The response of native Australian seedlings to heat and water stress

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A thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Applied Science (Honours) at the University of Canberra.

November 2014
The survival of plants depends on the ability of plants to cope with environmental stresses, often in combination and for different periods of time. Measuring the response of species and communities of plants to changes in environmental stresses is imperative given the predictions of widespread elevated temperatures, decreased rainfall and extreme events associated with climate change. Despite the wide range of research on plant physiology and the response of plants to particular stresses, there are still gaps in research, limited analysis into particular species, poorly understood responses of plants to a combination of stresses and limited information on the recovery of plants following stress (i.e. rewatering). The ability, accuracy and reliability of leaf health measurements to detect changes in particular species, in isolation and in competition to a range of stresses is not well studied for many species. Due to the expected effects of climate change, of particular concern are the potential effects of elevated temperature and decreased rainfall on ecologically vulnerable native conservation species in Australia.

This study examines the physiological and morphological response of eight non-commercial native Australian species (*Eucalyptus melliodora*, *Eucalyptus blakelyi*, *Acacia melanoxylon*, *Daviesia mimosoides*, *Dodonaea viscosa*, *Hardenbergia violacea*, *Poa sieberiana*, and *Poa labillardieri*) during seedling stage grown in competition to two heat (17-27°C and 23-35 °C) and two water (regularly watered and withheld water) conditions and a combination of these conditions for a 10 – 12 week duration. Along with this, the response of two of these species (*Eucalyptus melliodora* and *E. blakelyi*) to differing periods (7, 4 and 12 weeks) of heat and water stress and re-watering following 4 and 7 weeks stress was also examined. The experiments were conducted in conditions simulated in a glasshouse at the University of Canberra.

In order detect changes in leaf health this study examined a range of common leaf health measures: specifically visual leaf heath; photosynthesis; stomatal conductance; transpiration; photochemical reflectance index (PRI); normalised difference vegetation index (NDVI); simple ratio index (SR); and relative leaf chlorophyll content (CL) relative to time since last watering. Linear mixed effect models were used to analyse the effects of time of stress (water and heat), effect of species, experiment (competition and without competition) and
re-watering. Results were analysed for detection of change points in the measurements of leaf health and identification of any correlations between each measurements.

There were multiple key findings of the analysis of this study. Firstly, the results from this study suggest that heat and water stress have an additive effect where the effect of heat and water stress is increased when the stresses occur in combination than when in isolation. Specifically this study provided information into the use, reliability, correlation and effectiveness of multiple measurements of leaf health relative to multiple types of plant stresses (temperature, water). This study revealed that PRI, NDVI and SR are highly correlated indices which all reflect changes in leaf health following a period of water stress. These measures showed a steady decline with increased period of water stress in all species and were effective at detecting water stress. Results propose that any one of these leaf reflectance indexes could be used in isolation to detect change in leaf health. A change point in all species was detected in stomatal conductance and transpiration following 4-5 weeks without water, which suggests that these physiological processes cease when soil moisture is no longer available. Although these general trends exist, species differed in their response to stress with tree species generally less affected by water and heat stress when compared to shrub species.

Seedlings’ recovery following water stress was significantly improved following 4 weeks without water when compared to recovery following 7 weeks without water. This study suggests that the recovery of 6 month old seedlings following re-watering is likely to significantly decrease if periods of water and heat stress exceed five weeks.

In addition to these findings, this study can provide baseline information on the relative survival and leaf health of some seedlings within grassy-box woodland habitats in water and heat conditions predicted to occur (based on climate change predictions) within the next 100 years. Further analysis is needed to mirror this study in a natural habitat to fully understand the results demonstrated in this study taking into account other influences that occur in natural habitat (soil type, different aged plants, soil biota etc.).
ACKNOWLEDGEMENTS

I would like to thank my primary supervisor Dr Paul Downey for supporting me and giving me the opportunity to undertake my honours project. His assistance, guidance and feedback throughout the year, as well as encouragement to look at the big picture, has been invaluable. I would like to also thank my secondary supervisor Dr Bernd Gruber. His assistance with helping me grasp aspects of the wide range of statistical analysis and R coding techniques has certainly helped me develop skills and acquire knowledge which will undoubtedly stick with me throughout my career. Many thanks to Dr Bill Sea who assisted me with the beginnings of my project, including vital assistance with the use of the instruments and management of the glasshouse.

I would also like to thank Greening Australia for providing me with the seedlings for my experiment, without which this project would not have been possible.

I would like to acknowledge the financial, academic and technical support of the University of Canberra, particularly the Institute of Applied Ecology, for providing me with the IAE Honours scholarship as well as a variety of other avenues of support and assistance throughout the year.

A special thanks to my mum Shelley Owen, who has helped me on countless occasions with measurements, planting and editing. Her assistance and reassurance, along with always reminding me that no project is possible without hard work and a few hiccups, got me through the year. And finally I would like to thank Matthew Parker, who helped me sample on many occasions as well as providing me with moral support and encouragement during the project.
Table of Contents

ABSTRACT.............................................................................................................. ii
ACKNOWLEDGEMENTS ......................................................................................... v
List of tables............................................................................................................. x
List of figures............................................................................................................ xiv
CHAPTER 1 - Introduction ....................................................................................... 1
  1.1. Background ..................................................................................................... 1
  1.2. Plants and water stress .................................................................................... 2
    1.2.1. Defining water stress and drought ............................................................ 2
    1.2.2. Effects of water stress on plants ............................................................... 2
    1.2.3. Plants and recovery following water stress .............................................. 10
  1.3. Plants and temperature .................................................................................... 12
    1.3.1. Effects of temperature on plants ............................................................... 13
  1.4. Plant distribution and climate ........................................................................ 15
    1.4.1. Effects of temperature on plant distributions .......................................... 16
  1.5. Plants and seasonal light availability ............................................................... 16
  1.6. The effects of water stress on individual plant species relative to the effects on a
      vegetation community ....................................................................................... 17
  1.7. Measuring physiological and morphological changes in plants following water and
      heat stress ............................................................................................................ 18
    1.7.1. Comparing multiple measures of the physiological response of plants to stress ... 21
  1.8. Plants and drought in a changing climate: what are the future implications ....... 22
    1.8.1. Australian climate change predictions ...................................................... 22
    1.8.2. Effects of climate change on plants ........................................................... 24
  1.9. Research Gaps .................................................................................................. 24
  1.10. Thesis aims and objective .............................................................................. 25
CHAPTER 2 - Method ............................................................................................... 27
  2.1. Glasshouse ...................................................................................................... 27
    2.1.1. Operating conditions of the glasshouse (spring treatment) ...................... 27
2.1.2. Operating conditions of the cabinets (summer treatment)..........................27
2.1.3. Monitoring of operating conditions of the glasshouse .........................27
2.2. Study species .................................................................................................28
  2.2.1. Number of individual plants per species ..................................................28
2.3. Pre-treatment conditions ...............................................................................29
  2.3.1. Prior germination, growth and relocation to the Glasshouse ..................29
  2.3.2. Repotting of tubestock ready for experiments ........................................29
  2.3.3. Watering regime prior to the experiment, and for the control treatments ...30
2.4. The two experiments ......................................................................................31
  2.4.1. *Eucalyptus* only (single species) ............................................................31
  2.4.2. The competition experiment ....................................................................31
2.5. Treatments .......................................................................................................31
  2.5.1. Allocation of treatments and replication for the *Eucalyptus* only (single species) 33
  2.5.2. Allocation of treatments and replication for the competition experiment ....34
2.6. Sampling regime ............................................................................................34
  2.6.1. Selecting leaves and plants to sample ......................................................34
  2.6.2. Sampling interval and duration ................................................................36
2.7. Assessing the morphological and physiological responses .........................38
  2.7.1. Morphological measurements of leaf/plant health ..................................38
  2.7.2. Physiological measurements of leaf health .............................................39
  2.7.3. Abiotic measure .......................................................................................44
2.8. Data analysis ..................................................................................................44

CHAPTER 3 – Results ............................................................................................46
3.1. Description of data collected .........................................................................46
3.2. *Eucalyptus* only experiment .......................................................................46
  3.2.1. Seedling survival rates ............................................................................47
  3.2.2. Correlation plot of measurements in the *Eucalyptus* only experiment ......48
3.2.3. Photochemical Reflectance Index (PRI) leaf values .................................................49
3.2.4. Normalised Difference Vegetation Index (NDVI) leaf values .............................51
3.2.5. Simple Ratio (SR) .................................................................................................53
3.2.6. Leaf transpiration (T) rates ..................................................................................55
3.2.7. Stomatal Conductance (SC) ................................................................................56
3.2.8. Relative Leaf Chlorophyll Concentration (CL) ...................................................58
3.2.9. Percentage Soil Moisture (SM) content ...............................................................59
3.2.10. Summary ..............................................................................................................61
3.3. Rewatering the eucalypt plants after water stress .....................................................61
  3.3.1 Observations of plant recovery following rewatering ........................................61
  3.3.2. Rewatering ..........................................................................................................64
3.4. Competition experiment ..........................................................................................73
  3.4.1. Visual observation of leaf health ........................................................................74
  3.4.2. Correlation plot for leaf health values in the competition experiment ............76
  3.4.3. Photochemical Reflectance Index (PRI) leaf values .........................................77
  3.4.4. Normalised Difference Vegetation Index (NDVI) leaf values ...........................79
  3.4.5. Simple Ratio (SR) leaf values ............................................................................81
  3.4.6. Leaf Transpiration (T) rates ................................................................................82
  3.4.7. Leaf Stomatal Conductance (SC) rates ...............................................................84
  3.4.8. Relative leaf chlorophyll values (CL) .................................................................86
  3.4.9. Soil moisture percentage (SM) ..........................................................................88
  3.4.10. Summary ..........................................................................................................89
3.5. Comparing the results for Eucalyptus species between the Eucalyptus only experiment and the competition experiment ...............................................................89
  3.5.1. PRI leaf values from the Eucalyptus only experiment compared with those from the competition experiment .................................................................90
  3.5.2. Leaf transpiration rates from the Eucalyptus only experiment compared with those from the competition experiment .........................................................91
CHAPTER 4 - Discussion ................................................................. 94
4.1. Seedling health deterioration and mortality rates relative to water and heat stress ...... 94
  4.1.1. Seedling health and mortality relative to water stress .................................. 94
  4.1.2. Seedling health and mortality relative to heat stress ..................................... 100
  4.1.3. Seedling health and mortality relative to the combination of water and heat stress ................................................................. 100
4.2. Seedling recovery rates following different periods of water and heat stress........... 102
  4.2.1. Seedling recovery following water stress ..................................................... 102
  4.2.2. Seedling recovery following the combination of water and heat stress ........... 103
4.3. Seedling responses across multiple physiological measures to water stress, heat stress and re-watering following stress ................................................................. 104
  4.3.1. Comparing the results from multiple physiological measures to water stress, heat stress and re-watering ................................................................. 105
  4.3.2. An evaluation of the effectiveness and practical application of the physiological measures used relative to the response of seedlings to the different stresses examined 106
4.4. Australian native plants and other studies ....................................................... 107
  4.4.1. The vegetation community ................................................................. 107
  4.4.2. Species variation .................................................................................. 110
  4.4.3. Effects of competition .............................................................................. 110
  4.4.4. Effects of biotic stresses .......................................................................... 111
4.5. Implications of study and future plant conservation ............................................. 111
  4.5.1. Heat and water stress – a climate change perspective .................................... 111
  4.5.2. Revegetation and regeneration .................................................................... 112
  4.5.3. Remote Sensing ......................................................................................... 113
4.6. Limitations of study and suggestions for further research ..................................... 114
4.7. Conclusion ....................................................................................................... 116
CHAPTER 5 - References ....................................................................................... 117
List of tables

Table 1.1. A description of measurements used to monitor plant health and what each measurement shows. ..................................................................................................................................................20
Table 2.1. The number of individual plants needed for each of the eight Australian native species. .................................................................................................................................................................................................28
Table 2.2. Summary table of treatments used in the *Eucalyptus* only experiment (full details are outlined below). .........................................................................................................................................................................................32
Table 2.3. Summary table of the treatments used in the community experiment (full details are outlined below). .........................................................................................................................................................................................34
Table 2.4. The timetable developed for leaf health measurements and the associated time required to take each measurement relative to the period of optimal light available per day/week, from which the number of leaves per plants and experiment can be calculated. ...37
Table 3.1. Summary and sample size of both experiments in this study. ..................................................46
Table 3.2. Results of ANOVA of linear mixed effects model (LMM) of the changes in observed plant health relative to treatment type, time since last watering (week) and eucalypt species. ........................................................................................................................................................................48
Table 3.3. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the observation leaf health categories from summer and spring treatments for both species. ...............................................................................................................................................48
Table 3.4. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time since last watering and species on change in PRI leaf values. ...............................................................51
Table 3.5. Result of ANOVA of linear mixed effects model (LMM) of the relationship between the PRI leaf values from summer and spring treatments for both species. ...............................51
Table 3.6. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on change in NDVI leaf values. .........................................................................................................................53
Table 3.7. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the NDVI leaf values from summer and spring treatments for both species. .................................53
Table 3.8. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on simple ratio values. ..........................................................................................................................54
Table 3.9. Results of ANOVA of linear mixed effects model (LMM) of the relationship between simple ratio from summer and spring treatments for both species. ........................................54
Table 3.10. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf transpiration rates. .................................56

Table 3.11. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf transpiration rates from summer and spring treatments for both species. ...56

Table 3.12. Result of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on stomatal conductance rates. ...........................................57

Table 3.13. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the stomatal conductance values from summer and spring treatments for both species. .........................................................................................................................57

Table 3.14. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on the relative leaf chlorophyll content values...............................59

Table 3.15. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the relative leaf chlorophyll content from summer and spring treatments for both species. ........................................................................................................................................59

Table 3.16. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on soil moisture values. ..........................................................60

Table 3.17. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the soil moisture from summer and spring treatments for both species. .............60

Table 3.18. Summary table for Eucalyptus only experiment data......................................................61

Table 3.19. Analysis of deviance table of the results of binomial regression of the effects of treatment, time and species on location of new growth for the 4 weeks without water treatments.................................................................63

Table 3.20. Analysis of deviance table of the results of binomial regression of the effects of treatment, time and species on new growth for the 7 weeks without water treatments........63

Table 3.21. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf PRI from summer and spring treatments following rewatering. ..........66

Table 3.22. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the PRI leaf values from summer and spring treatments for both species. ..........66

Table 3.23. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf NDVI from summer and spring treatments following rewatering.............67

Table 3.24. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the NDVI leaf values from summer and spring treatments for both species.........68

Table 3.25. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf SR from summer and spring treatments following rewatering.............69
Table 3.26. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf simple ratio values from summer and spring treatments for both species.  

Table 3.27. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf transpiration from summer and spring treatments following rewatering.  

Table 3.28. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf transpiration rates from summer and spring treatments for both species.  

Table 3.29. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf stomatal conductance from summer and spring treatments following rewatering.  

Table 3.30. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf stomatal conductance rates from summer and spring treatments for both species.  

Table 3.31. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the relative leaf chlorophyll content from summer and spring treatments following rewatering.  

Table 3.32. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the relative LC values from summer and spring treatments for all species.  

Table 3.33. Summary table of re-watering data.  

Table 3.34. Results for ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on the observation leaf health category.  

Table 3.35. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the observation leaf health categories from summer and spring treatments for all species.  

Table 3.36. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on PRI leaf values. Note: species refers only to the five species which could be assessed for PRI (see text for details).  

Table 3.37. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the PRI leaf values from summer and spring treatments for all species.  

Table 3.38. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on NDVI leaf values.  

Table 3.39. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the NDVI leaf values from summer and spring treatments for all species.  

Table 3.40. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf simple ratio values.
Table 3.41. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the simple ratio from summer and spring treatments for all species. .................................82
Table 3.42. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf transpiration rates. ......................................................83
Table 3.43. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf transpiration rates from summer (heat and light) and spring treatments for all species. .........................................................................................................................83
Table 3.44. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf stomatal conductance rates. ........................................85
Table 3.45. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the stomatal conductance rates from summer and spring treatments for all species. 86
Table 3.46. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on relative leaf chlorophyll values. .............................................87
Table 3.47. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the relative leaf chlorophyll values from summer and spring treatments for all species. .........................................................................................................................88
Table 3.48. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment and time on percentage soil moisture. .........................................................89
Table 3.49. Summary table for competition experiment data ........................................89
Table 3.50. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on PRI leaf values between the *Eucalyptus* only and competition experiment ..................................................................................................................................................................................91
Table 3.51. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf transpiration rates between the *Eucalyptus* only and competition experiment ..................................................................................................................................................................................93
Table 4.1 A summary table of studies which have examined water stress, including the variables studies, length of stress, species examined and the results ................................ 97
Table 4.2. The known drought tolerances for the eight species used here from the White Box-Yellow Box-Blakely’s Red Gum Grassy Woodland ecological community, along with a description of their distribution (after Florabank (2013) and PlantNet (2014)). .................109
List of figures

Figure 2.1 (a-b). The position of each of the eight Australian native plant species within the square community pots. .................................................................................................................................................. 30
Figure 2.2. Photographic examples illustrating the six mortality index categories (see text for descriptions of all six categories). .................................................................................................................................................. 38
Figure 2.3. Photographic examples illustrating each of the three regrowth categories: (A) bottom, (B) middle and (C) top of the main stem observed (see text for descriptions of all six categories). .................................................................................................................................................. 39
Figure 2.4. A photograph showing how leaves were sampled with the PlantPen PRI 200® meter (the PolyPen RP 400® meter takes measurements in the same way). .......................................................................................................................... 41
Figure 2.5. A photograph showing how leaves are positioned in the leaf chamber of the CI – 340 Handheld Photosynthesis System during sampling ................................................................................................................. 44
Figure 3.1. Observed changes in Eucalyptus health for four different water stress durations 47
Figure 3.2. Bivariate correlation plots of measurements ................................................................................................................. 49
Figure 3.3. Changes in leaf PRI values of Eucalyptus species for four different water stress durations ................................................................................................................. 50
Figure 3.4. The variation of leaf PRI values for each Eucalyptus species over the control treatments .................................................................................................................................................. 50
Figure 3.5. Changes in NDVI leaf values of Eucalyptus species for four different water stress durations .................................................................................................................................................. 52
Figure 3.6. The variation of leaf NDVI values for each Eucalyptus species over the control treatments .................................................................................................................................................. 52
Figure 3.7. Changes in simple ratio values of Eucalyptus species for four different water stress durations .................................................................................................................................................. 54
Figure 3.8. Changes in leaf transpiration rates of Eucalyptus species for four different water stress durations .................................................................................................................................................. 55
Figure 3.9. Changes in stomatal conductance values of Eucalyptus species for four different water stress durations .................................................................................................................................................. 57
Figure 3.10. Changes in the relative leaf chlorophyll content for Eucalyptus species for four different water stress durations .................................................................................................................................................. 58
Figure 3.11. Changes in the percentage soil moisture content for pots of Eucalyptus species for four different water stress durations .................................................................................................................................................. 60
Figure 3.12. Probability of new growth following 4 weeks without water of *E. melliodora* and *E. blakelyi*. .................................................................62
Figure 3.13. Probability of new growth following 7 weeks without water of *E. melliodora* and *E. blakelyi*. .................................................................62
Figure 3.14. Bivariate correlation plot of measurements......................................................65
Figure 3.15. Change in the PRI leaf values post re-watering .............................................66
Figure 3.16. Change in the NDVI leaf values post re-watering...........................................67
Figure 3.17. Change in the SR leaf values post re-watering................................................68
Figure 3.18. Change in the leaf transpiration rates post re-watering ................................70
Figure 3.19. Change in the stomatal conductance rates post re-watering.......................71
Figure 3.20. Change in the relative leaf chlorophyll content post re-watering...............72
Figure 3.21. The observation leaf health category relative to summer and spring treatments, and water stress to survival$_{\text{max}}$ with time (weeks). .................................................................75
Figure 3.22. A binomial correlation plot of measurements ................................................76
Figure 3.23. PRI relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). ........................................................................78
Figure 3.24. NDVI relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). ........................................................................80
Figure 3.25. Leaf simple ratio values relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). .................................................................81
Figure 3.26. The leaf transpiration rates relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). .................................................................83
Figure 3.27. Leaf stomatal conductance relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). .................................................................85
Figure 3.28. Relative leaf chlorophyll content relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). .................................................................87
Figure 3.29. Percentage soil moisture relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). .................................................................88
Figure 3.30a. The PRI leaf values of *E. melliodora* with time for each treatment from the *Eucalyptus* only experiment (green dots) and the competition experiment (purple dots). ......90
Figure 3.30b. The PRI leaf values of *E. blakelyi* with time for each treatment from the *Eucalyptus* only experiment (green dots) and the competition experiment (purple dots). ......91
Figure 3.31a. The leaf transpiration rates of *E. melliodora* with time for each treatment from the *Eucalyptus* only experiment (green dots) and the competition experiment (purple dots). 92
Figure 3.31b. The leaf transpiration rates of *E. blakelyi* with time for each treatment from the *Eucalyptus* only experiment (green dots) and the competition experiment (purple dots). ....92
CHAPTER 1 - Introduction

1.1. Background

The susceptibility of seedlings of Australian native plants to abiotic and biotic disturbances is not fully understood. Stresses, disturbance and environmental fluctuations such as changes in water supply, temperature and wind, particularly in changing climates, can trigger large scale changes in seedling survival and mortality rates. An understanding of how specific disturbances affect seedling survival and mortality rates at a community level, particularly for those communities that have high risks of further species decline, is critical to the understanding and implementation of vegetation management. Vegetation management is particularly important in order to assist long term survival of a broad range of native species, species distribution, maintenance of soil layers, fauna and insect survival, with changing climate conditions.

Plants have adapted functionally and physiologically to a abiotic disturbances including to a range of water stress conditions (Bannister, 1976; Press et al., 1999; Huang, 2006). For example plants occur in regions with extremely low rainfall or water availability (e.g. Retama raetam which is native to Africa and the Middle East occurs in regions with an annual rainfall of ~400mm (Chaves et al., 2003)), through to regions with very high rainfall or water availability (e.g. Nauclea didderichii which is native to parts of tropical Africa occurs in regions with an annual rainfall of between 1600-3000mm (Press et al., 1999)). Plants have also developed a range of mechanisms to enable them to survive differing levels of water availability and stress (i.e. annual rainfall patterns, evapotranspiration, storms, flooding, drought (Adams, 2010)). Despite these development adaptations, there are physiological and chemical change points where too little or too much water is detrimental to a plant’s health (Huang, 2006). Whilst these change points are likely to be species specific and change with plant age (Crawley, 1997), for example the effects of water stress may be more evident in seedlings (Adams, 2010), understanding the change points is critical for determining how plants respond to water stress and drought, for more than just commercially valuable species. Given one of the predicted consequences of climate change is increased drought (Collins et al., 2013) it is imperative that we understand how plant species and plant communities (plants of different ages and functional groups) respond to extended periods of water stress and drought, and when change points occur. Water stress, its intensity and duration, can affect plants of different ages in different ways and plants in isolation in
different ways to plants in communities. Seedlings and saplings are the most susceptible to the stresses associated with drought (Duan et al., 2013). For this thesis I will be focusing on seedling survival.

In this chapter I will outline the research into plant physiology and morphology following heat and water stress. Specifically I will outline the stresses themselves, their known individual influence to plant health and their combined effects. I will also outline any research gaps in these areas, how changes in plant health can be measured and the need to study these stresses (i.e. climate change predictions).

1.2. Plants and water stress
1.2.1. Defining water stress and drought
Water availability covers a continuum from full hydration (complete water availability) through to drought (lack of water availability). Within this continuum water stress occurs when the demand for water by plants exceeds its availability (Munné-Bosch & Alegre, 2004). Whilst drought encompasses a temporary period without precipitation where plants are unable to access water (Larcher, 1980; Munné-Bosch & Alegre, 2004) and evaporation exceeds precipitation (Larcher, 1980), drought and severe water stress are often combined with high temperatures and high solar radiation (Munné-Bosch & Alegre, 2004).

Abiotic stresses such as water stress, salinity and extreme temperature (both high and low) can significantly impact the health (Valdés et al., 2013) or growth of plant species, without causing immediate death (Craine, 2009). Although some plants have adapted to living under extreme abiotic conditions (e.g. drought tolerance plants) (Huang, 2006), water stress (intensity and severity) can affect the productivity of ecosystems and thus the distribution of plant species within such ecosystems (O’Grady et al., 2013). Unlike extreme temperature and salinity, drought or some level of water stress is an abiotic stress that most plants experience at some point during their life (Press et al., 1999).

1.2.2. Effects of water stress on plants
Plant acclimation is a term used to describe initial structural, morphological and biological changes that occur within plants following water stress and other environmental stresses (Smith and Dukes, 2013). The capacity of plants to adjust and modify physiological and biochemical processes is related to the exposure to stimulus
(Smith and Dukes, 2013). Plants must maintain viable root water flow (through the xylem) and continue to extract water from the soil in water stress conditions to acclimate (adapt) to water stress (Costa E Silva et al., 2004). If plants are not able to adapt to the initial stages of water stress, other structural, morphological and biological changes will occur.

The effects of water stress on plants can influence the physiology and morphology of plants as well as their distribution. The response of seedlings to drought has been examined, mainly focused on the benefits for commercially valuable species in agriculture or forestry (e.g. Eucalyptus globulus (Duan et al., 2013)) or plants that may have invasive characteristics (e.g. Acacia saligna (Nativ et al., 1999)). However, it is also important to understand how native non-commercial seedlings respond to drought in current and changing climates so that predictions regarding species survival, movement (e.g. dispersal, colonisation, retreat, connectivity) and composition of native communities can be made. For many plant species however, the exact impacts of water or temperature stress are unknown, although plants generally follow similar physiological and biological changes to environmental stresses (Grime, 2001). How water stress affects plants and the importance of any such changes is discussed below.

1.2.2.1. Physiological effects of water stress on plants

Seedling leaf gas exchange is significantly altered by abiotic (e.g. water stress, climate, etc.) and biotic (e.g. insects, disease etc.) stress events (Duan et al., 2013). Water stress can cause significant physiological changes to occur in plants, particularly with respect to photosynthesis, respiration, carbohydrate metabolism and iron uptake, which can ultimately result in loss of development and function (Valdés et al., 2013). Many physiological processes control the balance of the supply and demand of water within plants (Hartmann et al., 2013). The main physiological effects of water stress in plants are changes in balance and supply of carbon and water resulting in changes to carbon assimilation and plant hydrology (Hartmann et al., 2013).

Adaptations which enable plants to change water use as the availability of water decreases (i.e. during the onset of drought) (Valdés et al., 2013) include the narrowing of stomata opening following the onset of drought which reduces CO₂ gas exchange that then reduces photosynthesis (Larcher, 1980). In plants, gas exchange is the biological process where gases are transferred across a concentration gradient through the cellular
wall of leaves and roots into cellular structures for use in physiological processes and functions (Press et al., 1999). Shifts in gas exchange as a result of water stress can impact the physiological functioning of plants and extreme changes can result in death.

1.2.2.1.1. Photosynthesis and stomatal conductance

Photosynthesis is the process by which plants absorb photosynthetic active radiation (PAR) (sunlight wavelengths) for conversion into energy (Bannister, 1976). PAR encompasses sunlight wavelengths between 400 and 700nm, spanning the visible light spectrum (CID, 2011). During photosynthesis plants use carbon dioxide (CO$_2$) and water to create carbohydrates and oxygen. Enzymes (rubisco and PEP carboxylase) catalyse the absorbed CO$_2$ or its hybrid form to produce 3-phosphoglycerate (PGA), which is then converted into energy (ATP) (Evert & Eichhorn, 2013). Therefore, photosynthetic response (production of energy) in plants is mediated by the availability of CO$_2$, water and temperature (Salazar-Parra et al., 2012). Studies have demonstrated decreased photosynthesis and increased respiration following moderate drought stress (Warren et al., 2011; Duan et al., 2013). The specific photosynthetic responses of individual plant species, as well as groups of plants, to changes in the variables that affect photosynthesis (light, temperature, water, CO$_2$) are not well known for most species and therefore the analysis of these changes across multiple species would enhance our understanding (refer Research Gap Section 1.9). The rate of photosynthesis is often associated with stress tolerance and is often viewed as a stress indicator (Huang, 2006).

Changes in stomatal conductance facilitated by drought cause a decrease in Rubisco CO$_2$ availability and a decrease in CO$_2$ chloroplast concentration (Salazar-Parra et al., 2012; Smith & Duke, 2013). Chloroplasts contain chlorophyll, which is the pigment which absorbs photosynthetic light. A reduction in stomatal conductance limits the concentration of CO$_2$ in chloroplasts, which limits photosynthesis.

In order to access CO$_2$ from the atmosphere for photosynthesis, plants need to open their stomata (specialised pores that occur mainly on leaves) to enable oxygen and carbon dioxide exchange between the plant and the surrounding air; a process called respiration. Opening the stomata also enables water and CO$_2$ exchange between the atmosphere and the plant’s cells. Through altering the structure of stomata pores a plant is able to regulate the intake and management of CO$_2$ concentration. Plants make a
‘trade-off’ between extracting enough CO₂ from the atmosphere (through stomatal pores in leaves) to photosynthesize while maintaining water potential (a concentration gradient of water in cells) to avoid desiccation (Adams, 2010). Stomata are open as light levels increase (morning) and close when light decreases (afternoon to night), or can also close when CO₂ levels are high or when temperatures exceed 30-35°C (Evert & Eichhorn, 2013). When stomata are open for respiration water vapour is lost: a process called transpiration (Wullschleger et al., 2002). Therefore water loss or transpiration is directly related to the time stomata are open and the content of CO₂ in the atmosphere (Ward et al., 1999). Drought tolerance in plants is influenced by their transpiration rates (Larcher, 1980), with higher transpiration rates in plants often causing less tolerance to drought (Milburn, 1979; Duan et al., 2013). The loss of water during transpiration does not pose a threat to plant health when a steady supply of water is available. The tolerance of plants to drought is largely based on the species’ ability to regulate stomatal opening, which is unknown for many species (Ward et al., 1999; Sperry, 2000). A multi-species analysis monitoring stomatal conductance may provide more information regarding the differences of stomata regulation between species (refer Research Gap Section 1.9).

There are a range of physiological processes that occur within plants affected by water stress, for example carbon metabolism and hydraulic maintenance (McDowell, 2011; Hartmann et al., 2013). These processes are critical to the survival and production of energy in plants. Commonly, the depletion of carbon metabolism and hydraulic failure are viewed as the main cause of plant mortality during water stress and often occur in combination (Hartmann et al., 2013; McDowell et al., 2013a; O’Grady et al., 2013). However, these processes are poorly understood with respect to water stress, in part because they are difficult to measure/determine within plants (O’Grady et al., 2013). Stomata generally remain open for longer periods of time during drought to enable carbon fixation, metabolism and maintenance (McDowell, 2011). This can however cause extensive water losses (McDowell, 2011). When the stomata remain open for longer periods of time xylem cavitation occurs (Fisher et al., 2006). The formation of cavities causes damage to photosynthetic machinery, negatively affecting phloem function and constraining energy transport in plants (Hartmann et al., 2013; McDowell et al., 2013a), and hydraulic failure. Often hydraulic failure more commonly occurs in anisohydric ‘optimistic’ plants that do not have a critical threshold for leaf water
potential and rely on available water resources, such as soil moisture, for survival (Schultz, 2003).

In contrast to hydraulic failure some isohydric ‘pessimistic’ plants have a critical minimum leaf water potential where, when reached, stomata close to reduce water loss which can cause the failure of plant metabolism as a result of prolonged negative carbohydrate balance, termed carbon starvation (Fisher et al., 2006; McDowell, 2011).

Hydraulic failure and carbon starvation are two plausible hypotheses which have been proposed to examine plant mortality associated with water stress. The success of individual species to abiotic stress depends on which strategy is used by the individual plant to combat stress such as the isohydric and anisohydric strategy (Molen et al., 2011). It is difficult to distinguish which of these processes causes final mortality in plants or if their combined effect contributes to death (Hartmann et al., 2013).

1.2.2.2. Morphological effects of water stress in plants
The physical form and structure of plants can be significantly impacted by water stress, and result in deficiencies in plant physiology. Despite the visible impacts of extreme drought on plants, limited studies have focused on plant health, performance and survival during and following drought events (Brodribb and Cochard, 2009) (refer Research Gap Section 1.9). Damage, death and/or change to the anatomical structures within plants can occur as a result of water stress conditions (Larcher, 1980). Below I outline a few of the key morphological effects of water stress on plants.

1.2.2.2.1. Impact on Roots
Water stress and low soil moisture can cause a reduction in root growth, which can limit the ability of plants to access soil water stores (Huang, 2006). Consequently root damage can lead to difficulties in plants completing physiological functions such as the accessing and taking up of nutrient supplies (Huang, 2006). Structural components such as the xylem, phloem, leaves, and roots of plants are important for managing control of water. Plant structures that are damaged from water stress include plant cellular walls, stomata, chloroplasts, leaves and root structure (Larcher, 1980; Xu et al., 2010). Structural damages can eventually lead to plant mortality. Plant mortality associated with drought is discussed in more detail below (see section 1.2.2.2.2).
1.2.2.2.2. Mortality

Understanding plant mortality associated with environmental stresses (drought, increased temperature etc.) is critical for predicting future vegetation communities under climate change (McDowell et al., 2013b). An essential component of which is the point at which plants can no longer recover from the stress (referred to as tipping points, thresholds or change point). Determining change points beyond which mortality is inevitable during periods of drought is critical to improve our understanding of the long-term effects of drought on plant communities (McDowell et al., 2013a) (refer Research Gap Section 1.9). Length and severity of abiotic stress events can also alter the success of individual species (Molen et al., 2011). When stresses occur many plants adapt to limited resources (e.g. isohydric plants) to prevent mortality. Thus, monitoring changes in physiological functions (i.e. stomatal conductance) during water stress may enable change points to be determined. There are numerous physiological measurements which could provide information as to the change points beyond which plant mortality is unavoidable.

1.2.2.2.2.1. Impact mortality has on communities

Drought can often be the cause of plant mortality. Understanding rates of mortality (e.g. recruitment, longevity patterns) and the differences between plant species is critical to predicting changes in future vegetation. Drought in eastern Australia during the 1900s caused large-scale die-off of many native plants (i.e. many species of Eucalyptus, Acacia and Callitris), as a result of multi-seasonal drought (very low mean precipitation) combined with increased heat (Allen et al., 2010).

Seedling survival at the establishment phase is usually extremely low/limited (Evert & Eichhorn, 2013; Gurvitch et al., 2006). Larger than normal seedling mortality can have extensive impacts on the function, composition, structure and health of ecosystems (McDowell et al., 2013b; Curran et al., 2013; Duan et al., 2013). Patterns of vegetation succession and ecosystem structure can be altered by the sufficient loss of seedlings (Duan et al., 2013). The establishment of seedlings is critical for maintenance of species range, survival and persistence (Polley et al., 1996). The survival of seedlings in drought conditions and their climate-induced survival adaptations shape the distribution and compositions of species at a regional scale (Curran et al., 2013). Plants are most susceptible to abiotic and biotic stresses during the seedling and sapling stages of their lifecycle (Polley et al., 1996; Duan et al., 2013; Will et al., 2013). Stresses such as low
soil moisture [drought] result in dehydration, which is one of the largest causes of seedling and sapling mortality (Polley et al., 1996; Hansen & Weltzin, 2000).

Understanding the survival of seedlings in drought conditions could have substantial implications for revegetation or restoration projects in changing climates (Ruthrof et al., 2010). Water stress can cause complete mortality or partial damage of all saplings and tubestock planted for revegetation within the first year of life (Duan et al., 2013). Planting of nursery raised tube-stock is the preferred method of rehabilitation in Mediterranean-type ecosystems such as south-eastern Australia, as direct seeding has limited success (Ruthrof et al., 2010). Knowing which seedlings perform better in water stress conditions, how often seedlings need to be watered and the predicted impacts of changing climates will contribute to the success of many restoration projects.

1.2.2.2.2. Mature trees
Mature trees have larger carbohydrate stores, more developed root systems, larger root exploration of soils and nutrient stores which enable them to have less sensitivity than seedlings to effects of water stress (Hanson & Weltzin, 2000; Curran et al., 2013). Seedlings on the other hand are more susceptible to cavitation, have non-developed roots, limited nutrient or carbohydrate stores, lower access to soil water and lower xylem water storage (McDowell et al., 2013). Mature trees also have increased resilience, larger respiratory mass and more complex canopies than seedlings (Hanson et al., 1994). The response of larger trees to stress is therefore a more complex procedure than seedlings and often differs between species, locations, soil types and nutrient availability. An extended drought period (over years and seasons) would be required to analyse the effect of water stress in adult individuals in different environments.

1.2.2.3. Plant adaptations to water stress
1.2.2.3.1. Water conservation strategies
The maintenance and recovery of living tissue requires adequate hydration [water concentration] (Mitchell et al., 2013). The strategy of individual plants to conserve water plays an important role in the conservation of water supply and preservation of hydraulic function (Mitchell et al., 2013). Leaf loss following a leaf water potential below 4Mpa allows some Eucalyptus species to focus energy on healthier leaves (Whitehead & Beadle, 2004). Maintaining the leaf water potential of non-damaged
leaves at appropriate levels enable these leaves to continue to regulate photosynthesis (Whitehead & Beadle, 2004). Homeostatic (regulation) adjustments of hydraulic properties enable *Eucalyptus* to conserve water-use in changing conditions (Whitehead & Beadle, 2004).

*Acacia saligna*, a potentially invasive and wood production species, was studied to determine the physiological and behavioural traits which were linked to the species’ high drought resistance (Nativ *et al*., 1999). Drought resistance traits increased stomatal regulation and chlorophyll content which enabled *A. saligna* to adjust leaves (e.g. stomata) in accordance with soil water availability (Nativ *et al*., 1999).

Some plants (e.g. rice (*Oryza sativa*)) have developed both deep as well as fine shallow root systems which collect water from multiple soil horizons during progressive drought (Huang, 2006). Such an adaptation enables some plants to combat low soil moisture by accessing larger quantities of available soil moisture.

The morphological characteristics of the leaves of some plants are also modified to combat damage from drought and temperature stresses. Some plants have developed adaptations to prevent water loss during transpiration (Osbourne & Sack, 2012), for example some plants have fewer stomata, smaller leaves, no leaves and thick waxy cuticles (e.g. *Eucalyptus*). *Eucalyptus* plants have characteristic long-lived, thick and waxy leaves that often hang vertically (Whitehead & Beadle, 2004). Leaf wax reduces water vapour losses during transpiration by creating a barrier, vertical hanging reduces the direct exposure to sunlight during the hottest time of day and long-lived leaves reduce the need to create new leaves (Whitehead & Beadle, 2004). Low leaf transpiration rates of *Eucalyptus* species such as *E. oliqua* have led to an increased tolerance of low water availability and higher survival in drought (Whitehead & Beadle, 2004). These adaptations enable the *Eucalyptus* species to have better success in limiting environments (low nutrients, low water availability etc.).

### 1.2.2.3.2. C3 and C4 plants and water stress

The majority of plants on earth are C<sub>3</sub> plants: these plants absorb CO<sub>2</sub> straight into the cells which then conduct the whole photosynthetic process (Adams, 2010). In each of these cells a three-carbon chain is created and hydrogen is provided by splitting water molecules (Adams, 2010). Hydrogen and carbon later combine to form energy. C<sub>3</sub>
plants lose some of their available CO₂ during this process and as a result their stomata need to remain open longer to combat the loss (Adams, 2010). These plants generally have a lower capacity to survive in water stress because they lose more water when their stomata are open.

C₄ plants, on the other hand, have separate cells which fix carbon and then convert it into a four-carbon chain which is transported as a molecule into the middle of the leaf (Adams, 2010). The inside of the cell is where the absorbed hydrogen and carbon are converted into sugars (Adams, 2010). C₄ plants are able to metabolise higher levels of CO₂ with less water loss when compared with C₃ plants (Press et al., 1999). Opening periods of stomata are reduced in C₄ plants, which can result in C₄ plants having higher drought tolerance when compared to C₃ plants. C₄ plants commonly occur in arid to semi-arid environments with low annual water supply (Adams, 2010). C₄ plants are more adapted to survive in drought conditions and therefore it is expected that their abundance and distribution will increase under conditions of water stress and/or elevated temperature. The abiotic stress of water loss is predicted to be the biggest constraint on the distribution and abundance of plants (Hanson & Weltzin, 2000). The climatic envelopes are derived mainly from precipitation and temperature gradients, as they control much of the distribution of both C₃ and C₄ plants (Paruelo & Lauenroth, 1996).

1.2.3. Plants and recovery following water stress
The response of plants to an increase in water availability following drought can be affected by the amount of water available and the extent of water stress experienced by the plant (i.e. to the duration of water stress relative to mortality). How plants recover from water stress is outlined below.

1.2.3.1. Physiological process of recovery
Physiological and morphological plant recovery is the process by which plant health is returned to a normal state (i.e. after a water stress period). Different parts and processes in plants can recover over different time periods following periods of water stress. For example, the recovery of plant gas exchange functions following water stress periods can range from one to one hundred days (Brodribb & Cochard, 2009). Recovery is dependent on the remaining water potential of a leaf which is altered by the intensity and duration of drought (Brodribb & Cochard, 2009). The ability of stomata to re-open
to begin photosynthesis is significantly reduced when leaf water potential is low (Brodribb & Cochard, 2009). The recovery of photosynthesis also relates to the restoration and recovery of chlorophyll content (Montagu & Woo, 1999). Recovery of species following re-watering can be monitored by recording the change in chlorophyll content in leaves. Low chlorophyll can be linked with low photosynthesis (Montagu & Woo, 1999).

Full plant recovery following re-watering (after water stress) can result in total net photosynthesis rates the same as those recorded in healthy non-stressed plants (Xu et al., 2010). Re-watering following extreme drought can lead to partial plant recovery only based on the changes in the net photosynthesis rate and observed stomatal conductance (Xu et al., 2010). Gas exchange and plant recovery has been analysed for a small subset of plants, many of which are crops or European species (Brodribb & Cochard, 2009) (refer Research Gap Section 1.9). Investigations into the recovery of a variety of native species following stress events have had little attention (Montagu & Woo, 1999).

Plants have multiple strategies for combating stress and prompting recovery. *Eucalyptus* plants typically survive under the resilience-type strategy, and as such have developed mechanisms to store and retrieve carbohydrates where stress is of relatively short duration (Mitchell et al., 2013). Although mechanisms exist (e.g. carbohydrate stores) for reducing the effects of stress and maintaining survival, there are change points if stress is extreme from which plants cannot recover (Crawley, 1997). The change point is different with each species and is not known for many plant species (Crawley, 1997). Further research is required to determine the nature of change points and their difference between species within the same ecological community (refer Research Gap Section 1.9). There may be a water stress change point beyond which recovery is not possible (Kirschbaum & Williams, 1988). The ecological significance of determining change points for multiple species is to determine whether stress causes a different response in different species or whether there is an exact point in every species where recovery cannot occur. If species that occur within a vegetation community have different change points to the same stress, such a stress could alter community dynamics and how the community as a whole tolerate the stress.
1.2.3.2. Recovery, stress intensity and duration

The duration and intensity of water stress has a significant effect on a plant’s ability to recover, both morphologically and physiologically. Water stress severity is often defined differently for different aged plants. Information present in this section is for seedlings. Recovery following mild water stress (i.e. ~1-30 days in seedlings) occurs much more rapidly in plants than following medium (moderate) (i.e. 30-63 days in seedlings) to intense drought stress (i.e. ~63-100 days in seedlings) (Brodribb & Cochard, 2009; Duan et al., 2013). For example, recovery of physiological functions from mild water stress in plants can occur as quickly as 1-2 days following rewatering (Chaves et al., 2009). Full recovery of some functions in plants, like net photosynthesis rates, rarely occurs following intense water stress (Chaves et al., 2009), implying that there are change points associated with the duration of water stress from which plants do not recover. The first stage of recovery following the end of a drought period (i.e. rewatering or the addition of water) is when stomatal conductance increases CO₂ assimilation (Kirshbaum, 1988). In the second stage, protein synthesis reverses the damage made to photosynthesis during the water stress event (Larcher, 1980; Kirschbaum & Williams, 1988; Chaves et al., 2009).

There is a cumulative effect of multiple stress events over time on plant health and survival, with each water stress event significantly affecting a plant’s ability to recover and survive subsequent stress events (Kirschbaum & Williams, 1988; Chaves et al., 2009). For example, seedlings that have only been exposed to one water stress event may recover more rapidly than those which have been exposed to multiple water stress events.

1.3. Plants and temperature

Along with water stress, temperature, particularly extreme temperatures (both high and low), can significantly impact the health of plant species (Valdés et al., 2013), although some plants have adapted to living under extreme temperatures (e.g. hot or cold tolerant plant species). Temperature adaptations in plants can (i) result in competitive advantages; (ii) help them establish in new environments; (iii) help them to cope with stresses; and consequently improve their survival (Huang, 2006). For example, the ability of a plant to survive in elevated temperatures is reliant on physiological and morphological adaptations like metabolic regulation and structural modification. However, how plants will respond physiologically to changes in climate is not well
known, especially in relation to the combined effects associated with changes in temperature and water availability (refer Research Gap Section 1.9) (Sperry, 2000; Hanson & Weltzin, 2000; Salazar-Parra et al., 2012; Way, 2013). The following sections describe the effects of temperature on plants.

1.3.1. Effects of temperature on plants
Temperature plays an important role in the regulation of many physiological and morphological processes which occur in plants such as enzyme regulation, membrane regulation and photosynthesis. Often plants have optimum temperature ranges based on the thermal kinetic window of their enzymes within which physical, chemical, functional and structural processes within the plant are performed without any adverse effects (Bannister, 1976; Huang, 2006). Modulative temperature adaptation, the process of change in enzymes to those which have better optima heat temperature resistance, can occur within hours of temperature stress (Larcher, 1980). Changing of enzymes enables plants to develop altered optimum temperature ranges (i.e. either highs or lows or both) which increases adaptive ability to temperature extremes or changes in temperature (Larcher, 1980; Smith & Dukes, 2013). For example, the optimum growth temperature for *Nothofagus cunninghamii* is from 5.8 to 37.2 °C, beyond which growth is decreased and plant performance is decreased (Cunningham & Read, 2002). Beyond optimal temperature ranges plant growth, efficiency and productivity can be reduced.

The study of temperature sensitive plant physiological processes (photosynthesis, stomatal conductance, transpiration) will help predict the vulnerability of specific plant species to the predicted temperature increases associated with climate change (O’Grady et al., 2013). The specific enzyme temperature adaptations and thermostolerances are only known for a few plant species, specifically those with a commercial value (i.e. cropping species such as wheat and cotton) (Huang, 2006). However, such information is critical for a broader suite of species if we are to understand the effects of climate change on plant species and vegetation communities (refer Research Gap Section 1.9).

1.3.1.1. Physiological and morphological effects
A range of changes to physiological and morphological processes can occur in plants during periods of increased temperature (or heat stress). For example, there are many metabolic processes (both chemical and physical) which can enhance plant survival to extreme temperatures including enzyme regulation, changing enzyme concentration and
changes in isozymes (Huang, 2006). Elevated temperatures beyond the optimal growth temperature for a specific plant species can modify enzyme stimulation (Smith & Dukes, 2013). The deactivation of enzymes may promote rubisco activase limitation, which limits the activation of photosynthesis energy and new tissue synthesis (Larcher, 1980; Lan & Mott, 1991; Smith & Dukes, 2013). As rubisco, a plant enzyme, assists with the uptake of CO₂ during photosynthesis, any limitation would affect CO₂ uptake and subsequently impair metabolism (Huang, 2006).

Many structural components of plants are important features that help them regulate temperature. For example, plant membranes separate the cells from the exterior environment and as such can prevent high temperatures and external disturbances from effecting interior structures of the plant (e.g. chloroplasts and mitochondria which are important to photosynthetic processes) (Huang, 2006). The physiology of plants is reliant on a delicate balance within cells and between the external abiotic environments. The external temperature is related to the fluidity and permeability of plant cells, such that under high temperatures (or heat stress) cells can increase their fluidity or permeability resulting in change in the movement of ions across membrane and changing gas exchange. Subsequently, increasing membrane fluidity can change the thermotolerance of the photosynthesis system and result in decreased heat and desiccation tolerances (Huang, 2006). During heat stress the lipid by-layer can melt causing membrane components to move around the cell, leaking into other structures and damaging enzyme efficiency (Huang, 2006). Whilst enzymes control temperature regulation inside cells to a point, the lack of enzyme efficiency can result in temperature induced mortality in plants. The outcome of elevated temperature results in changes to the physiology and morphology of plants, often resulting in diminished health and ultimately mortality.

Duan et al. (2013) studied the combined effects of temperature and elevated CO₂ on seedlings of Eucalyptus globulus; a species used extensively in commercial forestry operations. The authors found that photosynthesis, total non-structural carbohydrates and respiration in E. globulus seedlings were significantly negatively altered with increased temperature stress during exposure to progressive drought (i.e. up to 103 days) (Duan et al., 2013). The damage caused to E. globulus seedlings during the drought treatment was exacerbated with the addition of elevated temperature in moderate (63-79 days) and extreme (79-103 days) drought conditions (Duan et al.,
Other studies have also found a negative relationship between elevated temperature and level of water stress however these results were mainly focused on production species (Smith & Dukes, 2013).

Whilst plant growth is increased when water is readily available, elevated temperature (of ~5°C) without drought can cause an increase in leaf area, respiration and plant productivity (Duan et al., 2013). However, studies like Duan et al., (2013) illustrate the relationship between elevated temperature, water stress and decreasing plant health or increasing plant damage. Further studies are needed to determine the boarder trend across a wide range of plant species to determine whether the relationship is consistent over multiple species of different functional classifications (refer Research Gap Section 1.9).

1.3.1.1.1 Photosynthesis
High temperatures can cause a dramatic drop in the rate of photosynthesis in plants and an increase in the rate of respiration (Larcher, 1980; Chen et al., 2011a); under extreme temperatures photosynthesis is halted. Without photosynthesis plants cannot convert light into energy and cannot grow. The point where the temperature exceeds that at which a plant can appropriately photosynthesise is called the temperature compensation point (Larcher, 1980). The temperature compensation point is reached when stomata remain closed to prevent further water losses within the leaf, resulting in decreased photosynthetic ability (Adams, 2010). The temperature compensation point occurs in most plants between 30°C and 35°C (Evert & Eichhorn, 2013).

1.4. Plant distribution and climate
Climate is one of the best predictors for determining a plant species’ distribution. Six climatic variables have been widely used to determine a species’ climatic envelope, being annual precipitation, precipitation of the driest month, precipitation seasonality, mean temperature of the coldest quarter, maximum temperature of the hottest month, and mean diurnal temperature range (Beaumont et al. 2009). These six climatic variables illustrate the relationship between temperature and water availability in determining a species’ distribution.
1.4.1. Effects of temperature on plant distributions

The distribution of plants is highly dependent on temperature and climate (Adams, 2010), or their climatic envelope under which they can occur. The distribution limits in plants are largely determined by the maximum, mean and minimum summer and winter temperatures (Adams, 2010). Soil type, geology and other climatic variables, such as precipitation, also play a role in determining plant distribution limits. Changes in these temperatures will inevitably result in change in vegetation types and movement of species to different climatic envelopes (Adams, 2010). Species usually have particular heat tolerances. Climate envelopes describe the particular range of variation (temperature, rainfall, light) over which species can occur (Bakkenes et al., 2002). A temperature envelope is the temperature range over which a specific species can survive. Hughes (2003) summarised studies which suggest that eastern Australia will be very sensitive to environmental change as precipitation is already a major limitation to plant production. Unlike animals plants are not able to escape from the effects of abiotic stress (temperature, water, wind, light) and must rely on adaptations to survive such stresses (Huang, 2006). It is suggested by many authors that species may need to move location to maintain their current climatic envelope if climate conditions change (Bakkenes et al., 2002; McKenney et al., 2007; Beaumont et al., 2009; Lawing & Polly, 2011). It is predicted that increased temperature and decreased rainfall will result in the death or geographical movement of plants in the next 100 years (Hughes, 2003; Bakkenes et al., 2002; McKenney et al., 2007).

An increase in temperature of between 3-5°C would cause change in the distribution of temperature envelopes for around 50% of Eucalyptus species in Australia, given that climate is a major determinant on where species occur (Hughes et al., 1996). The soil and drainage patterns of the Australian landscape sharply define the geographical range and growth success of countless Eucalyptus species (Hughes, 2003). Changes in soil moisture, rainfall and temperature by as little as 10%, 15% and 20% respectively, could cause geographic conditions largely different to current climate envelopes and result in changes to Australian vegetation (Hughes, 2003).

1.5. Plants and seasonal light availability

Light availability changes during the year from summer to winter (seasonal), which effects how plants grow (Bannister, 1976). Seasonal variation in light (seasonal photoperiodism) controls the extent of processes such as photosynthesis, respiratory
activity, organic material transport and growth in plants through alterations in the intensity and duration of light (Larcher, 1980). Changes in seasonal light availability and intensity are intrinsically linked to changes in temperature (i.e. as daylight length and light intensity increases there is a correlative effect of increased temperatures) (Larcher, 1980).

Light availability plays an important role in plant growth and mortality (Finzi & Canham, 2000). For example, increased light can often increase the effects of elevated temperature (see section 1.5) as well as alter plant patterns of growth (i.e. seed production, flowering, leaf growth) (Finzi & Canham, 2000). High light conditions in combination with other stresses such as increased heat and drought can cause additional damage to plants than that experienced by each stress individually (Press et al., 1999).

Light and temperature control the amount of CO$_2$ which is taken up by plants and therefore they effect a plant’s ability to photosynthesise (Larcher, 1980).

1.6. The effects of water stress on individual plant species relative to the effects on a vegetation community

Studies on the effects of water stress on plants have mostly focused on single plant species (e.g. *Eucalyptus globulus* (Mitchell et al., 2013; Duan et al., 2013), *Vitis vinifera* (Salazae-Parra et al., 2012), *Eucalyptus smithi* (Mitchell et al., 2013) and *Plaseolous vulgaris* (Zlatev, 2013). These were all ex-situ pot experiments conducted in a glasshouse, growth chambers or a greenhouse (Salazae-Parra et al., 2012; Mitchell et al., 2013; Curran et al., 2013; Duan et al., 2013; Zlatev, 2013). Whilst you could combine the results for individual species that naturally co-occur in vegetation communities (if studies of co-occurring species, have been undertaken), studies that examine multiple species within a vegetation community are needed. One problem with the extrapolation of results from single species’ studies to community level trend, is that such comparisons do not account for the competitive interactions between species, particularly under stressed or resource limiting conditions. Plant competition occurs when plants interact for resources in limited supply (Hunt et al., 2006) (refer Research Gap Section 1.9). Resources that drive competition in plants are light, space, water, and nutrients (Hunt et al., 2006).

Competition can be more intense following a period of stress between plants that have the same adaptations to stress recovery (Hunt et al., 2006). The response of plants in a
particular community to water stress may be particularly dependent on resource limitations and the similarity of functional plant traits within that community.

Many plant competition studies focus on intraspecific competition of crop species in managed conditions to understand and increase crop productivity (Hunt et al., 2006). Studies which focus on interspecific competition of mixed forest communities are less common due to their increased complexity (Hunt et al., 2006). Different species have different stress tolerances (nutrient, water, and temperature), physiology and morphology, which allow them to behave differently in competitive circumstances (Unwin et al., 2006). The competitive performance of plants can be measured in many ways including competitive ability, fecundity, growth rate, photosynthesis, total leaf area, biomass, survival and germination rate (Daehler, 2003). The examination of the response and performance of plants to water stress in competitive environments has received little attention, highlighting a major research gap (Hanson & Weltzin, 2000) (refer Research Gap Section 1.9).

The stress-gradient hypothesis (SGH) predicts that as abiotic stress (i.e. water stress) increases, competition becomes less important and plants become more mutualistic (Bu et al., 2013). Studies have shown that when mild soil drying occurs, lack of moisture controls the effects of competition (Press et al., 1999). Competition may not have an effect on the survival of species in stress conditions because the limiting factor is the stress (i.e. water limitation) and not the competition. If you apply the SGH hypothesis in a circumstance where water stress is occurring there would be no difference between the survival of plants in a competitive environment compared with plants without competition. This is because water stress instead of competition controls the success of plants because effective competitive advantages of some plant species such as high growth rate become disadvantages in extreme stress (Grime, 1977).

1.7. Measuring physiological and morphological changes in plants following water and heat stress

An understanding of the physics and biochemistry underlying the physiological and visual changes in plants to environmental stress, can provide insights into changes in plant health (Barton, 2012) (Table 1.1). Plant health is a term which describes how an individual plant is performing functionally and physiologically, relative to the conditions under which it is growing (Doring et al., 2012). Plants often display
indications and symptoms when their health is decreasing (Huang, 2006), and as such there are many visual clues which are associated with the decrease in health (e.g. caused by change in environmental conditions, disease and pests (Barton, 2012)). How plants, more specifically leaves, use light is important in determining leaf health (Barton, 2012). For example, light use efficiency declines as plants become stressed (Barton, 2012). Thus measurement of the light use efficiency (through light reflectance) of plants can be used to determine leaf health (Table 1.1). In addition to visual and light reflectance changes there are a range of physiological changes associated with a decrease in plant health (Table 1.1). For example, complex leaf functions can now be assessed using empirical indices which describe mechanistic, physiological and biological processes (Barton, 2012) (Table 1.1). Monitoring these changes can help identify and quantify changes in plant health, either positive or negative.

Physiological measurements of leaves can be undertaken using a range of devices to determine changes in leaf physiology following exposure of plants to stresses, for example herbicides (Sea et al. 2013) and drought stress (Duan et al., 2013; Mitchell et al., 2013; Zlatev, 2013) (Table 1.1). These devices can measure photosynthesis, stomatal conductance, leaf chlorophyll content, photochemical reflectance, etc. A short description of how some of these physiological characteristics can be measured/assessed is presented in Table 1.1.

The surface properties, internal structure and biochemical components in a leaf directs how the reflectance of light is observed (Barton, 2012). Light reflectance from leaves [vegetation] can be observed and measured using multiple light spectrums, visual (400-700nm), near-infrared (700-1,300nm) and mid-infrared (1,300-3,000nm) (Barton, 2012). Each light reflectance spectrum can be used to describe the variation of a different leaf function (For examples see Table 1.1). Despite the use of leaf reflectance indexes as reliable water stress indicators, the assessment of multiple species relative to water stress has not been widely researched (Ripullone et al., 2011). By analysing the change in leaf reflectance of multiple species with regards to time of stress, time since planting and time following stress, it is possible for studies to follow the change in photosynthetic performance and cellular change (i.e. drop in leaf chlorophyll) of a vegetation community to stress, something which has rarely been analysed. By doing this it may be possible to determine whether the reflectance response is similar throughout species and whether it varies with stress type and duration.
### Table 1.1. A description of measurements used to monitor plant health and what each measurement shows.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Example of instrument used to measure</th>
<th>What is measured</th>
<th>What it shows</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis (or net photosynthesis)</td>
<td>Portable gas exchange system (e.g. CI-340 Handheld Photosynthesis System) or sealed growth chambers</td>
<td>The rate of CO₂ assimilation of a known leaf area over a given time</td>
<td>Rate of photosynthesis within leaf at given time or over a period of time</td>
<td>Montagu &amp; Woo (1999); Schultz (2003); Salazar et al. (2012); Duan et al. (2013)</td>
</tr>
<tr>
<td>Transpiration</td>
<td></td>
<td>Rate of loss of water (H₂O) vapour</td>
<td>Rate of transpiration within leaf at given time or over a period of time</td>
<td></td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td></td>
<td>Calculation including transpiration and leaf surface temperature</td>
<td>Rate of stomatal conductance within leaf at given time or over a period of time</td>
<td></td>
</tr>
<tr>
<td>Photosynthetic active radiation</td>
<td></td>
<td>Light wavelengths between 400 and 700nm, spanning the visible light spectrum</td>
<td>Available light for plants to use to photosynthesise</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td>Leaf temperature</td>
<td>Change in leaf temperature, used to measure stomatal conductance</td>
<td></td>
</tr>
<tr>
<td>Normalised Difference Vegetation Index (NDVI) and Simple Ration (SR)</td>
<td>PolyPen RP 400° or remote sensing</td>
<td>Light reflectance at 780nm and 630nm</td>
<td>Change in vegetation (cover, biomass, moisture content)</td>
<td>Chen et al. (2011b); Lakshmi Kumar et al. (2013); Chen &amp; Weber (2014); Photon Systems Instruments (2014)</td>
</tr>
<tr>
<td>Photochemical Reflectance Index (PRI)</td>
<td>PlantPen PRI 200° meter or remote sensing</td>
<td>Light reflectance at 531nm and 570nm</td>
<td>Change in the carotenoid pigment, xanophyll cycle and structure of leaves</td>
<td>Thenot et al. (2002); Guo &amp; Trotter (2006); Ripullone et al. (2011); Barton (2012); Sea et al. (2013); Photon Systems Instruments (2014)</td>
</tr>
<tr>
<td>Leaf chlorophyll content</td>
<td>Atleaf® Chlorophyll Meter</td>
<td>Reflectance of the red part of the light spectrum</td>
<td>Change in leaf chlorophyll cells and carotenoids cells in leaves</td>
<td>Larcher (1980); Munné-Bosch &amp; Alegre (2004); Barton (2012)</td>
</tr>
<tr>
<td>Visual leaf health</td>
<td>Visual observation, camera or leaf health software</td>
<td>Leaf wilting, leaf colour change, leaf shedding, leaf loss,</td>
<td>The observational leaf health</td>
<td>Engelbrecht &amp; Kursar (2003); Bakr (2005); Sea et al. (2013)</td>
</tr>
</tbody>
</table>
1.7.1. Comparing multiple measures of the physiological response of plants to stress

Many studies have used a single assessment or single type of assessment, either visual or physiological, to determine change in plant health following stress (e.g. Ni & Pallardy, 1990; Lima et al., 2003; Villagra & Cavagnaro, 2006; Zhao et al., 2013). Whilst these single assessment studies have examined a wide array of physiological processes (e.g. as listed in table 1.1), the comparison and combination of assessments using multiple measures has rarely been used to identify which is the best predictor of stress in plants or to determine change points in the decline in plant health (refer Research Gap Section 1.9). Notable exceptions include Sea et al. (2013), Duan et al. (2013) and Mitchell et al. (2012).

In those studies that examined multiple measures, the authors mentioned that one or more of the assessments displayed quite a different result. Such a difference might simply be due to the artefact of measuring a different variable. Sea et al., (2013) studied the physiological effect of herbicide application on four weed species and compared the results with visual assessments. The authors examined the physiological response by assessing stomatal conductance, leaf chlorophyll content and PRI, which they compared with the results from leaf area analysis from digital photographs of leaves and visual assessments. The key findings of Sea et al.’s (2013) study is that (i) visual observations can be very variable, particularly in the middle assessment values (i.e. between <25 damage and >90 damage), which was accentuated when the results from multiple samples were compared, (ii) comparisons between visual assessment and physiological responses showed the physiological responses were occurring before they could be detected through visual assessments, (iii) some leaf function occurred despite significant leaf damage, and (iv) the change in stomatal conductance generally occurred earlier than any other changes observed, suggesting that leaf function may decrease before it can be detected with other devices or visually.

In another study which examined multiple measures of leaf health, Duan et al. (2013) studied tree seedling growth, photosynthesis, respiration and total non-structural carbohydrate of seedlings following drought and rewatering. The authors found that changes in photosynthesis and respiration following drought were different, despite both measurements showing decreases. However, no change was observed in total non-structural carbohydrate.
Mitchell *et al.*, (2012) compared the response of three tree species to prolonged drought assessing growth rate, water relations, gas exchange (photosynthesis and respiration) and carbohydrate dynamics. Their study found that there was a significant depletion of total non-structural carbohydrate (TNC) in *P. radiata* following drought, contrasting with the results of Duan *et al.* (2013) who found no change in the TNC of *Eucalyptus* species. The physiological assessments measured by Mitchell *et al.*, (2012) all displayed significantly different patterns between the three species examined, suggesting species perform differently.

The assessment of leaf health can be measured in multiple ways, and therefore one measurement may not describe the range of changes occurring. Thus, using multiple forms of assessments of plant health (visual, physiological and morphological) following stress enables a more holistic understanding of the changes that may occur.

The use of multiple assessments of plant physiology, visual health and morphology provide the best evaluation of the likely effects of a stress on plant health. Examination of multiple measures may provide insights into which measure best detects the changes that occur, a greater understanding of leaf function and how quickly such changes occur with time since exposure to a stress, or duration of the exposure to a stress (Sea *et al.*, 2013).

1.8. Plants and drought in a changing climate: what are the future implications

1.8.1. Australian climate change predictions

Australia is currently one of the driest continents on earth. Increased temperature, decreased rainfall and increased light associated with climate change predictions will significantly alter Australian ecosystems (Hughes, 2003) which are already limited by nutrient supply, water and temperature stress (Hughes, 2003). Exact climate predictions and their predicted effects on plants are provided below. The drivers of global climate change are atmospheric concentrations of gases, solar radiation and human disturbance (Collins *et al.*, 2013). It is predicted that the increase in greenhouse gasses such as CO$_2$ will result in a global temperature increase of between 3 and 5°C by 2100 and changes in the patterns of seasonal rainfall (IPCC, 2013). Temperature and rainfall changes
beyond natural climatic variability may affect plant mortality and distribution and cause changes in vegetation biomes (IPCC, 2013).

1.8.1.1. Increased temperature
Climate change predictions hypothesise that the mean temperature will rise in most places in the world, with temperature extremes expected to become more frequent (Collins et al., 2013). Extreme change to rainfall and temperature may stretch from a single day event to multi-day, monthly or seasonal events (Collins et al., 2013). An increase of regular temperatures above the historical mean are also predicted to dramatically increase in Australia (Collins et al., 2013; DoECCW, 2010). The average temperature increase in eastern Australia is predicted to be 3-5°C by 2081-2100 (Collins et al., 2013; DoECCW, 2010). The temperature of Australia has increased on average by 0.8°C in the last 100 years (Hughes, 2003). Extended periods of elevated (above the mean) temperatures during drought have increased in the last 50 years (Hughes, 2003; Hennessy, 2011). The diurnal range of temperatures has decreased in Australia indicating that the range of temperatures between day and night temperatures has decreased (Hughes, 2003).

1.8.1.2. Increased drought/water stress
Under future climate change Australian yearly average precipitation is expected to become more varied between locations (geographically) suggesting that there will be a higher rainfall gradient between areas which receive high rainfall and those that receive low rainfall (Collins et al., 2013). In south-eastern Australia the duration of rainfall events (i.e. the number of consecutive days) is likely to decrease while the rainfall intensity is likely to increase (Collins et al., 2013; DoECCW, 2010). Rainfall in Australia has high inter-annual variability across the continent (Hughes, 2003). Generally rainfall is predicted to decrease in frequency and increase in severity in Australia under climate change (Hughes, 2003). Overall drought intensity and occurrence is expected to increase from 1% to 30% of total land area by 2100 due to the decrease in frequency of rainfall (Xu et al., 2010). Rainfall events in south-eastern Australia have become more varied with extreme rainfall events becoming more common (Ward et al., 1999; Hughes, 2003). The trend of decreasing rainfall observed is significantly linked to the El Niño Southern Oscillation (ENSO) phenomenon that alters the climatic patterns over much of Australia (Hughes, 2003), whilst La Niña (the opposite of El Niño) events are commonly linked to increased rainfall in south eastern
Australia. The decreased length of La Niña events and the increase of El Niño events as result of ocean warming in the Pacific may increase the drought durations in eastern Australia (Hughes, 2003).

Intensity and duration of drought play a significant role in altering the function and competition of plant behaviour (Press et al., 1999; Hughes, 2003), as outlined above (see Section 1.2.2 above). The increase in temperatures combined with decreased rainfall results in higher annual evaporation and increased water stresses on plants (Hughes, 2003).

The environmental fluctuations linked with climate change pose potentially severe changes to vegetation worldwide (Grime, 2001). The effect of environmental fluctuations associated with climate change, such as variations in water availability, light intensity and temperature, are not known for many Australian native plant species (Hughes, 2003).

1.8.2. Effects of climate change on plants
As outlined above elevated temperature and decreased rainfall are the predicted changes associated with climate change. Water and temperature stress results in changes in photosynthesis, plant structure, transpiration, and metabolism (Section 1.2. and Section 1.3.). Understanding how plants are likely to respond to decreased water availability (i.e. water stress or drought) and increased temperature following climate change is important for modelling future landscapes and planning conservation and management of vegetation (Hughes, 2003). Considering multiple climatic variables (temperature, precipitation, light) are predicted to change in the next 100 years over much of south eastern Australia (Hughes, 2003), studies that consider the effect, drivers and potential changes to native vegetation are essential (Adams, 2010; Collins et al., 2013) (refer Research Gap Section 1.9). This study seeks to determine the impact of increased water and temperature stress to inform vegetation management strategies.

1.9. Research Gaps
Following a review of the literature (see above), I have identified the following key research gaps with respect to water stress, heat stress and native Australian plants:

(i) information on how plants respond physiologically and morphologically to drought is limited;
(ii) how seasonal variations (i.e. changes in light and temperature) influence
(i) above has not been examined; and
(iii) the response of water stressed plants to the addition of water (i.e. re-watering) is poorly understood.
Within each of these broad research gaps there are a range of additional more specific research gaps, being:
(a) The point at which the effect of water stress becomes irreversible and plants die (or change points), or the degree to which plant species can recover if they do not cross such a change point;
(b) Comparisons across multiple physiological processes are needed for non-commercially valuable species, and in relation to a range of stresses;
(c) There are no comparisons across plant species or from multi-species studies – all the studies to date have been single species studies ex-situ; and
(d) Few non-commercial species have been studied.
Data collected from studies that fill the above research gaps can contribute to our understanding of how plant species and vegetation communities may respond under predicted climate change conditions (i.e. increased drought and temperature).

1.10. Thesis aims and objective
The main aim of my thesis is to examine the effects of increasing water stress combined with increased temperature and light (i.e. to compare summer and spring) and subsequent rewatering on (i) seedling leaf health and (ii) seedling mortality.
In order to achieve this aim this study will examine a range of common leaf health measures, specifically: photosynthesis; stomatal conductance; transpiration; photochemical reflectance of light transmission; absorbance of light across several light wavelengths; and leaf chlorophyll content relative to time since last watering.

The secondary aim of my thesis is to determine whether there are physiological change points from which plants cannot recover following water stress and temperature stress. The third aim is to examine the effects of the first two aims across seedlings of a range of non-commercial Australian native plants which occur within the same ecological community spanning three growth forms, being trees, shrubs and grasses through a competition experiment. Lastly the results will be discussed in the context of current and future climates and the conservation of south-eastern Australian native plants.
The general objective of this study is to determine the effectiveness and usability of a range of leaf health measurement techniques for detecting changes in leaf health as a result of water and heat stress in native Australian species. This study will act as an analysis of the application of each of these leaf health measurements over multiple species of different functional types with variable leaf structural characteristics.
CHAPTER 2 - Method

2.1. Glasshouse

The experiments outlined below were undertaken in a glasshouse at the University of Canberra, Canberra, Australia. The glasshouse has two sections, (i) a standard glasshouse; and (ii) six cabinets within the glasshouse. These sections operate under different conditions (temperature, light, etc.), which are detailed below.

2.1.1. Operating conditions of the glasshouse (spring treatment)

The glasshouse was set to operate at a temperature range of 25-27°C (day) and 17-20°C (night) for the duration of the experiment (in accordance with Canberra, ACT daily maximum spring temperature (+3°C)) (BOM, 2014a). The light within the glasshouse was left unregulated throughout the study, being approximately 8 hours of daylight and 16 hours of darkness. Relative humidity (RH) was not regulated within the glasshouse. Hereafter referred to as the spring treatment.

2.1.2. Operating conditions of the cabinets (summer treatment)

Cabinets within the glasshouse were used to increase the amount and level of light as well as temperature to simulate summer (hereafter referred to as the summer treatment) (in accordance with Canberra, ACT daily maximum summer temperature (+3°C)) (BOM, 2014a). To achieve this heat mats (Sage horticultural product: IP55) and grow lamps (Philips master HPI-T Plus 250w/645 globe, colour temperature: 200K, Luminous flux: 205000Lm) were used inside each cabinet to regulate the temperature at 33-35 °C (day) (12 hours) and 23-25 °C (night) (12 hours). Relative humidity (RH) was not regulated within the cabinets. The grow lamps were also used to regulate the light to 12 hours (day), as well as to increase the amount of light available by increasing the light levels to an average lux level of 7,452 lux.

2.1.3. Monitoring of operating conditions of the glasshouse

The cabinets and glasshouse were monitored using HOBO® temperature (C’) and light (Lux) sensors (Hobo® onset: Pendant temp/light: UA-002-64). The pendant is a waterproof miniature data logger which records temperature and light conditions on set intervals (once per minute). The glasshouse was also monitored for changes in temperature and light using Auto Grow systems – Multi-grow controller and heated or
cooled as appropriate. The intended temperature and light conditions were always met during the experiment.

2.2. Study species

Eight native Australian plant species that naturally occur within White Box-Yellow Box-Blakely’s Red Gum Grassy Woodland community (as described by NSW Scientific Committee, (2002)) were selected representing three life forms – tree, shrub and grass – to simulate a subset of the community. White Box-Yellow Box-Blakely’s Red Gum Grassy Woodland community is a commonly occurring community around the ACT region. The species were chosen as they are readily used for landscape restoration and conservation (e.g. by Greening Australia). The eight species used are *Eucalyptus melliodora* (Yellow Box) (tree), *Eucalyptus blakelyi* (Blakely’s Red Gum) (tree), *Acacia melanoxylon* (tree), *Daviesia mimosoides* (shrub), *Dodonaea viscosa* (shrub), *Hardenbergia violacea* (shrub), *Poa sieberiana* (grass), and *Poa labillardieri* (grass).

2.2.1. Number of individual plants per species

In order to run the experiments outlined below, 516 plants were needed across the eight species. As the two eucalypt species were used in both experiments higher numbers of each species were required (Table 2.1).

<table>
<thead>
<tr>
<th>Australian native plant species</th>
<th>Number of individuals needed for the experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eucalyptus blakelyi</em></td>
<td>162</td>
</tr>
<tr>
<td><em>Eucalyptus melliodora</em></td>
<td>162</td>
</tr>
<tr>
<td><em>Acacia melanoxylon</em></td>
<td>32</td>
</tr>
<tr>
<td><em>Daviesia mimosoides</em></td>
<td>32</td>
</tr>
<tr>
<td><em>Dodonaea viscosa</em></td>
<td>32</td>
</tr>
<tr>
<td><em>Hardenbergia violacea</em></td>
<td>32</td>
</tr>
<tr>
<td><em>Poa labillardieri</em></td>
<td>32</td>
</tr>
<tr>
<td><em>Poa sieberiana</em></td>
<td>32</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>516</strong></td>
</tr>
</tbody>
</table>
2.3. Pre-treatment conditions
2.3.1. Prior germination, growth and relocation to the Glasshouse
The eight plant species (listed above) were obtained from Greening Australia’s Capital Region nursery in February 2014, as approximately 6 month old tubestock. Seeds of all eight species were germinated, potted and grown as tubestock by staff and volunteers of Greening Australia Capital Region at their outdoor plant growing and nursery facilities in Aranda, ACT. The growing conditions at the Greening Australia facility were an average temperature range of 8-27˚C – spring/summer with a regular (daily) watering regime. At six months of age all plants were moved from Greening Australia to the University of Canberra glasshouse.

2.3.2. Repotting of tubestock ready for experiments
The tubestock from Greening Australia were re-potted at the University of Canberra glasshouse. Plants were re-potted into one of two pot sizes for use in two separate experiments – a Eucalyptus only experiment and a competition experiment (see section 2.4 and 2.5 for details). Each pot was filled with Debco® un-fertilised fine seedling commercial potting mix (product code 71428), comprising quartz, sand, cocopeat, Pinus radiata mix (composted grade 0-6mm).

2.3.2.1. Eucalyptus only (single species)
_Eucalyptus melliodora_ seedlings (tubestock) and _E. blakelyi_ seedlings were planted individually [transplanted] into tall cylindrical pots measuring 240mm deep with a radius of 85mm (130 plants of each species).

2.3.2.2. Competition experiment
Tubestock of one of each of the eight plant species was transplanted into cube shaped plastic pots (300mm deep and 290mm wide: hereafter square community pots). The eight species were all positioned in the same predetermined pattern within these square community pots (Figure 2.1a-b). The species’ arrangement was determined by firstly dividing the top of the pot into three planting rows, containing three, two and three plants (Figure 2.1b). The two grass species were allocated the centre row of two plants. The locations of the remaining six species were determined using a random number generator. A total of 32 square community pots were planted out with the tubestock, all with this same layout.

2.3.2.3. Pre-treatment

The repotted plants were placed in a random arrangement in the glasshouse for a four week period (i.e. pre-treatment) (March 2014) to ensure plants survived transplantation. The replanted seedlings were also fertilised once a week with Power Feed® Concentrate dynamic fertiliser and soil conditioner by Earthcare® (typical analysis of the concentration: Nitrogen (N) 12%, Phosphorous (P) 1.4%, Potassium (K) 7%; and Potassium humate 3.08%) at a rate of 20ml per 9L of water, with each plant receiving roughly equal quantities, during the first three weeks following replanting to optimise initial plant health and help the plants survive the transplanting process.

2.3.3. Watering regime prior to the experiment, and for the control treatments

All replanted seedlings across both experiments were watered on a regular basis (i.e. every 2-3 times per week) for four weeks (during March 2014) prior to the start of the experiment, to help them establish following re-potting into the larger pots for the experiment. The same watering regime was maintained for pots allocated to the control treatments in each experiment, being 2-3 times per week.
2.4. The two experiments
Two different experiments were used to test the aims of this thesis (see Section 1.10). A description of each experiment is provided below.

2.4.1. Eucalyptus only (single species)
The single species experiment examines eucalypt seedlings of both eucalypt species grown individually in tall cylindrical pots (hereafter referred to as: Eucalyptus only experiment). The Eucalyptus only experiment aims to determine the effect of water and heat stress and re-watering following stress on seedling survival and mortality. The Eucalyptus only experiment contained seedlings of *E. melliodora* (Yellow Box) and *E. blakelyi* (Blakely’s Red Gum), both of which are the dominant tree species of the White Box-Yellow Box-Blakely’s Red Gum Grassy Woodland community (Prober, 1996). One hundred and thirty seedlings (one per pot) of each of the two Eucalyptus species (*E. melliodora* and *E. blakelyi*) were used for the Eucalyptus only experiment. The 130 seedlings were split into eight treatments (see Section 2.5 below for details).

2.4.2. The competition experiment
This community experiment examines seedlings of all eight species grown together in square ‘community’ pots (see Section 2.3.2.2. for further details). The competition experiment aims to determine whether competition plays a role in seedling survival and response to water stress. As both experiments use the same two Eucalyptus species the results from both experiments can be compared to determine whether competition alters any of the results observed. As outlined in Section 2.3.2.2. above, one seedling of each of the eight species was planted into each square ‘community’ pot. In total 32 pots and thus 32 plants of each of the eight species were used in the competition experiment.

2.5. Treatments
Eight treatments were established to examine how water stress affected seedling survival across the eight Australian native plant species. A description of each treatment is provided below along with summary tables for each experiment (Tables 2.2 and 2.3). There were four treatments within two temperature treatments (eight treatments in total).
Treatment spC/suC : spring/summer – no water stress. Summer/Spring conditions with no water stress (i.e. experimental control). Spring and summer treatment conditions are described in Section 2.1. above. The watering regime for the control treatments is outlined in Section 2.3.3. above, being water applied every 1-2 days.

Treatment sp4/su4: spring/summer – 4 weeks water stress. Summer/spring conditions with a water stress period of 4 weeks without water (i.e. seedlings were not watered for 4 weeks). Spring and summer treatment conditions are described in Section 2.1. above.

Treatment sp7/su7: spring/summer – 7 weeks water stress. Summer/spring conditions with an extended water stress period of 7 weeks without water (i.e. seedlings were not watered for 7 weeks). Spring and summer treatment conditions are described in Section 2.1. above.

Treatment spM/suM: spring/summer - water stress until full mortality (Survival\textsubscript{max}). Spring conditions without water until all seedlings across all replicates die (i.e. extreme water stress). This treatment is aimed at determining the maximum longevity or survival period for seedlings of each species (hereafter referred to as Survival\textsubscript{max}). Spring and summer treatment conditions are described in Section 2.1. above.

Table 2.2. Summary table of treatments used in the Eucalyptus only experiment (full details are outlined below).

<table>
<thead>
<tr>
<th>Treatment combination</th>
<th>Season treatments</th>
<th>Re-watered after water stress treatment*</th>
<th>Number of replicates\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring#</td>
<td>Summer^</td>
<td></td>
</tr>
<tr>
<td>Water stress treatments</td>
<td>T1: Control</td>
<td>T5: Control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(regular watering) (spC)</td>
<td>(regular watering) (suC)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2: 4 weeks without water (sp4)</td>
<td>T6: 4 weeks without water (su4)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>T3: 7 weeks without water (sp7)</td>
<td>T7: 7 weeks without water (su7)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>T4: Survival\textsubscript{max} without water (spM)</td>
<td>T8: Survival\textsubscript{max} without water (suM)</td>
<td>15 each</td>
</tr>
</tbody>
</table>

\# simulated spring conditions (see text for details). \textsuperscript{a} simulated summer conditions (see text for details). \textsuperscript{b} the two defined period water stress treatments were re-watered to examine how the species/individuals responded to water after a period of water stress (see text for details). \textsuperscript{c} per species
2.5.1. Allocation of treatments and replication for the *Eucalyptus* only (single species)

The 130 *E. melliodora* and 130 *E. blakelyi* plants [pots] in the single species experiment were randomly allocated to one of eight treatments (T1-8) outlined above. Half of the pots were either placed on benches in the glasshouse (spring treatment – Section 2.1.1. above) or within cabinets inside the glasshouse (summer treatment – Section 2.1.2. above). Within each of the seasonal treatments plants/pots were assigned to one of four water stress treatments (Table 2.2).

The number of replicates differed between treatments, as follows: for the control treatments (T1 and T5) 10 replicates/plants were used for each. For treatments 2, 3, 6 and 7, twice the number of plants (20 replicates) were used to enable a re-watering component (or second treatment) to be undertaken after the initial water stress treatments were completed (see Section 2.5.1.1. below). The increased number was used to ensure that sufficient replicates were available after the water stress treatments to determine the results of the re-watering treatment, whilst 15 replicates were used in the Survival_{max} treatments (T4 and T8) to help determine maximum longevity without water (Table 2.2).

All pots were moved between and/or rotated (i.e. between allocated positions) within the benches in the glasshouse for the spring treatments and the cabinets for the summer treatments each week to minimise the effects of environmental variation within each location (sensu Duan et al., 2013).

2.5.1.1. Re-watering after water stress (second treatment)

Treatments 2, 3, 6 and 7 in the *Eucalyptus* only experiment contained a re-watering component (or second treatment – part b) which was applied after the initial water stress treatments of 4 weeks or 7 weeks were completed. Once the water stress treatment periods were completed all remaining plants in these four treatments were then given the normal watering regime (see Section 2.3.3. above, being water applied every 1-2 days). The re-watering component is examined independently being a) the water stress period; and b) re-watering. The re-watering component was included to determine if there are change points (i.e. water stress periods) from which seedlings could not recover from water stress, despite the availability of water.
2.5.2. Allocation of treatments and replication for the competition experiment

The 32 square community pots containing seedlings of all eight plant species were randomly allocated into one of four treatments (T1, 4, 5 and 8). Half of the pots were either placed on benches in the glasshouse (spring treatment – Section 2.1.1. above) or within cabinets inside the glasshouse (summer treatment – Section 2.1.2. above). Within each of the seasonal treatments pots were assigned to one of two water stress treatments (Table 2.3). Eight replicates were used for each of these four treatments (Table 2.3). Given the size of the square community pots and space restrictions, only the Survival\textsubscript{max} water stress treatments were used (T4 and T8); 4 weeks and 7 weeks water stress data can be readily extracted from the Survival\textsubscript{max} results. In addition, as Survival\textsubscript{max} was the only water stress treatment for the competition experiment there was also no re-watering component undertaken. As outlined in Section 2.5.1. above all pots were moved weekly to minimise the effects of environmental variation within any one location.

### Table 2.3. Summary table of the treatments used in the community experiment (full details are outlined below).

<table>
<thead>
<tr>
<th>Treatment Combination</th>
<th>Season treatments</th>
<th>Number of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring#</td>
<td>Summer^</td>
</tr>
<tr>
<td>Water stress treatments</td>
<td>Control (regular watering) (T1)</td>
<td>Control (regular watering) (T5)</td>
</tr>
<tr>
<td></td>
<td>Survival\textsubscript{max} without water (T4)</td>
<td>Survival\textsubscript{max} without water (T8)</td>
</tr>
</tbody>
</table>

# simulated spring conditions (see text for details). T1 and T4 refer to treatments 1 and 4.
^ simulated summer conditions (see text for details). T5 and T8 refer to treatments 5 and 8.

2.6. Sampling regime

This section outlines the sampling regime used to collect the morphological, physiological and abiotic measurements as outlined in Section 2.7 below. The sampling regime for each measurement is outlined below.

2.6.1. Selecting leaves and plants to sample

2.6.1.1. Eucalyptus only experiment

For each treatment of the *Eucalyptus* only experiment, 10 replicates or eucalypt plants were used. However, given time constraints with taking the measurements (see Section 2.6.2.2.1), physiological measurements could only be sampled for half (5) of these plants. The five pots (of each species) were randomly selected from the 10
replicates within each of the eight treatments in the Eucalyptus only experiment. All 10 replicates were assessed using visual assessments of the morphological changes.

2.6.1.2. Competition experiment
Physiological leaf health measurements for three of the eight community plant species could not be taken with the devices outlined in Section 2.6 due to the species small leaf/blade size, which prevented their measurement. These three plant species are: Dodonaea viscosa, Poa sieberiana and, Poa labillardieri. All physiological leaf health measurements were taken for the other five plant species. Half or four of the competition pots in each of the four treatments in the competition experiment were sampled for the physiological measurements. Visual assessments of leaf and plant health (i.e. morphological changes) were taken for all eight species in the competition experiment.

2.6.1.3. Selecting leaves to sample
For each selected plant (see above), two mature leaves were randomly selected and tagged loosely with different coloured sewing cotton strands about 10 cms in length, being leaf one pink cotton, and leaf two blue cotton, to enable the same leaf to be re-measured during the experiments (hereafter referred to as the tagged leaf or leaves). The samples for both leaves were then averaged on each sample data. Sampling from both leaves on a single plant was also undertaken to ensure that the same response was seen throughout the whole plant and values were averaged due to the strong correlation between values of leaves. These leaves were also used to monitor the change/response of individual leaves over time.

Due to time constraints with taking measurements with the CI – 340 Handheld Photosynthesis System leaf health measurements where only taken from one of the tagged leaves (refer Table 2.4 below).

If a tagged leaf died and fell off the plant, the leaf directly below or the closest leaf to the previously sampled leaf was then sampled. Any such leaf fall changes were recorded and the new leaf was tagged.
2.6.2. Sampling interval and duration

2.6.2.1. Morphological assessments

Visual assessments using the mortality index (see Section 2.7.1.1.1.) were collected once weekly for all treatments in each experiment, for the entire experimental period for the *Eucalyptus* only (12 weeks) and competition experiment (10 weeks). New growth or regrowth was sampled once weekly from the end of the water stress treatments, being either 4 weeks or 7 weeks without water, until the end of the *Eucalyptus* only (12 weeks).

2.6.2.2. Physiological assessments

Measurements of PRI, NDVI, SR, LC, SC, T and Pn (see Section 2.7.2.), were taken once weekly for the entire experimental period for the *Eucalyptus* only (12 weeks) and competition experiment (10 weeks).

2.6.2.2.1. Timing and number of physiological measurements

Many physiological measurements are influenced by changes in light and temperature, therefore measurements were collected under similar light and temperature conditions during the ‘optimal light conditions’ (i.e. between 10:30am-2:30pm) to standardise the effects of light patterns (e.g. angle of light, light intensity etc.) (Sea *et al.*, 2013; Duan *et al.*, 2013).

In order to determine how many samples for all physiological measurements could be undertaken each week, a timetable was developed, in which the average time required to take each physiological measurement was presented relative to the available time during the ‘optimal light conditions’ each day (Table 2.4). Given there were 480 minutes during the ‘optimal light period’ per day, and that each sample took between 1 and 3 minutes to determine, it was therefore only possible to sample ~120-240 leaves per day over 5 days, for a total of ~600+ leaves per week (as outlined in Table 2.4).
**Table 2.4.** The timetable developed for leaf health measurements and the associated time required to take each measurement relative to the period of optimal light available per day/week, from which the number of leaves per plants and experiment can be calculated.

<table>
<thead>
<tr>
<th>Physiological (◊), morphological (●) and abiotic (□) measurements</th>
<th>Estimated average time taken for each individual measurement per leaf (minutes)</th>
<th>Number of leaves per plant sampled</th>
<th>Eucalyptus only experiment</th>
<th>Competition experiment</th>
<th>Total time taken (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal Conductance◊, Transpiration◊, PAR◊, Leaf temperature◊, Net photosynthesis◊ (collected simultaneously)</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>PRI◊</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Chlorophyll Content◊</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>NDVI and SR◊ (collected simultaneously)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Visual leaf health●</td>
<td>1 (whole plant)</td>
<td>Whole plant</td>
<td>2</td>
<td>Whole plant</td>
<td>8</td>
</tr>
<tr>
<td>Soil Moisture□</td>
<td>30 secs</td>
<td>Each pot</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>560 (over all 8 treatments and both species)</td>
<td>5</td>
</tr>
</tbody>
</table>

* Please note that some abiotic and visual measurements could be taken whilst other instruments were stabilising, therefore the total time to sample would be reduced.

### 2.6.2.2.2. Standardising measurements across experiments

The same measurements were collected for both the *Eucalyptus* only and competition experiments to allow for comparisons between them.

In addition, the 260 plants in the *Eucalyptus* only experiment and 256 plants in the square pot competition experiment were all also assessed once per week for any diseases and insects/damage. The disease and insect assessment was conducted in order to assess the occurrence of any other stresses.
2.7. Assessing the morphological and physiological responses

The methodology used to assess or measure the morphological and physiological responses of the seedlings examined to each water stress treatment is outlined for each different measure below.

2.7.1. Morphological measurements of leaf/plant health

2.7.1.1. Visual assessments of leaf health and plant mortality

The morphological responses of each species to water stress, and season (i.e. heat stress in summer) were assessed visually, using a series of leaf/plant health categories, from health to dead. The leaf/plant health visual assessment categories or mortality index were based on signs of deteriorating plant/leaf health such as changes in leaf colour (i.e. green to brown), leaf desiccation (i.e. the drying out and associated deformation of leaves), leaf shrivelling, leaf loss and ultimately plant mortality (see below).

2.7.1.1.1. Mortality index

A mortality index was developed based around visual signs of deteriorating plant health and assigning six distinct stages or categories from healthy to dead. These categories were based on the number of present leaves, number of leaves lost, leaf browning, degree of damage and shrivelling and were placed in categories based on percentage damage. The six pre-determined categories (C) used in the mortality index are: (C1) no or 0% damaged or healthy; (C2) 25% damaged (minor damage); (C3) 25-50% damaged; (C4) 50-75% damaged; (C5) 75-90% damaged; and (C6) 90-100% damaged or dead.

Photo representations of the six categories are presented in Figure 2.2.

Figure 2.2. Photographic examples illustrating the six mortality index categories (see text for descriptions of all six categories). All six plants are from the *Eucalyptus* only experiment.
2.7.1.2. Visual assessments of new growth following the re-watering component of the *Eucalyptus* only experiment

The recovery of plants following re-watering was assessed to determine whether there was a point from which plant health could not recover from water stress.

As outlined above in Section 2.5.1.1., treatments 2, 3, 6 and 7 in the *Eucalyptus* only experiment were re-watered after the prescribed water stress period (i.e. 4 or 7 weeks without water). All *Eucalyptus* seedlings that survived the water stress treatments were visually assessed for signs of recovery (i.e. new plant growth) associated with the re-watering component, using three categories based on where the new growth occurred on the seedling. The three regrowth categories were: (A) new growth at the bottom or base of the main stem; (B) new growth in the middle of the main stem; and (C) new growth at the top of the main stem (Figure 2.4).

![Figure 2.3](image)

**Figure 2.3.** Photographic examples illustrating each of the three regrowth categories: (A) bottom, (B) middle and (C) top of the main stem observed (see text for descriptions of all six categories). Photos show *Eucalyptus blakelyi* seedlings from the *Eucalyptus* only experiment with regrowth after re-watering following a 4 week water stress treatment.

2.7.2. Physiological measurements of leaf health

The physiological responses of the leaves to water stress and season (i.e. heat stress in summer) were assessed using seven standard measures of leaf health. A description of each of the seven physiological measures of leaf health and the commercially available devices used to assess them is presented below.
The seven physiological measures of leaf health are:

1. Net Photosynthesis (Pn),
2. Stomatal Conductance (SC),
3. Transpiration (T),
4. Normalised Difference Vegetation Index (NDVI),
5. Simple Ratio Index (SR),
6. Photochemical Reflectance Index (PRI) and,
7. Relative Leaf Chlorophyll Content (LC).

Four devices were used to assess these seven physiological measures – 1 to 3 were assessed with the same device, 4 and 5 with another, and 6 and 7 were assessed separately. Thus these physiological measures are grouped below relative to the device used to assess each for ease of presentation.

2.7.2.1. NDVI and SR
Normalised Difference Vegetation Index (NDVI) and Simple Ratio (SR) measures were taken with a PolyPen RP 400® (Photon Systems Instruments, Drazov, Czech Republic). The PolyPen is used to measure the spectral reflectance, light transmittance and light absorbance of leaves (Photon Systems Instruments, 2014). The device uses spectral reference of an internal light source at 380 - 1050nm wavelength to measure light reflectance and absorbance. The device then uses incorporated formulas (based on specific light wavelengths: listed below) for commonly used light reflectance indexes such as NDVI and SR Index.

The NDVI formula (as described by Rouse *et al.*, (1974)) is:

\[
\text{NDVI} = \frac{R_{780} - R_{630}}{R_{780} + R_{630}}
\]

in which \(R_{780}\) and \(R_{630}\) indicate reflectance of wavebands of light at a wavelength of 850nm and 650nm respectively.

SR formula (as described by Jordan (1969) and Rouse *et al.*, (1974)) is:

\[
\text{SR} = \frac{R_{780}}{R_{630}}
\]

Both formulas are used by the PolyPen to formulate NDVI and SR values (Photon System Instruments, 2014).
The ‘typical’ range for NDVI measurements of leaves is between 0.2 and 0.9. A higher NDVI value (e.g. between 0.5 to 0.9) indicates healthy leaf function and a lower NDVI value (e.g. between 0.1 and 0.4) indicates unhealthy leaf function (i.e. diminished or lack of leaf function) (Anyamba & Tucker, 2005; Revandekar et al., 2012). The ‘typical’ range for SR measurements of leaves is between 10 and 1. An SR valve of 6.0 to 10.0 usually indicates healthy leaf function (green vegetation) and a value of 1.0 to 5.0 indicates unhealthy leaf function (Pinty & Verstraete, 1992).

Each measurement of NDVI and SR was taken by placing a leaf over the optical window at the top of the PolyPen, which was then held in place with the non-destructive leaf clip (Figure 2.4 – the PolyPen and the PlantPen (see below) take measurements in the same way). Each leaf was positioned with the lower leaf lamina over the optical window (i.e. the upper leaf lamina facing the clip), taking care to avoid placing the midrib over the optical window (Figure 2.4). Once a leaf was secured with the clip, the measure button was pressed and a measurement was made by assessing the light wavelength reflected into the optical window which was then converted into a numerical value using the formulas above. The numerical value for NDVI and SR were then displayed on the screen, along with a graph illustrating the NDVI pattern – which was then used to verify that the sample had been taken properly.

Figure 2.4. A photograph showing how leaves were sampled with the PlantPen PRI 200® meter (the PolyPen RP 400® meter takes measurements in the same way). Parts of the leaves and instrument are indicated: a) the upper leaf lamina; b) the non-destructive leaf clip; c) the lower leaf lamina; and d) the mid-rib (see text for further details).
2.7.2.2. PRI

Photochemical Reflectance Index (PRI) measurements were taken using a PlantPen PRI 200® meter (Photon Systems Instruments, Drazov, Czech Republic). PRI is a light reflectance index focused around a specific reflected light wavelength (being 531 and 570nm), from which a specific PRI value is calculated by using the following formula:

\[
PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}}
\]

in which \(R_{531}\) represents reflectance of wavebands of light at 531nm and \(R_{570}\) is used as a waveband reference, which is then used to assess/determine leaf health (Gamon et al., 1997; Thenot et al., 2002).

The ‘typical’ range for PRI measurements of leaves is between -0.1 and +0.1). A positive PRI value indicates healthy leaf function and a negative PRI value indicates unhealthy leaf function (i.e. diminished or lack of leaf function) (Sea et al., 2013).

PRI measurements were taken in a similar manner to NDVI and SR (see Section 2.7.2.1. above). Once a leaf was secured with the clip on the PlantPen PRI, the measure button was pressed and a measurement was made by assessing the light wavelength reflected into the optical window which was then converted into a numerical value using the formula above. The numerical value was then displayed on the screen.

2.7.2.3. Relative leaf chlorophyll

Relative leaf chlorophyll (LC) content was measured using Atleaf® Chlorophyll Meter (Agri-Lab Supplies, Jay, Maine, United States of America). LC content is measured by determining the optical density difference at two light wavelengths (660nm and 940nm) termed the chlorophyll light spectral region. Whilst the chlorophyll light spectral region corresponds with the 660nm wavelength, the 940nm wavelength is a used as a reference to compensate for leaf moisture, leaf thickness and other leaf factors to ensure values of chlorophyll are accurate and not a representation of other leaf characteristics (Zhu et al., 2012). The Atleaf® Chlorophyll Meter uses its own scale of 0 to 100 Atleaf+ values, a low value indicates low LC content and a high value indicates higher LC content.
The relative LC content was measured by inserting a leaf into the leaf aperture (or slot) on the top end of the Atleaf® Chlorophyll Meter, with the lower leaf lamina facing the bottom of the instrument (i.e. away from the display screen). Also care was taken to also avoid placing the midrib over the optical reader inside the leaf aperture. Once the leaf had been inserted into leaf aperture the measure button was pressed and a measurement was made by assessing the light wavelength reflected into the optical reader which was then converted into a numerical value using the AtLeaf+ index.

2.7.2.4. Pn, SC and T

Net Photosynthesis (Pn), Stomatal Conductance (SC), and Transpiration (T) were measured using a CI – 340 Handheld Photosynthesis System (Bio-science CID: WA, USA). The CI-340 handheld photosynthesis system measures Photosynthetic Active Radiation (PAR) (µmol/m²/s), Net Photosynthesis (Pn) (µmol/m²/s), Stomatal Conductance (SC) (mmol/m²/s), Transpiration (T) (mmol/m²/s), Leaf Temperature (Tleaf) (°C) and Internal T (temperature of internal compartment) (°C) values of a leaf in one sample. PAR is a measure of spectral light that is available for use in photosynthesis (Gurevitch et al. 2006). Net photosynthesis is the difference between total photosynthesis and respiration (Gurevitch et al. 2006). Transpiration and stomatal conductance are measures of absorbance, loss and flow of water vapour and CO₂ from leaves (Gurevitch et al. 2006).

Measurements were taken by placing a leaf into the leaf chamber on the top end of the CI – 340 Handheld Photosynthesis System and clipping the leaf into place, which then sealed the leaf lamina within the chamber (Figure 2.5). Once the leaf was in place, the measurement button was then pressed. It took approximately 3 minutes for all the measurements to be taken and their respective readings to stabilised. Measurements of SC, T, TLeaf, Pn and PAR were then recorded.
Figure 2.5. A photograph showing how leaves are positioned in the leaf chamber of the CI – 340 Handheld Photosynthesis System during sampling.

2.7.3. Abiotic measure

2.7.3.1. Soil moisture

A PMS-714 Soil Moisture Meter was used to measure the soil moisture of each pot over relative to the level of water stress. The soil moisture meter measures the percentage of moisture on a scale of 0 to 50%. The measurements were taken by inserting the heavy duty probe into the soil at a depth of approximately 20cm, waiting 30 seconds for the reading to stabilise and then recoding the soil moisture percentage off the display.

2.8. Data analysis

The following relationships for the Eucalyptus only experiment were analysed from the data: a) leaf health measurements against time; b) correlation of leaf health measurements; c) leaf health for each species and treatment; d) relative to re-watering. These relationships were plotted for each leaf health measurements and loess lines were fitted to demonstrate the polynomial fit of each variable (Cleveland, 1992). The same relationships (a, b and c) were also analysed for the competition experiment. Leaf health measurements (PRI, PAR, Pn, NDVI, SR, SC and T) (fixed factors), treatments (fixed factor) and species (fixed factor) were assessed using Linear Mixed Effect Models (LMM). LMM are used to analyse nested data with temporal correlation, spatial correlation or repeated measurements (Zurr et al., 2009). The random factor pot is not included in the analysis. The differences between fixed variables (leaf health measurement, treatment, time (weeks) and species) were then analysed using analysis of variance (ANOVA) in R (version 2.15.2, Vienna, Austria) (R Core team, 2013). The residuals were examined to ensure that the assumptions of normality and homogeneity of variances were satisfied. Significance levels were set at a p-value of <0.05. Additional data manipulations were conducted to analyse photosynthetic active
radiation to determine whether standardisation was required to allow for changing light levels. Binomial regression was conducted with the new growth following re-watering data to test the probability of new growth (top, middle or bottom) depending on species (fixed factor), week (fixed factor) and treatment (fixed factor).
CHAPTER 3 – Results

3.1. Description of data collected

Data were collected for a period of 12 weeks from April to August 2014. Visual observations and seven measures of leaf health were collected once weekly. A total of 5,420 visual observation and ~20,000 plant health measurements were collected over the 12 week period across the two experiments (Table 3.1).

Table 3.1. Summary and sample size of both experiments in this study.

<table>
<thead>
<tr>
<th>Type of measure</th>
<th>Measurement</th>
<th>Sampling period (weeks)</th>
<th>Number of leaves sampled</th>
<th>Total measurements over sample period *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E1</td>
</tr>
<tr>
<td>Visual</td>
<td>Visual observation</td>
<td>12</td>
<td>10</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Leaf reflectance</td>
<td>PRI</td>
<td>12</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>NDVI</td>
<td>12</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SR</td>
<td>12</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Physiological</td>
<td>Stomatal conductance</td>
<td>12</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Transpiration</td>
<td>12</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Photosynthetic active radiation</td>
<td>12</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Abiotic</td>
<td>Soil moisture</td>
<td>12</td>
<td>10</td>
<td>Whole pot</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>12</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

* = per leaf

3.2. Eucalyptus only experiment

Each of the following sections will show a plot of the data, then an ANOVA test using LMM (fixed factors were treatment, time, measurement and species). Pairwise tests of treatments (i.e. control, 4 weeks without water, 7 weeks without water and survival_{max}) are then shown to demonstrate the heat effect between the summer and spring replicate of each treatment. Where applicable a variant plot of control treatment is also included.
3.2.1. Seedling survival rates

Overall the relationship between observation leaf health category and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.693$, $F_{10, 683} = 157.6$, $p<0.001$) and each predictor itself was highly significant (Table 3.2). There was a significant decline in plant health with increased period of time since last watering (Figure 3.1) (Table 3.2). *Eucalyptus melliodora* and *E. blakelyi* had similar observational leaf health categories to one another over the experimental period (Table 3.2). In water stress treatments the observational leaf health category decreased over time which suggests that visual plant health declines following water stress. Summer control and survival$_{max}$ treatments were significantly different from the spring treatments in that their observation leaf health categories increased at a faster rate over the experiment suggesting that they decreased in observation leaf health at a faster rate (Table 3.3). Summer and spring 4 weeks and 7 weeks without water treatments did not differ (Table 3.3).

![Figure 3.1](image_url)

*Figure 3.1.* Observed changes in *Eucalyptus* health for four different water stress durations (no water stress (control) (a & e), 4 weeks (b & f), 7 weeks (c & g) and 12 weeks without water (d & h)). Observation leaf health categories were as follows: 1) 0% no damage or healthy, 2) 25% damaged, 3) 25-50% damaged, 4) 50-75% damaged, 5) 75-90% damaged, and 6) 90-100% dead. (a) *Eucalyptus blakelyi* = pink circles, and *E. melliodora* = blue circles. Spring treatments (a-d) and summer treatments (e-f). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.
Table 3.2. Results of ANOVA of linear mixed effects model (LMM) of the changes in observed plant health relative to treatment type, time since last watering (week) and eucalypt species.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.62</td>
<td>0.62</td>
<td>0.587</td>
<td>0.444</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>964.61</td>
<td>137.80</td>
<td>130.125</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>650.06</td>
<td>650.06</td>
<td>613.851</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time\</td>
<td>1</td>
<td>53.88</td>
<td>53.88</td>
<td>50.875</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>683</td>
<td>723.29</td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and E. melliodora
^ Eight water stress treatments (see text for details)
# Time since last watering in weeks

Table 3.3. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the observation leaf health categories from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatments compared with summer treatments</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (watered)</td>
<td>1</td>
<td>4.77</td>
<td>4.77</td>
<td>10.200</td>
<td>0.002</td>
</tr>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>0.17</td>
<td>0.17</td>
<td>0.392</td>
<td>0.533</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>0.18</td>
<td>0.18</td>
<td>0.251</td>
<td>0.617</td>
</tr>
<tr>
<td>Survival\max</td>
<td>1</td>
<td>33.39</td>
<td>33.39</td>
<td>31.082</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

3.2.2. Correlation plot of measurements in the *Eucalyptus* only experiment

Five of the seven measurements conducted in this study had a high degree of linear association with another variable. Stomatal conductance and transpiration are highly correlated (0.84) suggesting that an increase or decrease in one value would also result in an increase or decrease in the other value (Figure 3.2). All three leaf reflectance indexes (SR, NDVI and PRI) were highly correlated to one another (0.95, 0.91 and 0.84) (Figure 3.2). As these three indices are all calculated using leaf reflectance measurements and all had high correlation, many of the trends noted in the relevant sections below are similar. Net photosynthesis values were not correlated with other variables and also displayed odd trends and were therefore not used in the analysis for this study.
Figure 3.2. Bivariate correlation plots of measurements (Net photosynthesis (Pn), Stomatal conductance (SC), Transpiration (Trans), NDVI, SR, PRI and relative leaf chlorophyll content (CL)) taken in the *Eucalyptus* only experiment.

3.2.3. Photochemical Reflectance Index (PRI) leaf values

Overall the relationship between PRI and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.608$, $F_{9,660} = 116$, $p < 0.001$) and each predictor itself (aside from species) was highly significant (Table 3.4). The mean PRI leaf values from two sampled leaves (hereafter PRI leaf values) for each eucalypt species decreased over time in water stress treatments. The eight treatments were also significantly different with respect to time since last watering (Figure 3.3, Table 3.4). The longer water was withheld from plants, the larger the decrease in PRI values (Figure 3.3). There was significantly higher residual variation between PRI leaf values of *Eucalyptus blakelyi* when compared with *E. melliodora* in the control treatments (Figure 3.4). The PRI leaf values of each eucalypt species were not statistically different from one another relative to time in 7 week without water and survival$_{max}$ treatments, indicating a similar response between species (Table 3.4).

Spring control and summer control treatments remained more consistently positive during the study (Figure 3.3 a & e). PRI leaf values were consistently negative from week 6 to week 12 in the 7 weeks without water and survival$_{max}$ treatments for both
species (Figure 3.3 c, d, g & h). The PRI leaf values of the summer water stress treatments (Figure 3.3 e-h) decreased at a faster rate than the PRI leaf values of the spring water stress treatments (Figure 3.3 a-d) (Table 3.5).

**Figure 3.3.** Changes in leaf PRI values of *Eucalyptus* species for four different water stress durations (no water stress (control), 4 weeks, 7 weeks and 12 weeks without water). Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a-d) and summer treatments (e-f). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

**Figure 3.4.** The variation of leaf PRI values for each *Eucalyptus* species over the control treatments. Blue boxplot = *Eucalyptus blakelyi* and Pink boxplot = *E. melliodora*. Statistically significant variance between species (F=2.10, df=119, p-value <0.001).
Table 3.4. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time since last watering and species on change in PRI leaf values.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>1.264</td>
<td>0.261</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>0.18</td>
<td>0.03</td>
<td>71.662</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>0.19</td>
<td>0.19</td>
<td>541.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>660</td>
<td>0.23</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and E. melliodora
^ eight water stress treatments (see text for details)
# time since last watering in weeks

Table 3.5. Result of ANOVA of linear mixed effects model (LMM) of the relationship between the PRI leaf values from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>17.409</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>7.646</td>
<td>0.007</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>11.468</td>
<td>0.001</td>
</tr>
<tr>
<td>Survival_{max}</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>8.908</td>
<td>0.003</td>
</tr>
</tbody>
</table>

3.2.4. Normalised Difference Vegetation Index (NDVI) leaf values

Overall there was a highly significant relationship between NDVI and the predictors (time, treatment, species) ($R^2_{adj}$ =0.652, $F_{10,658}$ = 126, p<0.001) and each predictor itself was highly significant (Table 3.6). The NDVI leaf values (mean from two sampled leaves - hereafter NDVI leaf values) decreased over time in water stress treatments. There was a statistical difference between the NDVI leaf values among the eight treatments over time (Figure 3.5, Table 3.6). Control treatments remained at a consistent NDVI leaf value of between 0.6 and 0.8 whereas when the water stress treatments became progressively longer (4 weeks, 7 weeks and 12 weeks) the NDVI leaf values progressively decreased (Figure 3.5, Table 3.6). The NDVI leaf values for *Eucalyptus melliodora* and *Eucalyptus blakelyi* were statistically different from one another (Table 3.6). Post hoc Tukey Kramer analysis revealed that *E. blakelyi* values were relatively unchanged by summer and spring conditions however the NDVI values of *E. melliodora* leaves in summer 4 weeks and 7 weeks without water were consistently lower than the spring values of the same treatments. There was also significantly higher residual variation between the NDVI values of *E. blakelyi* when compared with *E. melliodora* in control treatments (Figure 3.6).

Spring control and summer control remained relatively consistent over time (Figure 3.5). In water stress treatments NDVI leaf values decreased as time since last water increased. Summer treatments were significantly different from the spring
treatments in the 7 week without water and the survival_{max} treatments with the NDVI leaf values decreasing at a faster rate (Table 3.7). There was no statistical difference between individual species’ NDVI leaf values of the summer and spring control and 4 weeks without water treatments (Table 3.7).

Figure 3.5. Changes in NDVI leaf values of Eucalyptus species for four different water stress durations (no water stress (control), 4 weeks, 7 weeks and 12 weeks without water). Blue dots = Eucalyptus blakelyi and pink dots = E. melliodora. Spring treatments (a-d) and summer treatments (e-f). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

Figure 3.6. The variation of leaf NDVI values for each Eucalyptus species over the control treatments. Blue boxplot = Eucalyptus blakelyi and Pink boxplot = E. melliodora. Statistically significant variance between species (F=2.512, df=118, p-value <0.001).
Table 3.6. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on change in NDVI leaf values.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.24</td>
<td>0.24</td>
<td>25.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>5.65</td>
<td>0.81</td>
<td>86.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>5.81</td>
<td>5.81</td>
<td>622.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time²</td>
<td>1</td>
<td>0.05</td>
<td>0.05</td>
<td>5.65</td>
<td>0.017</td>
</tr>
<tr>
<td>Residuals</td>
<td>658</td>
<td>6.14</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and E. melliodora
^ eight water stress treatments (see text for details)
# time since last watering in weeks

Table 3.7. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the NDVI leaf values from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>1.292</td>
<td>0.257</td>
</tr>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>1.813</td>
<td>0.182</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>0.06</td>
<td>0.06</td>
<td>11.402</td>
<td>0.001</td>
</tr>
<tr>
<td>Survival$_{max}$</td>
<td>1</td>
<td>0.04</td>
<td>0.04</td>
<td>6.318</td>
<td>0.013</td>
</tr>
</tbody>
</table>

3.2.5. Simple Ratio (SR)

Overall there was a relationship between SR and the predictors (time, treatment, species) that was highly significant ($R^2_{adj}=0.676$, $F_{10,659}=141$, p<0.001) and each predictor itself was highly significant (Table 3.8). The difference between the SR leaf values (mean value sampled from two leaves) among the eight treatments over time was significant (Figure 3.7) (Table 3.8). Control treatments remained at a SR leaf value of between 4.0 and 8.0 although as the water stress treatments became progressively longer (4 weeks, 7 weeks and 12 weeks) the SR leaf values progressively decreased. Each species also had statistically different SR values with SR leaf values of E. melliodora remaining comparatively frequently higher than the SR leaf values of E. blakelyi (Table 3.8). In water stress treatments SR leaf values decreased as the length of water stress increased. The SR leaf values of summer treatments declined at a faster rate and this was statistically different from the spring treatment (Table 3.9).
Figure 3.7. Changes in simple ratio values of Eucalyptus species for four different water stress durations (no water stress (control), 4 weeks, 7 weeks and 12 weeks without water). Blue dots = Eucalyptus blakelyi and pink dots = E. melliodora. Spring treatments (a-d) and summer treatments (e-f). The trend lines present on the graph are loess fit lines which for each species are polynomial fitting based on least squares.

Table 3.8. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on simple ratio values.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>65.87</td>
<td>65.87</td>
<td>60.060</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>620.23</td>
<td>88.60</td>
<td>80.788</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>803.39</td>
<td>803.39</td>
<td>732.527</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time^2</td>
<td>1</td>
<td>53.42</td>
<td>53.42</td>
<td>48.703</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>659</td>
<td>722.75</td>
<td>1.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and E. melliodora
^ eight water stress treatments (see text for details)
# time since last watering in weeks

Table 3.9. Results of ANOVA of linear mixed effects model (LMM) of the relationship between simple ratio from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.21</td>
<td>0.21</td>
<td>0.462</td>
<td>0.497</td>
</tr>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>1.91</td>
<td>1.91</td>
<td>3.069</td>
<td>0.084</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>9.34</td>
<td>9.34</td>
<td>13.369</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Survivalmax</td>
<td>1</td>
<td>11.25</td>
<td>11.25</td>
<td>13.328</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
3.2.6. Leaf transpiration (T) rates

Overall there was a highly significant relationship between leaf T and the predictors (time, treatment, species) ($R^2_{adj} = 0.661$, $F_{10, 655} = 131$, $p<0.001$) and each predictor itself was highly significant (Table 3.10). The difference between the leaf T rates among the eight treatments over time was significant (Figure 3.8) (Table 3.10). Leaf T rates remained varied in control treatments however a change point was observed in 4 week, 7 week and survival$_{max}$ treatments where beyond this point transpiration rates remained consistently close to zero (Figure 3.8). Each species had a statistically different leaf T rate, with Eucalyptus blakelyi usually having a higher leaf T rate (Table 3.10). In the 7 weeks without water and the survival$_{max}$ treatments leaf transpiration rates decreased with increasing time since last watering. Overall the leaf T rates of summer treatments were not significantly different from the spring treatments (Table 3.11).

Figure 3.8. Changes in leaf transpiration rates of Eucalyptus species for four different water stress durations (no water stress (control), 4 weeks, 7 weeks and 12 weeks without water). Blue dots = Eucalyptus blakelyi and pink dots = E. melliodora. Spring treatments (a-d) and summer treatments (e-f). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares. Change point is indicated with a black arrow.
Table 3.10. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf transpiration rates.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>29.80</td>
<td>29.80</td>
<td>32.573</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>160.63</td>
<td>22.95</td>
<td>25.078</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>425.07</td>
<td>425.07</td>
<td>464.545</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time$^2$</td>
<td>1</td>
<td>579.50</td>
<td>579.50</td>
<td>633.310</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>655</td>
<td>599.34</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and E. mellioidora
^ eight water stress treatments (see text for details)
# time since last watering in weeks

Table 3.11. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf transpiration rates from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.08</td>
<td>0.08</td>
<td>0.050</td>
<td>0.823</td>
</tr>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>14.20</td>
<td>14.20</td>
<td>12.999</td>
<td>0.001</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>0.95</td>
<td>0.95</td>
<td>0.729</td>
<td>0.395</td>
</tr>
<tr>
<td>Survival$_{max}$</td>
<td>1</td>
<td>0.77</td>
<td>0.77</td>
<td>0.555</td>
<td>0.457</td>
</tr>
</tbody>
</table>

3.2.7. Stomatal Conductance (SC)

Overall the relationship between SC and the predictors (time, treatment, species) was highly significant ($R^2_{adj} =0.506$, $F_{10, 655} = 69$, $p<0.001$) and each predictor itself was highly significant (Table 3.12). The difference between the SC values among the eight treatments over time was significant (Figure 3.9) (Table 3.12). The SC values of control treatments remained varied however a change point was observed in water stress treatments at around 4 weeks without water where SC remained consistently low following that period (Figure 3.9). Each species had a statistically different stomatal conductance value (Table 3.12). In the 7 weeks without water and the survival$_{max}$ treatments SC values decreased as time of water stress increased. The summer 4 weeks without water treatment was statistically different from the spring 4 weeks without water treatment (Table 3.13). All other summer treatments were not significantly different from the spring treatments (Table 3.13).
Figure 3.9. Changes in stomatal conductance values of *Eucalyptus* species for four different water stress durations (no water stress (control), 4 weeks, 7 weeks and 12 weeks without water). Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a-d) and summer treatments (e-f). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares. Change point is indicated with a black arrow.

Table 3.12. Result of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on stomatal conductance rates.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>676,539</td>
<td>676,539</td>
<td>44.013</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>2,117,361</td>
<td>302,480</td>
<td>19.678</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>4,674,655</td>
<td>4,674,655</td>
<td>304.113</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time^2</td>
<td>1</td>
<td>3,135,653</td>
<td>3,135,653</td>
<td>203.992</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>655</td>
<td>10,068,290</td>
<td>153,771</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and *E. melliodora*

^ eight water stress treatments (see text for details)

# time since last watering in weeks

Table 3.13. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the stomatal conductance values from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Normal treatment compared with heated</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>1,090.00</td>
<td>1,090.00</td>
<td>0.065</td>
<td>0.800</td>
</tr>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>286,969.00</td>
<td>286,969.00</td>
<td>9.382</td>
<td>0.003</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>15,406.00</td>
<td>15,406.00</td>
<td>0.838</td>
<td>0.361</td>
</tr>
<tr>
<td>Survival_{max}</td>
<td>1</td>
<td>209.00</td>
<td>209.00</td>
<td>0.014</td>
<td>0.905</td>
</tr>
</tbody>
</table>
3.2.8. Relative Leaf Chlorophyll Concentration (CL)

Overall there was a highly significant relationship between CL and the predictors (time, treatment, species) ($R_{adj}^2 = 0.276$, $F_{10, 665} = 26$, $p<0.001$) and each predictor itself was highly significant (Table 3.14). The difference between the CL values among the eight treatments over time was significant (Figure 3.10) (Table 3.14). Control treatments had a relatively consistent CL value, however in water stress treatments CL values increased during weeks four to eight (Figure 3.10) and in survival$_{max}$ treatments CL values then decreased. Each species had statistically different leaf chlorophyll content, with $E.~melliodora$ CL values generally higher throughout all treatments. Overall the leaf CL values of summer treatments were not significantly different from the spring treatments (Table 3.15).

*Figure 3.10.* Changes in the relative leaf chlorophyll content for *Eucalyptus* species for four different water stress durations (no water stress (control), 4 weeks, 7 weeks and 12 weeks without water). Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a-d) and summer treatments (e-f). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.
Table 3.14. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on the relative leaf chlorophyll content values.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>1,660.00</td>
<td>1,659.50</td>
<td>18.456</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>9,504.00</td>
<td>1,357.7</td>
<td>15.107</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>1,704.00</td>
<td>1,704.10</td>
<td>18.961</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time^2</td>
<td>1</td>
<td>10,806.00</td>
<td>10,806.50</td>
<td>120.242</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>780</td>
<td>58,957.00</td>
<td>89.90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and E. melliodora
^ eight water stress treatments (see text for details)
# time since last watering in weeks

Table 3.15. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the relative leaf chlorophyll content from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>9.10</td>
<td>9.12</td>
<td>0.281</td>
<td>0.597</td>
</tr>
<tr>
<td>4 weeks water stress</td>
<td>1</td>
<td>0.54</td>
<td>0.54</td>
<td>0.029</td>
<td>0.865</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>158.40</td>
<td>158.37</td>
<td>2.206</td>
<td>0.140</td>
</tr>
<tr>
<td>Survival_{max}</td>
<td>1</td>
<td>123.00</td>
<td>122.80</td>
<td>0.604</td>
<td>0.438</td>
</tr>
</tbody>
</table>

3.2.9. Percentage Soil Moisture (SM) content

The difference between the SM values among the eight treatments over time was significant (Figure 3.11) (Table 3.16). Control treatments varied in SM value over time, however spring control treatments had significantly higher SM than summer control treatments (Table 3.17) (Figure 3.11 a & e). In without water treatments SM decreased to 0% following 3-4 weeks without water in summer and spring treatments (Figure 3.11 b, c, d, f, g & h). Aside from the control treatment, the leaf SM values of summer treatments were not significantly different from the spring treatments (Table 3.17).
Figure 3.11. Changes in the percentage soil moisture content for pots of *Eucalyptus* species for four different water stress durations (no water stress (control), 4 weeks, 7 weeks and 12 weeks without water). Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a-d) and summer treatments (e-f). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

**Table 3.16.** Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on soil moisture values.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>68.20</td>
<td>68.24</td>
<td>4.001</td>
<td>0.046</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>7,060.20</td>
<td>1,008.61</td>
<td>59.134</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>1,762.90</td>
<td>1,762.88</td>
<td>103.356</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>410</td>
<td>6,993.10</td>
<td>17.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Eucalyptus blakelyi* and *E. melliodora*

^ eight water stress treatments (see text for details)

# time since last watering in weeks

**Table 3.17.** Results of ANOVA of linear mixed effects model (LMM) of the relationship between the soil moisture from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>compared with summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>407.37</td>
<td>407.37</td>
<td>31.917</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4 weeks water stress</td>
<td>1</td>
<td>2.61</td>
<td>2.61</td>
<td>0.4962</td>
<td>0.485</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>4.12</td>
<td>4.12</td>
<td>0.311</td>
<td>0.579</td>
</tr>
<tr>
<td>Survival$_{max}$</td>
<td>1</td>
<td>2.33</td>
<td>2.33</td>
<td>0.165</td>
<td>0.685</td>
</tr>
</tbody>
</table>
3.2.10. Summary

Seedling survival in both Eucalypt species decreased with extended periods of water stress periods (i.e. time since last watering) in both summer and spring conditions (Figure 3.1 - Figure 3.11) (refer Table 3.18). Complete seedling mortality was observed for both Eucalypt species within 12 weeks without water under both spring (Figure 3.1 a-d) and summer conditions (Figure 3.1 e - h). When looking at the seven leaf physiology measures transpiration and stomatal conductance showed clear change points where leaf health showed a dramatic decline and continual low following the change point (Figures 3.8 & 3.9). PRI, NDVI and SR leaf health measures showed a clear indication of declining leaf health values with an increasing period of water stress (Figures 3.3, 3.5 & 3.7).

Table 3.18. Summary table for Eucalyptus only experiment data

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Trend following water stress</th>
<th>Difference between summer and spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual leaf observation category</td>
<td>Decrease</td>
<td>Control and survival_{max}</td>
</tr>
<tr>
<td>PRI</td>
<td>Decrease</td>
<td>All</td>
</tr>
<tr>
<td>NDVI</td>
<td>Decrease</td>
<td>7 weeks without water, and survival_{max}</td>
</tr>
<tr>
<td>SR</td>
<td>Decrease</td>
<td>7 weeks without water, and survival_{max}</td>
</tr>
<tr>
<td>Transpiration</td>
<td>Decrease (\rightarrow ) change point</td>
<td>4 weeks without water</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>Decrease (\rightarrow ) change point</td>
<td>4 weeks without water</td>
</tr>
<tr>
<td>Relative leaf chlorophyll content</td>
<td>Increase (\rightarrow ) decrease</td>
<td>No</td>
</tr>
<tr>
<td>Soil Moisture</td>
<td>Decrease (\rightarrow ) change point</td>
<td>No</td>
</tr>
</tbody>
</table>

\(\rightarrow \) = followed by

3.3. Rewatering the eucalypt plants after water stress

3.3.1 Observations of plant recovery following rewatering

New growth observations

The probability of new growth occurring is significantly higher following 4 weeks without water when compared to new growth following 7 weeks without water (Figure 3.12 and 3.13). *Eucalyptus melliodora* has a higher probability of new growth at the top of the plant when compared to *E. blakelyi* following re-watering after both 4 weeks and 7 weeks without water (Figure 3.12 and 3.13). Heat (summer compared with spring) did not have a significant effect on the location of new growth so was not included as a variable on the figures (Table 3.19 and 3.20).
Figure 3.12. Probability of new growth following 4 weeks without water of *E. melliodora* and *E. blakelyi*. Lines represent the probability of new growth occurring at a particular location on the seedlings being top (crown) (red), middle (mid stem) (blue) and bottom (base of the stem) (black). *Eucalyptus melliodora* = dashed lines and *E. blakelyi* = solid lines.

Figure 3.13. Probability of new growth following 7 weeks without water of *E. melliodora* and *E. blakelyi*. Lines represent the probability of new growth occurring at a particular location on the seedlings being top (crown) (red), middle (mid stem) (blue) and bottom (base of the stem) (black). *Eucalyptus melliodora* = dashed lines and *E. blakelyi* = solid lines.
Table 3.19. Analysis of deviance table of the results of binomial regression of the effects of treatment, time and species on location of new growth for the 4 weeks without water treatments.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Deviance</th>
<th>Residual Df</th>
<th>Resid Dev.</th>
<th>Pr(&gt;Chi)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Null</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>New Growth Top</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td></td>
<td>639</td>
<td>846.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.107</td>
<td>638</td>
<td>846.69</td>
<td>0.744</td>
</tr>
<tr>
<td>Treatment^</td>
<td>1</td>
<td>52.560</td>
<td>637</td>
<td>749.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>37.963</td>
<td>636</td>
<td>756.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>New Growth Middle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td></td>
<td>639</td>
<td>887.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species*</td>
<td>1</td>
<td>8.117</td>
<td>638</td>
<td>879.09</td>
<td>0.004</td>
</tr>
<tr>
<td>Treatment^</td>
<td>1</td>
<td>12.297</td>
<td>637</td>
<td>866.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>190.518</td>
<td>636</td>
<td>676.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>New Growth Bottom</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td></td>
<td>639</td>
<td>855.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.007</td>
<td>638</td>
<td>855.45</td>
<td>0.935</td>
</tr>
<tr>
<td>Treatment^</td>
<td>1</td>
<td>12.202</td>
<td>637</td>
<td>843.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>67.192</td>
<td>636</td>
<td>776.06</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and E. melliodora
^ 4 weeks without water treatments (replicated for summer and spring conditions) (see text for details)
# time following re-watering in weeks

Table 3.20. Analysis of deviance table of the results of binomial regression of the effects of treatment, time and species on new growth for the 7 weeks without water treatments.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Deviance</th>
<th>Residual Df</th>
<th>Resid Dev.</th>
<th>Pr(&gt;Chi)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Null</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>New Growth Top</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td></td>
<td>479</td>
<td>353.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species*</td>
<td>1</td>
<td>1.259</td>
<td>478</td>
<td>352.58</td>
<td>0.262</td>
</tr>
<tr>
<td>Treatment^</td>
<td>1</td>
<td>34.227</td>
<td>477</td>
<td>318.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>2.204</td>
<td>476</td>
<td>315.45</td>
<td>0.088</td>
</tr>
<tr>
<td><strong>New Growth Middle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td></td>
<td>479</td>
<td>255.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.120</td>
<td>478</td>
<td>255.61</td>
<td>0.725</td>
</tr>
<tr>
<td>Treatment^</td>
<td>1</td>
<td>10.151</td>
<td>477</td>
<td>245.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>9.081</td>
<td>476</td>
<td>236.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>New Growth Bottom</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td></td>
<td>479</td>
<td>265.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.114</td>
<td>478</td>
<td>265.55</td>
<td>0.735</td>
</tr>
<tr>
<td>Treatment^</td>
<td>1</td>
<td>11.984</td>
<td>477</td>
<td>253.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>13.512</td>
<td>476</td>
<td>240.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and E. melliodora
^ 7 weeks without water treatments (replicated for summer and spring conditions) (see text for details)
# time following re-watering in weeks
3.3.2. Rewatering

The following section will show plots of the re-watering data from the *Eucalyptus* only experiment. Each section will show a plot of the re-watering data and then an ANOVA test using LMM (fixed factors treatment, time, species) to analyse the effects of re-watering between summer and spring treatments. Pairwise tests of treatments (i.e. control, 4 weeks without water, 7 weeks without water and mortality\textsubscript{max}) are then shown to demonstrate the heat effect between the summer and spring replicate of each treatment.

3.3.2.1. Correlation plot for re-watering values in the Eucalyptus only experiment

Five of the seven measurements conducted in this study had a high degree of linear association with another variable. Stomatal conductance and transpiration are highly correlated (0.84) suggesting that an increase or decrease in one value would also result in an increase or decrease in the other value (Figure 3.14). All three leaf reflectance indexes (SR, NDVI and PRI) were highly correlated to one another (0.95, 0.88 and 0.84) (Figure 3.14). As these three indices are all calculated using leaf reflectance measurements and all had high correlation, many of the trends noted in the relevant sections below are similar. Net photosynthesis values were not correlated with other variables and also displayed odd trends and were therefore not used in the analysis for this study.
Figure 3.14. Bivariate correlation plot of measurements (Net photosynthesis (Pn), Stomatal conductance (SC), Transpiration (Trans), NDVI, SR, PRI and relative leaf chlorophyll content (CL)) following re-watering in the *Eucalyptus* only experiment.

3.3.2.2. Photochemical Reflectance Index (PRI) leaf values following re-watering

Overall the relationship between PRI and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.457$, $F_{5,237} = 42$, $p<0.001$) and aside from species, each predictor itself was highly significant (Table 3.21). The recovery following re-watering of PRI leaf values was statistically different among the four treatments (Figure 3.15) (Table 3.21). The 7 weeks without water treatments had overall lower PRI leaf values when compared to 4 weeks without water treatments following 30 days of re-watering. The PRI leaf values of each species were not statistically different following re-watering (Table 3.21). The PRI leaf values of summer treatments (Figure 3.15 c & d) were lower than the spring treatments (Figure 3.15 a & b) (Table 3.22).
Figure 3.15. Change in the PRI leaf values post re-watering (0-50 days) for *Eucalyptus* species following 4 weeks (left) and 7 weeks (right) without water treatments. Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

Table 3.21. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf PRI from summer and spring treatments following re-watering.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.308</td>
<td>0.579</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>0.67</td>
<td>0.10</td>
<td>90.055</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>0.08</td>
<td>0.08</td>
<td>73.572</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>523</td>
<td>0.55</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Eucalyptus blakelyi* and *E. melliodora*  
^ two water stress treatments (see text for details)  
# time since re-watering in days

Table 3.22. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the PRI leaf values from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>0.04</td>
<td>0.04</td>
<td>19.354</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>0.08</td>
<td>0.08</td>
<td>143.275</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

3.3.2.3. Normalised difference vegetation index (NDVI) leaf values following re-watering

Overall there was a highly significant relationship between NDVI and the predictors (time, treatment, species) ($R^2_{adj} = 0.481$, $F_{5, 233} = 45$, $p<0.001$) and each predictor itself
was highly significant (Table 3.23). The recovery following re-watering of NDVI leaf values was statistically different among the four treatments (Figure 3.16) (Table 3.23). The 7 weeks without water treatments had overall lower NDVI leaf values when compared to 4 weeks without water treatments following 30 days of re-watering. The NDVI leaf values following re-watering of summer treatments (Figure 3.16 c & d) were lower than the spring treatments (Figure 3.16 a & b) (Table 3.24). The NDVI leaf values were statistically different between species with *E. melliodora* exhibiting higher NDVI leaf values in spring treatments (Figure 3.16 a & b) and *E. blakelyi* displaying higher NDVI leaf values in the summer 4 weeks without water treatment (Figure 3.16 c).

**Figure 3.16.** Change in the NDVI leaf values post re-watering (0-50 days) for *Eucalyptus* species following 4 weeks (left) and 7 weeks (right) without water treatments. Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

**Table 3.23.** Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf NDVI from summer and spring treatments following re-watering.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.47</td>
<td>0.47</td>
<td>28.158</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>13.15</td>
<td>1.88</td>
<td>112.098</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>1.05</td>
<td>1.05</td>
<td>62.637</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>519</td>
<td>8.70</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Eucalyptus blakelyi* and *E. melliodora*

^ two water stress treatments (see text for details)

# time since re-watering in days
Table 3.24. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the NDVI leaf values from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>0.08</td>
<td>0.08</td>
<td>3.392</td>
<td>0.067</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>0.35</td>
<td>0.35</td>
<td>26.022</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

3.3.2.4. Simple Ratio (SR) leaf values following re-watering

Overall the relationship between SR and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.422$, $F_{5, 233} = 36$, $p<0.001$) and each predictor itself was highly significant (Table 3.25). The recovery following re-watering of SR leaf values was statistically different among the four treatments (Figure 3.17) (Table 3.25). The 7 weeks without water treatments had overall lower SR leaf values when compared to 4 weeks without water treatments following 30 days of re-watering. The SR leaf values following re-watering of summer treatments (Figure 3.17 c & d) were lower than the spring treatments (Figure 3.17 a & b) (Table 3.26).

![Figure 3.17](image)

**Figure 3.17.** Change in the SR leaf values post re-watering (0-50 days) for *Eucalyptus* species following 4 weeks (left) and 7 weeks (right) without water treatments. Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.
Table 3.25. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf SR from summer and spring treatments following rewatering.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>78.07</td>
<td>78.08</td>
<td>53.193</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>7</td>
<td>1057.60</td>
<td>151.09</td>
<td>102.936</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>56.66</td>
<td>56.66</td>
<td>38.606</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time^2</td>
<td>1</td>
<td>12.21</td>
<td>12.21</td>
<td>8.320</td>
<td>0.004</td>
</tr>
<tr>
<td>Residuals</td>
<td>518</td>
<td>760.30</td>
<td>1.47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and E. melliodora
^ two water stress treatments (see text for details)
# time since re-watering in days

Table 3.26. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf simple ratio values from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>10.14</td>
<td>10.14</td>
<td>5.470</td>
<td>0.021</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>19.24</td>
<td>19.24</td>
<td>18.592</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

3.3.2.5. Leaf Transpiration (T) rates following re-watering

Overall there was a highly significant relationship between leaf T rates and the predictors (time, treatment, species) ($R^2_{adj} = 0.249$, $F_{5,226} = 16, p<0.001$) and each predictor itself (aside from species) was highly significant (Table 3.27). The recovery following re-watering of leaf T rates was statistically different among the four treatments (Figure 3.18) (Table 3.27). The 7 weeks without water treatments had relatively similar leaf T rates when compared to 4 weeks without water treatments following 30 days of re-watering. The leaf T rates following re-watering of summer treatments (Figure 3.18 c & d) were lower than the spring treatments (Figure 3.18 a & b) (Table 3.28). The leaf T rates were not statistically different between species (Table 3.27).
Figure 3.18. Change in the leaf transpiration rates post re-watering (0-50 days) for *Eucalyptus* species following 4 weeks (left) and 7 weeks (right) without water treatments. Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

Table 3.27. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf transpiration from summer and spring treatments following rewatering.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.33</td>
<td>0.33</td>
<td>0.451</td>
<td>0.502</td>
</tr>
<tr>
<td>Treatment^</td>
<td>3</td>
<td>42.57</td>
<td>14.19</td>
<td>19.581</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>25.85</td>
<td>25.85</td>
<td>35.678</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time^2</td>
<td>1</td>
<td>18.04</td>
<td>18.43</td>
<td>25.430</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>226</td>
<td>163.04</td>
<td>0.72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Eucalyptus blakelyi* and *E. melliodora*

^ two water stress treatments (see text for details)

# time since re-watering in days

Table 3.28. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf transpiration rates from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>0.06</td>
<td>0.06</td>
<td>0.064</td>
<td>0.801</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>0.87</td>
<td>0.87</td>
<td>2.793</td>
<td>0.099</td>
</tr>
</tbody>
</table>

3.3.2.6. Leaf Stomatal Conductance (SC) rates following re-watering

Overall the relationship between SC and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.258$, $F_{5,226} = 17$, $p<0.001$) and each predictor itself was highly significant (Table 3.29). The recovery following re-watering of leaf SC rates was statistically different among the four treatments (Figure 3.19) (Table 3.29). The 7 weeks
without water treatments had relatively similar leaf SC rates when compared to 4 weeks without water treatments following 30 days of re-watering. The leaf SC rates of summer survival\textsubscript{max} treatments (Figure 3.19 c & d) were lower than the spring survival\textsubscript{max} treatments (Figure 3.19 a & b) (Table 3.30).

**Figure 3.19.** Change in the stomatal conductance rates post re-watering (0-50 days) for *Eucalyptus* species following 4 weeks (left) and 7 weeks (right) without water treatments. Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

**Table 3.29.** Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf stomatal conductance from summer and spring treatments following rewatering.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>75,014.00</td>
<td>75,014.00</td>
<td>7.606</td>
<td>0.006</td>
</tr>
<tr>
<td>Treatment^</td>
<td>3</td>
<td>476,817.00</td>
<td>158,939.00</td>
<td>16.116</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>121,355.00</td>
<td>121,355.00</td>
<td>12.305</td>
<td>0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>226</td>
<td>2,228,853.00</td>
<td>9,862.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eucalyptus blakelyi and *E. melliodora*

^ two water stress treatments (see text for details)

# time since re-watering in days

**Table 3.30.** Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf stomatal conductance rates from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>compared with summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>15,480.00</td>
<td>15,480.00</td>
<td>1.202</td>
<td>0.275</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>5,168.00</td>
<td>5,167.70</td>
<td>4.431</td>
<td>0.039</td>
</tr>
</tbody>
</table>
3.3.2.7. Relative leaf chlorophyll content (CL) values following re-watering

Overall the relationship between CL and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.407$, $F_{5, 254} = 37$, $p<0.001$) and each predictor itself was highly significant (Table 3.31). The recovery following re-watering of leaf CL values was statistically different among the four treatments (Figure 3.20) (Table 3.31). The 7 weeks without water treatments had lower leaf CL values when compared to 4 weeks without water treatments following 30 days of re-watering. Generally leaf CL values decreased following a period of re-watering. *Eucalyptus melliodora* generally had higher CL values than those of *E. blakelyi* in each treatment (Figure 3.20) (Table 3.31). The leaf CL values of summer survival$_{\text{max}}$ treatments (Figure 3.20 c & d) were lower than the spring survival$_{\text{max}}$ treatments (Figure 3.20 a & b) (Table 3.32).

**Figure 3.20.** Change in the relative leaf chlorophyll content post re-watering (0-50 days) for *Eucalyptus* species following 4 weeks (left) and 7 weeks (right) without water treatments. Blue dots = *Eucalyptus blakelyi* and pink dots = *E. melliodora*. Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

**Table 3.31.** Results of ANOVA of linear mixed effects model (LMM) of the relationship between the relative leaf chlorophyll content from summer and spring treatments following rewatering.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>2,326.00</td>
<td>2,325.80</td>
<td>13.466</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>3</td>
<td>10,483.00</td>
<td>3,494.30</td>
<td>20.232</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>7,459.00</td>
<td>7,459.10</td>
<td>43.189</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>230</td>
<td>39,723.00</td>
<td>172.70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Eucalyptus blakelyi* and *E. melliodora*
^ two water stress treatments (see text for details)
# time since re-watering in days
Table 3.32. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the relative LC values from summer and spring treatments for both species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks without water</td>
<td>1</td>
<td>261.60</td>
<td>261.60</td>
<td>2.014</td>
<td>0.158</td>
</tr>
<tr>
<td>7 weeks without water</td>
<td>1</td>
<td>4,978.60</td>
<td>4,978.60</td>
<td>27.778</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

3.3.2.8. Summary

Generally plants showed signs of recovery following 4 weeks without water, but showed little recovery following 7 weeks without water (refer Table 3.33). If differences occurred between summer and spring treatments, summer treatments showed less signs of recovery.

Table 3.33. Summary table of re-watering data

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Overall trend following re-watering</th>
<th>Difference between summer and spring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks*</td>
<td>7 weeks*</td>
</tr>
<tr>
<td>PRI</td>
<td>Stable</td>
<td>Decrease</td>
</tr>
<tr>
<td>NDVI</td>
<td>Stable</td>
<td>Decrease</td>
</tr>
<tr>
<td>SR</td>
<td>Stable</td>
<td>Decrease</td>
</tr>
<tr>
<td>Transpiration</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Relative leaf chlorophyll content</td>
<td>Stable</td>
<td>Decrease</td>
</tr>
</tbody>
</table>

* Period of water stress before re-watering

3.4. Competition experiment

The competition experiment contained two seasonal treatments (spring and summer), two water stress treatments (no water stress, and survival_max time without water) and eight species encompassing three life forms (trees, shrubs and grasses), with each treatment combination replicated eight times. Unlike the Eucalypt (single species) experiment above, all eight species were grown together in ‘community’ pots and only water and survival_max were used to assess water stress, and thus there is not a re-watering treatment.

Specific leaf attributes were then examined in order to understand how plants were responding to water stress (Figures 3.21-3.28). The following sections give results for the observation leaf health categories, photochemical reflectance index (PRI), normalised difference vegetation index (NDVI), transpiration, stomatal conductance and simple ratio for the competition experiment. Please note that for all but the visual assessments of leaf health, only measurements for the following five species
(Eucalyptus melliodora, Eucalyptus blakelyi and Acacia melanoxylon, Daviesia mimosoides, and Hardenbergia violacea), were taken due to limitations on sampling species with small leaf areas/sizes (see methods section for further details).

3.4.1. Visual observation of leaf health

Overall the relationship between observational leaf health category and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.661$, $F_{9, 779} = 174$, $p<0.001$) and each predictor itself was highly significant (Table 3.34). The effect of water stress on seedling survival showed that following ten weeks of water stress complete seedling mortality occurred in summer and spring treatments as approximately 100% of plants were within observation leaf health category 6 (Figure 3.21). Survival$_{\text{max}}$ treatments lead to a decrease in observation leaf health category over time whereas control treatments had varied observation leaf health categories over time (Table 3.34) (Figure 3.21). In survival$_{\text{max}}$ treatments the visual signs of damage and mortality appeared to decrease the longer plants were subjected to water stress (Figure 3.21). The observation leaf health category of summer treatments decreased [higher percentage of observation leaf health damage] at a faster rate than those in spring treatments (Table 3.35). Each species also had statistically different observational leaf health category trends from one another; however species followed the general trends mentioned above (Table 3.34).
Figure 3.21. The observation leaf health category relative to summer and spring treatments, and water stress to survivalmax with time (weeks). Leaf health visual assessment categories: 1) 0% - no damage or healthy, 2) 25% damaged, 3) 25-50% damaged, 4) 50-75% damaged, 5) 75-90% damaged, and 6) 90-100% damaged or dead. The eight species used are represented by blue dots = *Acacia melanoxylon*, pink dots = *Daviesia mimosoides*, dark green dots = *Dodonaea viscosa*, red dots = *Eucalyptus blakelyi*, yellow dots = *Eucalyptus melliodora*, light green dots = *Hardenbergia violacea*, dark pink dots = *Poa labillardieri* and, darker blue dots = *Poa sieberiana*. Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares. Trend lines are not presented where a line pattern does not exist.

Table 3.34. Results for ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on the observation leaf health category.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>7</td>
<td>40.53</td>
<td>5.79</td>
<td>5.935</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment ^</td>
<td>3</td>
<td>2578.70</td>
<td>859.57</td>
<td>881.164</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time #</td>
<td>1</td>
<td>1798.58</td>
<td>1798.58</td>
<td>1843.772</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>2545</td>
<td>2482.62</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Acacia melanoxylon, Daviesia mimosoides, Dodonaea viscosa, Eucalyptus blakelyi, E. melliodora, Hardenbergia violacea, Poa labillardieri, and Poa sieberiana.*

^ Four treatments (two stress and two control)
# time since last watering in weeks
**Table 3.35.** Results of ANOVA of linear mixed effects model (LMM) of the relationship between the observation leaf health categories from summer and spring treatments for all species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>4.20</td>
<td>4.20</td>
<td>12.428</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Survival&lt;sub&gt;max&lt;/sub&gt;</td>
<td>1</td>
<td>49.81</td>
<td>49.81</td>
<td>96.153</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### 3.4.2. Correlation plot for leaf health values in the competition experiment

Five of the seven measurements conducted in this study had a high degree of linear association with another variable. Stomatal conductance and transpiration are highly correlated (0.84) suggesting that an increase or decrease in one value would also result in an increase or decrease in the other value (Figure 3.22). Both measurements describe physiological leaf changes. All three leaf reflectance indexes (SR, NDVI and PRI) were highly correlated to one another (0.93, 0.83 and 0.70) (Figure 3.22). Net photosynthesis values were not correlated with other variables and also displayed odd trends and were therefore not used in the analysis for this study.

**Figure 3.22.** A binomial correlation plot of measurements (Net photosynthesis (Pn), Stomatal conductance (SC), Transpiration (Trans), NDVI, SR, PRI and relative leaf chlorophyll content (CL)) taken in the competition experiment.
3.4.3. Photochemical Reflectance Index (PRI) leaf values

Overall there was a highly significant relationship between PRI and the predictors (time, treatment, species) ($R^2_{adj} = 0.466$, $F_{9,779} = 77$, $p<0.001$) and each predictor itself was highly significant (Table 3.36). The PRI leaf values sampled from two leaves (hereafter PRI leaf values) for each species between the four treatments were significantly different over time (Figure 3.23) (Table 3.36). PRI leaf values decreased as the length of water stress increased (Figure 3.23). Spring control and summer control remained relatively consistent over time for most species, with few outliers (Figure 3.23). On the whole all PRI leaf values in the controls were positive. However, by 8 weeks without water virtually all PRI values were negative across the five species. Each species had statistically different PRI leaf values from one another (Table 3.36). The PRI leaf values for *Daviesia mimosoides* was significantly lower than the other four species throughout the survival$_{max}$ summer treatment (Figure 3.23). *Daviesia mimosoides* also had significantly lower PRI leaf values for survival$_{max}$ treatments although *Hardenbergia violacea* also had low PRI values. Summer treatments were significantly different from the spring treatments in that the PRI values declined at a faster rate and had more overall variation (Table 3.37).
Figure 3.23. PRI relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). The five species used are represented by Blue dots = *Acacia melanoxylon*, pink dots = *Daviesia mimosoides*, red dots = *Eucalyptus melliodora*, green dots = *Eucalyptus blakelyi* and yellow dots = *Hardenbergia violacea*. Note: PRI values could not be taken for three species as their leaves were not of sufficient size (see methods section for further details). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

Table 3.36. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on PRI leaf values. Note: species refers only to the five species which could be assessed for PRI (see text for details).

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>4</td>
<td>0.10</td>
<td>0.03</td>
<td>35.769</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>3</td>
<td>0.19</td>
<td>0.06</td>
<td>88.681</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>0.20</td>
<td>0.20</td>
<td>278.617</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time$^2$</td>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>8.328</td>
<td>0.004</td>
</tr>
<tr>
<td>Residuals</td>
<td>779</td>
<td>0.55</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Acacia melanoxylon, Daviesia mimosoides, Eucalyptus blakelyi, E. melliodora and Hardenbergia violacea.*

^ Four treatments (two stress and two control)

# time since last watering in weeks

Table 3.37. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the PRI leaf values from summer and spring treatments for all species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>17.071</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Survival$_{\text{max}}$</td>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>20.330</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
3.4.4. Normalised Difference Vegetation Index (NDVI) leaf values

Overall the relationship between NDVI and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.587$, $F_{9,779} = 126$, $p<0.001$) and each predictor itself was highly significant (Table 3.38). There was a statistical difference between the NDVI leaf values sampled from two leaves (hereafter NDVI leaf values) for each species between control treatments and the survival$_{max}$ treatments over time (Figure 3.24) (Table 3.38). The NDVI leaf values of control treatments remained at a relatively consistent value of between 0.6 and 0.8 throughout the experimental period (Figure 3.24). Survival$_{max}$ treatments conversely showed a continual decrease to a NDVI leaf value of 0.2 at the end of the experimental period (Figure 3.24). Each of the five species had statistically different NDVI leaf values from one another although followed the same general trends of change in NDVI leaf values (Table 3.38). The NDVI leaf values for Daviesia mimosoides were significantly lower than the other four species throughout the survival$_{max}$ summer treatment (Figure 3.24). Daviesia mimosoides also had significantly lower NDVI leaf values for survival$_{max}$ treatments in spring conditions although Hardenbergia violacea also had low NDVI leaf values (Figure 3.24). The NDVI leaf values of Acacia melanoxylon remained consistently higher than all other species throughout the sampling period regardless of treatment.

Survival$_{max}$ summer treatments were significantly different from the survival$_{max}$ spring treatments with NDVI leaf values decreasing at a quicker rate in summer treatments (Table 3.39). Control spring and summer treatments were not statistically different from each other (Table 3.39).
Figure 3.24. NDVI relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). The five species used are represented by Blue dots = *Acacia melanoxylon*, pink dots = *Daviesia mimosoides*, red dots = *Eucalyptus melliodora*, green dots = *Eucalyptus blakelyi* and yellow dots = *Hardenbergia violacea*. Note: NDVI values could not be taken for three species as their leaves were not of sufficient size (see methods section for further details). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

Table 3.38. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on NDVI leaf values.

<table>
<thead>
<tr>
<th>Species*</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment$^\wedge$</td>
<td>4</td>
<td>1.27</td>
<td>0.32</td>
<td>34.330</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment$^\wedge$</td>
<td>3</td>
<td>4.69</td>
<td>1.56</td>
<td>169.317</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time$^#$</td>
<td>1</td>
<td>4.38</td>
<td>4.38</td>
<td>474.824</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time$^2$</td>
<td>1</td>
<td>0.09</td>
<td>0.09</td>
<td>9.229</td>
<td>0.002</td>
</tr>
<tr>
<td>Residuals</td>
<td>779</td>
<td>7.19</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Acacia melanoxylon, Daviesia mimosoides, Eucalyptus blakelyi, E. melliodora and Hardenbergia violacea.
$^\wedge$ Four treatments (two stress and two control)
$^\#$ time since last watering in weeks

Table 3.39. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the NDVI leaf values from summer and spring treatments for all species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>1.107</td>
<td>0.293</td>
</tr>
<tr>
<td>Survival$_{\text{max}}$</td>
<td>1</td>
<td>0.41</td>
<td>0.41</td>
<td>64.160</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
3.4.5. Simple Ratio (SR) leaf values

Overall the relationship between SR and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.634$, $F_{9, 779} = 153$, $p<0.001$) and each predictor itself was highly significant (Table 3.40). There was a statistical difference between the SR leaf values sampled from two leaves (hereafter SR leaf values) for each species between control treatments and the survival$_{max}$ treatments over time (Figure 3.25) (Table 3.40). Spring and summer control treatments remained relatively consistent over time for most species, with few outliers (Figure 3.25) (Table 3.41). The SR leaf values of spring survival$_{max}$ treatment decreased at a slower rate than the SR leaf values of summer survival$_{max}$ treatment, although both treatments showed a decrease of leaf SR as the length of withheld water increased (Figure 3.25) (Table 3.41). Each species was had statistically different SR leaf values from one another (Table 3.40). The SR for Daviesia mimosoides was significantly lower than other species throughout the water stress survival$_{max}$ summer treatment (Figure 3.25). *Hardenbergia violacea* had lower average SR leaf values for all other treatments (Figure 3.25).

![Figure 3.25](image)

**Figure 3.25.** Leaf simple ratio values relative to summer and spring treatments and water stress to survival$_{max}$ with time (weeks). The five species used are represented by Blue dots = *Acacia melanoxylon*, pink dots = *Daviesia mimosoides*, red dots = *Eucalyptus melliodora*, green dots = *Eucalyptus blakelyi* and yellow dots = *Hardenbergia violacea*. Note: SR values could not be taken for three species as their leaves were not of sufficient size (see methods section for further details). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.
Table 3.40. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf simple ratio values.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>4</td>
<td>442.91</td>
<td>110.71</td>
<td>85.182</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>3</td>
<td>661.30</td>
<td>220.24</td>
<td>169.576</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>682.24</td>
<td>682.24</td>
<td>524.839</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>780</td>
<td>1,013.93</td>
<td>1.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Acacia melanoxylon, Daviesia mimosoides, Eucalyptus blakelyi, E. melliodora and Hardenbergia violacea.

^ Four treatments (two stress and two control)

# time since last watering in weeks

Table 3.41. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the simple ratio from summer and spring treatments for all species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.11</td>
<td>0.11</td>
<td>0.145</td>
<td>0.704</td>
</tr>
<tr>
<td>Survival_{max}</td>
<td>1</td>
<td>49.23</td>
<td>49.23</td>
<td>65.013</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

3.4.6. Leaf Transpiration (T) rates

Overall there was a highly significant relationship between leaf T rates and the predictors (time, treatment, species) (R^2_{adj} =0.530, F_{9, 778} = 100, p<0.001) and each predictor itself was highly significant (Table 3.42). There was a statistical difference between the leaf T rates for each species between control treatments and the survival_{max} treatments over time (Figure 3.26) (Table 3.42). The leaf T values of control treatments varied throughout the experimental period ranging between 0 and 6 (Figure 3.26).

Conversely there was a change point in the leaf T rates of survival_{max} treatments between weeks four and five where beyond this point leaf T rates remained consistently close to zero (Figure 3.26).

Spring treatments generally had higher leaf T rates (over time) than summer treatments and each of the five species also had a statistically different transpiration value over time when compared to one another (Table 3.43). E. blakelyi overall had higher leaf T rates than all of the other species and the leaf T rates of each other species varied from each other another. In water stress treatments leaf T rates for all species decreased as the time without water increased. Leaves in summer treatments had a significantly lower transpiration rate when compared with the spring treatment (Table 3.43). Between week four and five of the survival_{max} treatments in both summer and spring conditions leaf transpiration rates dropped to zero and remained at or close to zero for the remainder of the sampling period, regardless of species indicating a change point.
**Figure 3.26.** The leaf transpiration rates relative to summer and spring treatments and water stress to survival\(_{\text{max}}\) with time (weeks). The five species used are represented by Blue dots = *Acacia melanoxylon*, pink dots = *Daviesia mimosoides*, red dots = *Eucalyptus melliodora*, green dots = *Eucalyptus blakelyi* and yellow dots = *Hardenbergia violacea*. Note: Transpiration values could not be taken for three species as their leaves were not of sufficient size (see methods section for further details). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares. Change point is indicated with a black arrow.

**Table 3.42.** Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf transpiration rates.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>4</td>
<td>83.34</td>
<td>20.83</td>
<td>17.736</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>3</td>
<td>620.27</td>
<td>206.76</td>
<td>177.993</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>232.23</td>
<td>232.23</td>
<td>199.922</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time(^2)</td>
<td>1</td>
<td>205.95</td>
<td>105.95</td>
<td>91.206</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>778</td>
<td>903.73</td>
<td>1.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Acacia melanoxylon, Daviesia mimosoides, Eucalyptus blakelyi, E. melliodora and Hardenbergia violacea.*

^ Four treatments (two stress and two control)

# time since last watering in weeks

**Table 3.43.** Results of ANOVA of linear mixed effects model (LMM) of the relationship between the leaf transpiration rates from summer (heat and light) and spring treatments for all species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>23.91</td>
<td>23.91</td>
<td>9.309</td>
<td>0.002</td>
</tr>
<tr>
<td>Survival(_{\text{max}})</td>
<td>1</td>
<td>5.66</td>
<td>5.66</td>
<td>3.860</td>
<td>0.050</td>
</tr>
</tbody>
</table>
3.4.7. Leaf Stomatal Conductance (SC) rates

Overall the relationship between SC and the predictors (time, treatment, species) was highly significant ($R^2_{adj} = 0.485$, $F_{9, 778} = 83$, $p<0.001$) and each predictor itself was highly significant (Table 3.44). There was a statistical difference between the leaf SC rates for each species between control treatments and the survival_{max} treatments over time (Figure 3.27) (Table 3.44). The leaf SC values of control treatments varied throughout the experimental period ranging between 0 and 600 (Figure 3.27). Conversely there was a change point in the leaf SC rates of survival_{max} treatments between weeks four and five where beyond this point leaf SC rates remained consistently close to zero (Figure 3.27). Each species was also statistically different from one another in control treatments and before week 5 in survival_{max} treatments (Table 3.44). *Eucalyptus blakelyi* leaf SC values were consistently higher than other species and *Daviesia mimosoides* and *Acacia melanoxylon* generally had lower leaf SC values (Figure 3.27). The leaf SC values of summer treatments were not significantly different from the spring treatment (Table 3.45).
Figure 3.27. Leaf stomatal conductance relative to summer and spring treatments and water stress to survival$_{\text{max}}$ with time (weeks). The five species used are represented by Blue dots = *Acacia melanoxylon*, pink dots = *Daviesia mimosoides*, red dots = *Eucalyptus melliodora*, green dots = *Eucalyptus blakelyi* and yellow dots = *Hardenbergia violacea*. Note: Stomatal conductance values could not be taken for three species as their leaves were not of sufficient size (see methods section for further details). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares. Change point is indicated with a black arrow.

Table 3.44. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf stomatal conductance rates.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>4</td>
<td>1,243,490</td>
<td>310,872</td>
<td>27.037</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>3</td>
<td>5,051,378</td>
<td>1,683,793</td>
<td>146.430</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>2,244,781</td>
<td>2,244,781</td>
<td>195.229</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time^2</td>
<td>1</td>
<td>75,977</td>
<td>75,977</td>
<td>6.608</td>
<td>0.0103</td>
</tr>
<tr>
<td>Residuals</td>
<td>778</td>
<td>8,945,604</td>
<td>11,498</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Acacia melanoxylon, Daviesia mimosoides, Eucalyptus blakelyi, E. melliodora and Hardenbergia violacea.*

^ Four treatments (two stress and two control)

# time since last watering in weeks
**Table 3.45.** Results of ANOVA of linear mixed effects model (LMM) of the relationship between the stomatal conductance rates from summer and spring treatments for all species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>3,125</td>
<td>3,125</td>
<td>0.203</td>
<td>0.652</td>
</tr>
<tr>
<td>Survival\textsubscript{max}</td>
<td>1</td>
<td>12,534</td>
<td>12,534</td>
<td>1.845</td>
<td>0.175</td>
</tr>
</tbody>
</table>

### 3.4.8. Relative leaf chlorophyll values (CL)

Overall there was a highly significant relationship between CL and the predictors (time, treatment, species) ($R^2_{adj} = 0.309$, $F_{9, 779} = 40$, $p<0.001$) and each predictor itself was highly significant (Table 3.46). There was a statistical difference between the CL leaf values sampled from two leaves (hereafter CL leaf values) for each species between control treatments and the survival\textsubscript{max} treatments over time (Figure 3.28) (Table 3.46). Spring and summer control treatments remained relatively consistent over time for most species, with few outliers (Figure 3.28) (Tables 3.46 & 3.47). The CL leaf values for *Acacia melanoxylon*, *Eucalyptus melliodora* and *E. blakelyi* (the tree species) for the survival\textsubscript{max} summer treatment started to increase following four weeks without water and started to decrease following eight weeks without water (Figure 3.28 d). The same trend occurred in the spring survival\textsubscript{max} treatment without the decline following eight weeks without water. The shrub species (*Daviesia mimosoides* and *Hardenbergia violacea*) followed a different trend in survival\textsubscript{max} treatments with a decrease following 4 weeks for the summer and 6 weeks for the spring survival\textsubscript{max} treatment (Figure 3.28 d and 3.28 b). Each species was had statistically different leaf CL values from one another in each treatment (Table 3.46). The CL values for *Daviesia mimosoides* and *Hardenbergia violacea* were significantly lower than other species throughout the water stress survival\textsubscript{max} summer and spring treatment (Figure 3.28 b & 3.28 d).
Figure 3.28. Relative leaf chlorophyll content relative to summer and spring treatments and water stress to survival_{max} with time (weeks). The five species used are represented by Blue dots = *Acacia melanoxylon*, pink dots = *Daviesia mimosoides*, red dots = *Eucalyptus melliodora*, green dots = *Eucalyptus blakelyi* and yellow dots = *Hardenbergia violacea*. Note: Relative leaf chlorophyll content could not be taken for three species as their leaves were not of sufficient size (see methods section for further details). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.

Table 3.46. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on relative leaf chlorophyll values.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>4</td>
<td>31,706.00</td>
<td>7,926.40</td>
<td>75.397</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>3</td>
<td>3,151.00</td>
<td>1,050.20</td>
<td>9.990</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>670.00</td>
<td>669.80</td>
<td>6.371</td>
<td>0.012</td>
</tr>
<tr>
<td>Residuals</td>
<td>780</td>
<td>82,001.00</td>
<td>105.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Acacia melanoxylon, Daviesia mimosoides, Eucalyptus blakelyi, E. melliodora and Hardenbergia violacea.*

^ Four treatments (two stress and two control)

# time since last watering in weeks
Table 3.47. Results of ANOVA of linear mixed effects model (LMM) of the relationship between the relative leaf chlorophyll values from summer and spring treatments for all species.

<table>
<thead>
<tr>
<th>Spring treatment compared with summer</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>1,646.70</td>
<td>1,646.74</td>
<td>65.694</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Survival&lt;sub&gt;max&lt;/sub&gt;</td>
<td>1</td>
<td>827.00</td>
<td>826.70</td>
<td>5.805</td>
<td>0.016</td>
</tr>
</tbody>
</table>

3.4.9. Soil moisture percentage (SM)

There was a statistical difference between the soil moisture percentage of control treatments and the survival<sub>max</sub> treatments over time (Figure 3.29) (Table 3.48). The soil moisture percentage for spring and summer control treatments varied over the experiment period however soil moisture percentage remained between 5-20% (Figure 3.29 a & c) (Table 3.48). There was a change point observed in soil moisture percentage at four weeks without water for spring and summer survival<sub>max</sub> treatments where soil moisture dropped to 0% and remained there for the rest of the experimental period (Figure 3.29 b & d).

Figure 3.29. Percentage soil moisture relative to summer and spring treatments and water stress to survival<sub>max</sub> with time (weeks). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines which are polynomial fitting based on least squares. Trend lines are not presented where a line pattern does not exist.
Table 3.48. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment and time on percentage soil moisture.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment*</td>
<td>3</td>
<td>2,305.00</td>
<td>786.51</td>
<td>96.544</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>1,168.00</td>
<td>1,167.95</td>
<td>146.724</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>780</td>
<td>1,233.00</td>
<td>7.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* Four treatments (two stress and two control)
# time since last watering in weeks

3.4.10. Summary

Seedling survival in all eight species decreased with extended periods of water stress (i.e. time since last watering) in both summer and spring conditions (Figure 3.21- Figure 3.28). Almost complete seedling mortality was observed for all species within 10 weeks without water under both spring (Figure 3.21 a-d) and summer conditions (Figure 3.21 e-h). When looking at the seven leaf physiology measures, transpiration and stomatal conductance showed clear change points where leaf health showed a dramatic decline and continual low following the change point (Figures 3.26 & 3.27.) (Table 3.49). PRI, NDVI and SR leaf health measures showed a clear indication of declining leaf health values with increasing period of water stress (Figures 3.23, 3.24 & 3.25) (Table 3.49).

Table 3.49. Summary table for competition experiment data

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Trend following water stress</th>
<th>Difference between summer and spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual leaf observation category</td>
<td>Decrease</td>
<td>All</td>
</tr>
<tr>
<td>PRI</td>
<td>Decrease</td>
<td>All</td>
</tr>
<tr>
<td>NDVI</td>
<td>Decrease</td>
<td>Survival\text{max}</td>
</tr>
<tr>
<td>SR</td>
<td>Decrease</td>
<td>Survival\text{max}</td>
</tr>
<tr>
<td>Transpiration</td>
<td>Decrease → change point</td>
<td>All</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>Decrease → change point</td>
<td>No</td>
</tr>
<tr>
<td>Relative leaf chlorophyll content</td>
<td>Increase → decrease</td>
<td>All</td>
</tr>
</tbody>
</table>

\* = followed by

3.5. Comparing the results for *Eucalyptus* species between the *Eucalyptus* only experiment and the competition experiment

The results from *Eucalyptus blakelyi* and *E. melliodora* control and survival\text{max} treatments from the *Eucalyptus* only experiment and the competition experiment are presented in the sections below. The following section compares the results for the two eucalypt species across the two experiments, being the *Eucalyptus* only experiment and
the competition experiment. PRI and transpiration were chosen to represent leaf health measurements as these measurements represent one reflectance index and one physiological measure which provided clear results.

### 3.5.1. PRI leaf values from the *Eucalyptus* only experiment compared with those from the competition experiment

The difference between the PRI leaf values of the control and survival$_{\text{max}}$ treatments over time was significant for both experiments (Figure 3.30a & 3.30b) (Table 3.50). The PRI leaf values of the summer survival$_{\text{max}}$ treatments were statistically different between the *Eucalyptus* only experiment and the competition experiment (Figure 3.30a & 3.30b). PRI leaf values were generally higher in the competition experiment when compared to the *Eucalyptus* only experiment (Figure 3.30a & 3.30b).

![Graph](image)

**Figure 3.30a.** The PRI leaf values of *E. melliodora* with time for each treatment from the *Eucalyptus* only experiment (green dots) and the competition experiment (purple dots). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each experiment which are polynomial fitting based on least squares.
Figure 3.30b. The PRI leaf values of *E. blakelyi* with time for each treatment from the *Eucalyptus* only experiment (green dots) and the competition experiment (purple dots). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each experiment which are polynomial fitting based on least squares.

Table 3.50. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on PRI leaf values between the *Eucalyptus* only and competition experiment.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>25.038</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>0.17</td>
<td>0.06</td>
<td>134.833</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species*</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.461</td>
<td>0.497</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>0.21</td>
<td>0.21</td>
<td>498.771</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time^2</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>4.904</td>
<td>0.027</td>
</tr>
<tr>
<td>Residuals</td>
<td>779</td>
<td>0.32</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

◊ *Eucalyptus* only experiment and the competition experiment
* *Eucalyptus blakelyi* and *E. melliodora*
^ Four treatments (two stress and two control)
# time since last watering in weeks

3.5.2. Leaf transpiration rates from the *Eucalyptus* only experiment compared with those from the competition experiment

The difference between the leaf transpiration (T) rates of the control and survival$_{\text{max}}$ treatments over time was significant for both experiments (Figure 3.31a & 3.31b) (Table 3.51). The leaf T rates of the summer survival$_{\text{max}}$ treatments were statistically different between the *Eucalyptus* only experiment and the competition experiment (Figure 3.31a & 3.31b). Leaf T rates of the control treatments were generally higher in the competition experiment when compared to the *Eucalyptus* only experiment although survival$_{\text{max}}$ treatments had little variation (Figure 3.31 & 3.31b).
Figure 3.31a. The leaf transpiration rates of *E. melliodora* with time for each treatment from the *Eucalyptus* only experiment (green dots) and the competition experiment (purple dots). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each experiment which are polynomial fitting based on least squares.

Figure 3.31b. The leaf transpiration rates of *E. blakelyi* with time for each treatment from the *Eucalyptus* only experiment (green dots) and the competition experiment (purple dots). Spring treatments (a & b) and summer treatments (c & d). The trend lines present on the graph are loess fit lines for each species which are polynomial fitting based on least squares.
Table 3.51. Results of ANOVA of linear mixed effects model (LMM) of the effects of treatment, time and species on leaf transpiration rates between the *Eucalyptus* only and competition experiment.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species*</td>
<td>1</td>
<td>19.97</td>
<td>19.97</td>
<td>16.390</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment^</td>
<td>3</td>
<td>249.81</td>
<td>83.27</td>
<td>68.332</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time#</td>
<td>1</td>
<td>527.11</td>
<td>527.11</td>
<td>432.549</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Experiment◊</td>
<td>1</td>
<td>98.72</td>
<td>98.72</td>
<td>81.011</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time²</td>
<td>1</td>
<td>216.19</td>
<td>216.19</td>
<td>177.405</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>694</td>
<td>845.71</td>
<td>1.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

◊ *Eucalyptus* only experiment and the competition experiment
* *Eucalyptus blakelyi* and *E. melliodora*
^ Four treatments (two stress and two control)
# time since last watering in weeks
CHAPTER 4 - Discussion

4.1. Seedling health deterioration and mortality rates relative to water and heat stress

4.1.1. Seedling health and mortality relative to water stress
Increasing water stress had a negative effect on seedling leaf health and seedling mortality. Multiple leaf health measurements (PRI, NDVI, SR, transpiration and stomatal conductance) showed similarities and differences when comparing the effects of water stress on seedling survival. Conversely there were unexpected results for relative leaf chlorophyll content of leaves from multiple species within both experiments, which will be discussed below.

There was little difference between seedlings of the two eucalypt species grown in isolation (i.e. single species experiment) and those grown in competition with other co-occurring native plant species (i.e. the competition experiment) in their response to water stress, suggesting that the trends observed for the two eucalypt species were unaffected by competition and pot size (i.e. the competition pots held 4.6 times the soil therefore more water holding capacity although had higher quantities of plants). Any significant differences will be presented in the competition section of this discussion (Section 4.4.3.).

4.1.1.1. Comparisons across species and differing durations of water stress
Whilst there was some variation between the seedlings of the eight native species examined, they all exhibited the same overall decline in health with increasing duration of water stress. Warren et al. (2011) suggested that the response of species to water stress is generally consistent if they originate from the same habitat type, a result which to a degree is also reflected in this study. A similar decline in the response of multiple species to water stress was also seen in Lima et al. (2003). Lima et al. (2003) conducted a study on the response of five common plantation *Eucalyptus* species to water stress and found that following stress similar decreasing trends in photosynthesis, stomatal conductance and transpiration occurred between all the species although the rate actual rate of the measurements differed.
In this study the length of water stress (4 weeks, 7 weeks and 12 weeks) significantly impacted the degree of plant damage, with longer water stress periods leading to more extensive damage (i.e. seedling leaf health) and high mortality. Variations in seedling health between the eight plant species were greatest during periods of mild water stress (i.e. between 4 and 6 weeks without water). Duan et al. (2013) found similar results with temperature (combined with water stress) effects being greatest in mild water stress (63 days) however in extreme drought (103 days) any temperature effect was unseen and seedlings began to die.

4.1.1.1.1. Transpiration and stomatal conductance

A change point was observed in the leaf transpiration and stomatal conductance rates of all eight species between four and five weeks without water. Transpiration is the process where water is moved from the soil into roots, through the body of the plant and passed out through stomatal pores of the leaves as vapour (Larcher, 1980). To regulate the amount of water lost through transpiration, the stomata can, to a degree, close (Jones, 1998; Ward et al., 1999). The rate of water or CO₂ movement through the stomata is termed stomatal conductance. Transpiration and stomatal conductance are therefore largely regulated by the availability of soil moisture (Guswa, 2005; Nourtier et al., 2012). Thus water stress and drought can affect both transpiration and stomatal conductance (Nourtier et al., 2012).

Between three to five weeks without water the soil moisture declined to the point where it was not measurable, which in turn triggered a physiological response in the leaves of the seedlings resulting in the change point observed in the transpiration and stomatal conductance rates, at which point they both ceased. A threshold or change point is a biological response or abrupt change that can be detected as a result of a disturbance or stress (Groffman et al., 2006). Whilst there is a degree of resilience (flexibility, adaptability etc.) to stresses or disturbances (e.g. changes in climate), a point is reached where resilience can no longer account for the stress and a change occurs (Groffman et al., 2006). A decline in stomatal conductance has also been shown in several other studies which analysed the response of plants to water stress (Fotelli et al., 1999; Lima et al., 2003; Gindaba et al., 2004). For example Fotelli et al. (1999) found that stomatal conductance declined after nine weeks without water for five Mediterranean species, a longer period than this study likely due to the increased age of seedlings (2 years compared with 7 months), older seedlings generally have more advanced roots and
higher numbers of leaves than smaller seedlings. Gindaba et al. (2004) also found that stomatal conductance of one year old deciduous and *Eucalyptus* seedlings declined following 12 days of water stress, although that study did not monitor the response after this period and therefore it is unclear whether the stomatal conductance remained low in the following weeks. A change point is something which is evident in Fotelli et al. (1999) although the results from the Gindaba et al. (2004) study conducted on similar age seedlings did not follow the response for an adequate length of time to define a change point. The change point observed in this study and elsewhere (e.g. Fotelli et al., 1999), shows that under different lengths of time without water (depending on age of plants), the effect of water stress drives detrimental changes in plant physiology. Other studies also report a decrease in transpiration rates following water stress (Lima et al., 2003; Nourtier et al., 2012). For instance in Nourtier et al. (2012) transpiration severely decreased in *Abies alba* trees in response to water deficits over an extended period (years) with little recovery, which also suggests the presence of a change point. The results from Nourtier et al. (2012) are similar to the results of this study suggesting that the detection and application of transpiration values to detect change points in multiple species may be able to be applied in natural habitats.

Stomatal conductance and transpiration have been shown to change as a result of abiotic and biotic environmental stresses (Evert & Eichhorn, 2013). Decreases of stomatal conductance and transpiration (as observed here) are strongly linked with the decrease in photosynthetic ability and therefore linked to low growth and productivity (McDowell, 2011; Salazar-Parra et al., 2012; Smith & Duke, 2013). Such changes in growth were observed (from the visual observations) in this study after the change point in stomatal conductance and transpiration. Similar changes have also been detected in other studies which identified decreases in plant physiology (although not visual assessments) following water or drought stress (Lima et al., 2003; Sun et al., 2008; Warren et al., 2011; Duan et al., 2013) (Table 4.1). These studies showed that some small physiological (i.e. photosynthesis, stomatal conductance, CO₂ movement) variability occurs between species, with *Acacia* species showing a slightly different pattern to *Eucalyptus* species (Warren et al., 2011) (Table 4.1). As found here, despite such variability declines in physiological health are evident following periods of drought stress (e.g. 5 days (Lima et al., 2003); 15 days (Warren et al., 2011); and 30-63 days (Duan et al., 2013) (Table 4.1).
Table 4.1 A summary table of studies which have examined water stress, including the variables studies, length of stress, species examined and the results.

<table>
<thead>
<tr>
<th>Number of variables measured</th>
<th>Types of physiological variable measured</th>
<th>Species examined</th>
<th>Stress measured</th>
<th>Length of experiment</th>
<th>Result across measures</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Growth (G) Photosynthesis (Pn) Respiration (R) Total non-structural carbohydrate (TNC)</td>
<td><em>Eucalyptus globulus</em></td>
<td>Water Heat CO₂</td>
<td>63-100 days</td>
<td>Reduction in G, Pn while increase in R. TNC differential according to stress</td>
<td>Duan <em>et al.</em> (2013)</td>
</tr>
<tr>
<td>4</td>
<td>Pn Stomatal Conductance (SC) Phyllode chlorophyll Soluble protein</td>
<td><em>Acacia auriculiformis</em></td>
<td>Water</td>
<td>4-5 months</td>
<td>Decrease following stress</td>
<td>Montagu &amp; Woo (1999)</td>
</tr>
<tr>
<td>3</td>
<td>Transpiration (T) SC Pn Trans</td>
<td><em>Thuja occidentalis</em></td>
<td>Water Heat</td>
<td>34 days</td>
<td>Decrease in all measurements following stress</td>
<td>Zhao <em>et al.</em> (2013)</td>
</tr>
<tr>
<td>4</td>
<td>Photochemical reflectance index (PRI) Florescence reflectance index Pn Chlorophyll concentration (CLc)</td>
<td><em>Olea europaea</em></td>
<td>Water</td>
<td>15 days</td>
<td>Rapid decrease</td>
<td>Sun <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>3</td>
<td>SC Pn Trans</td>
<td><em>Eucalyptus gradis, E. urophylla, E. camaldulensis, E. torelliana, E. phaeotrica</em></td>
<td>Water CO₂</td>
<td>5 days</td>
<td>Decrease. Trend was similar between species.</td>
<td>Lima <em>et al.</em> (2003)</td>
</tr>
<tr>
<td>4</td>
<td>CLc Pn Acids Sugars</td>
<td>Five Eucalyptus and Two Acacia sp.</td>
<td>Water</td>
<td>12 days</td>
<td>Decrease in Pn and CLc. Similar changes to acid and sugars. Some variability between sp.</td>
<td>Warren <em>et al.</em> (2011)</td>
</tr>
</tbody>
</table>
4.1.1.1.2. Leaf reflectance indexes

Drought resistance and tolerances are largely linked with physiological and morphological characteristics of species (Engelbrecht & Kursar, 2003). Some species, in particular shrub species *D. mimosoides* and *H. violacea* appeared to be more affected by the water stress, for example after 4 weeks without water seedlings of both species exhibited lower leaf reflectance (PRI, NDVI and SR) values suggesting these species have less tolerance to the water stress, compared to *E. blakelyi*, *E. melliodora* and *A. melanoxylon*. Conversely tree species, *E. blakelyi*, *E. melliodora* and *A. melanoxylon* exhibited slightly higher leaf reflectance values (PRI, NDVI and SR) suggesting that these species may be able to better withstand the effects of extended durations of water stress. These results suggest that there might be a broad distinction between the tolerances of seedlings to water stress based on their life form or functional type. Other studies have shown that a range of plant traits (genetic variability, age, species, vegetation community) and other disturbances (pathogens, fungus, insects, nutrient, light) could also influence how individual plants respond to water stress (Schupp, 1995; He et al., 2014). Seedlings of tree species (*Acacia* and *Eucalyptus*) generally survived for the longest periods without water and exhibited consistently higher levels leaf health measurements. It is suggested that long-lived tree species have a larger degree of genetic variation which can result in more resilient and resistant capability to cope with drought stress although in long-duration water stress tree species are increasingly more vulnerable (McDowell et al., 2008; Mitchell et al., 2012).

4.1.1.1.3. Relative leaf chlorophyll content

Differences between the results of trees and shrubs species were also seen in another measurement of leaf health in this study, relative leaf chlorophyll content. In some studies which analyse the change in chlorophyll content relative to water stress, the chlorophyll (green pigment) content tended to have a more rapid decline than carotenoids (which can be measured by PRI) due to a reduction of chlorophyll in plants during stress (Hsiao, 1973; Alberite & Thornber 1977; Sims & Gamon, 2002; Munné-Bosch & Alegre, 2004; Efeoğlu et al., 2009). In contrast to previous findings which were conducted on a variety of different species (Hsiao, 1973; Alberite & Thornber 1977; Sims & Gamon, 2002; Munné-Bosch & Alegre, 2004; Efeoğlu et al., 2009) the current study found that relative leaf chlorophyll content first rose considerably (from week 1-7) in tree species before slightly declining (from week 8 onwards) in water stressed treatments. Other findings showed an almost immediate drop in relative leaf
Chlorophyll, or chlorophyll content (Alberte & Thornber, 1977; Efeoğlu et al., 2009). In
the current study the shrub species followed the expected trend based on analysis of
literature, with a decline in leaf chlorophyll content after 6 weeks without water, which
also was reflected in the discoloration of the leaves.

In contrast to shrub species, the relative leaf chlorophyll content of tree species rose
between weeks 6-10. Multiple hypotheses have been developed to explain the result of
atLEAF relative leaf chlorophyll content (CL) of trees in this study. The peak in CL
values between weeks 6-10 could be due to: i) machine faults (could not detect change,
light errors); ii) large variation of chlorophyll content during this period; iii) wax or oils
within leaves effecting the reflectance of leaves during a transition stress period; or
iv) values may represent different sections of the leaf such as the midrib (Zhu et al.,
2012). It is unlikely that iv) occurred as, if a measurement was observed to be an
extremely high or low value, the leaf was re-tested twice in different locations to ensure
correct measurements were taken. Chlorophyll is the green pigments within leaves,
therefore the chance of brown (dead) leaves recording high concentrations of relative
leaf chlorophyll is low (Larcher, 1980; Evert & Eichhorn, 2013). It is therefore unlikely
that ii) was the cause of the peak in atLEAF relative chlorophyll content. The most
probable reason for the observed high relative leaf chlorophyll content is either machine
inaccuracy, machine faults or the inability of the machine to measure response of
Eucalyptus or Acacia seedlings following water stress. The inability of the machine to
pick up these changes is likely due to the oil and wax contents of the leaves changing
the values of the leaf reflectance. The leaf structure (eg. waxy, tough, thin) and the
quantity and location of waxes on leaf cuticles of Eucalypts leaves can result in
variation in leaf capacity of leaves to absorb light and this may affect light reflectance
measurements (Cameron, 1970; Sims & Gamon, 2002). The waxes of E. blakelyi are an
almost complete cover of irregularly shaped plates while E. melliodora have an almost
complete cover of simple long relatively unbranched tubes as well as angular plates
(Hallam & Chambers, 1968). The reliability of the measurements of the relative leaf
chlorophyll content of leaves in this study is therefore uncertain.

4.1.1.2 Maximum water stress duration relative to seedling mortality
The majority of changes in leaf health occurred between 4-6 weeks of withheld water
suggesting that beyond this point leaves are displaying significant signs of visual,
morphological and physiological stress. Complete visual mortality in most species
occurred between eight and ten weeks of the experiment, however a drop in leaf reflectance index below healthy values occurred between weeks five and seven, and was relatively consistent between species. Mitchell et al. (2012) found that mortality occurred from a range of 90 days to 130 days in two Eucalyptus species following water stress. Unlike this study, those plants were watered at decreasing rates followed by a without water period (Mitchell et al., 2012). The presence of some soil water may be indicative of the longer survival time of seedlings in that study (Mitchell et al., 2012).

4.1.2. Seedling health and mortality relative to heat stress
Seedling health for all eight Australian native plant species was only slightly affected by heat stress across the 10 weeks of the study. The majority of leaf health measurements (NDVI, SR, Transpiration, Stomatal conductance) showed no difference between the heated (summer) control and the spring control values. These results suggest that heat stress (up to 30-35˚C) alone does not cause critical damage to the leaf health of this subset of Australian native plants. These results also suggest that plant water status is also linked with temperature stress and that if water was limited a temperature effect occurred (Section 4.1.3) (Wahid et al., 2007). Although the results suggested little heat effect, it is predicted that if temperatures were to exceed 35-40˚C for a period of time a heat compensation point would be reached beyond which photosynthesis, cellular homeostasis and related measurements (stomatal conductance, transpiration) decrease dramatically and thus result in changes to leaf health and subsequently mortality (Larcher, 1980; Cunningham & Read, 2002; Kotak et al., 2007). A heat compensation point has been demonstrated in the results of many other studies including Cunningham & Read (2002) who demonstrated that temperate and tropical species have different optimum temperature ranges for their photosynthetic function and Battaglia et al. (1996) who demonstrated that two eucalyptus species had optimum temperature for photosynthesis over a period of months.

4.1.3. Seedling health and mortality relative to the combination of water and heat stress
The combined effect of water stress and elevated temperature (heat stress) caused a significant decline in seedling leaf health for all eight Australian native plant species. Whilst the trend of the combined effect of heat and water stress was similar to water stress alone on seedling health, the combined effect results in a faster deterioration of leaf health and quicker mortality (see section 4.1.1 for water stress effects).
accelerated deterioration of seedling health following the combination water and heat stress highlights the importance of examining the effects of multiple stresses, something few studies have done.

4.1.3.1. Differing durations of water and heat stress
In this study elevated heat in combination with water stress generally led to a greater mortality rate, a faster drop in leaf health parameters and lower soil moisture in most treatments. The following sections will address in greater detail the response of different measures of leaf health and provide a discussion of the results of this study.

Elevated temperature [heat] has generally exacerbated the effects of water stress in previous studies (Duan et al., 2013; Dreesen et al., 2012; Will et al., 2013; Zhao et al., 2013). Duan et al. (2013) conducted a study on the effects of water stress in combination with elevated heat and increased CO₂ on Eucalyptus globulus. The study found that elevated heat increased the negative effects of water stress on leaf physiological health during the moderate drought period (63-79 days) however when plants were then subjected to extreme drought the temperature effect was eliminated (Duan et al., 2013). The current study also demonstrated that the elevated heat contributed to increased physiological leaf health damage in the moderate period of stress (weeks 4-8) while in weeks 8-10 any temperature effect appeared to decrease. These results along with the results of Duan et al. (2013) suggest that temperature generally exacerbates the effects of water stress in moderate stress periods.

The same patterns in relative leaf chlorophyll content (CL) that was seen in the water stress treatment were present and more defined in the treatments which combined water and heat stress (see section 4.1.1.1.3.). The CL values of tree species significantly increased (week 4) before decreasing (week 8). The increase in CL leaf values occurred earlier in elevated temperature treatments suggesting that these plants were most likely experiencing a quicker change in leaf health than plant which were not subject to temperature stress.

4.1.3.2. Maximum water and heat stress duration relative to seedling mortality
The current study found that complete mortality occurred approximately one week earlier in seedlings subjected to high temperatures and water stress in combination. A study conducted on seedlings of 10 tree species from grassland ecotone (USA) also
found that elevated heat in combination with water stress caused seedling mortality to occur two days prior than water stress without elevated heat (Will et al., 2013). The results from Will et al. (2013) in combination with the results from this study suggest that generally elevated heat (in combination with water stress) causes earlier mortality in seedlings than water stress alone.

4.2. Seedling recovery rates following different periods of water and heat stress

The recovery of the two eucalypt seedlings (i.e. in terms of leaf health and resprouting) differed with duration of water stress, with both species showing a longer lag to extended periods of water stress. Although some studies demonstrate recovery following short water withholding periods (2-7 days), the degree, speed and ability of plants to recover following longer periods of water stress is lacking (Flexas et al., 2004; Miyashita et al., 2005). In Miyashita et al. (2005) it is suggested that studies which monitor longer periods of recovery following water stress are needed. The current study monitored a longer stress (water withholding) and response (recovery following re-watering) period (4 weeks and 8 weeks) of plants than other studies, which studied short term responses (2-7 days) (Ronde et al., 2004; Miyashita et al., 2005).

Recovery following re-watering of stomatal conductance and transpiration following multiple water stress conditions is largely unknown (Flexas et al., 2004; Warren et al., 2011). This study analysed the recovery of different photosynthetic components (transpiration and stomatal conductance) upon re-watering in multiple conditions (spring and summer) of multiple plants (Eucalyptus blakelyi and E. melliodora) in different water stress conditions (4 weeks and 7 weeks without water).

4.2.1. Seedling recovery following water stress

4.2.1.1. Recovery following different durations of water stress
Re-watering promoted recovery in seedlings following water stress during this study. The process of recovery following re-watering occurred over a period of weeks following re-watering and differed between plants which were re-watered following 4 weeks without water and those which were re-watered following 7 weeks without water. The recovery of species was statistically different between the two Eucalyptus species with E. blakelyi generally recovering more in summer and E. melliodora more in spring conditions (refer to Chapter 3).
4.2.2. Seedling recovery following the combination of water and heat stress

4.2.2.1. Recovery following different durations of water and heat stress

4.2.2.1.1 Transpiration, stomatal conductance and leaf reflectance

The rewatering following summer 7 weeks without water treatments had lower stomatal conductance, PRI, SR and NDVI rates than spring re-watering following 7 weeks without water treatments, suggesting that heat impacted the recovery of plants following 7 weeks without water. A study conducted by Gallé & Feller (2007) on the photosynthetic response of beech saplings to heat and water stress and recovery following re-watering showed following 14 days of water stress stomata remained closed and after re-watering stomatal conductance values did not recover to pre-water stress values. The current study demonstrated similar results with stomatal conductance values remaining lower than control values. It is suggested that following 7 weeks without water in this study, irreversible damage occurred to the photosynthetic apparatus (i.e. stomata, chlorophyll, cellular components, xylem) often causing the leaf desiccation. During periods of water stress a range of physiological alterations occur in plants, triggering a decrease in plant function (i.e. energy production and growth), ultimately resulting in mortality (McDowell, 2011). Due to the damaged photosynthetic apparatus seedlings were unable to produce new leaves or were unable to continue to produce energy from damaged leaves therefore suffering mortality.

4.2.2.1.2. New growth following re-watering

Heat did not cause a significant change to new growth following re-watering in this study. There is a greater possibility for new growth following re-watering after 4 weeks without water when compared with re-watering following 7 weeks without water. Generally the location and amount of new growth differed between *E. melliodora* and *E. blakelyi*. New leaf sprouting following disturbances in plants is a sign of regeneration, regaining of biomass and is associated with high initial growth rate (Bellingham & Sparrow, 2000; Teixeira *et al.*, 2002). The capacity for new sprouting leaves to remain healthy is highly dependent on the available nutrient reserves, soil fertility and environmental conditions (Teixeira *et al.*, 2002). It is possible that plants where new sprouting occurred in multiple locations (top, middle, bottom) had greater nutrient stores than those where new sprouting only occurred in one location. Generally the location of plant re-sprouting will follow a hierarchical trend where disturbance level regulates the location of new growth (Bellingham & Sparrow, 2000). Commonly
plant degradation occurs in sequences: first leaf; then twig; branch; stem; and trunk (Bellingham & Sparrow, 2000). Re-sprouting should first occur in the bud of the leaf axil if a plant is less damaged from disturbance. As damage increases the re-sprouting occurs in the bud of the twig axil on the branch and the branch axil on the trunk and so on (Bellington & Sparrow, 2000). Although not directly studied in this paper, the location of new growth is largely linked with a hierarchical sequence, and generally healthier seedlings are more likely re-grow from higher up on the plant.

4.3. Seedling responses across multiple physiological measures to water stress, heat stress and re-watering following stress

Results from this project to assess the physiological response in five Australian native plants to water and heat stress, using a range of different commercially available tools, has shown that similar trends can be obtained from the measurement of NDVI, PRI and SR using handheld devices. In addition, physiological changes (stomatal conductance and transpiration) in the leaves, while correlated with the leaf damage observed through visual assessments, occurred earlier, suggesting that substantial change points in physiological leaf health were occurring which was later reflected on visual leaf degradation. The results from this study suggest that mortality occurs over a prolonged period of weeks with declines in leaf function and structure relating to changes in available water, heat severity and light intensity.

One aim of this thesis was to determine whether leaf health measurements were able to detect physiological changes in *Eucalyptus melliodora*, *Eucalyptus blakelyi*, *Acacia melanoxylon*, *Daviesia mimosoides*, *Dodonaea viscosa* and *Hardenbergia violacea* seedlings as a result of water stress. Photochemical reflectance index (PRI), Normalised difference vegetation index (NDVI), Simple Ratio (SR), Transpiration (T) and Stomatal Conductance (SC) appeared to accurately monitor changes in plant health and the response of seedlings to water stress. As previously discussed (Section 4.1.1.1.3) relative leaf chlorophyll content (CL) conversely displayed trends which did not directly reflect results demonstrated in other studies, likely due to incorrect measurements due to device failure or inaccuracy.
4.3.1. Comparing the results from multiple physiological measures to water stress, heat stress and re-watering

Of the six physiological measures examined, five (PRI, NDVI, SR, Stomatal Conductance and Transpiration) consistently detected the decline in seedling leaf health across all eight plant species, despite some variation between species. These five physiological measures also detected the declines in seedling leaf health at the same time as or quicker than they could be detected visually. Whilst the sixth physiological measure examined (leaf chlorophyll) did detect changes which could be attributed to changes in leaf health following water stress, the pattern between species was significantly different, with seedlings of tree species showing an increase, whilst shrubs showed a decrease. The ability for physiological measures to detect changes in seedling leaf health before visual observation was also a finding in Sea et al. (2013). This allows for detection of plant stress earlier and could be used as a monitoring/management tool for replanted seedlings to maximise replantation success.

4.3.1.1. Physiological measures that showed declining leaf health relative to duration of water stress

Whilst other studies have found similar results, for individual physiological measures, for example Thenot et al. (2002) and Ripullone et al. (2011) found that PRI could detect water stress in Chenopodium quinoa, Arbutus unedo and forest species and Revadekar et al. (2012) found NDVI could detect water stress in the Indian region, very few studies have shown the comparative ability of, or value in using, multiple physiological measures to detect the changes in leaf health following stress. Notable exceptions include Sea et al., (2013) who looked at PRI, stomatal conductance and leaf chlorophyll content following the application of herbicide on the leaves of four weed species. Sea et al.’s, (2013) results support those presented here in that the four measures could be used to detect changes, although not all were needed. The results of this study demonstrate the effectiveness and usability of a range of leaf health measurement techniques for detecting changes in leaf health as a result of water and heat stress in native Australian species. Although the use and efficiency of vegetation indices such as PRI has previously been uncertain (Sun et al., 2008; Sarlikioti et al., 2010), their application to show changes in plant physiology following water stress is demonstrated in this study (See section 4.3.2 below).
The use of the atLEAF chlorophyll meter to detect changes in leaf chlorophyll of Australian tress species may not be possible or may be limited. There is only one review of the success of the atLEAF chlorophyll instrument which analyses the use of its values compared with SPAD (another leaf chlorophyll index) values on crop species following nitrogen changes (Zhu et al., 2012). It is unclear from available data as to the extent of previous study of the use of this instrument to follow changes in chlorophyll in response to drought stress. It is recommended that further studies on native Australian species use another meter such as the SPAD-502 or CCM-200 or reflectance indexes such as Chl NDI to determine chlorophyll content following drought stress, as more research has been conducted on their reliability (Richardson et al., 2001). It is also recommended further research be conducted into the effectiveness of the atLEAF chlorophyll meter in testing the relative leaf chlorophyll content of native Australian species with and without stress to examine the usefulness and application of this device in Australia.

4.3.1.2. Physiological measures that showed deterioration of leaf function
As discussed earlier, transpiration and stomatal conductance are largely regulated by the availability of soil moisture to plant roots, and are therefore reliant on the availability of soil moisture. This project confirmed that stomatal conductance and transpiration decreased as a direct result of water stress. The importance of the use of this measure was to identify the change point in physiology which explains the point at which plants stop transpiring/ conducting therefore stopping photosynthetic activity and demonstrating deterioration in leaf function.

4.3.2. An evaluation of the effectiveness and practical application of the physiological measures used relative to the response of seedlings to the different stresses examined
Overall, most instruments in this study gave an indication of plant physiological and observational changes following water stress and re-watering. Although not on the same timescale and degree the instruments all picked up the changes related to overall leaf health decline and leaf recovery. NDVI, PRI and SR were able to detect plant physiological changes in response to water and heat stress with good accuracy and precision indicating a declining trend which could be used as suitable rapid direct indicators of plant physiological status (Dobrowski et al., 2005). If plants recovered from stress PRI, NDVI and SR increased, indicating that these measures could be used
to demonstrate recovery. Stomatal conductance and transpiration results showed a change point as response to water stress. It is suggested by Sun et al. (2008), that the reduction in photosynthetic processes (stomatal conductance and transpiration) causes a change in the ratio of chlorophyll to carotenoid content which then results in the change to light reflectance. Stomatal conductance or transpiration could give a better indication of water stress if measured over a continuous period over multiple days or weeks to assess the short-term progression of physiological responses to water stress (Fotelli et al., 1999). While the morphological and leaf reflectance (NDVI, PRI and SR) changes could be monitored over a longer timescale to provide an indication of photosynthetic decline over a longer period. The two leaves sampled from the one plant were also highly correlated suggesting that sampling one leaf from a given plant will give an indication of overall plant health.

In future studies it would be recommended by this author to use one reflectance index (NDVI, PRI or SR), one physiological measure (Stomatal conductance or transpiration) sampled from a single leaf per plant and one visual (visual observation or digital photographs) to gage an overall indication of leaf health changes. Although this is recommended there is the potential to use a reflectance index or physiological measurements in isolation from one another to detect water stress by using healthy range information. An example of this would be the use of health ranges for reflectance indexes for example a PRI of between 0 and 1 is healthy and a PRI of between 0 and -1 is unhealthy and these may give indications of the level of stress in each plant. PlantPen PRI 200® is an effective and efficient way of using handheld instruments to measure PRI; this instrument (in contrast to others used in this study) is relatively cost effective ($1,500AUD for PlantPen PRI 200® compared with ~$25,000AUD for the handheld photosynthesis system CI-340).

4.4. Australian native plants and other studies

4.4.1. The vegetation community

The focus of many studies on Australian plants, particularly *Eucalyptus* and acacia species is on those such as *Eucalyptus globulus* which are used as a source of hardwood or production of resources (Way et al., 2013). Research tends to focus on highly productive, fast growing, economically important species rather than species with conservation priorities, often due to lack of funding and the long growth periods of species (Valdés et al., 2013; Zlatev, 2013).
White Box – Yellow Box – Blakely’s Red Gum Grassy Woodland (Box – Gum grassy woodland) is an ecological community listed as Critically Endangered under the Environment Protection and Biodiversity Conservation Act (EPBC Act) (NSW Scientific Committee, 2011). Box – Gum grassy woodlands are also listed as an ecologically endangered community under NSW (Threatened Species Conservation Act) and ACT legislation (Nature Conservation Act) and listed within endangered communities in Queensland and Victoria legislation (Prober et al., 2002). Greening Australia uses many species listed in this community to rehabilitate degraded areas and to promote landscape recovery (Greening Australia, 2014). The planting of seedlings is a technique used to promote the repair of Australian landscapes (Greening Australia, 2014).

Historically the Box – Gum Grassy Woodlands have a habitat from the Great Dividing Range to Central Victoria up through ACT and NSW to Southern Queensland (NSW Scientific Committee, 2011). Box – Gum grassy woodland is an ecological community that occurs along eastern Australia in areas with rainfall between 400 and 1200mm per annum (NSW Scientific Committee, 2011). Box – Gum grassy woodlands occur on moderate to highly fertile soils within altitudes of 170m to 1200m (NSW Scientific Committee, 2011). Given that the soils where this ecological community occur are highly fertile, much of the known occurrence and range is un-reserved and occurs on agricultural land (Prober et al., 2002). Box- Gum woodlands are considered to be one of the most poorly conserved ecosystems (Prober, 1996). The known dominant grasses within Box – Gum grassy woodlands, Kangaroo Grass (Themeda triandra or Themeda australis) and Snow Grass (Poa sieberiana), have been significantly degraded in many sites from agricultural cropping and grazing (Prober, 1996; Prober et al., 2002). Three Eucalyptus species are common within these ecosystems Eucalyptus albens (White Box) in higher areas with shallower soils and E. melliodora (Yellow Box) and E. blakelyi (Blakely’s Red Gum) in areas which have deeper soils or those areas which lay on watercourses (Prober, 1996). Many areas of Box – Gum woodlands which were once healthy are now significantly degraded and do not retain sufficient values and therefore are not conserved under the EPBC Act as part of this community.

The known drought tolerances for many species are usually related to adults of that species (i.e. given as drought tolerance in Table 4.2). The tolerance of seedlings to...
drought is important as seedling survival is imperative for re-establishing a community as well as for long-term success. The results of this study demonstrate that seedlings of tree species generally displayed higher tolerances to drought over shrub species, which differed from the expected tolerances given for adult plants (Table 4.2). This therefore signifies that once particular species are established plants their drought tolerance could alter.

**Table 4.2.** The known drought tolerances for the eight species used here from the White Box-Yellow Box-Blakely’s Red Gum Grassy Woodland ecological community, along with a description of their distribution (after Florabank (2013) and PlantNet (2014)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution/Description</th>
<th>Growth rate †</th>
<th>Drought tolerance*</th>
<th>Seedling drought tolerance*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eucalyptus blakelyi</em></td>
<td>Common throughout south eastern Australia. Commonly found on lower slopes and water passages.</td>
<td>moderate</td>
<td>moderate to high</td>
<td>moderate to high</td>
</tr>
<tr>
<td><em>Eucalyptus melliodora</em></td>
<td>Medium to large tree which is common in grassy woodlands throughout eastern Australia.</td>
<td>slow</td>
<td>moderate to high</td>
<td>moderate to high</td>
</tr>
<tr>
<td><em>Acacia melanoxylon</em></td>
<td>Medium size tree common along eastern Australia through to Tasmania. Shallow rooted</td>
<td>moderate</td>
<td>sensitive</td>
<td>moderate to high</td>
</tr>
<tr>
<td><em>Poa sieberiana</em></td>
<td>Grass which occurs in various habitats along the eastern side of Australia</td>
<td>fast</td>
<td>moderate</td>
<td>moderate</td>
</tr>
<tr>
<td><em>Poa labillardieri</em></td>
<td>Widely distributed grass across south eastern Australia.</td>
<td>fast</td>
<td>moderate</td>
<td>moderate</td>
</tr>
<tr>
<td><em>Daviesia mimosoides</em></td>
<td>Common shrub across south eastern Australia.</td>
<td>fast</td>
<td>moderate to high</td>
<td>moderate to low</td>
</tr>
<tr>
<td><em>Dodonaea viscosa</em></td>
<td>Widely spread shrub across Australia.</td>
<td>fast</td>
<td>moderate</td>
<td>moderate</td>
</tr>
<tr>
<td><em>Hardenbergia violacea</em></td>
<td>Wide spread shrub along eastern Australia.</td>
<td>fast</td>
<td>moderate</td>
<td>moderate to low</td>
</tr>
</tbody>
</table>

* Drought tolerances based on information on adults: sensitive = can tolerate little water stress, moderate = can tolerate some water stress, high = can tolerate extended water stress
† Growth rate: slow, moderate, fast
* Drought tolerances of seedlings based on this study (moderate to high surviving the longest in water stress and moderate to low demonstrating the highest damages from stress)
4.4.2. Species variation

The leaf health measurements of species *Eucalyptus melliodora* and *E. blakelyi* were mostly statistically different over the course of the study. *Eucalyptus blakelyi* had more variability in PRI and NDVI than *E. melliodora* in control water treatments over the length of the experiment. In other treatments this pattern was not observed suggesting that the water stress diminished any previous effects. There are various possible hypothesis as to why the variability of plant health measurements being greater in one species than another. The first hypothesis is that the seed from one species had higher genetic variation and therefore these plants behave differently to stress (Ellstrand & Elam, 1993). It is possible that seeds from different sites with different genetic make-up have slightly different adaptations, structure, plant traits (floral morphology, allelic diversity) which cause greater variation between plants from the one species (Loveless & Hamrick, 1984; Ellstrand & Elam, 1993). Greater variation can enable particular species to persist when subject to abiotic and biotic disturbances (Ellstrand & Elam, 1993). Genetically inferior seed results in plants which have a low genetic variability are generally not recommended for propagation (Burrows, 2000). The lack of knowledge on the genetic origin of the seed for this study means that this hypothesis cannot be discounted. The cost of seed collection along with the timing and duration of government funding is being linked to an inability to have a large range of diverse high quality seed for re-vegetation projects (GACR, 2012). The location and accessibility of sites where seed can be collected is also a constraint to the collection of genetically diverse seed (GACR, 2012). The second hypothesis is that *E. blakelyi* normally has more variation between individual plants and although not genetically more diverse from one another. Species variation is noted in this study and results suggest that the variation can alter the response of species to mild stress (4 weeks without water). It is recommended by this author that further studies focus on genetic diversity of Australian native seed or directly concentrate on increasing genetic diversity in seedbanks.

4.4.3. Effects of competition

Competition between plants did not negatively affect the leaf health of *Eucalyptus blakelyi* and *E. melliodora* following a 10 week period of water stress and heat stress in this study. Leaf health values closely mirrored or were more positive than leaf health values of each species within the *Eucalyptus* only experiment. Competition resulted in less overall decline of leaf health over time for both *Eucalyptus* species. The results of the study demonstrate that the response of plants in stressed conditions is largely related
to the larger stress [water] over competition for resources (Press et al., 1999; Bu et al., 2013). These results are consistent with results hypothesised using the Stress-gradient hypothesis (SGH) and the Competitive, stress, ruderal (C-S-R) hypothesis which state that extreme environmental stresses can cause a mutualistic relationship between plants or can eliminate the advantage of species with strong competitive traits (fast growth rate) (Grime, 1977; Bu et al., 2013).

The slower leaf health decline seen in the competition experiment may also be attributable to the increase in soil volume within each pot leading to more soil water holding capacity, however plants within competition pots had less soil for individual growth (approximately half the amount soil than the Eucalyptus only experimental plants).

4.4.4. Effects of biotic stresses

Biotic stresses such as insects, parasitic plants and pathogens (fungal, bacterial or viral) have been known to re-direct resources away from plant growth and cause changes to plant health (Polley et al., 1996; Craine, 2009). Towards the end of this study there was some evidence of green leafroller caterpillar which damaged some leaves on some plants, mainly those plants which were not sampled with leaf health instruments. Plants were treated with Yates® Pyrethrum Insect Pest Gun (Active constituents: 30g/L Pyrethrins, 1.2g/L Piperonyl Butoxide) to kill the pests. Two plants within the control non-sampled plants exhibited signs of rust. These biotic stresses occurred toward the end of the experiment, and due to their small scale, were unlikely to affect the results. As seedlings become more stressed they are generally more susceptible to biotic stresses (Polley et al., 1996; Mitchell et al., 2012). It is important to note that although in a controlled environment, biotic stresses are generally always present and usually contribute to decline in leaf health along with abiotic stresses.

4.5. Implications of study and future plant conservation

4.5.1. Heat and water stress – a climate change perspective

Most regions of south-eastern Australia are currently subject to summer dominated rainfall patterns (Suppiah et al., 2007; DoECCW, 2010). Rainfall patterns are expected to alter in climate change scenarios, with an increase in extreme events, and a decrease in spring, winter and autumn rainfall patterns (Suppiah et al., 2007; DoECCW, 2010; Fisichelli et al., 2014). The increase in temperature predicted in climate change models
 (>2-3°C by 2070) along with an increase in days above average maximum temperature (>35°C) will undoubtedly combine with altered rainfall patterns to have a greater impact of water stress (Hughes, 2003; Suppiah et al., 2007).

The co-occurrence of high temperature and water stress is largely associated with negative influences on plant physiology (McDowell et al., 2008; Dreesen et al., 2012). As demonstrated in this study, heat and water stress have an additive effect where the effect of heat and water stress is larger when the stresses occur in combination than when in isolation (McDowell et al., 2008; Dreesen et al., 2012). Generally the response of plants to heat stress is largely dependent on the soil moisture (linked with rainfall) conditions at the time of stress (Myers & Landsberg, 1988; Dreesen et al., 2012; Fisichelli et al., 2014). Elevated temperature will likely have the greatest impact in summer when temperatures rise above 30-35 °C (Dreesen et al., 2012). If in future climates there is a shift between summer and spring/autumn rainfall patterns, plants may be subject to additive effects from lack of water combined with extreme temperatures. Water stress in summer (increased heat and light) months may result in a greater decrease in survival of seedlings than if water stress were to occur in spring/autumn.

Periods of absent rainfall for a period greater than 21 consecutive days already occurs in Australia, for example in in the Canberra region where grassy box woodland is native a 20 day period without water occurred in January (summer) 2014 (BOM, 2014b). In some areas of the NSW/ACT region the length of days without rain per year has increased by 40 days (per/yr) from 1970 to 2013 (BOM, 2014b). If the incidence of these events increased in future climates it is likely that seedling survival rates would significantly decrease.

4.5.2. Revegetation and regeneration

Revegetation, regeneration and restoration of endangered communities are becoming an increasing focus due to the widespread clearing of temperate woodlands for agriculture (Prober et al., 2002). Many patches of Box – Gum Grassy Woodlands could benefit from rehabilitation and regeneration schemes however hot dry summers can cause challenging conditions for seedling development (Ruthrof et al., 2010). Planting of nursery raised tube-stock is the preferred method of rehabilitation in Mediterranean-type ecosystems such as south-eastern Australia as direct seeding has limited success (Ruthrof et al., 2010). The success of the early establishment of ecosystems could
depend on the types of species, climate conditions and adaptability to drought. The planting regime for Box-Gum woodland seedlings may need to be adapted to the climate regime. By understanding which species will respond to drought, and the timing and susceptibility of species to stress, it may be possible to estimate the success of revegetating with the species as well as the climate adaptability of the species.

A likely consequence of severe water stress (drought) is mortality of seedlings and saplings. Seedlings and saplings are critical for the regeneration of vegetation ecosystems following stress (Will et al., 2013). Understanding the success of seedlings in drought conditions could have substantial implications for revegetation projects in changing climates (Ruthrof et al., 2010). Currently more than $3,500,000 has been spent on the management, conservation and rehabilitation of Box-Gum woodlands in Australia and the success as well as ongoing implementation of these projects is a critical component of management (Australian Government, 2006). Low seedling survival, as a response of water or heat stress, in future climates may not sufficiently support regeneration, recruitment and migration of vegetation communities (Will et al., 2013). Revegetation of plant communities, often those which are endangered, is significantly affected by the occurrence of drought, in varying intensities and durations (Duan et al., 2013). Generally the recruitment and establishment phase of plants is most affected by changes in water supply (Hanson & Weltzin, 2000). Drought can cause complete or partial mortality of all regenerating saplings and tubestock, as well as those planted for revegetation, within the first year of life (Duan et al., 2013). Water stress induced plant mortality and loss of seedlings can lead to a change in ecosystem structure (demography and composition) and result in different patterns of succession (Jentsch et al., 2007; Duan et al., 2013). This study confirms that predicting the point at which seedlings cannot recover using minimum physiological measurements (refer section 4.3.2) with sufficient time is needed to implement a re-watering or management strategy. Seedlings should also be planted at times where they are most likely to not be subject to periods of longer than 4 weeks without water (based on regional climate studies). However, experimental testing in natural habitats would be required to determine the length of time seedlings can survive without water (in natural habitats).

4.5.3. Remote Sensing

Understanding the changes and applications of the leaf reflectance indices could be particularly important as each index can be non-invasively remote censored (Sun et al.,
Remote sensing the degree of water stress in plant communities, their physiological condition and recent changes, particularly newly planted, regenerating or endangered communities, could improve conservation activities, target areas, vegetation surveying and species recovery. PRI and NDVI can be used to show rapid changes in plant stress and photosynthetic status as well as the detection, predictions and impacts of drought events (Dobrowski et al., 2005; Pettorelli et al., 2005). Monitoring PRI, NDVI and SR through remote sensing could improve the identification of vegetation stress and therefore improve procedures and targeted responses to stress events predicted with climate change. Other authors suggest the development of new methods, tools and techniques for tracking ecosystem changes and maintenance of resilient ecological systems is needed in the anticipation of global climate change (Jentsch et al., 2007; Mooney et al., 2009).

Community specific large scale analysis of remote sensed reflectance indexes could provide an efficient and accurate analysis of the status (healthy, stressed) of that community without conducting physical assessments and site visits. Site visits and physical assessments are time consuming and expensive tasks with often inconclusive results. There are already nationwide databases which analyse NDVI as a guide, such as the Vegetation greenness climate maps which can be accessed from the Bureau of Meteorology website (http://www.bom.gov.au/jsp/awap/ndvi/index.jsp) (Commonwealth of Australia 2014). Targeting these maps to a smaller scale based on vegetation community would enable more effective use and targeted management. Future research could target whether remote sensing of leaf reflectance indexes could detect changes in the health of seedlings or smaller plants rather than or in-combination with canopy health.

4.6. Limitations of study and suggestions for further research

There are several limitations of the use of data gathered in this thesis. The results gathered in this thesis were from a glasshouse study where climate parameters (wind, soil water holding capacity, soil biota etc.) were controlled and environment was simulated. Glasshouse studies are generally a good starting point to understand general interactions and initial responses in seedlings. Glasshouse experiments enable control of variables (water stress, temperature) when these conditions do not yet exist in the natural field locations at the current time. A longer study could be conducted in field conditions with planted or re-generation seedlings to see responses in a natural habitat.
with other interactions and landscape based variability such as soil (biota, leaf litter, texture, holding capacity), wind, different aged communities (fully grown etc.), different species, vegetation aspect, vegetation sub-climates and other disturbances (seasonal variability, fire) (Dreesen et al., 2012; Fisichelli et al., 2014). Soil biota including microbes, fungi and soil invertebrates has been shown to alter the response of communities to extreme weather events and contribute to the complexity of a whole community response (Jentsch et al., 2007). The responses of plants to stresses under natural conditions (non-laboratory or non-glasshouse) need to be clarified to increase the strength and reliability of experimental results which suggest significant interactions between species, heat and water stress. Studies in natural environments would require more resources and time and would largely be dependent on natural climate or the development of open air temperature and precipitation controlled chambers. These studies are important as many studies currently focus on the development of innovative, sustainable and effective management for production and crop environments as opposed to natural environments important for conservation purposes (Panda et al., 2004). Using broad vegetation classifications (functional type, ecological community, height, species) to model changed climate conditions and their impact on vegetation (succession, survival, movement, emergence, growth) may be possible with future research (Fisichelli et al., 2014).

The analysis of the leaf health measurement on blades of grass and small leafed shrubs was not conducted in this experiment due to their small size and equipment capacity. Further research could analyse the leaf health measurement of grasses and small leafed shrubs to determine whether they differ from tree and larger leafed shrub species in their physiological response. The research would address the results which suggest a broad difference between the responses of different vegetation functional types.

The normal range of leaf health measurements is not known for many species. Mapping the normal ranges of PRI, NDVI, SR, SC, T, CL and Pn for individual species and communities could provide information which would contribute to the effective and efficient analysis of stress levels and enable quicker response to the decline in leaf health of vital species in native communities.
4.7. Conclusion

This study provided new insights into the physiological and morphological response of multiple non-commercial native Australian seedlings to water and heat stress and a combination of these stresses. As demonstrated in this study, heat and water stress have an additive effect, where the effect of heat and water stress is larger when the stresses occur in combination than when in isolation. Specifically this study provided information into the use and suitability/veracity of multiple measurements of leaf health relative to multiple types of plant stresses (temperature, water). This study also provided information into the subsequent leaf health of two species following re-watering after two different periods of water stress. This study revealed that PRI, NDVI and SR are highly correlated indices which all reflect changes in leaf health following a period of water stress. A change point was detected in stomatal conductance and transpiration following 4-5 weeks without water which was consistent over the five species analysed. In addition to these findings, this study is able to provide baseline information on the relative survival and leaf health of some seedlings within grassy-box woodland habitats in water and heat conditions projected to occur within the next 100 years based on climate change predictions. However, further analysis is needed to mirror this study in a natural habitat to fully understand the results demonstrated in this study such as time to mortality and soil moisture in a habitat which has other influences (soil biota, wind etc.).
CHAPTER 5 - References


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