Variation in the Photosynthetic Heat Tolerance of Plants from Extreme Environments: The Influence of Heat Exposure and Environmental Context

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Alicia Cook declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, 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.

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Table 1.1. Selection of extrinsic and intrinsic factors that alter the photosynthetic or leaf heat tolerance of plants. Focuses on species that have not been bred extensively for agriculture, with a few agricultural examples included for specific factors. T_{50} is the temperature threshold at which there is a 50% decline in F_V/F_M; T_{crit} is the inflection point of F_0 measured with continuously increasing temperature of a F_0-T curve; T_{max} here is the peak temperature of dark respiration; and LT_{50} is the temperature at which 50% of visual mortality of a leaf is evident. Assays conducted under light (L) vs dark (D) conditions may have different implications with respect to the ability for photosystem repair during and following stress.

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Table A2.2. Temperature, duration, repeats and trials model random parts estimates modelling the heterogeneity between groups.

Table A2.3. Temperature, duration, repeats and trials model build of the factors and their interactions with the -2* log likelihood IGLS value (-2ll IGLS), difference in model (Δ-2ll IGLS) and a 1-sided p-value on the difference between models calculated on a χ^2 distribution. The *"i"* notation indicates where the variances within factor levels were allowed to vary. Final model (no. 38) included the non-significant 3-way interaction between temperature-reprieve-trial as it contained a significant value, which was of key interest to the study. To control for leaf collection over multiple days within each trial, which may affect the heat stress response, the model build incorporated the environmental temperature of either the cumulative mean temperature of one to five days prior, or the maximum or minimum temperatures within the five days prior to the day of leaf collection (Table S3). The minimum temperature of two days prior singularly had the strongest effect on the model and was included as a co-variable.

Table A2.4: Post-hoc Wald contrast tests of model effects. This table corresponds to the high-level data presented in Table 1 and Figure 3 & Figure S1. Two controls were used in analyses: 1) contrasts at high temperature stress treatments (48, 50 and 52°C) have been corrected, by excluding handling effects within a given experiment (see Methods); 2) all F_V/F_M responses were compared to the average control F_V/F_M of 0.784 (SE ± 0.008). The table is organised into sections addressing an overall question with a joint Chi Square Statistic results for the following contrasts in that group. Functional effect (f-k= Δ) indicates the effect size which is the difference in means between the tested groups.

Table A2.5. Weighted linear regression model results of the relationship of thermal tolerance thresholds with duration curve (Figure 2.6A), describing the relationship between heat tolerance and stress duration for *M. montanum* (*M. mon*) and *E. socialis* (*E. soc*). *M. montanum* was measured in three trials (1, early January; 2, late January; 3, early February, 2016); *E. socialis* was measured in Trial 1 only. Bold p-values indicate significance at α=0.05. $T_{50:1'}$ refers to the predicted heat tolerance at a 1-min stress duration.

Table A2.6. Comparisons of threshold temperature ($T_{50:1'}$ and its equivalent, CT_{max}) and thermal sensitivity *(z)* of plant species from this experiment and estimated from the literature. The plant form, method of damage quantification and growth conditions are also reported. CT_{max} and thermal sensitivity estimates were back-calculated from previous reported leaf damage when responses to three or more temperature-duration combinations were reported at similar duration scales.

Table A3.1. Number of leaves (n) per temperature treatment measured at each measurement period (occasion) within each trial for linear models 'Stress' and 'Combined stress and reprieve' with response variables EQY and NPQ. Repeated three times as 'Trials'.

Table A3.2. Model estimates for 'Stress' models which model the EQY and NPQ response pre-stress through to stress six, excluding reprieve measurements. Models accompany Figure 3.2.

Table A3.3. Model estimates for 'Combined stress and reprieve' models with separate responses of EQY and NPQ between Stress 1 to Stress 6 including the reprieve measurements. Models accompany Figure 3.3.

Table A3.4. Correlations among EQY responses to re-occurring stress and reprieve exposures for the Combined stress and reprieve model. The level of photoinhibition (decline in EQY) with subsequent high temperature exposure was more strongly correlated with the response of previous high temperature exposures than prior cool reprieves. The influence of previous exposures diminishes with further stresses (0.840 to 0.396) while the influence of reprieves diminishes at a lesser rate (0.689 to 0.403). Bold = >0.5 correlation.

Table A3.5. Correlations among NPQ responses to re-occurring stress and reprieve exposures for the Combined stress and reprieve model. The level of non-photochemical quenching with subsequent high temperature exposure was strongly correlated with the response of previous high temperature exposures and cool reprieves with previous reprieves, except for the response after the first stress. The influence of previous exposures diminishes with further stresses (0.843 to 0.100) while the influence of reprieves diminishes to a lower level (0.843 to -0.03). Bold = >0.5 correlation.

Table A3.6. Pearson's moment correlation and p-values for recovery metrics of NPQ relaxation and EQY recovery at each Temperature, Repeat and Trial between the last stress and 90-mins post heat stress as well as 90-mins to day two. Significant values bolded at alpha 0.05 level.

Table A4.1. Habitat details of the area where seeds were collected for *Poa costiniana*, *Luzula modesta* and *Oreomyrrhis ciliata*.

Table A4.2. Growth conditions and the temperature regimes including the duration of each 'season' and temperature step, temperature ranges and the day/night hour ratio (Satyanti, 2018). The thermal tolerance threshold (T_{50}) was measured at the end of the second experimental summer. The difference in season length was changed by reducing the number of days comprising 'winter' and increasing the number of days in the 'summer' season.

Table A5.1. Leaf water potential Tukey post-hoc contrasts of well-watered leaves *vs* water-stressed leaves.

Table A5.2. Tukey post-hoc contrasts between maximum air and leaf temperatures for well-watered (HW) and water-stressed (LW) plants.

ABSTRACT

Photosynthesis supports life on earth and is highly temperature dependent. Extreme temperatures can inhibit photosynthesis and damage the photosystem machinery, potentially limiting future productivity and plant survival. With increasing risk of extreme temperature exposure under climate change, plants may be pushed to the edge of their thermal limit, but at what point is a complex question. Temperatures that cause substantial damage to photosystems, encapsulated by heat tolerance thresholds, help to answer this question. On hot days leaf temperatures can spike multiple times, yet what we know of the variability of heat tolerance often comes from tests that vary in only one dimension – temperature. In Chapter 2, I demonstrated that varying combinations of heat characteristics can accumulate as heat doses and reveal multiple heat tolerance thresholds. By varying the heat dose, the thermal sensitivity of tolerance can also be examined, which is a first in plants. In Chapter 3, I followed the temporal effects of multiple exposures to extreme high temperatures, which potentially both reduced and delayed the capacity for repair to Photosystem II (PSII) with sustained photoinhibition present on the following day. Examining plants in less obviously extreme environments, alpine summers are predicted to be warmer and longer under climate change, potentially increasing heat stress for alpine plants. In Chapter 4, I explored the scarcely studied effect of elevated and extended growth temperature on the heat tolerance of Australian alpine species. While alpine plant species maintained surprisingly high photosynthetic heat tolerance, they only marginally increased their tolerance in response to warming, suggesting increased vulnerability to heat stress with long term climate change. The application of plant physiological heat tolerance in assessing future vulnerability to increasing temperatures under climatic change, however, is complicated. As I showed in Chapter 5, water availability plays an important role in the relationship between leaf and air temperature, which also influences the level of heat tolerance. Considered together, these components can considerably alter species predicted vulnerability assessments to high temperature. Combined, this work demonstrates that considering the multiple dimensions of high temperatures as heat doses, and the effects of complex relationships of water availability and temperature sources, widens the frame of photosynthetic thermal limits in plants experiencing extreme environments.

Declaration of Contribution to each chapter

The following chapters have recently been reviewed or are prepared for imminent submission for publication. Contribution of co-authors and myself are detailed below.

Chapter 2: Beyond a single thermal threshold: varying the characteristics of temperature stress creates a landscape of plant heat tolerance*. Alicia M. Cook, James Brown, Neil G. Berry, Katherina Petrou, Andrea Leigh.*

I designed and lead the project, collected field data, analysed and wrote the paper (80%), with contributions from co-authors as follows. Brown guided mathematical model building and interpretation (5%), Berry helped to collect field data (2%), Petrou contributed technical advice on chlorophyll fluorescence and reviewed manuscript drafts (3%) and Leigh contributed conceptually to the study design, biological interpretation of results and reviewed manuscript drafts (10%). Two anonymous reviewers provided constructive advice on the original manuscript, which was submitted to *Functional Plant Ecology*.

Chapter 3: Heat dose influence on the temporal dynamics of effective quantum yield and non-photochemical quenching of Photosystem II. *Alicia M. Cook, James Brown, Katherina Petrou, Andrea Leigh.*

I designed and lead the project, collected field data, analysed results, biologically interpretated results and wrote the chapter (80%), with contributions from co- authors as follows. Brown guided linear model building and interpretation (3%), Petrou contributed technical advice on chlorophyll fluorescence, aided biological interpretation and reviewed manuscript drafts (7%) and Leigh contributed conceptually to the study design and analysis, biological interpretation of results and reviewed manuscript drafts (10%).

Chapter 4: Alpine plant species only marginally increase their heat tolerance with experimental warming. *Alicia M. Cook, Susanna Venn, Annisa Satyanti, Adrienne B. Nicotra and Andrea Leigh.*

I designed assay sampling, ran T_{50} assays, analysed and wrote the paper (75%), with contribution from co-authors as follows. Venn aided data collection and reviewed drafts

(5%), Satyanti co-designed and ran the growth project, aided data collection and reviewed drafts (5%), Nicotra co-designed the growth project and reviewed drafts and Leigh assisted with assay sampling (5%), contributed to analysis and interpretation and reviewed drafts (10%). This project was part of another PhD student's (Satyanti) larger experiment at the Australian National University, looking at the effects of warmer longer summers on Alpine species phenological stages which was almost complete when I was invited to be involved. Satyanti was supervised by Nicotra and Venn. Two anonymous reviewers provided constructive advice on the original manuscript, which was submitted to *Conservation Physiology*.

Chapter 5: A higher heat tolerance does not always mean lower thermal vulnerability: why water availability matters to leaves. *Alicia M. Cook, Neil Berry, Kirsty Milner, Andrea Leigh.* This chapter has been published in *Functional Ecology* as 'Water availability influences thermal safety margins for leaves'. Published online 19 June 2021.

I co-lead conceptual design, data analysis, interpretation and lead the writing (75%), with contributions from the co-authors as follows. Berry collected the data and conducted preliminary data analysis (15%), Milner co-lead conceptual design, contributed to drafts of the manuscript chapter (5%) and Leigh co-lead conceptual design, aided interpretation, and reviewed drafts of the chapter (10%). Note that the data collection for this research was undertaken as part of an Honours student (Berry) project, which I co-designed with Leigh, and co-supervised with Leigh and Milner. Berry has left science and I have lead the manuscript for publication, for which I completely re-analysed the data, added new conceptual components (e.g. T_{air}:T_{leaf} relationships and leaf homeothermy) and wrote the manuscript from scratch..

Finally, because it has direct relevance to my thesis, I include as an appendix parts of a review paper for a large project in which I was invited to be involved, based on my expertise in plant heat tolerance. Titled 'The thermal tolerance of photosynthetic tissues: a global systematic review and roadmap for future research'. *Sonya R. Geange, Pieter A. Arnold, Alexandra A. Catling, Onoriode Coast, Alicia M. Cook, Kelli M. Gowland, Andrea Leigh, Rocco F. Notarnicola, Bradley C. Posch, Susanna E. Venn, Lingling Zhu, Adrienne B. Nicotra.* Published as a Tansley Review in *New Phytologist (2021, vol 229: 2497-2513)*.

I was actively involved throughout the entire review process, at regular meetings and several weekend research team retreats. I contributed conceptual ideas and direction initially, screening protocols, screening articles and recording data attributes, result selection and writing and revisions of the manuscript.

GLOSSARY OF KEY TERMS

Acquired tolerance	Increases in heat tolerance in response to immediate prior exposure to sub-lethal high temperatures or priming conditions. Often involving the upregulation of HSP. In some contexts, the term 'acclimation' will be used to describe the process of reaching acquired tolerance.
Assay	The use of increasing intensity or concentration of an abiotic variable to determine organism responses. Herein, it refers to exposing leaves to a range of high temperatures used to determine the temperature at which a particular change in PSII function occurs, either a 50% decline in F_V/F_M (T_{50} threshold) or the temperature at which F_0 increases (T_{crit}).
Basal tolerance	Thermal tolerance without priming. Used here to describe sampled thermal tolerance measurements *without* pre-treatment designed to induce acquired tolerance.
HSP	*Heat shock proteins*. Here, HSP is used to refer to the entire family of proteins, including small heat shock proteins (sHSP), induced as a response to stress and serving a range of functions to protect and repair proteins from aggregation and denaturation.
PSII	*Photosystem II,* located in the thylakoid membrane, is responsible for light reactions of photosynthesis. PSII oxidizes water and is at the beginning of the electron transport chain.
Priming	Conditions that can induce acquired tolerance mechanisms.
Thermal tolerance metric	A specific temperature at which some physiological threshold is reached (e.g. 50%). Such a metric is based on the physiological response of an organism or organ (e.g. animal or leaf chlorophyll fluorescence) assayed across multiple temperatures. In this thesis, T_{50} and T_{crit} are the metrics used. See Appendix 1, Table A1.1 for further examples of plant thermal tolerance metrics.

T_{50} threshold	A 50% decline in the chlorophyll fluorescence measurement F_V/F_M post temperature exposure. A metric used to compare the temperature tolerance of PSII in leaves.
Chlorophyll fluorescence terms:	
F_0	*Minimum fluorescence* of chlorophyll in *dark* adapted leaves, under a minimal measuring light. All PSII reaction centres open.
F	The *fluorescence level* of chlorophyll in *light* adapted leaves, under a minimal measuring light. Some PSII reaction centres closed (quenched).
F_M	*Maximum fluorescence* of chlorophyll in *dark* adapted leaves post-saturating light flash. All PSII reaction centres closed.
F_M'	*Maximum fluorescence* of chlorophyll measured on *light* adapted leaves post saturating light flash. All PSII reaction centres closed.
F_V	*Variable fluorescence* is the maximum (F_M) minus the minimum (F_0) fluorescence of chlorophyll in *dark* adapted leaves.
F_V/F_M	*Maximum quantum yield of PSII*. A chlorophyll fluorescence parameter measured on dark adapted leaves.
EQY ($\Delta F/F_M'$)	*Quantum yield or effective quantum yield of PSII*. A chlorophyll fluorescence parameter measured on *light* adapted leaves.
NPQ (F_M-F_M'/F_M')	*Non-photochemical quenching*. A unitless value on a scale of 0-infinity, which describes the difference between maximum dark-adapted fluorescence and the maximum fluorescence under light conditions. This difference is due to non-photochemical quenching and is often an indication of diversion of energy to protective mechanisms. NPQ is in direct competition with EQY and is a linearly related to heat dissipation.