The price of heat stress: functional and resource constraints to thermal tolerance in arid zone plants

Kirsty Milner
Bachelor of Science (Hons)

2020

School of Life Sciences,
University of Technology Sydney

A thesis submitted in fulfilment of the requirements for the degree
Doctor of Philosophy in Science

Certificate of original authorship

I, Kirsty Milner declare that this thesis, is submitted in fulfilment of the requirements for the award of, in the School of Life Sciences/Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

Signature:

Production Note:
Signature removed prior to publication.

Date: May 2020

Acknowledgements

First of all, I would like to thank my supervisors Andy Leigh, Kris French and Stella Valenzuela. It has been an honour to work with such brains and leaders in science. It is especially important that I acknowledge they had to work against/with my leave-it-till-the-last-minute nature and handled it with aplomb. I have been lucky enough to have had Andy as a supervisor from Honours. I am grateful her approach to supervising includes the whole human, her kindness and guidance were much appreciated. Her enjoyment of the desert is quite infectious. Thanks to Kris who seemed to know exactly when a check-in was required to keep me on track. I learned a lot about analyses and targeted discussion writing from you. Thank you Stella for getting me into, through and understanding the lab work.

My PhD would not have been possible without the help of Alicia Cook. She has been friend, fellow student, driver, teacher and entertainment provider all while in Port Augusta.

For those in Port Augusta - I would like to thank the Port Augusta City Council, Friends of the AALBG and the Australian Arid Lands Botanic Garden for their financial support, but more valuable was their help and support during my field work. I would especially like to thank the employees at AALBG; Cherie Gerlach and nursery staff Cory Keenan, Ryan Hayward, Ryan Blash and Bernie Haase and The Friends of the Gardens and AALBG volunteers Brian Reichelt, Peter Langdon, Peter and Ronda Hall. Without Ronda in particular we would never have got started on that very first day. My thanks extends to the Work for the Dole crews who helped establish and maintain the experimental garden. To those who helped me in the field Neil Berry and Melinda Cook. Thanks in particular to Neil for keeping me from going crazy next to a noisy generator and daring to take on his own field research at the AALBG. To Mel, thanks for helping train sleepy lizards.

Back in Sydney and in the laboratory I would like to thank all those who stepped me through the skills required for protein work. To go from field ecology to molecular biology was a leap for me. My thanks are extended to those who helped with Western Blotting; from French's lab Mike Davies and from Valenzuela's lab Claudia D'Amario, Rufaka Hossain and Leonie Herson. There are those from Macquarie University who assisted me with Steve Van Sluyter's thorough protein protocol; Katy Breakwell, Michelle Demers, Aaron Phillips and James Lawson. And none of that would have been possible without Steve himself. Working in the lab with him was a treat, I just wish I could remember half the stuff he taught me. Big thanks to the tech staff at UTS, Gemma Armstrong, Lucia Bennar and Sue Fenech, for helping with equipment both at UTS and in the field. Importantly I have to thank Paul Brooks and Graeme Poleweski for letting me use their courier account and dealing with sending and receiving

dangerous goods. Not a small feat. My hat goes off to Dan Krix and Mick Ashcroft for help with data analaysis.

Last but not least to those who have maintained by sanity during my PhD. This includes friends at and outside of UTS. To my all my family, but especially Karen, Nick, Jenna, Tai and Charles for providing endless support, but not endlessly asking about my research. To David, who inspired me to return to study, thanks for the inspiration and for all the support in getting me through. Hopefully your patience in me studying finally pays off. For my all my football friends, you cannot imagine how important it was to let me kick things during this time. To my friends who provided ample, but necessary distractions and cuddles, may the camping trips continue. And to my friends and colleagues at UTS, I especially enjoyed the lively lunchtime banter, Sci-League futsal and Friday night drinks. I have studied for so long at UTS my time is now divided as pre- and during UTS. Hopefully post-UTS is just as rewarding.

Thanks to anyone I've forgotten, including those who have simply asked about my research or checked on me.

Statement of thesis format

This thesis is submitted as a thesis by compilation. Chapter 1 provides a general introduction to the background literature, the gaps in the field and my research questions. The following three data chapters have been prepared as standalone manuscripts for publication. Chapter 5 provides a synthesis of my research and highlights the contribution this thesis makes to our knowledge on the thermal tolerance of desert plants, as well as identifying future directions.

Declaration of contribution to each publication

1. Chapter 2. Milner KM, French K, Ashcroft MB, Valenzuela SM, Leigh A (In preparation for submission). Capacity to tolerate, acclimate and recuperate. In preparation for submission to *Plant, Cell and Environment*.

I designed the experiment in consultation with my supervisors, Leigh and French. I conducted the field and laboratory work, collected and analysed the data. Ashcroft wrote the R scripts for Bayesian analysis of data. Valenzuela aided with laboratory work. I led the writing, with all authors contributing to the text.

2. Chapter 3. Milner KM, French K, Krix DW, Valenzuela SM, Leigh A (Submitted for revision). Plant stress under spring or summer extreme heat events**.** For resubmission to *New Phytologist.*

I designed the experiment in consultation with Leigh and French. I conducted the field work, including the development of the open-topped heat-stress chambers, collected and analysed the data. The Australian Arid Lands Botanic Garden nursery staff provided help with growing experimental plants in Port Augusta and watered them in my absence. Krix wrote the R scripst, which contributed to the data analysis. Valenzuela aided with laboratory work. I led the writing, with all authors contributing to the text.

3. Chapter 4. Milner KM, French K, Van Sluyter S, Leigh A (In preparation). Seasonal changes in functional protein groups, in contrasting desert plants

I designed the experiment in consultation with Leigh and French. I conducted the field and nearly all of the laboratory work, collected and analysed the data. Van Sluyter had previously developed the leaf proteomics methodology and aided with protein extraction and analysis, including running the samples and protein calculations. I led the writing, with all authors contributing to the text.

Preface

Plants! Why study them?

The more we learn about the way plants sense and respond to the environment the more interesting they become to me. Plants do not exist in isolation and despite appearing unchangeable are highly responsive to the conditions in which they grow. The close interactions between fungi and bacteria mean that plants are inextricably linked to one another. The sharing of nutrients and carbon between plants through mycorrhizal fungi moves resources from high to low gradients benefiting individuals, and not always of the same species (Arnebrant et al. 1993, Simard et al. 1997). And while the continued movement of water to a leafless-stump of *Agathis australis* to keep it alive does not appear advantageous, it is likely beneficial for its congeneric neighbours during water limitation (Bader and Leuzinger 2019). Plants sense their environment; detect emissions from other plants and prime themselves against herbivore attack (Frost et al. 2008). They share among themselves but exploit animals, including attracting parasitoid wasps to fend off herbivores (van Poecke and Dicke 2002), tricking animals into pollination (Jersáková et al. 2006) and in the extreme cases, eating them, a trait so good it evolved multiple times (Albert et al. 1992).

Despite all the interesting adaptations plants use to survive, plus making up ~ 80% of the 550 Gt of carbon of biomass on Earth (Bar-On et al. 2018) and providing vast ecosystem services (Costanza et al. 1997), plants suffer from being overlooked. The term ‘plant blindness’ was coined because plants go unnoticed, are not recognised as important, or less important than animals (Wandersee and Schussler 1999). The concern is that conservation funding for plants is lower than for animals (Balding and Williams 2016). All the while natural ecosystems face enormous pressure due to human activity; thus far resulting in 600 seed plant species having gone extinct (Humphreys et al. 2019). Threats to plants are mounting under climate pressure. Yet land plants currently draw down 30% of carbon emissions per year (Ciais et al. 2013) and re-forestation projects have the potential to further drawdown CO_2 emissions (Bastin et al. 2019). If re-vegetation is to work, the identification of appropriate species, ones that can cope with temperature change under climate change, is paramount.

Table of Contents

List of Figures

Figures in main text

logistic curves using Bayesian Monte Carlo iterative modelling for *Solanum oligacanthum* (blue) and *S. orbiculatum* (purple) in winter (full circles, solid line) and summer (empty circles, dashed line).

Figure 2.5. Upward adjustment in temperature thresholds of two Australian desert *Solanum* species from winter to spring. Thresholds of $F_v'/F_m'_{90}$, $F_0'_{90}$, Recovery of F_0 and membranes (MSI) are given for *S. oligacanthum* (top) and *S. orbiculatum* (bottom). For comparisons between seasons (within species), thresholds that were significantly higher in summer than winter are denoted with *. For comparisons between species (within seasons), significantly different thresholds are denoted with #. Thresholds were found using Bayesian modelling and differences were considered significant when curves (Figure 2.4) were different ≥95% of 20 0000 iterations. Boxplots are described in Figure 2.3.

Figure 2.6. Seasonal relative expression of Hsp70 (Hsp70 relative to total protein; mean ± SE) from leaves of *Solanum oligacanthum* (blue) and *S. orbiculatum* (purple) following exposure to treatment temperature for 15 min plus 2 h recovery (a). Species were sampled in winter and summer. Dashed vertical lines show the temperature thresholds of $F_v'/F_m'_{90}$. Example immunoblots of Hsp70 expression in *S. oligacanthum* from winter and summer samples (b).

Figure 2.7. Seasonal relative expression of chloroplastic sHsp24 (chl-sHsp24 relative to total protein; mean ± SE) in detached leaves of *Solanum oligacanthum* (blue) and *S. orbiculatum* (purple) after 15 mins at treatment temperature plus 2 h recovery. Details same as for Figure 2.6.

Figure 2.8. Relationships between chlorophyll *a* fluorescence and physiological parameters of *Solanum oligacanthum* (a, b) and *S. orbiculatum* (c, d) following 15 min heat treatment at six temperatures in winter (a, c) and summer (b, d). Significant Spearman's rank correlations are indicated by solid red lines. chl-sHsp24 was not detected (n.d.) in any samples in winter. For Spearman's rank correlation coefficients (R^2) and p values see Table S2.2.

Figure 3.1. Distribution **(a)** and appearance of the two study *Solanum* species, *Solanum orbiculatum* **(b)** and *Solanum oligacanthum* **(c).** Distribution is displayed in relation to the major classes of the Köppen climate classification of Australia (BoM 1990), showing the common arid zone distribution of the two species. Within this broader classification, *S. orbiculatum* (purple points) has a wider distribution and is found in 'drier' microhabitats, while *S. oligacanthum* (blue points) grows in 'wetter' microhabitats. Species distribution map was produced using the Atlas of Living Australia (ALA 2018).

Figure 3.2. Example of ramping of leaf temperatures (red points) during imposed heat stress using IR ceramic lamps in open top chambers (data shown are all measured plants from spring and summer). Lamp temperature in the chambers was ramped over the first hour and then adjusted manually to maintain leaf temperatures at ~45°C until completion of heat stress at 180 min. Leaf temperature was monitored with a thermographic camera and infrared thermometer. Mean leaf temperature is the average of three leaves per plant. The leaf temperature of ambient plants (blue points) outside and adjacent to the chambers was not controlled and depended on the environmental conditions on that day. Solid lines show the loess smoothing of leaf temperatures.

Figure 3.3. Mean maximum leaf temperatures (± SD, n = 4) recorded during a seasonal heat stress experiment on *Solanum oligacanthum* and *S. orbiculatum* in southern arid Australia. Plants were placed in one of two chambers for imposed heat stress (red) using IR lamps or left in ambient conditions (blue). Different letters indicate significant ($P < 0.05$) differences between the means of treatment responses.

Figure 3.4. Mean (± SD) short-term physiological responses of *Solanum oligcanthum* (pale blue) and *Solanum orbiculatum* (purple) during a heat stress experiment in southern arid Australia. Significant interactions of membrane stability (MSI; a,b), relative expression of Hsp70 (c,d) and relative expression of chl-sHsp24 (e) are plotted. The colours of symbols are indicative of whether plants were grown in high or low nutrients (dark green and yellow respectively) and exposed to ambient (light blue) or heat stress (red) conditions in either spring (green) or summer (orange). Different lower-case letters above symbols indicate significant differences (p <0.05) among the means of treatments. Note that panels c and g represent three-way interactions, d-f show two-way interactions and a and b are main factors.

Figure 3.5. Responses of growth and allocation of biomass in two species of *Solanum* subject to heat stress (mean ± SD). *Solanum oligacanthum* and *S. orbiculatum* were grown in high or low nutrients and subjected to heat stress or ambient conditions in either spring or summer. Colours are described in Fig. 3.4. Variables are: LMA (a); stem to leaf ratio (b); and relative growth rate of leaves (RGR_{leaf}, g day^{-1}). Significant interactions are plotted e and f. Different lower-case letters above symbols indicate significant ($P < 0.05$) differences between the means of treatments. Note that panels e and f show two-way interactions and a-d,g are main factors. Means of main factors of aboveground biomass can be seen in Table S3.2.

Figure 3.6. Visible damage and survival of desert *Solanum* species following heat stress treatment (mean ± SD). Colours are explained in Fig. 3.4. Proportion of plants with visible damage greater than 10% (a); proportion of surviving plants (b-d). Significant two-way interactions are shown in panels a, c and d and three-way interaction in panel b. Different

lower-case letters above symbols indicate significant ($P < 0.05$) differences between the means of treatments. Note that panel b represents a three-way interaction and a,c and d show two-way interactions.

Figure 3.7. Fitness and allocation of resources to reproductive structures of desert *Solanum* species in response to nutrient availability and seasonal heat stress (mean ± SD). Colours are explained in Fig. 3.4. Number of flowers produced per day following heat stress treatment (a-c); flower mass to aboveground (AG) biomass (d); Number of fruits produced per day following heat stress treatment (e); flower mass to AG biomass (f). Note, panels c and e show main factors, two-way interactions are shown in panels a,b,f and a three-way interaction in panel d. Different letters indicate significant ($P < 0.05$) differences between the means of treatments. Relative proportional representation of estimated seed output of *S. oligacanthum* (g) and *S. orbiculatum* (h). Fruit were harvested following heat stress treatment (ambient, A; or heat stress, HS) on plants grown in low (LN) or high (HN) nutrients in spring or summer. The mean number of seeds plant^{-1} was calculated using the mean number of seeds fruit^{-1} x number of fruit plant^{-1} day^{-1}. Note that the panel on the right contains both species, with *S. oligacanthum* represented by the very narrow strip at bottom, which is magnified on the left to show *S. oligacanthum* seed output only. Mean seed output by each species by factor is shown in Table S3.2.

Figure 4.1. Proteins required for the stress response and involved in acquired thermal tolerance (blue boxes) of photosynthesis (green box) and membranes. When temperatures cross thermal thresholds, stress occurs, including increased reactive oxygen species (ROS) production, and damage to proteins and membranes (red boxes). Photo: Annie Spratt.

Figure 4.2. Species distributions according to occurrences recorded in Atlas of Living Australia (ALA 2018). The shrubs *Acacia ligulata* (red points; a) and *Myoporum montanum* (green points; b) are widely distributed, while the herb or sub-shrub *Solanum oligacanthum* (blue points; c) has a narrow distribution.

Figure 4.3. Hourly air temperature (°C) in Port Augusta, South Australia. Arrows indicate sampling points of protein samples (grey arrows), species thresholds (colours follow Figure 4.2). Air temperature collected using i-Button placed in the experimental garden.

Figure 4.4. Seasonal membrane stability (MSI, a) and PSII efficiency (F_v/F_m, b) threshold temperatures of three species of desert plants (*A. ligulata* in red, *M. montanum* in green and *S. oligacanthum* in blue). Box and whisker plots (in the style of Tukey: interquartiles with whiskers extending to lowest and highest datum within 1.5*IQR of lower and upper quartiles respectively). Small black diamonds represent the mean. Different letters show groups with significantly different means ($p < 0.05$). Lower-case letters above plots indicate tests within a

species among seasons and upper-case letters below plots indicate differences within a season among species (winter in light blue, spring in light green, summer in orange).

Figure 4.5. Species comparison of total and photosynthetic proteins within the leaf proteome of *Acacia ligulata*, *Myoporum montanum* and *Solanum oligacanthum*. Amounts (mg m^{-2}) of total protein (a) and total Rubisco (c) and the make-up (percentage of total protein) of photosynthetic protein (b) and Rubisco (d) are shown. Each protein group was analysed separately, and different letters signify significant differences ($p < 0.05$) among means. Boxplots explained in Figure 4.4.

Figure 4.6. Principal Component Analysis (PCA) of the leaf proteomes of three desert plants. These first two axes (PC1 and PC2) explain 77.3% of the variance. Species are shown as coloured symbols (*Acacia ligulata* (red), *Myoporum montanum* (green) and *Solanum oligacanthum* (blue)) and symbol shapes represent season (winter (diamond), spring (square) and summer (circle)). Variables are proteins (blue numbers) grouped at two levels of hierarchy according MapMan BINs (see Table S4.3 for protein function). Green lines show the strength of the influence of a protein on the principal component.

Figure 4.7. The proportion of the top three most influential proteins in each functional protein group contributing to dissimilarities amongst seasons in Australian arid zone plants using SIMPER analysis. Average dissimilarities between seasons are given in the left hand coumn but see Table S4.4. for complete SIMPER output. Functional protein groups are: photosynthesis (a), lipid metabolism (b), Redox homeostasis (c) and external stiumli response (d).

Figure 5.1. Visual damage and membrane stability index (MSI) of *Myoporum montanum* leaves. Heat stress treatment was applied to whole plants by submerging in temperature baths for 15 min. Temperatures used were above and below the T_{50} (PSII threshold) of ~48°C, plus a control and an extreme high temperature. For MSI, a leaf was detached and electrical conductivity measured 300 min after heat stress. Photos were taken the day after treatment. Tukey boxplots show the mean and variance of three replicate experiments.

Figures in supplementary

Figure S2.1. Leaf mass per area (LMA; g m^{-2}) of *Solanum oligacanthum* and *S. orbiculatum* in winter and summer. Different letters signify significant differences ($p < 0.05$) among groups.

Figure S2.2. Dot blots showing the reactivity of antibody raised against consensus region III of chloroplastic small heat shock protein Hsp21. Each column represents a serum tested (pre-bleed: before rabbit was given peptide; first-bleed: after rabbit was injected with peptide; and

kill bleed: after a booster of peptide) and each row represents a different dilution of primary antibody (1:500, 1:1000 and 1:2000). Each dot blot was loaded with 1 µl of synthetic peptide, membranes were blocked, then incubated with appropriate dilution of sera, washed, incubated with secondary antibody, and imaged.

Figure S2.3. Trial for optimal dilution of anti-chl-sHSP21 in So*lanum orbiculatum*. Each immunoblot was loaded with a protein standard ladder (L) and two samples under different heat stress treatments: sample 1 was heated at 50°C for 3 h; sample 2 was heated for 3 h at 50°C following natural priming. Intensities of bands within an immunoblot differ due to unequal loading.

Figure S2.4. Immunoblot for test of reactivity of pre-immune and kill bleed serum used at the optimal dilution of 1:5000. There was no reaction of proteins with pre-immune serum (left), whereas chl-sHsp24 is detected on the immunoblot incubated with kill serum (right). Protein extracted from leaf of *Solanum orbiculatum* heated for 3 h at 50°C following natural priming was loaded in a 2-fold serial dilution 60 to 7.5 µg.

Figure S2.5. Optimisation of total protein loading amount for detection of Hsp70. Mean standard curve (error bars show SE, n = 2) of Hsp70 intensity *versus* total protein loading (a) and example membrane probed with Hsp70 antibody (b).

Figure S2.6. Total protein standard curve. Standard curve of mean (± SE; n = 2) total protein loading intensity, produced using 2-fold serial dilution starting at 80 ug of leaf protein sample (*S. orbiculatum* primed and heated for 3 h) (a). Example image of membrane stained with Amido black (b). Total protein intensity in each lane was estimated by selecting a narrow strip as shown in example red rectangle.

Figure S2.7. Example immunoblot of heat-treated total leaf proteins probed for CLIC1. The protein detected is ~28 kDa in size. To illustrate the differences in expression of Hsp70 and CLIC1 they are both shown taken from the same image, hence Hsp70 is oversaturated.

Figure S3.1. Timeline of seasonal heat stress experiment. Plants were grown from cuttings and allocated to nutrient treatments (green points); a sub-set of plants were harvested prior to the heat stress treatments (pre-harvest; blue points); heat stress treatments were imposed on four consecutive days (red points) in Austral spring (October) and summer (February). After the heat stress treatments, plants were left to grow, and a sub-sample was destructively harvested for biomass and fitness (post-harvest; black points). Non-destructive sampling for visible damage, survival and numbers of flowers and fruit of all remaining plants were counted (dark blue points).

Figure S3.2. Nitrogen status of *Solanum oligacanthum* (left) and *Solanum orbiculatum* (right). Total leaf protein concentration (mean ± SD, n = 15) in plants following application of fertiliser (green points) or growth in sand and potting mix alone (yellow points). Nutrient status was influenced by species and time in a three-way interaction (ANOVA $F_{1,112} = 7.31$, $p = 0.007$). Different letters indicate significant ($P < 0.05$) differences between the means of treatments.

Figure S3.3. Maximum quantum yield (F_v/F_m) of *Solanum* plants pre- and post-heat stress. *Solanum oligacanthum* (top panels) and *Solanum orbiculatum* (bottom) plants were grown in high or low nutrients. In spring (left panels) or summer (right) plants were water stressed before exposure to heat stress (red) or ambient conditions (blue). F_v/F_m was measured pre-dawn on the mornings pre- and post-heat stress. Boxplots include all individual plants (n = 24, except *S. oligacanthum* high nutrient summer = 18). Box and whisker plots (in the style of Tukey: interquartiles with whiskers extending to lowest and highest datum within 1.5*IQR of lower and upper quartiles respectively).

Figure S3.4. Ambient air temperature and VPD at Australian Arid Lands Botanic Gardens, Port Augusta, South Australia. Data for the five days preceding, four days during (shaded area) and five days following heat stresses in spring (a) and summer (b).

Figure S3.5. Air temperature (°C) and VPD (kPa) during four replicate heat stress treatments (one replicate per row) imposed in spring (a, c) and summer (b, d). Heat stress conditions are shown within open top chambers (red lines) and ambient conditions adjacent to chambers (blue lines). No data were collected for the first replicate treatment in spring due to non-functional data loggers.

Figure S3.6. Example immunoblots of HSP expression in Solanums. Hsp70 (a) and chlp-sHsp24 (b) expression are shown for *S. oligacanthum* (left) and *S. orbiculatum* (right). Immunoblots shown are representative of all blots, each row is from a single membrane. Some lanes have been reordered for ease of interpretation (borders show where image was spliced).

Figure S3.7. Resprouting *Solanum oligacanthum* following heat stress.

List of Tables

Tables in main body

given followed by the protein BIN number from MapMan (see Table S4.6 for list of proteins). Colours are not representative of any metric, simply a visual aid.

Table 4.4. Proteins that correlate with threshold adjustment patterns across season for *Acacia ligulata*, *Myoporum montanum* and *Solanum oligacanthum.* Proteins with significant positive or negative correlations that match the changes in membrane and photosynthetic thresholds are given. Analyses were conducted separately for each species. For protein details, see Table S4.6, for a full list of proteins with significant correlations see Table S4.7.

Tables in supplementary

Table S2.1. Temperature thresholds of photosynthesis and membranes in two Solanums in winter and summer. Thresholds of effective quantum yield (F_v'/F_m'), membrane stability (MSI), minimal fluorescence (F_0') and Recovery of F_0 (R_{Fo}) found using sigmoidal curves fit with Bayesian models. Thresholds were set at 88% of low temperature asymptote. Thresholds were compared between species (*Solanum oligacanthum* and *S. orbiculatum*) within season, or within season between species. Threshold differences were considered significant one threshold higher in >95% of 20 000 iterations and are indicated in bold. Confidence intervals of 2.5 and 97.5% are contained within parentheses.

Table S2.2. Spearman Rank correlation coefficients and *p* values between chlorophyll *a* fluorescence and physiological parameters of *Solanum oligacanthum* and *S. orbiculatum* following 15 min heat treatment at six temperatures in winter and summer.

Table S2.3. Sequence of UniProtKB – P31170 (HS25P_ARATH) heat shock protein 21, compared with the highly conserved amino acid sequence from consensus region III (methionine-rich domain) published by Downs et al. (1998) (underlined section). The leucine, at position 89, in P31170 sequence has been replaced by a methionine in the sequence by Downs et al. (1998).

Table S3.1. Air temperature and VPD during heat stress treatments in spring and summer.

Table S3.2. Main factor means (± SE) short- and long-term responses to heat stress experiment during spring *versus* summer.

Table S4.1. F- and p-values of species and seasonal comparisons of thermal thresholds of membrane (MSI) and PSII (F_v/F_m) thresholds.

Table S4.2. F- and p-values of amounts and percentages of leaf proteins. Proteomes of *Acacia ligulata*, *Myoporum montanum* and *Solanum oligacanthum* were measured in winter, spring and summer and amounts of total proteins and Rubisco and percentages of photosynthetic proteins and Rubisco to total proteins compared.

Table S4.3 Leaf proteins of *Acacia ligulata, Myoporum montanum* and *Solanum oligacanthum* arranged at two levels of hierarchy according to functional protein BINs in MapMan.

Table S4.4 Top three most influential proteins in each functional protein group contributing to dissimilarities amongst seasons in Australian arid zone plants using SIMPER analysis.

Table S4.5. Pseudo-F and p-values (Monte Carlo; MC) of PERMANOVA analysis of species and seasonal expression of entire leaf proteome. Pair-wise t-tests identified where the species*season interaction lay.

Table S4.6. Leaf proteins of interest detected in *Acacia ligulata, Myoporum montanum* and *Solanum oligacanthum* grouped into complexes or functional groups, up to four levels of hierarchy according to functional protein BINs in MapMan.

Table S4.7. Protein correlation with expression patterns across season for *Acacia ligulata*, *Myoporum montanum* and *Solanum oligacanthum.*

Abstract

Understanding how plants cope with extreme temperatures is key to determining species distribution under climate change. Plants possess an inherent ability to withstand high temperatures and acquire greater thermal tolerance seasonally. The membranes and photosynthetic apparatus in leaves are particularly susceptible to heat damage and likely to respond to different environmental cues. The question arises as to how these two systems differ in acquiring thermal tolerance and what roles proteins have in raising thresholds. As part of the stress response and to aid in thermal tolerance, heat shock proteins (HSP) are upregulated, but there are associated resource costs, of particular concern for natural populations. In extreme environments, like deserts, the additional stressors of water and nutrient limitation may affect how plants allocate resources to growth, reproduction and survival. My thesis is important in linking ecology, plant physiology and molecular biology over seasonal time scales in wild Australian desert plant species *in situ* in desert conditions. I estimated temperature thresholds of photosystem II (PSII, using chlorophyll *a* fluorescence) membrane stability (via electrolyte leakage) and fitness (via reproductive output) in response to heat stress across seasons. To determine how relative protein expression changes with conditions, I also quantified the complete proteome using shotgun proteomics with tandem mass spectrometry. Overall, species acquired higher thresholds of PSII and membranes and HSP expression was dependent upon season, with little sHSP detected in winter. Cost of three-hour heat stress was reduced in plants with access to additional nutrients, but unexpectedly, heat stress in spring was found to be less costly than in summer, likely due to more severe summer conditions making recovery hard. I show that changes to the proteome are complex, but consistent patterns emerged, with lipid metabolism, ROS homeostasis and HSPs meeting expectations of higher expression during summer. Also, regardless of species or heat-stress treatment, small HSPs were detected in greatest amounts in summer, emphasising the importance of small-HSPs for acquired thermal tolerance in desert species. Importantly, species differences were highlighted throughout the research. Across broad climatic zones, species have many modes for achieving the same outcome and microhabitat likely has an effect on driving adaptation. My work underscores the temporal dynamics of plant thermal tolerance in non-crop species in the environment and how this is achieved through proteome changes. However, my findings suggest that for species from harsh microhabitats, increasing heat stress in summer may have particularly severe consequences.