal damage threshold can be a useful index for gauging high-temperature tolerance. The thermal damage threshold is the temperature causing a 50% decline in maximum quantum yield of PSII, T_{50}; corresponding to the onset of irreparable thermal damage (Knight & Ackerly, 2003; Curtis *et al.*, 2014). The critical thermal threshold of plants is highly dynamic and varies, not only with species, but also through time and space (Knight & Ackerly, 2003; Curtis *et al.*, 2016; thesis Chapter 4). Spatial variation reflects adaptation to a particular thermal environment represented by different biomes (Knight & Ackerly, 2003) and microhabitats within biomes (Curtis *et al.*, 2016). What is not known is whether leaf thermal thresholds are influenced by finer scale environmental changes; for example, within a single plant canopy.

Tree crowns are spatially heterogeneous, sometimes resulting in significant variation in the microclimate of individual leaves across a given canopy. Incident sunlight is highest for equatorial-facing foliage; whereas wind speed, air temperature and sunlight typically increase, and humidity decreases along a vertical profile from the bottom to the top of a canopy (Russell *et al.*, 1990; Niinemets & Valladares, 2004). Therefore, in the southern hemisphere, upper-canopy and north-facing foliage is expected to be exposed to higher average air temperatures and greater vapour pressure deficits (VPD) than foliage elsewhere in the canopy (Eamus *et al.*, 2006; Laisk & Nedbal, 2009; Niinemets, 2012). Because wind speed also strongly affects the thermal environment of a leaf, it too is an important factor influencing leaf temperature (Niinemets *et al.*, 1999). Even gentle wind speeds, e.g., above 0.5 m s^{-1}, are sufficient to disturb the leaf boundary layer and increase the rate at which heat is transferred away from the surface of a leaf via convection. Yet wind speed is highly dynamic, fluctuating on the order of seconds (Vogel, 2009). When air movement drops, leaf temperatures may increase rapidly (Leigh *et al.*, 2012). The latter scenario is more likely if the thermal time constant of a leaf is short and/or if a protracted lull in wind speed is coupled with conditions of high light and/or high temperature. The diversity of fine-scale environmental conditions drives significant intracanopy variation in leaf morphology and can profoundly influence leaf-level physiological and developmental processes (Zwieniecki *et al.*, 2004; Bauerle *et al.*, 2007; Niinemets, 2007). For example, due to microclimatic changes in light, temperature and VPD, leaf photosynthetic capacity can vary two- to four-fold along a vertical gradient within a canopy (Meir *et al.*, 2002; Niinemets, 2012) and transpiration rates can vary among branches (Frak *et al.*, 2002; Zweifel *et al.*, 2002). Documenting gradients in leaf-level responses to changes in the microclimate has contributed predictive insight into a range of processes that ultimately effect whole plant productivity, e.g., the influence of light on leaf development (Niinemets, 2007), variability of water transport (Zwieniecki *et al.*, 2004) and photosynthetic carbon gain and respiratory carbon release from leaves (Niinemets, 2007; Laisk & Nedbal, 2009).

Profiling of within-canopy variation in leaf traits is often done in vegetation communities with small inter-crown gaps (e.g., dense, closed forests) or contrasts inner and outer canopy positions. These studies frequently employ the dichotomy in light environments across a canopy to explain intracanopy leaf trait variation, e.g., sun *versus* shade leaves (Pearcy *et al.*, 1990; Niinemets & Valladares, 2004; Laisk & Nedbal, 2009). In arid environments, where individual trees are widely spaced, the within-plant canopy microclimate profile can vary considerably from that of closed-canopy communities. Typically, air temperature decreases rapidly with height above ground in a desert environment, due to intense, unabating solar radiation (Whitford, 2002). For Australian deserts in summer, for example, mean maxima near-surface air temperatures of 65 – 70 °C have been recorded (Mott, 1972; Cook et al. unpublished data). In contrast with the extreme temperatures measured at the soil-air interface, at two meters above the ground, ambient air temperature can be as much as 20 °C lower (e.g., Sonoron Desert, Fig. 6.6 in Warner, 2009). The high thermal loading of exposed surface soils drastically alters the thermal environment for near-surface vegetation (Warner,

2009) and can contribute as much as 10 – 30% of a canopy's total energy budget in hot, dry, arid environments (Eamus *et al.*, 2006). In these environments, high surface temperatures often are coupled with naturally low soil water, which lead to high VPD (Macinnis-Ng & Eamus, 2009). A common consequence of the combined high temperatures, high VPD and an inadequate source of available water, typical of deserts, is that plants will limit stomatal conductance. Reduced stomatal conductance can cause leaf temperatures to rise rapidly by restricting transpiration and influencing the energy budget of a leaf (Macinnis-Ng & Eamus, 2009; Teskey *et al.*, 2015).

Given the many drivers of potential variation in canopy temperature, particularly in an extreme desert system, we might expect physiological adjustments to occur across a single plant canopy, such that leaves are best adapted to a given level of thermal stress. Therefore, this study sought to investigate how microclimatic variation across the canopy of a dominant tree species of Australia's southern arid-zone may result in localised variation in leaf thermal tolerance. We asked: 1) to what extent does the microclimate vary within the canopy of *Acacia papyrocarpa* Benth? and 2) do these microclimatic differences equate to varied thermal tolerances among canopy positions of height and/ or aspect?

6.3 METHODS

6.3.1 Site and study species

The study site was located at the Australian Arid Lands Botanic Garden (AALBG) in Port Augusta, South Australia, within Australia's southern arid region (32°27'56.3"S 137°44'40.7"E). Sampling was conducted throughout the 2013/14 austral summer. The AALBG covers an area exceeding 250 hectares, of which a significant portion includes a natural stand of western myall *(Acacia papyrocarpa* Benth.). Mean annual rainfall is approximately 250 mm and mean maximum summer temperature is approximately 31.3 °C, but maximum temperatures frequently exceed 45 °C (AGBoM).

Acacia papyrocarpa is a large evergreen perennial desert shrub to small tree (3 – 8 m high), with a dense spreading canopy (8 – 10 m diameter), with foliage that consists of phyllodes rather than true leaves (Fig. 6.1) (World Wide Wattle V2, 2016), but hereafter referred to as 'leaves'. This species is slow-growing and long-lived, with lifespans exceeding 300 yrs. Although evergreen, foliage is lost cyclically, with new growth occurring in spring and summer and net leaf losses commencing in late summer (Maconochie & Lange, 1970). Large expanses of mature *A. papyrocarpa* occur throughout Australia's southern arid region, where it forms sparse open woodlands with an understorey dominated by chenopod shrublands. For the current study, we selected five *A. papyrocarpa* plants categorised as mature (Lange & Purdie, 1976), which had no visible signs of damage. In contrast to juvenile growth stages, mature *A. papyrocarpa* have a dense, billowing canopy with foliage that droops near to ground level and even rests on the ground in mature trees.

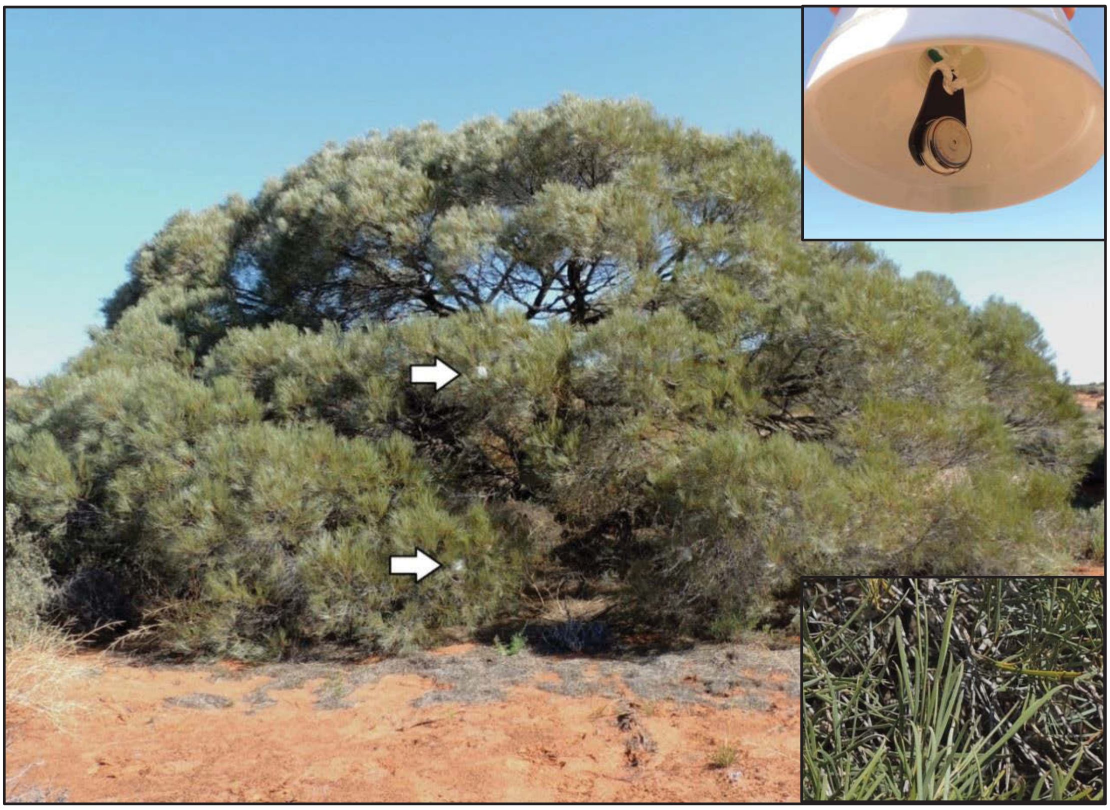

Figure 6.1 Example of the placement of data loggers within the canopy of the study species, *Acacia papyrocarpa* Benth. Inset upper right: close-up of temperature/ humidity data loggers and housing, shallow enough to allow adequate air flow around the sensor. Inset lower right: close-up of phyllodes.

All study plants experienced similar environmental conditions, grew on sandy soils surrounded by low growing herbs and shrubs, with no shading of the canopy by neighbouring plants. Exposure to daily light levels was similar between north and south-facing canopy positions (Supporting information, Fig. S6.1). Measurements were taken at four canopy positions, selected to compare differences in height and aspect. Four key microclimatic variables that significantly influence canopy temperature were also measured: ambient maximum air temperature, maximum vapour pressure deficit, minimum humidity, and wind speed. To provide an indication of how closely leaf tissue temperatures track ambient air temperatures, the predicted thermal time constant was estimated for foliage from each canopy position. Micrometeorological measurements were logged between December and February (austral summer), with leaf physiological and morphological measurements made over 12 days during the peak of summer beginning in late January.

6.3.2 Microclimate measurements

6.3.2.1 Temperature, humidity, VPD

Forty temperature/ humidity (°C/ %) data loggers (DS1923 iButtons®, Alfa-Tek Australia) were placed at one of four positions in the outer canopy of five replicate plants: the upper north-facing (UN), upper south-facing (US), lower north-facing (LN) and lower south-facing canopy (LS). Lower and upper canopy were defined here as a height of approximately 0.4 m and 2 m above ground level, respectively. Prior to canopy positioning, all forty data loggers were pre-programed using the Express Thermo 2007 Basic Software (http://www.eclo.pt/home), set to record ambient air temperature and humidity every 45 minutes for a period of 11 weeks during summer. Data loggers were individually attached to device mounts with recess (DM9000 Touch device mounts, Alfa-Tek Australia) and suspended inside a custom-built, white plastic housing that shielded the sensor from overhead and lateral light, whilst being shallow enough to allow adequate air flow around the sensor (Fig. 6.1). To account for potential instrument failure two data loggers were placed adjacent to one another at each of the four positions, equating to eight data loggers per replicate shrub. As all of the devices remained functional throughout the measurement period, data points of both loggers at each canopy position were averaged. For each position, the maximum ambient air temperature and minimum per cent humidity were determined for each measurement day (n = 80 days). Measurements from the eighty days were averaged to provide overall summer maximum ambient air temperature and minimum per cent humidity for the four canopy positions. This process was repeated for each of the five replicate plants.

Average summer maximum vapour pressure deficit (VPD_{max}) was estimated for the four canopy positions from ambient air temperature and humidity data using the equation:

$$\mathrm{VPD\ (kPa)} = e_s - e_a \qquad \textbf{eq. 6.1}$$

where e_s and e_a are the saturated and actual vapour pressure of air, respectively, estimated here following the Penman-Monteith model for determining evapotranspiration (Zotarelli *et al.*, 2013):

$$es = 0.6108e^{\frac{17.27\times T}{T+237.3}} \qquad \textbf{eq. 6.2}$$

and

$$e_a = e_s(T) \times \frac{RH}{100} \qquad \textbf{eq. 6.3}$$

where T is a point measurement of ambient temperature at the location of interest and RH is a point measurement of relative humidity corresponding to the time of T. Equivalent with steps outlined above for determining temperature and humidity, VPD was first determined for each measurement point. Subsequently, the daily maximum VPD was determined (n = 80 days), followed by averaging daily measurements for a summer maximum VPD for the four canopy positions, and repeated for each of the five study plants.

Another indicator of stressful microclimatic conditions is the frequency with which air temperature at a given canopy position reaches a known critical threshold. For each canopy position, therefore, we determined the number of days that maximum ambient air temperature exceeded the previously recorded T_{50} threshold of 49 °C for north-facing *A. papyrocarpa* foliage (i.e., 49.6 °C, Curtis *et al.*, 2014), here termed AT_{49}.

6.3.2.2 Wind speed

Near to the ground, wind speeds tend to approach zero and increase approximately logarithmically with height above the canopy (Warner, 2009). Here, of interest were the potential differences that may arise in wind speed with small-scale (< 2 m) changes in height and aspect within the canopy of a plant. We recorded wind speed adjacent to the canopy and at a height similar to positions where leaves were sampled: 0.4 cm and 2 m above the ground. Measurements were obtained using a Testo 435 multifunction anemometer with hot wire probe attachment (m s^{-1}, °C) positioned adjacent to the foliage of a representative *Acacia papyrocarpa* tree at each of the four canopy positions. Measurements were recorded at one second intervals for a period of five minutes between 1600 – 1700 hr on each of three days in late summer. From these data, a mean wind speed for the three days was recorded and the proportion of time wind speed dropped below 0.5 m s^{-1} at each canopy position was determined. Long-term meteorological records for the Port Augusta region indicate that at a height of 7 m above sea level summer winds prevail from a southerly direction and that winds originating from the south frequently exceed speeds reached in any other direction (AGBoM, 2016).

6.3.3 Thermal response indices

6.3.3.1 Leaf thermal damage thresholds

To assess variation in leaf-level physiological thermal protection across positions of the canopy, T_{50} was measured at each position for each plant following the protocol of Curtis *et al.* (2014). This method uses chlorophyll *a* fluorescence to measure the temperature at which F_V/F_M drops to 50% of prestress levels using a pulse modulated fluorometer (HeinzWalz, Effeltrich, Germany). Briefly, for each canopy position ten leaves were treated with one of three heat treatments using temperature controlled water baths, accurate to ± 0.2 °C (60 leaves per canopy position). Of the four temperature treatments, one was a control treatment (28 °C) and the other three were heat stress treatments increasing by 2 °C increments: 50 °C – 54 °C. This range of treatments encompassed the temperatures that bracketed the lowest and highest T_{50} for all canopy positions across all replicates. Leaves were exposed to a subsaturating light level of ca. 280 µmol photons m^{-2} s^{-1} throughout the treatment process.

6.3.3.2 Predicted leaf thermal dynamics

Using leaves immediately adjacent to those used to measure thermal thresholds, a series of morphological measurements were made for estimating leaf boundary layer thickness and subsequent thermal time constants. Leaf boundary layers are defined as the still air situated adjacent to the surface of a leaf (Nobel, 2012). The estimated boundary layer thickness (δ) can be used to predict the thermal time constant (τ) of leaves. Here, the average thickness of the leaf boundary layer was estimated for leaves at each of the four canopy positions using the following equation for a flat leaf presented in Leigh et al. (2017, and refs within):

$$\delta = 4.0\sqrt{(w_e/\mu)}, \qquad \textbf{eq. 6.4}$$

where δ is the average boundary layer thickness in mm, the factor 4.0 is a constant, with units of mm $s^{-0.5}$; μ is the average wind speed in m s^{-1} recorded for that canopy position; w_e is the effective leaf width in unit meters. Effective leaf width, the diameter of the largest circle that can be placed within the leaf margin (Leigh *et al.*, 2017), was measured for ten leaves and then averaged for each canopy position. Effective leaf width and leaf area (for eq.6.5, see below) were obtained from scanned images using the graphic software program ImageJ (version 1.50a, United States National Institute of Health, Bethesda, MD, USA). This process was repeated for each of the five plant replicates. Estimated thickness of the boundary layer for leaves at each position in the canopy was subsequently used to predict τ using the following equation (Leigh *et al.*, 2017):

$$\tau = C\delta/2_k, \qquad \textbf{eq. 6.5}$$

where C is the heat capacity of the leaf per unit area, obtained by multiplying the water content (g) per unit area for each leaf by the heat capacity of water (4.18 Joules g^{-1} $°C^{-1}$); k is the thermal conductivity coefficient of air (2.6×10^{-2} Joules $°C^{-1}$ m^{-1} s^{-1}); the multiplier 2 accounts for the two sides of the leaves. Leaf water content was measured as: ((fresh weight - dry weight)/ leaf area). All fresh and dry weights were measured using a precision analytical balance sensitive to 0.001 g (Mettler Toledo, city).

6.3.4 Data analyses

Temperature, vapour pressure deficit and relative humidity usually vary in tandem and have compounding effects on leaf physiology. We therefore used principal component analysis (PCA) with direct oblimin oblique rotation to extract a single composite variable from the canopy microclimatic variables: average summer maximum temperature, maximum VPD, and minimum RH. Following the method of Curtis et al. (thesis Chapter 5), each variable was standardised using *z*–transformation prior to the PCA. Component scores for the composite variable were extracted and used as an index of climatic stress (C_{STRESS}) in subsequent analyses comparing differences among canopy positions. Note that wind speed does not necessarily vary concomitantly with temperature, VPD or RH, so was not included as part of this composite climatic stress variable.

Individual two-factor ANOVA were conducted to investigate differences in C_{STRESS}, predicted τ, wind speed, and AT_{49} among the four canopy positions of *A. papyrocarpa*: UN, US, LN, and LS). Aspect (north and south) and height (upper and lower) were considered as fixed factors. A generalised linear model (GzLM) approach with Gaussian distribution and identity link function was applied to investigate the influence of height or aspect and microclimatic covariates on T_{50}. Specifically, the climatic stress index (C_{STRESS}) and predicted τ were included as a covariate in two separate models: Model 1 and Model 2, respectively. To simplify the models, and being already captured by C_{STRESS} and predicted τ temperature, RH, VPD and wind speed were not included as covariates. Initially, models consisted of the full factorial design, including all main effects and interaction terms. Models were reduced by eliminating all nonsignificant interaction terms until only significant interactions remained (Engqvist, 2005). The goodness-of-fit for each model was assessed using Akaike's information criteria corrected for small sample sizes (AICc, with low AICc indicating a better model fit) (Garson, 2013b). The most parsimonious reduced models consisted of all main effects and the height × C_{STRESS} interaction for Model 1, or aspect × predicted τ interaction for Model 2.

Due to the high number of zero-values, a nonparametric Kruskal-Wallis test with Dunn-Bonferroni post-hoc tests was used to evaluate differences in AT_{49} among the same four canopy positions. To evaluate effects of only height (with aspect pooled) or aspect (with height pooled), individual nonparametric Mann-Whitney U tests were used. For all analyses, differences were considered significant at $\alpha = 0.05$. All data analyses were carried out using the statistical software IBM SPSS® (v23).

6.4 RESULTS

6.4.1 Drivers of thermal stress

Temperature, VPD, and humidity fluctuated greatly over the study period, both daily and among canopy positions (Fig. S6.2, S6.3). When average daily summer temperatures increased, VPD also increased and average daily summer humidity declined. PCA produced a single principal component, C_{STRESS}, which explained 80.2% of the total variability of the original data. High positive component scores along the C_{STRESS} axis represented higher average maximum ambient summer temperatures and VPD, and lower average minimum summer humidity (Fig. 6.2a). Results of two-factor ANOVA indicated that C_{STRESS} was significantly higher in the lower than upper canopy and higher in the north-facing than south-facing canopy, but the interaction between height and aspect was non-significant (Table 6.1). For predicted τ, height, aspect and the interaction between height and aspect had a significant influence, such that overall, τ was predicted to be longer for north-facing foliage and lower canopy foliage, but within canopy height, the effect of aspect on predicted τ was only significant for the lower canopy positions (Table 6.1, Fig. 6.2b). Wind speed was significantly higher in upper canopy positions than lower canopy positions, but there was no significant effect of aspect and the interaction between height and aspect was nonsignificant (Table 6.1, Fig. 6.2c). In contrast with average wind speed results, the proportion of time that wind speed dropped to ≤ 0.5 m s^{-1} was not significantly influenced by height or aspect as main effects, but the interaction between these two factors was significant (Table 6.1). That is, in the north-facing canopy, lower positions reached wind speeds of ≤ 0.5 m s^{-1} proportionally more often than upper positions (Fig. 6.2d).

The four canopy positions could be ranked, from the highest to lowest frequency with which AT_{49} was exceeded, as: LN > LS > UN > US. A Kruskal-Wallis test indicated a significant difference among the four canopy positions in the median number of days that the maximum temperature breached the critical threshold temperature of 49 °C (χ^2

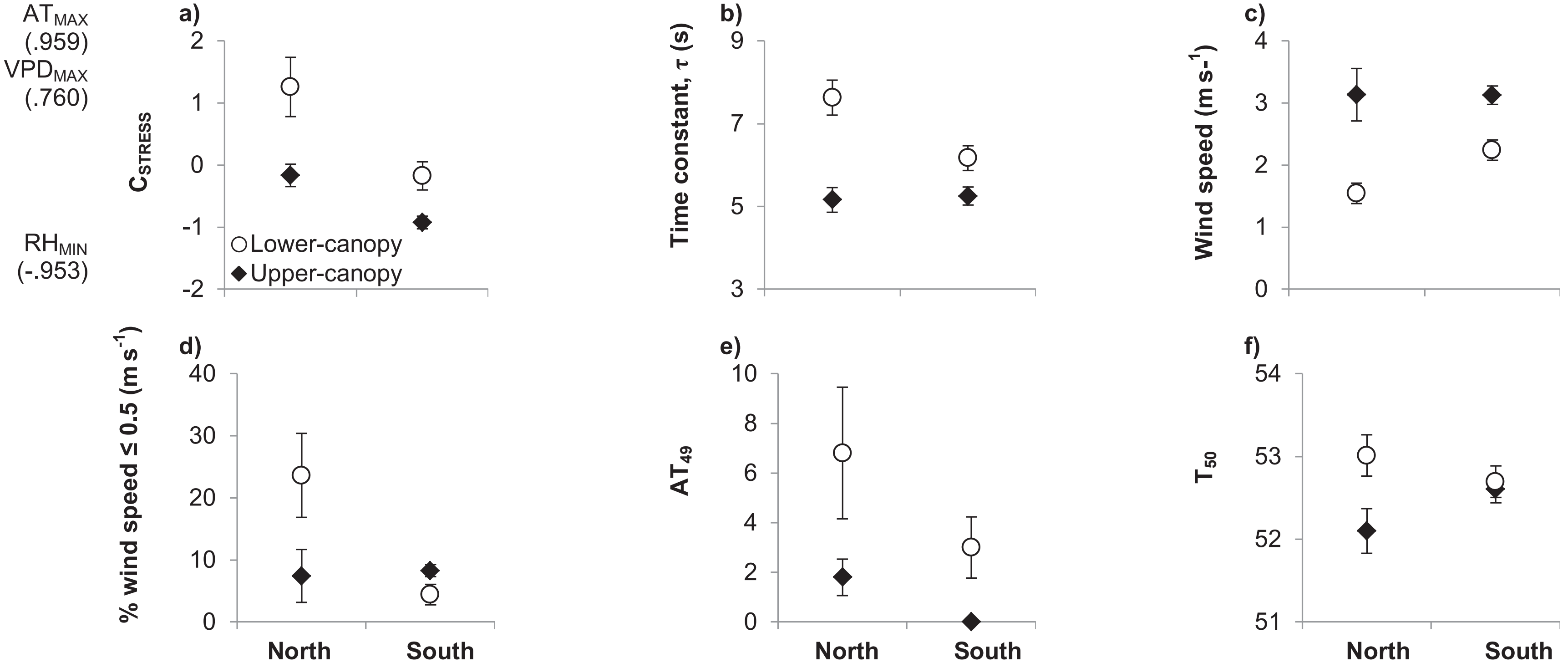

Figure 6.2 Effect of within-canopy height and aspect on a range of microclimatic indicators and leaf physiological response in *Acacia papyrocarpa* plants (*n* = 5). PCA-determined climatic stress index (C_{STRESS}) **a),** predicted thermal time constant in seconds (τ) **b),** wind speed (m s^{-1}) **c),** frequency with which wind speeds drop ≤ 0.5 (m s^{-1}) **d),** frequency of days that maximum temperatures exceeded the critical threshold temperature of 49 °C (AT_{49}) **e),** and thermal damage threshold (T_{50}) **f)** for outer canopy leaves at four positions: upper north-facing, UN; lower north-facing, LN; upper south-facing, US; lower south-facing canopy, LS. PCA variable loadings are presented left of C_{STRESS}, where AT_{MAX}, VPD_{MAX}, and RH_{MIN} are mean daily maximum ambient temperature (°C) and vapour pressure deficit (kPa), and mean daily minimum relative humidity (%), respectively (for loading interpretation see, Methods). See Tables 6.1 and 6.2, as well as text for statistical results.

Table 6.1 Summary of two-way ANOVA tests for the effect of canopy position on the climatic stress index (C_{STRESS}), wind speed (m s^{-1}), frequency with which wind speeds drop ≤ 0.5 m s^{-1}, and predicted leaf time constant (τ) in five replicate *Acacia papyrocarpa* plants. The canopy positions were: upper north-facing, UN; lower north-facing, LN; upper south-facing, US; lower south-facing outer canopy, LS. Significant differences in bold (α = 0.05) and the directions of significant effects for height and aspect are indicated with arrows.

Variable	Height *n* = 10	Height effects	Aspect *n* = 10	Aspect effects	Interaction Height × Aspect
C_{STRESS}	$F_{(1,16)} = 14.677$ **(*P* = 0.001)**	Upper < Lower	$F_{(1,16)} = 14.948$ **(*P* = 0.001)**	South < North	$F_{(1,16)} = 1.422$ (*P* = 0.250)
Wind speed (m s^{-1})	$F_{(1,8)} = 24.048$ **(*P* = 0.001)**	Upper > Lower	$F_{(1,8)} = 1.831$ (*P* = 0.213)	-	$F_{(1,8)} = 1.973$ (*P* = 0.198)
% wind speed ≤0.5 m s^{-1} (arcsin transformed)	$F_{(1,8)} = 1.923$ (*P* = 0.203)	-	$F_{(1,8)} = 4.306$ (*P* = 0.072)	-	$F_{(1,8)} = 7.538$ **(*P* = 0.025)**
Predicted time constant, τ (s)	$F_{(1,16)} = 28.435$ **(*P* < 0.001)**	Upper < Lower	$F_{(1,16)} = 4.649$ **(*P* = 0.047)**	South < North	$F_{(1,16)} = 5.947$ **(*P* = 0.027)**

$_{(3, 20)}$ = 8.354, *P* = 0.039). Subsequent Mann-Whitney U pairwise comparisons signified that only differences between LN and US were statistically different (*P* = 0.036), a result possibly driven by the excessive zero-values recorded for US (Fig. 6.2e). Nevertheless, the average frequency that the LN position exceeded AT_{49} was more than twice that of LS, more than three times UN and more than six times the frequency of the US position, which did not exceed AT_{49} during the 11 week study period. Pooling positions to compare differences in height and aspect indicated that AT_{49} was significantly greater at lower positions than upper positions (U = 78.00, z = 2.197, *P* = 0.035), whereas differences in AT_{49} between north and south-facing positions were nonsignificant (U = 28.500, z = -1.687, *P* = 0.105).

6.4.2 Thermal damage thresholds

Thermal damage thresholds, T_{50}, were significantly higher in north-facing canopy positions near to the ground, compared with upper and south-facing locations (Fig. 6.2f). Accounting for the influence of the covariate C_{STRESS} on the variation in $T_{50,}$ the main effect of height was significant, as was the interaction between height × C_{STRESS}, while the main effect of aspect and C_{STRESS} were nonsignificant (Table 6.2, Model 1). Additionally, when accounting for the influence of predicted τ on the variation in T_{50}, the main effect of aspect and the interaction between aspect × predicted τ were statistically significant, while the main effects of height and predicted τ were nonsignificant (Table 6.2, Model 2).

6.5 DISCUSSION

Coupled with an expected widespread increase in the frequency and intensity of high-temperature days and more variable rainfall is an increase in atmospheric ‘stilling’ or the slowing of wind speeds at a continental scale (McVicar *et al.*, 2008). When combined, these conditions can exacerbate the damaging effects of high-temperature on plants, particularly in already harsh ecosystems. Plants in desert systems are sparsely spaced and have more exposed canopies than those in dense, closed-canopy forests, which can reduce exposure of all but the very upper canopy leaves to extreme high temperatures via self-shading (De Frenne *et al.*, 2013). Overlaying regional-scale fluctuations in climate is the influence on individual plants of their local microclimate. Leaf photosynthetic function is particularly sensitive to fine-scale variation in environmental conditions within a canopy; this leaf-level sensitivity can in turn govern whole plant performance by influencing overall canopy carbon balance (Laisk & Nedbal, 2009). In this study, we found significant variation in microclimate across the canopy of the arid-zone species, *A. papyrocarpa*, and this variation related to differential leaf physiological function.

Table 6.2 Generalised liner models predicting the influence of four canopy positions and one of two covariates on the thermal damage threshold (T_{50}) of *Acacia papyrocarpa* leaves. Height and aspect were factors and the climatic stress index, C_{STRESS} (Model 1) and predicted thermal time constant of a leaf, predicted τ (Model 2) were covariates. Results are for the most parsimonious models, assuming Gaussian distributions with identity link functions. Significant differences in bold ($\alpha = 0.05$). Omnibus tests confirmed that each fitted model was significantly different from its null model.

	Model 1: C_{STRESS}			Model 2: Predicted τ		
Model parameters	df	Wald χ^2	*P*	df	Wald χ^2	*P*
Main Effects						
Height	1	8.969	**0.003**	1	2.677	0.102
Aspect	1	0.271	0.603	1	4.944	**0.026**
Covariate	1	2.025	0.155	1	0.049	0.825
Interactions						
Height x covariate	1	5.830	**0.016**	-	-	-
Aspect x covariate	-	-	-	1	4.539	**0.033**

As expected for plants in an open-canopy community, intracanopy temperature, VPD, and relative humidity varied with height above ground and differences in aspect (Fig. 6.2a, Fig. S6.3). Also as predicted, wind speed was slowest at lower canopy positions on the northern, leeward side of plants. Slower wind speeds resulted in significantly longer predicted thermal time constants for leaves at these positions. The co-occurrence of high-stress micrometeorological conditions, coupled with significantly slower thermal response times, suggests that leaves in lower north-facing positions are more likely to exceed ambient temperature than are leaves elsewhere in the canopy (Niinemets & Valladares, 2004; Vogel, 2009; Leigh *et al.*, 2012). Small differences in leaf temperature can mean the difference between recovery and irreparable damage of the photosynthetic apparatus (Curtis *et al.*, 2014). In response to spatially patchy environmental conditions within a canopy, we expected that leaf thermal tolerance would adjust at a local scale within a single canopy. Indeed, we found that the more thermally stressful conditions experienced by leaves in the lower north-facing canopies of *A. papyrocarpa* equated to significantly higher T_{50} than for leaves elsewhere in the canopy (Fig. 6.2). Exposure of leaves to sub-lethal high-temperature events can trigger a stream of protective pathways, including the synthesis of heat-shock proteins (HSPs) and also reactive oxygen species (ROS), which although toxic in large amounts, play an important dual role in signal transduction. The accumulation of ROS during heat stress therefore can have a protective role, activating stress-response pathways involved in the acquisition of thermal tolerance (Larkindale *et al.*, 2005a; Gechev *et al.*, 2006; for review, Suzuki & Mittler, 2006; Miller *et al.*, 2008). Similarly, HSP production can increase the critical thermal limits of plants, for example, by stabilising the photosynthetic apparatus, rendering it more resistant to damage from subsequent stress events (Bita & Gerats, 2013). Here, the higher T_{50} for leaves nearest the ground suggests that the thermal response pathways of *A. papyrocarpa* operate effectively to manage the higher risk of thermal damage at these positions through localised thermal acclimatisation. Upregulating the physiological processes associated with damage prevention and repair can be costly (Leroi *et al.*, 1994; Hoffmann, 1995; Loeschcke & Hoffmann, 2002). By limiting thermal damage in high risk positions, while maintaining lower thresholds in cooler regions of the canopy, whole-plant carbon-gain would be maximised, as occurs for key functional traits in other species (Sack *et al.*, 2006). Our findings therefore point to an important thermal optimisation strategy for this – and potentially many other – species.

Notwithstanding the idea of whole-plant optimisation, a critical thermal event such as a sudden heat wave, when plants may have little time to acclimatise, may result in significant loss of photosynthetic function and even leaf death. This scenario may be especially damaging to upper-canopy leaves, which have an innately lower critical thermal limit than leaves near the ground. For example, during a recent summer heatwave event, where maximum temperatures across the study site exceeded 46 °C (AGBoM 2017), leaf temperatures of 50 °C were recorded for another southern Australian arid-zone *Acacia* species, *A. ligulata*, accompanied by visible signs of leaf damage including discolouration and leaf shedding, throughout the canopy (A. Cook et al., 2017 unpublished data). Notably, these leaf temperatures come close to the previously recorded critical thermal threshold for *A. ligulata* (i.e., 51.1 °C, measured in summer, Curtis et al. 2014), suggesting that even species with reasonably high thresholds risk severe thermal damage to their photosynthetic apparatus during such events, allowing little time for acclimatisation. Summer is when damage thresholds are naturally expected to be maximally acclimatised. Therefore, while plants may optimise thermal protection during *average* summer conditions, during severe thermal events, lethal thermal damage will inevitably occur.

6.5.1 Ecological significance

Through their roles as climate moderators (i.e., as nurse plants) and 'fertile islands', individuals of *A. papyrocarpa* facilitate the growth and establishment of other plant species, help shape plant community composition and may increase local diversity (Facelli & Brock, 2000; Prider & Facelli, 2004). For example, certain shrub species restrict growth almost exclusively to beneath the canopies of individual *A. papyrocarpa* plants (Prider & Facelli, 2004), which create a below-canopy microclimate that is more conducive to the establishment of seedlings. This species therefore is a valuable woody overstorey plant dominating wide expanses of the Australian arid landscape. Our finding of significant intracanopy variation in the microclimate could provide insight into the potential effects of increased temperature on plant community dynamics. For instance, low lying branches of mature *A. papyrocarpa* often touch the ground, where leaves would be exposed to exceptionally high re-radiated surface temperatures. During particularly severe conditions, e.g., a heat wave coupled with wind stilling, leaves near to ground level may near critical thermal limits. Leaf death may be substantial under such a scenario, and could potentially expose thermally vulnerable species beneath the canopy to heat stress. Yet, the picture is more optimistic in light of the apparent plastic response by this species to protect its lower leaves by adjusting its leaf-level thermal threshold. What is clear from these findings is that using only microclimatic variation as a guide to thermal damage could greatly underestimate species' abilities to cope with heat stress. Similarly, sampling leaves from different positions in the canopy for temperature response measurements could greatly vary the outcome of predictive carbon balance models. Higher leaf temperatures not only increase heat stress of a leaf, but also can reduce their rate of photosynthetic carbon assimilation (Farquhar &

Sharkey, 1982), a key input to carbon balance models. If leaf-level variation in plant physiological responses to the vertical distribution of temperature gradients is disregarded, canopy flux models may overestimate carbon uptake by as much as 25% (Bauerle *et al.*, 2007). If the variation in T_{50} for *A. papyrocarpa* were found to be consistent across a range of dominant species, this information could improve attempts to model carbon flux dynamics at an ecosystem-level within arid biomes.

Chapter 7

Synthesis

Predicted changes to the Australian continent under climate change are expected to vary with region (CSIRO and Bureau of Meteorology, 2015). In contrast with coastal regions, the already hot and dry interior of Australia is predicted to warm faster with climate change. Especially across Australia's arid southern rangelands, winter and spring rainfall are projected to decline, and the frequency and duration of severe droughts increase (CSIRO and Bureau of Meteorology, 2015; Australian Bureau of Meteorology and CSIRO, 2016). Currently, long-term ambient temperature records are being surpassed annually, signalling for many parts of Australia that conditions are becoming hotter and drier, potentially already placing species at risk of thermal damage (CSIRO and Bureau of Meteorology, 2016; AGBoM, 2017b). Persistence of a given species under rapid climate change will depend on its ability to disperse to more suitable conditions, adjust or acclimatise to new environmental conditions *in situ*, and to recover function when critical tissues are damaged during a stress event. Naturally extreme temperature environments and the species therein, can therefore provide important insight into the processes underlying thermal tolerance and species persistence; yet few studies extend focus to these regions (Lindgren *et al.*, 2016). The overall objective of my PhD research was to provide insight into leaf-level thermal responses of plants under extreme high temperatures in light of a warming climate. My research demonstrates how dynamic and varied the heat stress response can be, including cross-species variation of critical thermal limits, heat stress recovery, and acclimatisation patterns within and among species over time, and spatial differences relating to native microhabitat. In doing so, the broad objective of this research was fulfilled. In this final chapter, I provide a synthesis of these key results, highlighting their ecological and functional significance. Moving forward from this thesis, I offer suggestions for future research directions.

7.1 SUMMARY AND ECOLOGICAL IMPLICATIONS

A challenge in measuring plant thermal responses is making them relevant to field conditions, especially if such measures are to be used for parameterising predictive models. When I began my research, existing plant thermal response protocols suitable for coordinated, multi-species studies used unrealistically long heat stress times (often hours) and unnatural light conditions (dark acclimatised samples). It is well established, however, that low, sub-saturating light levels during and following heat stress play an important role in plant recovery processes (Havaux *et al.*, 1991; Marutani *et al.*, 2012; Buchner *et al.*, 2015), whereas temperature changes due to lulls in wind speed, rapid sun flecks and changes in cloud cover can fluctuate at the rate of seconds or minutes (Pearcy *et al.*, 1990; Vogel, 2009). In **Chapter 2** I described the development of a novel, highly repeatable and relatively rapid procedure for determining thermal damage thresholds, T_{50}. By incorporating sub-saturating light during stress and recovery and shorter, more realistic treatment times, this protocol, provides a more biologically relevant estimate, of the critical thermal limits of plants. Using this method, I conducted a comparison of high-temperature damage and recovery of PSII for 41 Australian desert trees, shrubs, grasses and forbs, spanning 21 families. Not only is this the first time that the critical thermal limits for these desert species have been documented *in situ* and within a given season, the large diversity of species sampled revealed that critical thermal thresholds can vary by as much as 6 °C among species within a single summer. This result was despite species being from the same desert biome and exposed to a similar thermal environment, indicating genetic differences among species' abilities to tolerate high-temperature stress.

The finding that summer thermal thresholds can vary so widely among species within a single biome prompts the question: to what extent do thresholds vary across biomes? In a recent, large-scale study, O'Sullivan *et al.* (2017) found that thresholds vary linearly from polar to equatorial latitudes by approximately 8 °C. Notwithstanding the value of this significant work, it is important to note that this variation represented a single snapshot in time for each species measured and therefore does not account for their potential to acclimatise (but see Table S6 in O'Sullivan *et al.*, 2017). As shown in **Chapter 4** for my desert species, from the coolest to warmest season, average T_{50} can increase by over 5 °C – and remarkably, by more than 8 °C for a single species, i.e., T_{50} of *Atriplex vesicaria* increased by 8.3 °C. The average increase further masks the complexity of interspecific differences in the trajectory of thermal acclimatisation over time (see Fig. 4.4 Chapter 4). Australia-wide, unusually warm events are increasing in frequency and showing distinct seasonal trends. Increases in average temperatures have been more notable across autumn, winter and spring, with the smallest trends occurring in summer; meteorological observations in recent years (2013 – 2015) have recorded the warmest spring seasons yet (Australian Bureau of Meteorology and CSIRO, 2016). These temperature changes are coupled with predicted reductions in soil moisture and less runoff due to reductions in rainfall and more sunshine, particularly in winter and spring (CSIRO and Bureau of Meteorology, 2015). The frequency with which a plant exceeds its baseline thermal damage threshold therefore is also likely to increase. Understanding species' seasonal thermal acclimatisation patterns could help elucidate which groups are more vulnerable to thermal damage during critical thermal events occurring in typically cooler months. In Chapter 4 I found that the different trajectories of altering thresholds during spring ranged from almost no to large increases in T_{50} between winter and spring or spring and summer. Given a sudden heatwave, Late Jump and No Response species may have insufficient time to acclimatise, resulting in tissue damage or death. Avoiders, with their restricted or absent summer growth, may fail to flower or set seed before dying off. Steady Increasers, on the other hand, would have a better chance at survival and Early Jumpers may be unaffected. This detail provides a more nuanced understanding of species' thermal response through time. The extent to which differences in species' temporal thermal acclimatisation trajectories ameliorate adverse effects from anomalous extreme temperature events remains uncertain and deserves further examination.

It is becoming increasingly clear that an organism's physiological response closely parallels fine-scale differences in its microclimate (Somero, 2002; Ashcroft & Gollan, 2013; Pincebourde & Casas, 2014; Curtis *et al.*, 2016), yet modelling efforts largely focus on linking coarse-scale spatial and climatic data to species' distribution records (e.g., Thuiller *et al.*, 2005; Elith *et al.*, 2010). Such an approach does not address the importance of the mechanistic links between species' functional traits and their native microhabitat (Kearney & Porter, 2009). For instance, high-temperature stress in plants is exacerbated by limited available water, which varies both temporally and spatially across a species' distribution range. In **Chapter 3**, I reiterate the importance of understanding plant-environment interactions at a local-scale. Specifically, I asserted that local drivers, like water availability, are more important for determining species' critical thermal limits than are metrics that incorporate broad-scale environmental conditions, e.g., warming tolerance (Curtis *et al.*, 2016). Notably, I showed a clear difference in innate thermal tolerance between xeric-adapted and mesic-adapted desert species. It is possible that this result reflects generational adaptation to different 'hydrologic microrefugia' (McLaughlin *et al.*, 2017) within an arid biome, with xeric-adapted species best adapted to cope with conditions of thermal stress where water is most scarce. While these findings suggest that species are well-adjusted to the temperature conditions of their native microhabitat, further research is needed to clarify the extent to which adjustments in T_{50} among species can be considered truly adaptive. To address the question of adaptation, a series of reciprocal transplant experiments (Givnish & Montgomery, 2014) that incorporate measures of species' growth and competitive ability under a range of conditions would be a valuable extension of this current work.

So far, the findings discussed have related to cross-species' patterns. Of potentially equal importance are patterns of variation in leaf-level thermal tolerance within the canopy, something that is rarely looked at in open canopy communities typical of desert ecosystems. In **Chapter 6** I demonstrated that T_{50} of *Acacia papyrocarpa* may be significantly influenced by the thermal time constant of a leaf (τ) and localised canopy microclimatic conditions. This is the first study to document intracanopy variation in T_{50}, thereby contributing new insight into within-plant thermal tolerance dynamics and potentially revealing an important thermal optimisation strategy. Because T_{50} represents a leaf-level thermal response index relating to the functional state of the photosynthetic machinery, this result reveals potential implications of varying temperature stress on whole-plant productivity and growth. By reducing electron transport capacity and increasing CO_2 evolution from photorespiration, higher leaf temperatures not only increase heat stress of a leaf, e.g., via increase tissue damage from toxic reactive oxygen species (ROS, Gill & Tuteja, 2010; Sharma *et al.*, 2012), but also can reduce the rate of photosynthetic carbon assimilation (Farquhar & Sharkey, 1982). Differences in the photosynthetic capacity of leaves across a canopy can substantially influence whole-canopy photosynthetic productivity and growth (Niinemets & Valladares, 2004). When incorporated into predictive models, this information could greatly vary scenario outcomes. For instance, if the variation in leaf-level physiological responses with vertical temperature gradients is disregarded, canopy flux models may overestimate carbon uptake by as much as 25% (Bauerle *et al.*, 2007). This is because plant growth is the result of the balance between photosynthetic carbon gains and respiratory losses, including both dark- (i.e., non-photorespiratory mitochondrial CO_2 release (Atkin *et al.*, 1997)) and photo-respiration (Chapin *et al.*, 2006). Because processes including protein synthesis and replacement and membrane repair vary exponentially with temperature, the maintenance component of dark respiration is especially sensitive to environmental change (Ryan, 1991). An increase in maintenance respiration at higher temperatures therefore results in an increase of carbohydrates being used for sustaining existing biomass, and significantly affects the total carbon budget of a plant (Amthor, 1984). The temperature at which maximum dark-respiration is reached represents the break-point before it rapidly declines, termed T_{max} and corresponding with the critical temperature for PSII stability: Tc or Tcrit (Katja *et al.*, 2012; O'Sullivan *et al.*, 2013). Given the strong relationship between Tc and T_{50} (Knight & Ackerly, 2002; Knight & Ackerly, 2003; Lin, 2012; Marias *et al.*),T_{50} could operate as a proxy index for the temperature at which both the photosynthetic apparatus and respiratory processes are disrupted by high-temperature (O'Sullivan *et al.*, 2013). The work presented in Chapter 6 prompts the need for further research to quantify the relationship between T_{50} and respiratory processes as well as cost-benefit analyses for a range of functional types. Such research would enhance our understanding of the functional importance of within-canopy T_{50} variation and could help in generalising present findings across species to elucidate the resource implications of maintaining a higher thermal tolerance. In a practical sense, this information could be applied to understand growth costs under higher ambient temperatures for species holding high agricultural importance, as well as for dominant native species like *A. papyrocarpa*, the survival of which can influence the establishment and growth of other species (Prider & Facelli, 2004).

As discussed, the rate and optimisation of key physiological functional processes including photosynthesis and respiration are profoundly affected by heat stress and this impacts whole-plant growth and development. A species can reduce its risk of incurring thermal damage by increasing its physiological thermal tolerance; however the processes involved in maintaining a higher thermal threshold or repairing heat associated damage can be costly (Mitra & Bhatia, 2008). Previously, I and co-authors found evidence to suggest arid-zone plants may exploit suites of morphological and spectral traits to confer increased protection against thermal damage (Curtis *et al.*, 2012). In that study, we postulated that by investing more in structural traits, a species may reduce its need for increased physiological thermal protection: physiological tolerance may trade-off with structural traits conferring increased thermal protection. In **Chapter 5**, however, I found evidence to suggest that species complement, rather than trade-off, innate physiological thermal tolerance with two alternative leaf-level morphological pathways of thermal protection, falling along two clear principle components. Both axes described a spectrum of thermal protection or resistance, with less protected species at one end and well-protected species at the other. The first strategy coupled physiological protection with leaf traits that can increase structural strength, but which also have a role in buffering leaves against reaching high temperatures, including high leaf mass per area or LMA. Because LMA is a central trait of the leaf economics spectrum, this finding provides a quantitative link between thermal protection traits and plant resource use strategies (Michaletz *et al.*, 2015), adding impetus for including metrics of thermal tolerance along the global leaf economics spectrum. This result also raises the possibility that other thermal protective processes, e.g., heat shock protein production, may sit along this axis of thermal resistance. The strong and consistent association of LMA along the first principal axis also extends the findings of Chapters 3 and 4 relating to the relationship between native microhabitat and innate thermal tolerance. Namely, that long-lived xeric-adapted species invest more in long-term protection of their leaves than short-lived, mesic-adapted species. The second strategy revealed in Chapter 5 established a link between protective spectral traits, increased visible and near infrared reflectance, and physiological protection, particularly during warmer periods of the year. Again, contrary to previous expectations that traits may trade off with one another (Curtis *et al.*, 2012), here morphological and structural traits coincided, such that species investing in fewer protective structural traits were also less likely to protect themselves physiologically. This work suggests that leaf-level morphological traits have the potential to be used to characterise species as less or more physiologically vulnerable with regard to thermal damage. To establish the robustness of the thermal protection strategies found here, further research assessing their generality across species and sites and within species across provenances is needed. Such work could also include other key physiological traits, such as membrane stability and the production of heat shock proteins. Once a strong link between these easily measured leaf traits and physiological protection is shown to be generalisable, this research has practical use in that it could provide an accessible method for estimating the thermal vulnerability of a wide range of species.

To summarise the key findings of my thesis, I developed a novel protocol for measuring biologically relevant, species-specific thermal damage thresholds, which I subsequently used to demonstrate seasonal and spatial effects on species' thermal responses. The latter findings emphasise that a deeper understanding of plant thermal responses requires a concerted effort to obtain not only static measures of species' critical thermal limits, but also their capacity to shift their thermal response over time and space. I then showed that species' innate physiological thermal tolerance aligns in multi-trait space with two alternative leaf-level morphological pathways of thermal protection. The latter raises the possibility that other thermal protective processes, e.g., heat shock protein production and increased membrane stability, may also sit along this axis of thermal resistance. Lastly, I highlighted that to expand our mechanistic understanding of plant-environment interactions, accounting for intracanopy variation in leaf-level physiological response would benefit models seeking to predict the cost to species of a warming climate. Excitingly, the findings presented in this body of work raise further questions. Below I discuss potential future research directions stemming from my work.

7.2 FUTURE RESEARCH

7.2.1 Intensity, duration, and frequency of heat stress

My thesis highlights significant spatiotemporal variation in thermal damage thresholds, yet knowledge of plant thermal responses remains limited by a porous understanding of repeat heat stress events. The task, therefore, of establishing well-defined and biologically meaningful critical thermal thresholds for any given species remains an elusive but nonetheless needed area of research. In particular, one area complicating our understanding of a plant's thermal response is its ability to recover following heat stress. In Chapter 2, for example, I demonstrate how transient exposure to high temperatures can lead to the slowing of PSII recovery, and that recovery time increases significantly with an increase in treatment temperature of only ≤ 2 °C. In this experiment I was not able to consider the cumulative effects of short-term, consecutive heat stress events, nor the effect of altered recovery times between stress events. Further questions to be addressed include: how long do plants need to recover, what are the implications of repeated heat stress events for PSII damage, and how do the frequency and magnitude of stress events affect recovery times?

Clearly some species can tolerate single episodes of high temperatures that well-exceed 40 to 45 °C (see, Curtis *et al.*, 2014): temperatures commonly considered to be the upper optimal range for photosynthesis (Larcher, 2003) and the point at which PSII is damaged (Sharkey, 2005), respectively. *Triodia irritans*, for example, recorded a summer T_{50} of 54.3 °C after a single 15 minute heat stress episode (Curtis *et al.*, 2014). Short, single-measurement thresholds do not account for the fact that heat stress could arise from an otherwise non-damaging temperature if a thermal event is sustained for lengthy periods of time. Likewise, thermal events much shorter than 15 minutes could result in damage of the photosynthetic apparatus if extreme or if short, rapid consecutive thermal events provide insufficient time for recovery. Highly dynamic conditions occur daily, resulting in thermal episodes of infinite combinations of duration, intensity and frequency, with which plants must contend. Understanding the effect of magnitude, i.e., intensity and duration, and the frequency of thermal events on leaf damage recovery is thus essential for understanding species-specific thermal tolerance. A key question I originally asked in my PhD was: How do changes in the frequency and magnitude of repeated heat stress events affect a plant's thermal damage response? To address this question, I designed and completed an experiment in which temperature treatments differed in three distinct ways: 1) number of stress events, i.e., one *vs* three, 2) treatment temperature, i.e., 28 (control), 48, 50, 52 °C and 3) the duration of recovery following each temperature stress event, i.e., 10, 30, and 90 minutes recovery. These experiments took place over two-weeks, with three replicate runs for each experiment, using *Acacia papyrocarpa*. A change in ambient temperature part-way through experiments caused unequal variance in replicates among treatments, which will require a careful statistical approach to analyse. Due to constraints on time these results were not detailed in the main thesis. Yet, preliminary data exploration suggests that at high temperatures, thermal damage increased exponentially with temperature (Supporting information, Fig. S7.1). Subsequently, the time required for the photosynthetic apparatus to recover also increased at higher stress temperatures; however, complete recovery appeared to require a period of non-stress substantially longer than the duration of the damage-inducing event. Ultimately, these data suggest that a longer initial recovery period between stress events results in better recovery of the photosynthetic apparatus and fewer instances of permanent damage. My preliminary investigations emphasise the challenge that researchers face in utilising easily measured, yet still ecologically meaningful traits and treatment scenarios (Hoffmann *et al.*, 2013). Laboratory based experiments are useful for controlling test conditions, but often do not adequately reflect field conditions or capture species' plastic response through time. Similarly, the thermal response can be sensitive to methodological procedures (Terblanche *et al.*, 2007). Understanding the effect of dynamic temperature regimes on plant thermal responses, therefore, remains a key area of future study, which is currently being explored by two colleagues within our research team at UTS.

7.2.2 Forces driving variation in plant thermal tolerance

When the environment in which growth is established alters, a plant can acclimatise to an extent, thereby improving its tolerance to a novel set of conditions. The work presented in this thesis established significant differences in the physiological thermal tolerance of plant groups originating from the same arid biome but varying along a microhabitat spectrum, contrasting mesic- with xeric-adapted species. To extend this work, future research could investigate the forces driving this disparity in plant thermal tolerance between microhabitat groups. For example, are they the result of differences in stress responses among species in the amount or type of stress proteins expressed? (Wahid *et al.*, 2007; Mathur *et al.*, 2014). In developing an understanding of the mechanisms contributing to thermal tolerance, the cost of having greater protection may also be quantified. Such information may pave the way for the development of heat tolerant plant varieties. Engineering stress tolerance in plants is typically considered for crop species (e.g., Bita & Gerats, 2013), but it also could benefit conservation efforts if applied to increase the thermal resistance of native populations (van Oppen *et al.*, 2015). Such an approach has recently been suggested for natural coral populations to improve the resistance of at risk groups already undergoing rapid population declines due to anthropogenic disturbance (van Oppen *et al.*, 2015). These authors suggest that via human-assisted acceleration of naturally occurring processes, including epigenetics (i.e., human-assisted evolution), the stress tolerance of such groups may be enhanced, thereby building their resistance to future climate change. Although the work presented in this thesis has touched upon the roles of adaptation and acclimatisation, I did not specifically explore the heritability of environmentally induced nongenetic changes; that is, 'epigenetic acclimatisation (Mirouze & Paszkowski, 2011). Again, epigenetic procedures are typically manipulated for generating commercially advantageous phenotypic outcomes, with limited application to natural ecosystems, but could play a pivotal role in the restoration of heavily disturbed ecosystems (Jones & Monaco, 2009).

Further work is needed to determine the feasibility and ethical standing of such processes.

7.2.3 Thermal priming and acclimatisation

I return now to the noteworthy relationship between T_{50} and priming temperature underlying results observed in **Chapter 4**. That T_{50} was slightly more strongly correlated with PT_{min} than with PT_{max} is of particular interest, especially as T_{50} was not related to the *range* in PT (the difference between PT_{min} and PT_{max}). Daily maximum and minimum temperatures typically represent day and night, respectively, and have been shown to have differential effects on plant thermal responses. For example, Krause *et al.* (2013) presented evidence for the thermal acclimatisation of key physiological processes in a tropical plant species, *Ficus insipida*, exposed to warmer night-time temperatures. These authors found that CO_2 assimilation increased while dark respiration decreased under elevated day- and night-time temperatures, contrasting the results of treatments where only an increase in day-time temperature was applied. The effect of these physiological adjustments was a remarkable increase in plant biomass production (Krause *et al.*, 2013), suggesting that warmer night-time temperatures primed the plants for acclimatisation to warmer days. By contrast, others have noted a decrease in growth and yield production in response to higher minimum temperatures (e.g., wheat, Prasad *et al.*, 2008; e.g., rice, Welch *et al.*, 2010). Many studies that incorporate a day/night, max/min diurnal treatment comparison are of agricultural species, for which favourable conditions, such as irrigation, are actively maintained, effectively reducing heat stress during growth (Hatfield *et al.*, 2011). Australia's desert regions have a broad diurnal temperature range (e.g., as large as 15 – 20 °C, Trewin, 2006), yet the thermal response of most native species remains largely unknown. In particular, future research could examine how and what physiological mechanisms are stimulated by maximum *vs* minimum priming temperatures, thereby refining our understanding of plant critical thermal thresholds and the processes governing acclimatisation. Research examining species' lower thermal limits in conjunction with their upper thermal limits, would also strengthen our understanding of species' thermal response. Again, this idea is beginning to be explored by colleagues in my research group, along with a group at The Australian National University. This aspect of thermal tolerance is particularly pertinent, given the predicted rise in both maximum and minimum temperatures expected with global warming.

7.2.4 Invasion ecology

Climate change will undoubtedly create new environmental niches for species to occupy, prompting the question of whether these niches will favour invasive plants (Laube *et al.*, 2015). Compared with their native counterparts, successful establishment of invasive or exotic species in general has been associated with their high phenotypic plasticity and broad environmental tolerance (Van Kleunen *et al.*, 2010; Godoy *et al.*, 2011). Others, however, have found no difference in performance between native and non-native species under a combination of stress conditions, including combined drought and frost (Laube *et al.*, 2015). Research into the thermal tolerance of invasive species is rare, although recently, invasive species have been predicted to be more successful than native species as temperature increases (Liu *et al.*, 2017). One means of understanding species' invasiveness has been to establish which traits facilitate the establishment and persistence of exotic *vs* native species (Phillips *et al.*, 2010; Davidson *et al.*, 2011). For example, leaf attributes relating to resource use and carbon capture and growth strategies are often compared (Smith & Knapp, 2001; Leishman *et al.*, 2007). The typically shorter generation times of invasive species are also given as a reason for their ability to quickly capture and maintain space when advancing into new areas. The rapid growth rates and short generation times of invasive or exotic species suggests that they should be positioned closer to the low-LMA, fast-return end of the leaf economics spectrum (for review, see Funk, 2013). Interestingly, it is that end of the spectrum that I show in **Chapter 5** aligns with lower thermal damage thresholds in desert plants. Aligning invasive plants onto the fast turnover end of my thermal protective axis would challenge both the theory of their broad environmental tolerance and the prediction that they will thrive as temperature increases. Phenology, and in particular whether plants employ an annual or perennial life-history, likely plays a key role in the invasiveness of many exotic species (Funk *et al.*, 2016). Similarly, the availability of specialised microenvironments could help invasive species establish growth outside of their natural ranges. To expand the findings I outline in Chapter 5 to develop our understanding of invasion ecology, I suggest that future research should compare invasive *vs* native arid-zone species along a multi-trait thermal protection axis. Doing so may serve to identify a general suite of traits linking invasiveness and thermal resistance, extending current trait theories. This information could be incorporated into current and future distribution models, helping improve predictions of future plant invasions.

7.2.5 Combined influences on heat stress

Temperature is a primary driver of plant functional traits influencing growth and survival. Yet it does not operate in isolation; other key climatic drivers vary concomitantly with changes in temperature. How a plant responds to multiple stresses is different from how it responds to an individual stress, with each response being unique to a given suite of environmental conditions (for review, see Mittler, 2006; Atkinson & Urwin, 2012; Mittler *et al.*, 2012). To appropriately detect and respond to compound stress conditions, plants have developed a complex network of signalling pathways and molecular and physiological mechanisms (Atkinson & Urwin, 2012). It therefore is not possible to directly extrapolate a plant's response to multiple stresses from its response to the same stresses applied individually (Mittler, 2006). The substantial and rapid climatic changes predicted for the near future potentially will result in a plethora of new high stress conditions to which plants must respond to survive. For example, an increase in both drought and heat waves is predicted with climate change. These two abiotic stresses often co-occur and are common to desert environments. Yet plant responses to drought and heat stress are frequently examined separately. Those studies that have investigated these factors together, suggest that the negative effect on plants of heat stress and drought combined is significantly greater than if each stress is applied in isolation (Rizhsky *et al.*, 2004). In particular, plants subjected to water stress at high temperatures experience enhanced heat stress, resulting in greater photorespiration and reduced plant productivity (Lindner *et al.*, 2010). Together with more frequent and intense occurrences of drought and heat stress, atmospheric CO_2 is also increasing with global climate change (Field *et al.*, 2014). Although higher levels of CO_2 may benefit the growth of some species (Liu *et al.*, 2017), when coupled with increased temperatures, higher CO_2 concentrations can negatively affect plant productivity (Baker *et al.*, 1992; Morison & Lawlor, 1999). For instance, commercially important cultivars like rice have shown decreased yield under combined high CO_2 and high-temperature conditions (Baker *et al.*, 1992; Morison & Lawlor, 1999), suggesting an increased stress response. These studies highlight the importance of expanding plant thermal tolerance research to incorporate responses to an array of combined stresses. Clearly, plant responses are treatment-, as well as species- and system-specific. Although this challenges our ability to generalise the results from multiple studies across species and biomes, it is an important consideration for understanding how sensitive species will be to changing climatic conditions. Further, insight into species' capacities to shift their functional response under a range of combined stresses remains an open, yet key area of future research.

7.2.6 Conclusion

The work presented in this thesis enhances our understanding of leaf-level thermal responses of plants to extreme high temperatures. In doing so, this body of work contributes information that could be applied to identify species or groups of species most at risk from high-temperature stress now and into the future. In particular, the suggestion of a multi-trait thermal protection axis that is centred on easily measured leaf traits inferring differences in resource use and stress resistance could serve as a tool for understanding responses of species – and groups of species – to climate warming. This thesis also reiterates the importance of measurement timing for species' thermal responses and trait expression, something that must be considered when attempting to interpret or generalise results, both for single and across species. Finally, this work has identified that species accustomed to a more reliable source of water for growth have innately lower physiological thermal tolerance and potentially will be most at risk from changing climatic conditions. These findings emphasise the importance of interpreting species' thermal responses in context of time and space, and provide impetus to explore new cross-species' patterns in plant thermal responses at various temporal and spatial scales. For example, using spring thermal thresholds to predict the risk posed by increased heat waves in summer is likely to lead to inaccurate assessments and either under or overestimating species' thermal vulnerability. Further, failing to understand species-specific temporal trajectory of acclimatisation limits our potential to anticipate which species may suffer most during aseasonal heat waves. Given the relative paucity of work focusing on thermal response patterns among, desert and arid-zone plants especially, this body of work contributes to closing gaps in knowledge relating to the 'spatiotemporal dynamics of high-temperature tolerance in Australian arid-zone plants'.

Appendix

SUPPORTING MATERIAL: CHAPTER 2

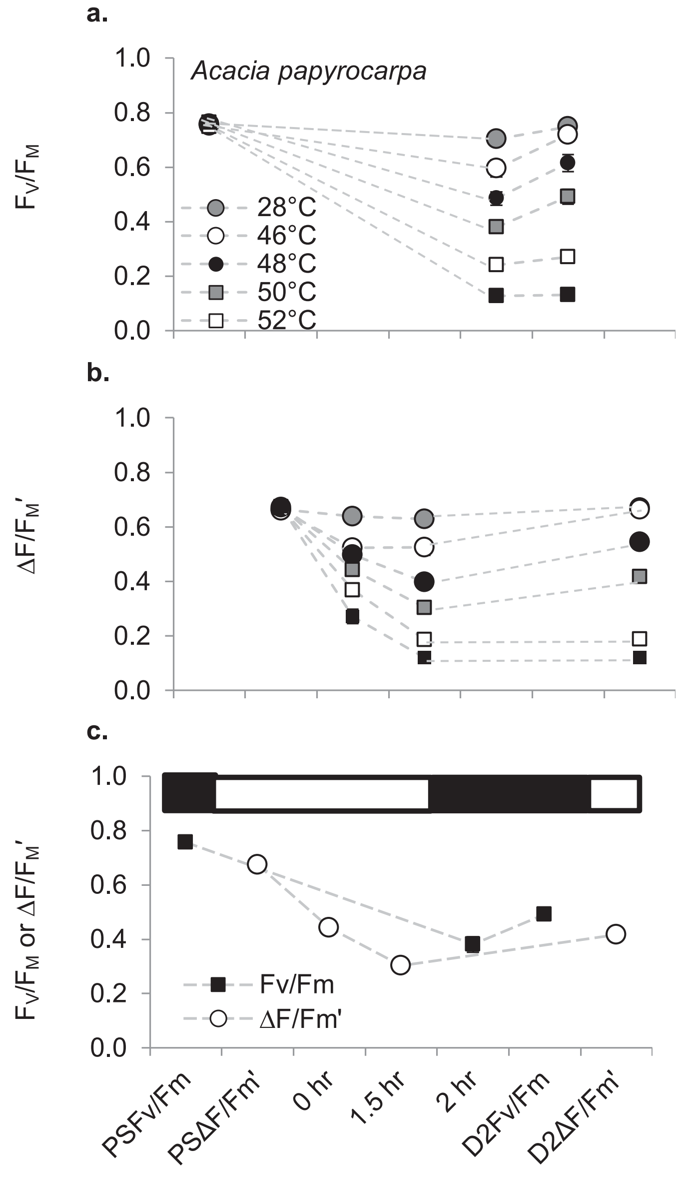

Figure S2.1 Photochemical quantum yield in response to heat stress treatments, as demonstrated in *Acacia papyrocarpa* during summer. Control measurements of maximum quantum yield of PSII ($PS_{FV/FM}$) and effective quantum yield ($PS_{\Delta F/FM'}$) were measured prior to heat stress. F_V/F_M was measured two hours after stress treatment and after a further recovery period of ca. 16 hours ($D2_{FV/FM,}$ indicating day two of measurements) at 46, 48, 50, 52 to 54 °C and a control temperature of 28 °C **a)**. $\Delta F/F_M'$ was measured immediately following stress treatment, 1.5 hours after and on day two following dark-adapted measurements and an additional 15 minutes under control conditions in order to light-adapt samples **b)**. The difference between pre- stress and day two maximum quantum yield (F_V/F_M) was used as a simple measure of damage (D_{PSII}) to PSII where $D_{PSII} = 1- (D2_{FV/FM}/ PS_{FV/FM})$, solid symbols. Recovery ($R_{\Phi PSII}$) from heat stress was measured as the proportion of initial loss of photosynthetic efficiency ($\Delta F/F_M'$) that was recovered by day two of measurements, i.e., $R_{\Phi PSII} = (D2_{\Delta F/FM'} - 1.5 \text{ hr.})/ (PS_{\Delta F/FM'} - 1.5 \text{ hr.})$, open symbols **c)**. For all data points $n = 10 \pm$ SE. Dashed lines are for ease of reading patterns and not representative of continuous time.

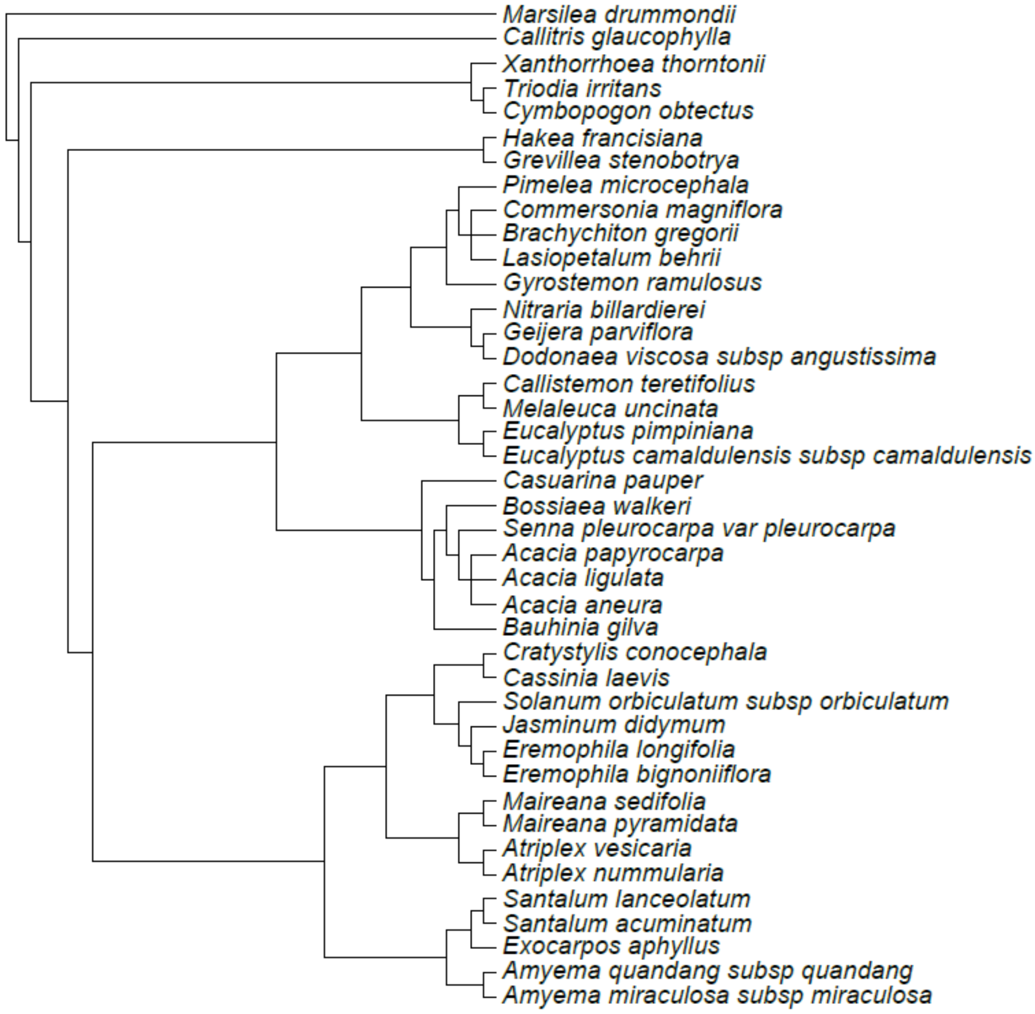

Figure S2.2 Phylogenetic tree showing the relatedness among the 41 Australian southern desert plant species used in the study.

Table S3.1 Pearson correlations (*r* values) of T_{50} with the minimum, maximum, range and mean of three climate-based parameters: mean annual rainfall, mm; solar radiation, MJ.m^{-2}.day; mean annual aridity index. Results shown are for all 42 species and individually for W_{high} (n = 10), W_{var} (n = 14), and W_{low} (n = 18) species. $P > 0.05$ in all cases except where indicated in bold ($P < 0.05$).

T_{50}	**Mean annual rainfall, mm**				**Solar radiation, MJ.m^{-2}.day**				**Mean annual aridity index**			
	Min.	Max.	Range	Mean	Min.	Max.	Range	Mean	Min.	Max.	Range	Mean
Across microhabitats												
All Species (n = 42)	.060	.079	-.130	.103	.211	.003	-.243	.113	-.171	-.304	**-.309**	-.095
Within microhabitats												
W_{high} (n = 10)	.586	.613	-.596	.609	-.030	.135	-.516	.116	-.194	-.160	-.361	-.081
W_{var} (n = 14)	.109	-.354	-.346	-.035	.148	-.160	-.374	-.068	.133	-.398	-.395	.009
W_{low} (n = 18)	-.233	.147	.160	.228	.167	.107	-.122	.253	-.255	-.044	-.031	-.003

Table S3.2 Species rank according to their warming tolerance (WT), calculated as the difference between a species' physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on the highest value across each species' Australia-wide distribution using four different thermal indices. Here, T_{hab} values were based on the highest annual maximum mean temperature (WT_{amm}).

Species	**T_{hab}**	**WT_{amm}**
Eremophila bignoniiflora	35.4	12.5
Pimelea microcephala	34.8	13.4
Marsilea drummondii (terrestrial form)	35.1	13.6
Bauhinia gilva	34.4	15.0
Dodonaea viscosa subsp *angustissima*	34.8	15.0
Eremophila longifolia	35.3	15.3
Amyema quandang subsp *quandang*	32.8	15.5
Gyrostemon ramulosus	33.4	16.2
Acacia ligulata	34.8	16.3
Cymbopogon obtectus	34.2	16.7
Senna pleurocarpa var *pleurocarpa*	33.2	17.3
Grevillea stenobotrya	34.5	17.3
Jasminum didymum	35.3	17.5
Santalum lanceolatum	35.4	17.7
Sida ammophila	34.2	18.0
Santalum acuminatum	33.2	18.5
Atriplex nummularia	31.6	18.6
Melaleuca uncinata	32.4	18.9
Hakea francisiana	29.9	19.0
Exocarpos aphyllus	31.1	19.5
Atriplex vesicaria	33.0	19.5

Table S3.2 continued.

Geijera parviflora	30.9	19.8
Acacia papyrocarpa	29.7	19.9
Maireana pyramidata	32.5	20.1
Eucalyptus camaldulensis subsp *camaldulensis*	29.5	20.5
Nitraria billardierei	30.2	20.6
Callitris glaucophylla	31.6	20.8
Solanum orbiculatum subsp *orbiculatum*	30.5	21.1
Brachychiton gregorii	30.9	21.1
Cassinia laevis	29.1	21.6
Commersonia magniflora	29.8	22.0
Amyema miraculosa subsp *miraculosa*	26.8	22.1
Casuarina pauper	29.5	22.1
Triodia irritans	30.9	23.4
Eucalyptus pimpiniana	27.1	23.6
Xanthorrhoea thorntonii	29.4	24.1
Bossiaea walkeri	27.6	24.5
Maireana sedifolia	28.7	24.5
Callistemon teretifolius	26.5	25.3
Acacia aneura	26.8	25.5
Cratystylis conocephala	27.2	25.5
Lasiopetalum behrii	24.3	27.9

Table S3.3 Species rank according to their warming tolerance (WT), calculated as the difference between a species physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on the highest value across each species' Australia-wide distribution using four different thermal indices. Here, T_{hab} values were based the highest annual mean temperature.

Species	**T_{hab}**	**WT_{am}**
Marsilea drummondii (terrestrial form)	29.5	19.2
Eremophila bignoniiflora	28.4	19.5
Pimelea microcephala	28.0	20.2
Amyema quandang subsp *quandang*	26.7	21.6
Dodonaea viscosa subsp *angustissima*	28.0	21.8
Eremophila longifolia	28.0	22.6
Cymbopogon obtectus	27.9	23.0
Gyrostemon ramulosus	26.5	23.1
Acacia ligulata	28.0	23.1
Jasminum didymum	29.6	23.2
Grevillea stenobotrya	27.8	24.0
Exocarpos aphyllus	26.4	24.2
Senna pleurocarpa var *pleurocarpa*	26.2	24.3
Santalum lanceolatum	28.8	24.3
Sida ammophila	27.41	24.8
Melaleuca uncinata	26.1	25.2
Santalum acuminatum	26.3	25.4
Atriplex nummularia	24.7	25.5
Atriplex vesicaria	27.0	25.5
Callitris glaucophylla	26.9	25.5
Hakea francisiana	23.1	25.8

Table S3.3 continued

Acacia papyrocarpa	23.1	26.5
Eucalyptus camaldulensis subsp *camaldulensis*	23.4	26.6
Geijera parviflora	24.0	26.7
Bauhinia gilva	22.6	26.8
Maireana pyramidata	25.3	27.3
Nitraria billardierei	23.5	27.3
Solanum orbiculatum subsp *orbiculatum*	24.0	27.6
Cassinia laevis	22.9	27.8
Brachychiton gregorii	24.1	27.9
Amyema miraculosa subsp *miraculosa*	20.7	28.2
Casuarina pauper	22.9	28.7
Commersonia magniflora	22.8	29.0
Triodia irritans	23.8	30.5
Eucalyptus pimpiniana	20.0	30.7
Xanthorrhoea thorntonii	22.8	30.7
Maireana sedifolia	22.3	30.9
Bossiaea walkeri	21.0	31.1
Callistemon teretifolius	20.6	31.2
Acacia aneura	20.7	31.6
Cratystylis conocephala	20.2	32.5
Lasiopetalum behrii	17.9	34.3

Table S3.4 Species rank according to their warming tolerance (WT), calculated as the difference between a species' physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on the highest value across each species' Australia-wide distribution using four different thermal indices. Here, T_{hab} values were based on the highest warmest maximum period (WT_{wmp}).

Species	$\mathbf{T_{hab}}$	$\mathbf{WT}_{wmp}$
Eremophila bignoniiflora	41.2	6.7
Pimelea microcephala	41.0	7.2
Marsilea drummondii (terrestrial form)	41.2	7.5
Amyema quandang subsp *quandang*	40.1	8.2
Gyrostemon ramulosus	41.2	8.4
Bauhinia gilva	41.0	8.4
Dodonaea viscosa subsp *angustissima*	40.9	8.9
Eremophila longifolia	41.6	9.0
Hakea francisiana	39.5	9.4
Cymbopogon obtectus	41.4	9.5
Senna pleurocarpa var *pleurocarpa*	40.9	9.6
Acacia papyrocarpa	39.9	9.7
Acacia ligulata	41.2	9.9
Atriplex nummularia	40.0	10.2
Grevillea stenobotrya	41.5	10.3
Nitraria billardierei	40.5	10.3
Exocarpos aphyllus	40.0	10.6
Santalum acuminatum	40.8	10.9
Eucalyptus camaldulensis subsp *camaldulensis*	39.1	10.9
Maireana pyramidata	41.5	11.1
Solanum orbiculatum subsp *orbiculatum*	40.5	11.1
Jasminum didymum	41.5	11.3

Table S3.4 continued

Santalum lanceolatum	41.7	11.4
Melaleuca uncinata	39.9	11.4
Sida ammophila	40.6	11.6
Atriplex vesicaria	40.8	11.7
Amyema miraculosa subsp *miraculosa*	37.1	11.8
Casuarina pauper	39.8	11.8
Brachychiton gregorii	40.1	11.9
Geijera parviflora	38.7	12.0
Callitris glaucophylla	39.7	12.7
Cassinia laevis	37.9	12.8
Commersonia magniflora	38.1	13.7
Maireana sedifolia	39.2	14.0
Bossiaea walkeri	38.1	14.0
Eucalyptus pimpiniana	36.3	14.4
Xanthorrhoea thorntonii	38.5	15.0
Triodia irritans	39.2	15.1
Acacia aneura	37.1	15.2
Callistemon teretifolius	36.4	15.4
Cratystylis conocephala	36.3	16.4
Lasiopetalum behrii	33.8	18.4

Table S3.5 Species rank according to their warming tolerance (WT), calculated as the difference between a species' physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on the highest value across each species' Australia-wide distribution using four different thermal indices. Here, T_{hab} values were based on the highest warmest quarter (WT_{wq}).

Species	$\mathbf{T_{hab}}$	$\mathbf{WT}_{wq}$
Eremophila bignoniiflora	32.7	15.2
Pimelea microcephala	32.7	15.5
Marsilea drummondii (terrestrial form)	32.7	16.0
Bauhinia gilva	32.8	16.6
Amyema quandang subsp *quandang*	31.4	16.9
Dodonaea viscosa subsp *angustissima*	32.5	17.1
Gyrostemon ramulosus	32.7	17.1
Eremophila longifolia	32.8	17.8
Senna pleurocarpa var *pleurocarpa*	32.3	18.2
Hakea francisiana	30.6	18.3
Cymbopogon obtectus	32.6	18.3
Acacia ligulata	32.6	18.5
Acacia papyrocarpa	31	18.6
Atriplex nummularia	31.5	18.7
Eucalyptus camaldulensis subsp *camaldulensis*	31.1	18.9
Melaleuca uncinata	32.2	19.1
Grevillea stenobotrya	32.6	19.2
Exocarpos aphyllus	31.4	19.2
Santalum acuminatum	32.5	19.2
Nitraria billardierei	31.3	19.5
Sida ammophila	32.5	19.7
Geijera parviflora	30.7	20.0

Table S3.5 continued

Maireana pyramidata	32.5	20.1
Jasminum didymum	32.7	20.1
Atriplex vesicaria	32.4	20.1
Solanum orbiculatum subsp *orbiculatum*	31.3	20.3
Santalum lanceolatum	32.8	20.3
Brachychiton gregorii	31.5	20.5
Casuarina pauper	30.9	20.7
Callitris glaucophylla	31.5	20.9
Cassinia laevis	29.6	21.1
Amyema miraculosa subsp *miraculosa*	27.4	21.5
Commersonia magniflora	29.8	22.0
Bossiaea walkeri	28.9	23.2
Triodia irritans	31.0	23.3
Maireana sedifolia	29.8	23.4
Xanthorrhoea thorntonii	29.8	23.7
Eucalyptus pimpiniana	26.6	24.1
Callistemon teretifolius	27.4	24.4
Acacia aneura	27.4	24.9
Cratystylis conocephala	26.8	25.9
Lasiopetalum behrii	25.5	26.7

SUPPORTING MATERIAL: CHAPTER 4

Figure S4.1 Species used in the current study were grown in a common environment at the Australian Arid Lands Botanic Garden (AALBG), located in Port Augusta, within the southern arid region of South Australia. Plants were sourced by the AALBG from locations throughout Australia's southern arid-zone, where the average annual rainfall is < 250 mm (information sourced: AALBG, 2016).

Table S4.1. Pearson correlations between species' thermal damage thresholds and the daily minimum, daily maximum, daily cumulative minimum, daily cumulative maximum and daily range temperatures obtained one to ten days prior to physiological measurements; here termed 'priming temperature'. Analyses revealed few significant relationships between T_{50} and priming temperature for the days preceding our experiment in winter and spring. For summer measurements, statistically significant correlations were consistent across priming temperature metrics by the third day prior to T_{50} measurements (indicated by dashed line); with the exception of daily temperature range, which remained nonsignificant irrespective of day or season. Results suggest ambient temperature has a stronger influence on T_{50} during summer than typically cooler seasons. From these analyses, we chose to incorporate daily ambient temperature recordings from day three as a proxy for priming temperature in all generalised linear models. Statistically significant relationships appear in bold. $\alpha = 0.05$.

	Winter (*n* = 23)				
Days Prior	**Min.**	**Max.**	**C.Min.**	**C.Max.**	**Range**
1	-0.074 (*P* = 0.738)	-0.197 (*P* = 3.68)	NA	NA	-0.044 (*P* = 0.842)
2	0.270 (*P* = 0.213)	0.106 (*P* = 0.63)	0.130 (*P* = 0.553)	-0.062 (*P* = 0.777)	-.192 (*P* = 0.380)
3	-0.299 (*P* = 0.165)	0.196 (*P* = 0.369)	-0.072 (*P* = 0.743)	0.035 (*P* = 0.875)	0.375 (*P* = 0.078)
4	-0.243 (*P* = 0.264)	-0.240 (*P* = 0.270)	-0.180 (*P* = 0.412)	-0.047 (*P* = 0.832)	0.130 (*P* = 0.554)
5	-0.366 (*P* = 0.086)	0.132 (*P* = 0.549)	-0.295 (*P* = 0.172)	-0.006 (*P* = 0.980)	0.388 (*P* = 0.067)
6	-0.221 (*P* = 0.312)	-0.130 (*P* = 0.554)	-0.365 (*P* = 0.087)	-0.035 (*P* = 0.874)	0.160 (*P* = 0.465)
7	0.198 (*P* = 0.364)	-0.367 (*P* = 0.085)	-0.268 (*P* = 0.217)	-0.131 (*P* = 0.550)	-0.310 (*P* = 0.150)
8	0.144 (*P* = 0.513)	-0.216 (*P* = 0.323)	-0.189 (*P* = 0.389)	-0.225 (*P* = 0.303)	-0.234 (*P* =0.282)
9	**0.478** **(*P* = 0.002)**	-0.038 (*P* = 0.862)	-0.285 (*P* = 0.188)	-0.269 (*P* = 0.215)	0.397 (*P* = 0.061)
10	0.118 (*P* = 0.591)	0.088 (*P* = 0.689)	-0.250 (*P* = 0.249)	-0.250 (*P* = 0250)	-0.065 (*P* = 0.769)

Table S4.1 continued. Spring Pearson correlations. Description as above.

	Spring (n = 22)				
Days Prior	**Min.**	**Max.**	**C.Min.**	**C.Max.**	**Range**
1	0.196 (P = 0.382)	-0.136 (P = 0.546)	NA	NA	-0.205 (P = 0.360)
2	-0.125 (P = 0.581)	-0.090 (P = 0.692)	0.044 (P = 0.847)	-0.155 (P = 0.491)	-0.014 (P = 0.950)
3	-0.323 (P = 0.143)	-0.320 (P = 0.147)	-0.188 (P = 0.403)	-0.299 (P = 0.177)	-0.021 (P = 0.928)
4	0.193 (P = 0.390)	-0.302 (P = 0.172)	-0.046 (P = 0.839)	**-0.479** **(P = 0.024)**	-0.400 (P = 0.065)
5	**0.565** **(P = 0.006)**	0.172 (P = 0.445)	0.184 (P = 0.413)	-0.363 (P = 0.097)	-0.157 (P = 0.484)
6	**0.478** **(P 0.024)**	0.411 (P = 0.057)	0.364 (P = 0.096)	-0.159 (P = 0.480)	0.062 (P = 0.785)
7	0.239 (P = 0.285)	0.281 (P = 0.205)	**0.532** **(P=0.011)**	-0.031 (P = 0.892)	0.023 (P = 0.919)
8	-0.156 (P = 0.489)	0.202 (P = 0.367)	**0.428** **(P = 0.047)**	0.073 (P = 0.748)	0.300 (P = 0.175)
9	-0.101 (P = 0.655)	0.025 (P = 0.912)	0.397 (P = 0.067)	0.098 (P = 0.664)	0.092 (P = 0.683)
10	-0.057 (P = 0.802)	-0.065 (P = 0.775)	0.317 (P = 0.150)	0.077 (P = 0.735)	-0.006 (P = 0.977)

Table S4.1 continued. Summer Pearson correlations. Description as above.

	Summer (*n* = 42)				
Days Prior	**Min.**	**Max.**	**C.Min.**	**C.Max.**	**Range**
1	**0.334** **(*P* = 0.031)**	0.237 (*P* = 0.131)	NA	NA	0.105 (*P* = 0.510)
2	**0.396** **(*P* = 0.009)**	0.246 (*P* = 0.116)	**0.398** **(*P* = 0.009)**	0.252 (*P* = 0.108)	0.174 (*P* = 0.271)
3	**0.437** **(*P* = 0.004)**	**0.384** **(*P*= 0.012)**	**0.437** **(*P* = 0.004)**	**0.333** **(*P* = 0.031)**	0.222 (*P* = 0.157)
4	**0.450** **(*P* = 0.003)**	**0.36** **(*P* = 0.019)**	**0.468** **(*P* = 0.002)**	**0.392** **(*P* = 0.010)**	0.130 (*P* = 0.412)
5	0.197 (*P* = 0.212)	**0.305** **(*P* = 0.050)**	**0.437** **(*P* = 0.004)**	**0.431** **(*P* = 0.004)**	0.232 (*P* = 0.140)
6	**0.308** **(*P* = 0.047)**	0.129 (*P* = 0.416)	**0.437** **(*P* = 0.004)**	**0.424** **(*P* = 0.005)**	-0.037 (*P* = 0.818)
7	0.201 (*P* = 0.201)	0.176 (*P* = 0.265)	**0.432** **(*P* = 0.004)**	**0.419** **(*P* = 0.006)**	0.066 (*P* = 0.678)
8	0.129 (*P* = 0.414)	0.079 (*P* = 0.618)	**0.411** **(*P* = 0.007)**	**0.411** **(*P* = 0.007)**	0.012 (*P* = 0.939)
9	0.096 (*P* = 0.544)	0.222 (*P* = 0.157)	**0.394** **(*P* = 0.010)**	**0.411** **(*P* = 0.007)**	0.204 (*P* = 0.196)
10	0.303 (*P* = 0.051)	0.052 (*P* = 0.745)	**0.404** **(*P* = 0.008)**	**0.399** **(*P* = 0.009)**	-0.143 (*P* = 0.368)

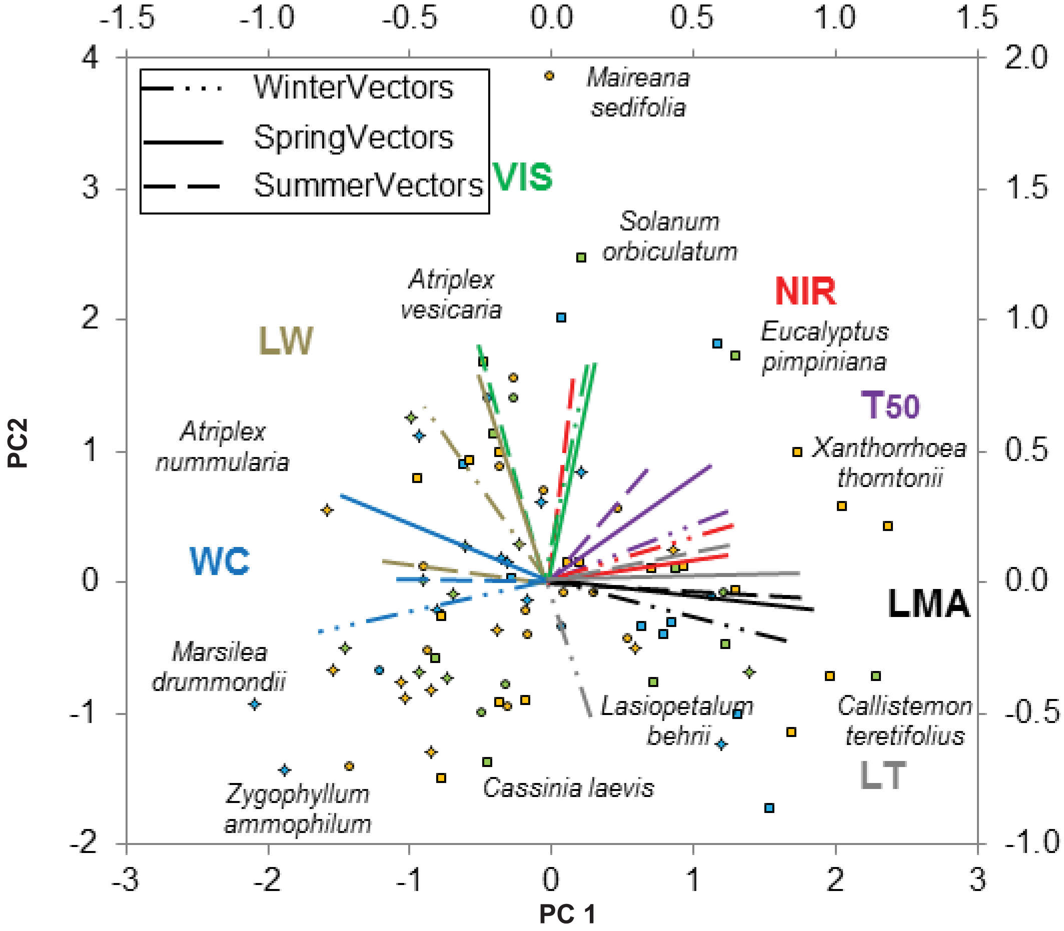

Figure S5.1 PCA biplot combining species data from all three seasons, winter (blue symbols), spring (green symbols), summer (orange symbols). Species grouped by preferred native microhabitat: diamond symbol, W_{high}; round symbols, W_{var}; Square symbols, W_{low}. Lines indicate direction and weighing of vectors per season for the seven traits considered: Leaf thickness, LT; leaf mass per area, LMA; near infrared reflectance, NIR; thermal damage threshold, T_{50}; visible reflectance, VIS; effective leaf width, LW; water content, WC. The approximate positions of some example species are shown.

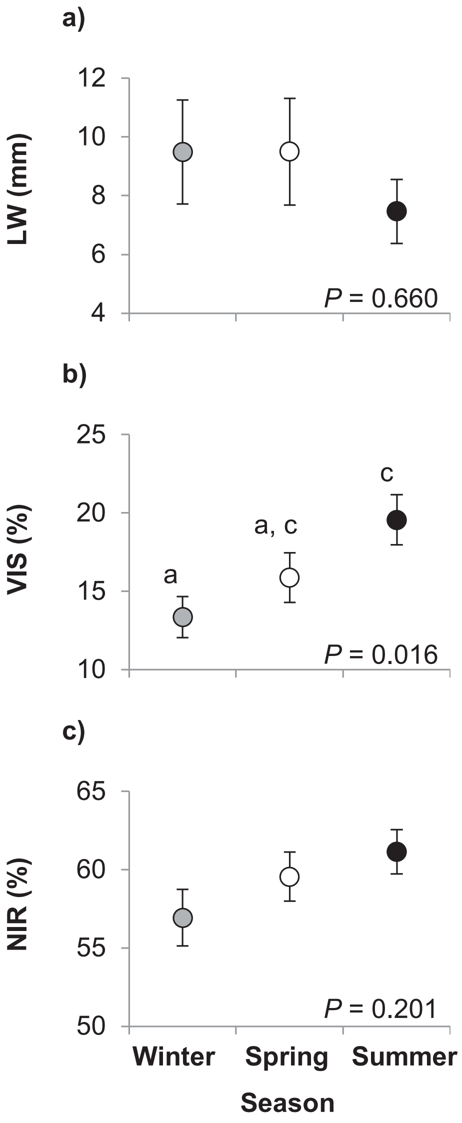

Figure S5.2 Mean (± SE) effective leaf width **a)**, percentage of visible reflectance **b)**, and percentage of near infrared reflectance **c)** for Australian arid-zone plant species from three seasons: winter (*n* = 23), spring (*n* = 21), summer (*n* = 41). Results show a general tendency for effective leaf width to decrease and spectral parameters to increase over the course of the year, from winter to summer. Results shown inset are for Welch's ANOVA with post-hoc comparisons based on the Games-Howel test. Data points with different letters above differed significantly at * $P < 0.05$.

Table S5.1 Seasonal descriptive statistics including minimum, maximum and mean (± SE) values for six leaf morphological and structural traits: leaf thickness, leaf mass per area, leaf water content, effective leaf width, and visible and near infrared reflectance. Values are for all species within each season (total) and for species grouped by microhabitat: high water, W_{high}; low water, W_{low}; variable water availability, W_{var}.

		Winter				**Spring**				**Summer**			
Leaf Trait	**Statistic**	**W_{high} $n = 10$**	**W_{low} $n = 9$**	**W_{var} $n = 4$**	**Total $n = 23$**	**W_{high} $n = 9$**	**W_{low} $n = 9$**	**W_{var} $n = 4$**	**Total $n = 22$**	**W_{high} $n = 9$**	**W_{low} $n = 18$**	**W_{var} $n = 14$**	**Total $n = 41$**
Leaf thickness (LT), mm	Minimum	0.16	0.36	0.49	0.16	0.22	0.39	0.45	0.22	0.21	0.33	0.19	0.19
	Maximum	1.52	1.67	0.77	1.67	1.03	1.55	0.81	1.55	1.63	1.96	1.52	1.96
	Mean	0.63	0.75	0.59	0.67	0.42	0.75	0.57	0.58	0.60	0.87	0.79	0.78
	SE	0.15	0.13	0.06	0.08	0.08	0.11	0.08	0.07	0.16	0.13	0.11	0.07
Leaf mass per area (LMA), g/m	Minimum	51.96	68.20	65.16	51.96	57.91	15.84	122.9	15.84	66.74	89.91	84.02	66.74
	Maximum	778.30	973.29	457.2	973.29	445.16	677.3	405.5	677.37	501.27	1247.1	426.86	1247.1
	Mean	166.77	384.77	240.6	264.92	154.87	278.7	227.5	218.77	224.11	420.66	234.26	313.87
	SE	69.66	111.69	81.98	56.99	39.22	72.21	63.34	36.15	51.81	71.00	25.65	36.81
Leaf water content (WC), %	Minimum	53.11	41.99	46.37	41.99	44.42	39.62	43.23	39.62	42.63	27.96	32.06	27.96
	Maximum	99.55	89.49	95.57	99.55	79.58	89.29	70.20	89.29	73.18	79.39	86.75	86.75
	Mean	70.94	58.71	71.90	66.32	63.00	55.14	57.85	58.85	59.59	46.36	58.01	53.24
	SE	4.52	5.24	11.60	3.53	3.84	5.34	5.57	2.86	2.86	2.69	4.61	2.24
Effective leaf width (LW), mm	Minimum	1.12	0.71	1.49	0.71	1.21	0.82	1.89	0.82	1.14	0.91	1.89	0.91
	Maximum	20.90	28.54	12.23	28.54	24.62	28.71	10.35	28.71	18.33	29.41	27.01	29.41
	Mean	9.48	11.07	5.96	9.49	10.79	10.04	5.39	9.50	9.37	6.37	7.52	7.42
	SE	1.96	3.92	2.26	1.77	2.56	3.62	1.78	1.82	1.99	1.80	2.09	1.14
Visible reflectance (VIS), %	Minimum	5.32	6.09	6.52	5.32	7.69	7.89	6.90	6.90	10.12	8.56	6.62	6.62
	Maximum	18.09	26.85	26.68	26.85	19.53	33.04	32.58	33.04	30.03	42.19	56.74	56.74
	Mean	11.32	14.59	15.64	13.35	14.42	16.64	17.34	15.86	17.53	19.26	21.28	19.57
	SE	1.41	2.23	4.54	1.31	1.55	2.77	5.74	1.58	1.88	2.37	3.50	1.62
Near infrared reflectance (NIR), %	Minimum	44.12	48.56	48.76	44.12	49.49	53.90	53.13	49.49	48.85	46.64	43.46	43.46
	Maximum	60.44	78.83	60.30	78.83	63.55	72.22	62.01	72.22	67.86	74.50	89.72	89.72
	Mean	52.07	63.39	54.58	56.93	54.55	65.54	57.38	59.56	58.60	60.75	62.70	60.94
	SE	1.57	3.08	3.31	1.82	1.43	2.20	2.42	1.57	1.99	2.00	3.18	1.45

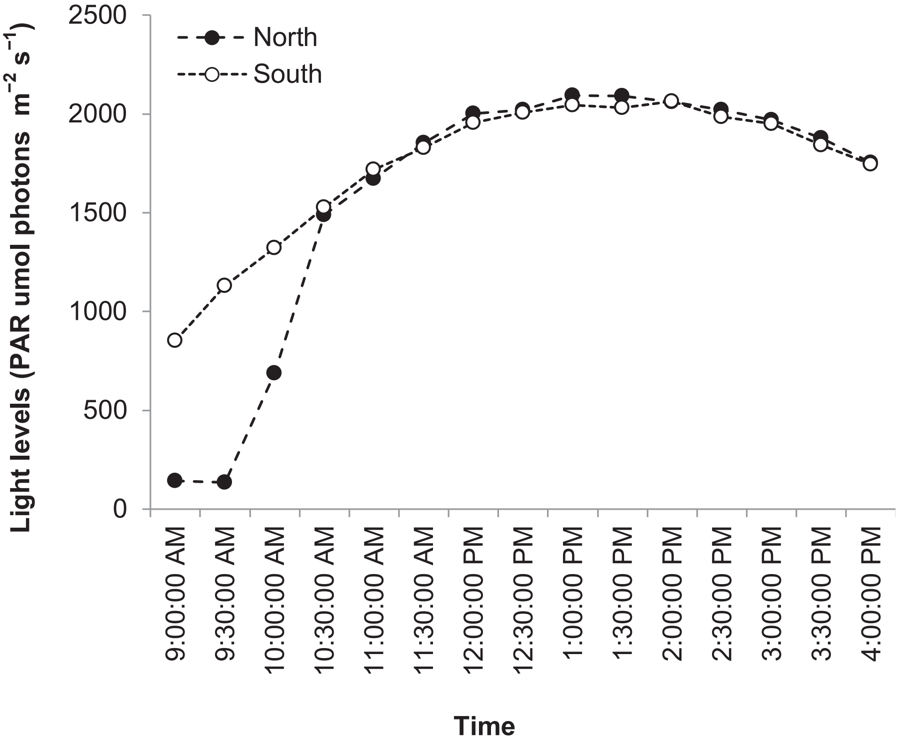

Figure S6.1 Half-hourly measurements of light levels (PAR µmol photons m^{-2} s^{-1}) adjacent to the canopy for a representative *Acacia papyrocarpa* tree. Measurements shown are for a single day between 900 to 1600 hrs for the north- and south-facing canopy.

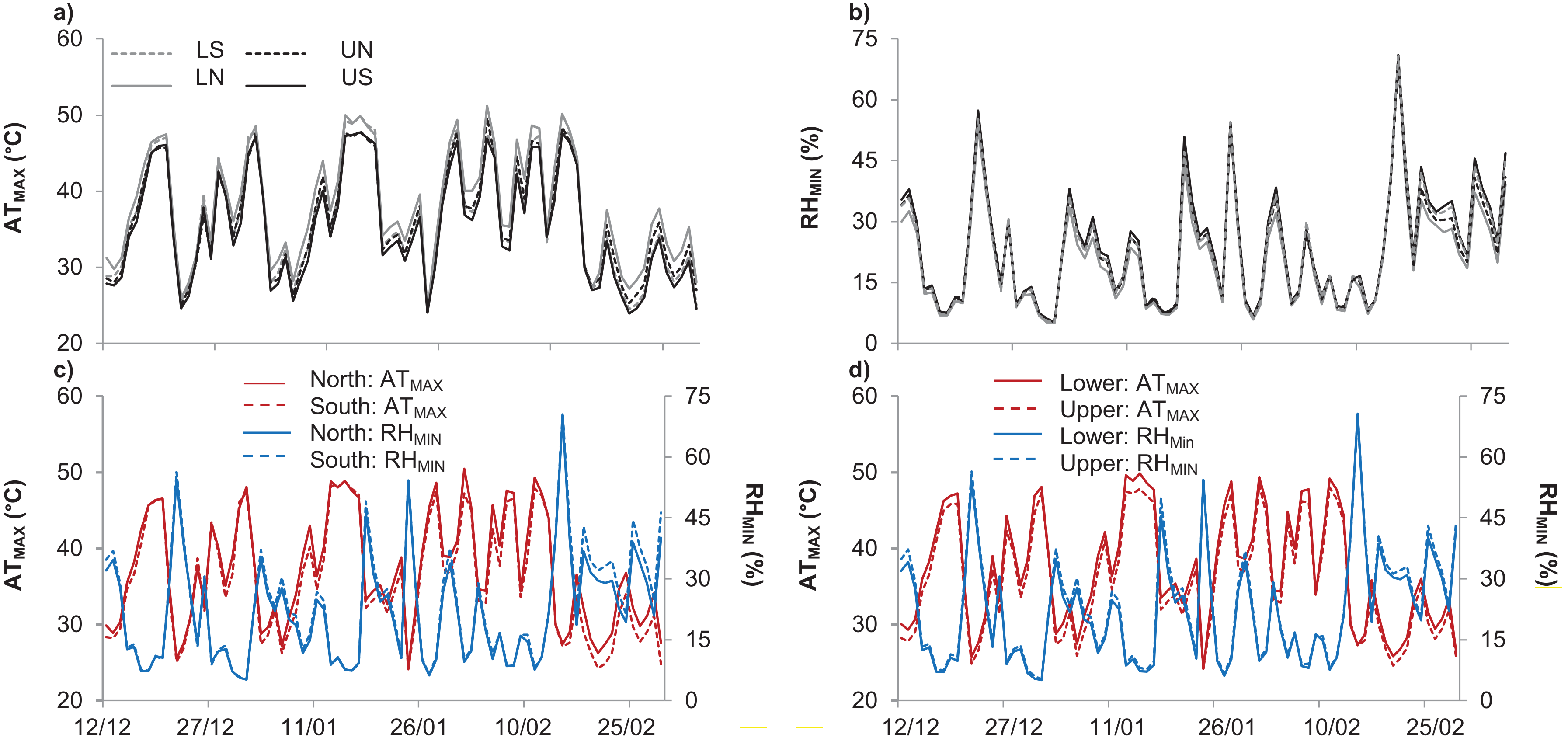

Figure S6.2 Mean daily maximum ambient temperature (AT$_{MAX}$, °C) **a)** and daily minimum per cent relative humidity (RH$_{MIN}$, %) **b)** at four positions in the outer canopy of *Acacia papyrocarpa*: upper north, lower north, upper south, and lower south canopy (UN, LN, US, LS) (n = 5). Data also presented as north- and south-facing positions combined (n = 10) **c)**, and upper and lower positions combined (n = 10) **d)**. Mean maximum daily vapour pressure deficit is not presented, but mirrored temperature trends.

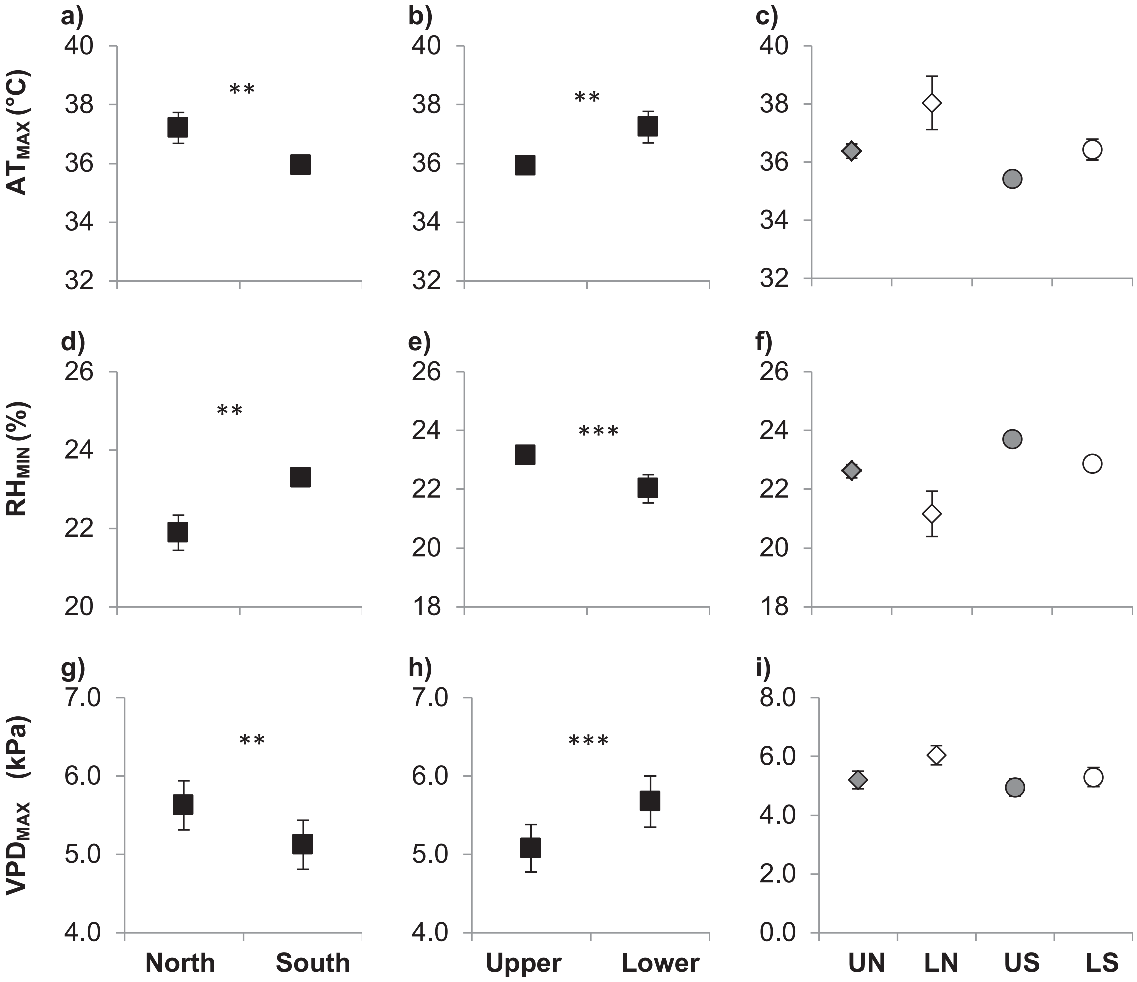

Figure S6.3 Mean daily maximum ambient temperature (AT_{MAX}, °C) **(a – c)**, daily minimum per cent relative humidity (RH_{MIN}, %) **(d – f)**, and mean daily maximum vapour pressure deficit (VPD_{MAX}, kPa) **(g – i)** measured at four positions of height (upper, lower) and aspect (north, south) and jointly: upper north canopy, UN; lower north canopy, LN; upper south canopy, US; lower south canopy, LS. The significance of main effects for factorial ANOVA is indicated: $\alpha = 0.05$, *** $P < .001$, ** $P < .01$, * $P < .05$. Interaction effects were nonsignificant.

SUPPORTING MATERIAL: CHAPTER 7

Supporting information, Figure S7.1 Preliminary results from an experiment not reported elsewhere in the thesis, which asked: How do changes in the frequency and magnitude of repeated heat stress events effect species' thermal damage response?

To address this question, I designed and completed an experiment in which temperature treatments differed in three distinct ways: 1) number of stress events, i.e., one *vs* three, 2) treatment temperature, i.e., 28 (control), 48, 50, 52 °C and 3) the duration of recovery following each temperature stress event, i.e., 10, 30, and 90 minutes recovery. These experiments took place over two-weeks, with three replicate runs for each experiment, using the Australian southern arid-zone dominant tree species, *Acacia papyrocarpa* Benth. Following the protocol of Curtis *et al.* (2014), heat stress and short-term recovery took place under conditions of subsaturating light followed by an extended overnight dark recovery phase. Responses were measured using chlorophyll *a* fluorometry to investigate differences in the proportion of thermal damage at the site of photosystem II, D_{PSII} (thesis Chapter 2). Briefly, the maximum quantum yield of PSII or F_V/F_M was measured under prestress control conditions prior to each stress treatment ($PS_{FV/FM}$), then again following an extended recovery period in the dark ($D2_{FV/FM}$). From these values the proportion of damage incurred, D_{PSII}, was quantified following the protocol outlined in Curtis *et al.* (2014) as:

$$D_{PSII} = 1 - (D2_{FV/FM}) / (PS_{FV/FM}) \qquad (eq.2\ 1)$$

Preliminary results suggest a complex interaction of temperature duration, intensity and frequency, including recovery time, on the plant thermal damage response (Fig. S7.1a, b). At high temperatures, thermal damage appears to have increased exponentially with an increase in temperature (Fig. S7.1c). Consequently, the time required for the photosynthetic apparatus to recover also increased; however, complete recovery appeared to require a period of non-stress substantially longer than the duration of the damage-inducing event. Compare, for example, values of D_{PSII} for leaves that received either a single or multiple applications of heat stress at 50 °C for durations of 3 or 15 min (Fig. S7.1d). Regardless of treatment duration, a single heat treatment immediately

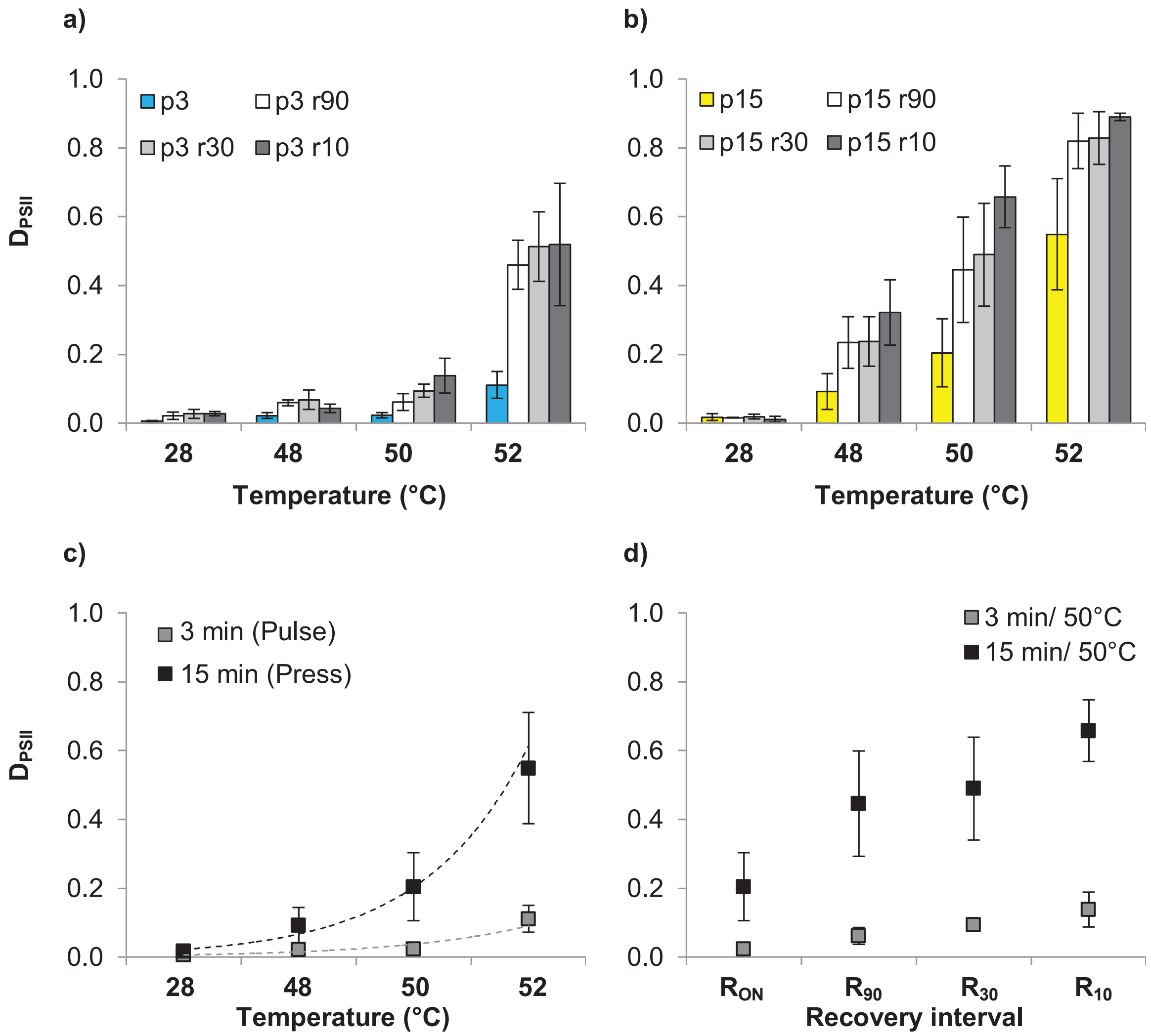

Figure S7.1 Damage (being the difference between pre- and post-stress levels of photosystem health, D_{PSII}, ± SE) of *Acacia papyrocarpa* leaves exposed to 28 (control), 48, 50, 52 °C treatment temperatures for three **a)** or fifteen minutes **b)** duration. Note, higher D_{PSII} values indicate greater long-term damage. Treatments varied in the number of stress events and duration of recovery phases. For instance, comparisons in panels **a** – **b** are for a single stress event followed by a single 90 min recovery phase under sub-saturating conditions and an extended overnight recovery phase (R_{ON}), or three consecutive heat stress events interspersed with recovery phases varying in duration: 90 minutes (R_{90}), 30 minutes (R_{30}) and 10 minutes (R_{10}). In all instances, final recovery phases under sub-saturating light were followed by an extended overnight recovery phase. Comparison of D_{PSII} after single 3 (grey symbols) and 15 minute (black symbols) heat stress at control and treatment temperatures **c)**. Comparison of D_{PSII} after a single heat stress event at 50 °C followed by an overnight recovery phase (R_{ON}) and three consecutive heat stress events of 3 and 15 minutes, also at 50 °C **d)**. Recovery phases for consecutive stress treatments are as described above. All treatment combinations were replicated three times randomly over the course of the 2-week measurement period.

followed by an extended recovery phase (R_{ON}) resulted in leaves sustaining markedly lower levels of damage compared with leaves that received consecutive heat stress applications. Similarly, by increasing the interval between multiple heat stress events from 10 to 90 minutes (R_{10}, R_{30}, R_{90}), leaves sustained lower levels of D_{PSII} (Fig. S7.1d). Not only the duration of the period between treatments, but also the duration of the treatment itself affected recovery, e.g., with more damage sustained after a 15, compared with 3 minute, heat treatment.

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