Drought traits of Eucalyptus gomphocephala in Yalgorup National Park

Paul L. Drake

Edith Cowan University

Follow this and additional works at: https://ro.ecu.edu.au/theses

Part of the Forest Sciences Commons

Recommended Citation
https://ro.ecu.edu.au/theses/188

This Thesis is posted at Research Online.
https://ro.ecu.edu.au/theses/188
Edith Cowan University
Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study.

The University does not authorize you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following:

- Copyright owners are entitled to take legal action against persons who infringe their copyright.

- A reproduction of material that is protected by copyright may be a copyright infringement. Where the reproduction of such material is done without attribution of authorship, with false attribution of authorship or the authorship is treated in a derogatory manner, this may be a breach of the author’s moral rights contained in Part IX of the Copyright Act 1968 (Cth).

- Courts have the power to impose a wide range of civil and criminal sanctions for infringement of copyright, infringement of moral rights and other offences under the Copyright Act 1968 (Cth). Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.
Drought Traits of *Eucalyptus gomphocephala* in Yalgorup National Park

Paul Laurence Drake BEnvSc (Hons) (MSc)

Thesis Submitted in Partial Fulfilment for the Degree of Doctor of Philosophy in the School of Natural Sciences, Faculty of Computing, Health and Science, Edith Cowan University

January 2008
USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.
SUMMARY

The Mediterranean climate of south western Australia has undergone a significant reduction in annual rainfall potentially associated with global climate change. The resulting decrease in rainfall recharge of aquifers has been considered a cause of decline in canopy condition of several phreatophytic species of the region. The purpose of this study was to explore the possibility that a similar climate change induced decline in canopy condition has occurred in a large, iconic eucalypt of south western Australia: *Eucalyptus gomphocephala* D. C. (known commonly as Tuart).

The hydrological environment of *Eucalyptus gomphocephala*, while well characterised from a hydrogeological perspective, remains understudied in the context of the traits that underpin a tolerance to plant tissue dehydration. Over two successive wet-dry seasonal cycles three *E. gomphocephala* populations, located at various positions in the landscape, exhibited a seasonal oscillation in pre-dawn and midday leaf water potential (Ψ_pd and Ψ_md respectively), estimated leaf transpiration rate (*E* *) and stomatal conductance to water vapour (g_s). The amplitude of this oscillation depended on the proximity of the water table from the natural surface. This dependency was reflected by the ability of trees growing low in the landscape to express a lower turgor loss point (Ψ_TL) and leaf water use-efficiency (quantified as a higher leaf carbon isotope discrimination, Δ_leaf). Over the study period native leaf water potential did not fall below Ψ_TL but it is likely that substantial xylem embolisms occurred during the hot dry conditions of summer, as estimated by xylem vulnerability curves. Using landscape position as a proxy for eco-hydrological niche, it is concluded that the current hydrological habitat of Yalgorup National Park is compatible for the survival of *E. gomphocephala*.

Natural variation in stable isotopes of hydrogen were exploited to estimate the relative contribution of a given water source in *E. gomphocephala* populations across stage of maturity and season and at different proximities to groundwater. The linkage between use of a given water source and canopy physiological
processes was also explored. Across stages of maturity and location, *E. gomphocephala* can be classified as an opportunist, able to express a phreatophytic state when conditions favour this strategy. Canopy processes suggest that, at a given site, seedlings were more stressed than saplings or trees. Large trees growing high in the landscape were surprisingly less stressed than other individuals. It is argued that this observation could reflect the advantage of a long term adaptation to dry conditions that are now experienced generally across the landscape as a result of a stepped reduction in annual rainfall.

An interspecific study of vulnerability to water stress-induced xylem embolisms in *Eucalyptus gomphocephala* and several co-occurring canopy species in Yalgorup National Park was also undertaken. Under the auspice of this physiological comparison, the pre-European geographical distribution on the Swan Coastal Plain of the studied species was examined to comment on the role of drought tolerance and species distribution. Vulnerability to xylem embolisms did vary across the small but diverse group of species in this study. However, there was no relationship between this variability and the minimum rainfall at which an individual species occurs. A rationale for this is discussed in terms of growth rate and plant organ longevity.

A preliminary investigation of root hydraulic and anatomic properties of *E. gomphocephala* roots in Yalgorup revealed that a small average hydraulically weighted vessel diameter (*d*) was associated with a greater native specific hydraulic conductivity and lower percentage loss hydraulic conductivity. Combined, these findings suggest that, under the current conditions of Yalgorup National Park, a conservative root anatomy provides the most effective hydraulic transport system in roots.

Across the three core *E. gomphocephala* populations used in this study, multiple linear regression models were used to examine the possibility that dry season physiological traits were associated with an individual the canopy condition of an individual tree. Overall, dry season physiological traits were poor predictors of *E. gomphocephala* canopy condition. However, excluding water stress as an underlying cause of the current decline syndrome, centred on Yalgorup National
Park, on this basis alone should be done so with caution. Two theoretical explanations that retain the integrity of a drought-induced canopy decline scenario but also provide a basis for the poor correlations between dry season physiological traits and canopy condition are: 1) the temporal separation from the initial event and physiological measurements could preclude an interpretable correlation with current instantaneous dry season physiological traits, and 2) through attrition, the retention of resilient individuals could mask the undying effect of water stress.

In a glasshouse experiment the role of contrasting growth conditions (light and soil water) on *E. gomphocephala* mature leaf physiology was investigated. Under a water deficit during plant development *E. gomphocephala* seedlings were capable of manipulating leaf epidermal morphology (increasing the density of stomata and reducing their anatomical dimensions) and expressing conservative leaf gas exchange properties. While specific hydraulic conductivity of petioles ($K_s$) was weekly related to the average hydraulically weighted mean vessel diameter ($d$), $K_s$ appeared to be affected more by irradiance during plant development than water availability. Surprisingly this affect meant that seedlings that were developed under 2% of full sun exhibited a higher $K_s$. The consistency of leaf-area-specific hydraulic conductivity ($K_L$) across treatments would imply that leaf area scales according the hydraulic efficiency of the supplying petiole.

*Eucalyptus gomphocephala* is an opportunist, capable of exploiting a range of water sources in the rhizosphere depending on temporal and spatial patterns of soil water distribution. The species is also adept at modifying its phenotype when developed under contrasting soil water environments, a conclusion drawn from both field and glasshouse experiments. These two properties, no doubt, contributed to the observation that the turgor loss point was not breached at any field location in Yalgorup National Park. While these conclusions, alone, do not preclude water stress as an underlying cause of the species’ decline, they do imply that the current ecohydrological niche of Yalgorup National Park is compatible for its survival.
ACKNOWLEDGEMENTS

This study was supported by an Australian Research Council grant (Grant Number LP0346931) under the auspice of Edith Cowan University and in collaboration with Murdoch University, The Department of Environment and Conservation, Alcoa and Mandurah Shire Council. Additional financial assistance was provided by The Centre for Ecosystem Management at Edith Cowan University.

I am indebted for the advice and guidance provided by my supervisors: Associate Professor Ray Froend (Edith Cowan University) and Dr. Peter Franks (James Cook University). Discussions with Professor Will Stock, Associate Professor Giles Hardy, Dr. Paul Barber, Professor Bernie Dell, Dr. Robert Archibald and Drew Hasswell were invaluable. I am also grateful for assistance with field and laboratory work from Patrick Mitchell, Caroline Mc Cormick, Dr. Alexander Watson, Anya Lam, Todd Edwards and Rebecca Kenna. Murray Banks from The Department of Environment and Conservation ensured that my rustic accommodation in Yalgorup National Park remained habitable and Linda and Bill provided insightful editorial comment on earlier drafts. Finally, thanks to family and friends for support throughout the project.
TABLE OF CONTENTS

LIST OF FIGURES viii

LIST OF TABLES xviii

LIST OF APPENDICES xix

GLOSSARY AND ABBREVIATIONS xx

CHAPTER 1: INTRODUCTION, RATIONALE AND OBJECTIVES 1

1.1 A LINKAGE BETWEEN CLIMATE CHANGE AND TREE DECLINE 3

1.1.1 CLIMATE CHANGE IN SOUTH WESTERN AUSTRALIA 3

1.1.2 OCCURRENCE OF CANOPY DIEBACK 4

1.2 A PRELIMINARY CLASSIFICATION OF THE ECOHYDROLOGICAL NICHE OF EUCALYPTUS GOMPHOCEPHALA 5

1.3 YALGORUP NATIONAL PARK: A CENTRE FOR EUCALYPTUS GOMPHOCEPHALA CANOPY DIEBACK 7

1.4 SOIL WATER AND PLANT WATER RELATIONS 10

1.5 OBJECTIVES 12

CHAPTER 2: A DESCRIPTION OF THE STUDY SPECIES AND REGION 14

2.1 BACKGROUND 15

2.1.1 A DESCRIPTION OF THE STUDY SPECIES 15

2.1.2 THE PRICE OF CLEARING 17

2.1.3 PLANT COMMUNITY STRUCTURE 18
CHAPTER 5: VULNERABILITY TO XYLEM EMBOLISM IN EUCALYPTUS GOMPHOCEPHALA AND ITS CO-OCCURRING CANOPY SPECIES OF YALGORUP NATIONAL PARK

5.1 INTRODUCTION

5.2 MATERIAL AND METHODS

5.2.1 PLANT MATERIAL AND FIELD SITE

5.2.2 VULNERABILITY CURVES

5.3 RESULTS

5.4 DISCUSSION

5.5 CONCLUSIONS
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.2.2 LEAF WATER POTENTIAL</td>
<td>162</td>
</tr>
<tr>
<td>9.2.3 LEAF GAS EXCHANGE</td>
<td>162</td>
</tr>
<tr>
<td>9.2.4 CARBON ISOTOPE DISCRIMINATION</td>
<td>163</td>
</tr>
<tr>
<td>9.2.5 LEAF NUTRIENTS</td>
<td>164</td>
</tr>
<tr>
<td>9.2.6 STATISTICAL ANALYSES</td>
<td>164</td>
</tr>
<tr>
<td>9.3 RESULTS</td>
<td>165</td>
</tr>
<tr>
<td>9.4 DISCUSSION</td>
<td>171</td>
</tr>
<tr>
<td>9.5 CONCLUSIONS</td>
<td>176</td>
</tr>
</tbody>
</table>

CHAPTER 10: GENERAL DISCUSSION AND CONCLUSIONS 177

10.1 OBJECTIVE 1: HOW DO THE WATER RELATIONS PROPERTIES AND HYDRAULIC ARCHITECTURE OF *E. GOMPHOCEPHALA* DIFFER WITH PROXIMITY TO GROUNDWATER AND SEASON (CHAPTER 3)? 178

10.2 OBJECTIVE 2: WHAT ARE THE WATER SOURCES OF *E. GOMPHOCEPHALA* AND DO THESE SOURCES DIFFER WITH STAGE OF MATURITY, PROXIMITY TO GROUNDWATER AND SEASON (CHAPTER 4)? 179

10.3 OBJECTIVE 3: DOES VULNERABILITY TO DROUGHT-INDUCED XYLEM EMBOLISM DIFFER BETWEEN *E. GOMPHOCEPHALA* AND CO-OCCURRING CANOPY SPECIES (CHAPTER 5)? 182

10.4 OBJECTIVE 4: ARE ROOT ANATOMICAL FEATURES RELATED TO XYLEM HYDRAULIC PROPERTIES IN *E. GOMPHOCEPHALA* (CHAPTER 6)? 183

10.5 OBJECTIVE 5: ARE CURRENT PLANT WATER RELATIONS PROPERTIES RELATED TO THE DEGREE OF CANOPY DIEBACK IN *E. GOMPHOCEPHALA* (CHAPTER 7)? 184

10.6 OBJECTIVE 6: HOW DO *E. GOMPHOCEPHALA* SEEDLINGS RESPOND TO DEVELOPMENT UNDER A SUBOPTIMAL SOIL MOISTURE ENVIRONMENT (CHAPTER 8)? 185
10.7 OBJECTIVE 7: HOW DOES DEVELOPMENT ON ASH-BEDS INFLUENCE LEAF-AREA-SPECIFIC PROPERTIES OF \( E. GOMPHOCEPHALA \) (CHAPTER 9)?

10.8 CONCLUSIONS

REFERENCES

APPENDICES
Figure 1.1 Diagrammatic representation of the possible difference in *E. gomphocephala* root distribution in relation to landscape position and proximity to the water table (A). This model implies that in some areas a phreatophytic state may exist. Under the scenario of a declining water table (B) phreatophytes may lose contact with the water source.  

Figure 1.2 Examples of *E. gomphocephala* canopy decline in Yalgorup National Park. Symptoms range from dieback of terminal branches and associated epicormic resprouting (top two images) to complete mortality (bottom two images).  

Figure 1.3 An example of a comparison of change in groundwater level from a monitoring bore in Yalgorup National Park (line demarked with symbols) and the CDFM rainfall model (unbroken line) over the same period. Note that after an initial training period the bore hydrograph data and rainfall model oscillate (and decline) in synergy. Adapted from Lindsay (2002).  

Figure 1.4 A thesis outline (presented as a flow chart) relating aspects of the biotic and abiotic environment of *E. gomphocephala*. In this model climate change would invoke a decline in *E. gomphocephala* canopy condition by its influence on available soil water and associated physiological impact. The relative severity of this shift in available soil water is likely to depend on proximity to groundwater and tree size class (synonymous with stage of maturity). This thesis investigates several aspects of plant water relations, hydraulic architecture and water source partitioning. These are considered in the context of: 1) landscape position (Chapter 3), 2) water sources and stage of maturity (Chapter 4), 3) inter-specific variation (Chapter 5), 4) rooting properties (Chapter 6), 5) canopy condition (Chapter 7), and 6) development under water deficit (Chapter 8).  

Figure 2.1 The geographical distribution of *E. gomphocephala* prior to European settlement. Overlaid are mean annual rainfall isohyets. Data were supplied by the Bureau of Meteorology and the Department of Conservation and Land Management (Sheperd 2003). Datum = GDA 94.  

Figure 2.2 Mean monthly rainfall and average maximum monthly temperature in the vicinity of the study (Mandurah 32.52°S; 115.75°E) as a representative of the overall climatic pattern of south western Australia. Data represent the mean of 117 years (Australian Bureau of Meteorology, 2006).
Figure 2.3 The average annual rainfall for Perth (32.92°S; 115.87°E) has shown a stepped-change since the mid 1970's. In graph A horizontal lines depicting the average rainfall between the period 1876-1975 and 1976-2002 are included. Perth 1, Perth 2 and Perth Airport represent relocations of Perth's monitoring station. In graph B data for Perth 1 and Perth 2 are reproduced as a percentage deviation of the mean annual rainfall over the period 1876-1975 and overlaid with a 10 year running mean. Data obtained from the Australian Bureau of Meteorology.

Figure 2.4 Mean maximum annual temperature for Perth (32.92°S; 115.87°E) over the period 1876-2003. Monitoring stations are as described in Figure 1.3. Data supplied by the Australian Bureau of Meteorology.

Figure 2.5 A schematic diagram of the subsurface profile of Yalgorup National Park representing the below ground wet bodies that occur.

Figure 2.6 Remnant *E. gomphocephala* (Tuart) woodland in Yalgorup National Park. The park is comprised of three parallel lake systems. These lakes form groundwater sinks for the regional freshwater superficial aquifers. They are made hypersaline by evaporation from their surface and interactions with underlying hypersaline groundwater. The three sites indicated are presented here in preparation for reference below and in subsequent chapters. Datum = GDA 94.

Figure 2.7 A diagrammatic representation of the groundwater flow systems in Yalgorup National Park. Rainfall in the catchment contributes to fresh groundwater flow into lakes which are subsequently transformed into a hypersaline state due to evaporation from their surface and interaction with underlying hypersaline groundwater. The vertical scale (in metres) portrays the approximate distance from sea level to various hydrological features of Yalgorup National Park. Adapted from Commander (1988). An estimate of the distribution of *E. gomphocephala* in this system is indicated by the images of trees.

Figure 2.8 Vertical profiles of soil conductivity (A) and pH (B) from cores collected during summer 2004 from Yalgorup National Park. Depth to groundwater from the natural surface was 2.30 m, 8.04 m and 21.73 m for sites 1, 2, and 3 respectively. The vertical lines dissociated from data points represent the values for groundwater at the respective sites. The inset in A incorporates both groundwater and soil electrical conductivity (note the change of scale). The differing salinity of soil and groundwater is typical of a system where leached salts from soil accompany groundwater recharge.

Figure 2.9 A description of the soil profiles of three sites in Yalgorup National Park. The arrows indicate the approximate position of the water table.
Figure 3.1 The thesis outline highlighting the relevant processes covered in this chapter. Root zone water potential is determined partly by proximity to groundwater. Proximity to groundwater varies spatially due to topography and geology, and temporally due to seasonal rainfall and climatic variability. Root zone water potential, hydraulic conductance and transpiration rate are all integral to canopy water potential, which ultimately determines canopy condition. This chapter quantifies and integrates these characteristics in order to assess the impact of future climate scenarios on *E. gomphocephala* populations in Yalgorup National Park.

Figure 3.2 Mean (± s.e., *n* = 12) pre-dawn and midday leaf water potentials (Ψ<sub>pd</sub> and Ψ<sub>md</sub> respectively) for *Eucalyptus gomphocephala* growing at different depths to the water table. Distances from the natural surface to the water table for sites 1, 2 and 3 respectively were 2.30 m, 8.04 m and 21.73 m. Also shown is monthly rainfall for the same location over the study period and the monthly long-term average. Arrows indicate the range of pre-dawn leaf water potentials observed over the study period.

Figure 3.3 Plot of midday estimated transpiration rate (*E*) versus midday stomatal conductance to water vapour (*g*) for *Eucalyptus gomphocephala* (*n* = 12 for each point). The fitted line is an exponential function: 

\[ g_s = 321.05 - 233.11e^{-0.71t_{pd}} \]  

\( r^2 = 0.44, P < 0.05 \). Sites 1, 2 and 3 are as in Figure 3.2.

Figure 3.4 Plot of midday stomatal conductance to water vapour (*g*) versus pre-dawn leaf water potential (Ψ<sub>pd</sub>). A. and *g*<sub>s</sub> versus midday leaf water potential (Ψ<sub>md</sub>). B (*n* = 12 for each point) for *Eucalyptus gomphocephala* growing at different depths to groundwater (refer to Figure 3.2 for respective depths). Fitted line in A is a linear model: 

\[ g_s = 372.24 + 262.08\Psi_{pd} \]  

\( r^2 = 0.57, P < 0.001 \). Stomatal conductance to water vapour was weakly correlated with Ψ<sub>md</sub> (in a linear fashion) in B: 

\[ g_s = 370.34 + 113.22\Psi_{md} \]  

\( r^2 = 0.23, P < 0.001 \).

Figure 3.5 Pressure volume curves, plots of 1/Ψ versus the relative water content (RWC), for leaves collected from the 12 individuals of each site. The fitted linear models (refer to methodology for fitting procedure) have the following equations: 1/Ψ = 0.04 - 0.52RWC, 1/Ψ = 0.19 - 0.69RWC and 1/Ψ = 0.15 - 0.55RWC for graphs A (\( r^2 = 0.69; P < 0.001 \)), B (\( r^2 = 0.71; P < 0.001 \)) and C (\( r^2 = 0.73; P < 0.001 \)) respectively. Refer to Figure 3.2 for respective depths to groundwater.

Figure 3.6 The water potential at full turgor (\( \pi_i \)) was positively correlated with the turgor loss point (Ψ<sub>tl</sub>) across sites. The fitted linear model has the equation: 

\[ \pi_i = -0.73 + 0.87\Psi_{tl} \]  

\( r^2 = 0.75, P < 0.0001 \). Refer to Figure 3.2 for site depths to groundwater.
Figure 3.7 Both $\Psi_{11}$ (A) and $\Delta_{\text{leaf}}$ (B) were correlated with mean depth to groundwater in a convergent linear manner. The linear fit for A has the equation: $\Psi_{11} = -2.30 - 0.03 \times \text{Depth to groundwater}$ ($r^2 = 0.82$, $P < 0.0001$). The linear fit for B has the equation: $\Delta_{\text{leaf}} = 21.88 - 0.11 \times \text{Depth to groundwater}$ ($r^2 = 0.31$, $P < 0.001$).

Figure 3.8 Vulnerability curves, plots of percentage loss hydraulic conductivity (PLC) versus xylem water potential ($\Psi_\text{x}$), for *Eucalyptus gomphocephala* growing at different depths to groundwater (refer to Figure 3.2 for respective depths). Fitted lines are exponential-sigmoidal models (Pammenter and Vanderwilligen 1998) with the equation:

$\text{PLC} = 100 \left[1 + \exp\left(0.67(\Psi_\text{x} + 2.23)\right)\right]$ ($r^2 = 0.80$) for Site 1,
$\text{PLC} = 100 \left[1 + \exp\left(0.72(\Psi_\text{x} + 2.13)\right)\right]$ ($r^2 = 0.75$) for Site 2,
$\text{PLC} = 100 \left[1 + \exp\left(0.70(\Psi_\text{x} + 2.29)\right)\right]$ ($r^2 = 0.75$) for Site 3.

Figure 3.9 Plots of the midday leaf water potential ($\Psi_{\text{md}}$) versus pre-dawn leaf water potential ($\Psi_{\text{pd}}$) for *Eucalyptus gomphocephala* growing at different depths to groundwater (refer to Figure 1 for respective depths). Fitted lines are linear models: $\Psi_{\text{md}} = -0.62 + 1.27\Psi_{\text{pd}}$ ($r^2 = 0.26$, $P = 0.03$), $\Psi_{\text{md}} = -0.68 + 0.98\Psi_{\text{pd}}$ ($r^2 = 0.75$, $P < 0.01$) and $\Psi_{\text{md}} = -0.91 + 0.88\Psi_{\text{pd}}$ ($r^2 = 0.71$, $P < 0.01$). The dashed line in each graph represents the 1:1 ratio.

Figure 4.1 The thesis outline highlighting the relevant processes covered in this chapter. The role of transition to maturity, quantified by size class, and proximity to groundwater are assessed in terms of the spatial and temporal partitioning of water sources. Differences in water source strategies, and accompanying distinction in rooting properties, are associated with plant water relations and canopy hydraulic architecture.

Figure 4.2 Groundwater depth from the natural surface at site 1 (A) and site 2 (B) and daily rainfall for the area over the same period (C). Note that both water tables responded rapidly to rainfall recharge but that the magnitude of this response was greater at site 1.

Figure 4.3 Plots of soil depth versus volumetric water content ($\theta$) (A and C) and soil water $\delta^2$H (B and D) for the periods February, July and October. The vertical shaded bars of B and D represent the mean $\pm$ s.e. ($n = 3$) of groundwater $\delta^2$H from samples collected in February, July and October.

Figure 4.4 Proportion of water source used with transition from the summer dry season (February) to winter wet season (July) and spring (October) for seedlings (A) saplings (C) and mature trees (E) at site 1 ($n = 6$ for each stage of maturity). Pre-dawn leaf water potential ($\Psi_{\text{pd}}$) over the same
period for seedlings (B), saplings (D) and mature trees (F) are also shown \((n = 4\) for each stage of maturity). 68

Figure 4.5 Proportion of water source used with transition from the summer dry season (February) to winter wet season (July) and spring (October) for seedlings (A) saplings (C) and mature trees (E) at site 2 \((n = 6\) for each stage of maturity). Pre-dawn leaf water potential \(\left(\Psi_{pd}\right)\) over the same period for seedlings (B), saplings (D) and mature trees (F) are also shown \((n = 4\) for each stage of maturity). 69

Figure 4.6 Correlation between the maximum yield of photosystem II \(\left(F_v/F_m\right)\) and the Huber value \((H)\) for data collected over the study period. The linear fit follows the equation: \[ \frac{F_v}{F_m} = 0.78 + 435.78H \] \((r^2 = 0.81; P = 0.02)\). Seedlings = •, saplings = • and trees = ▲. Closed symbols are plants from site 1, open symbols are plants from site 2. Each point is the mean ± s.e. \((n = 36\) for each). 70

Figure 4.7 Leaf-area-specific hydraulic conductivity \(\left(K_L\right)\) was positively correlated with stem-area-specific hydraulic conductivity \(\left(K_S\right)\) in \(E. gomphocephala\) stems. Each point represents the mean \((± s.e., n = 36);\) closed symbols are data from samples collected at site 1, open symbols are data from samples collected at site 2. The data corresponds to branches collected over the study period and therefore \textit{in situ} \(K_S\) and \(K_L\) integrated across the dry to wet seasonal transition. The fitted linear model has the equation: \[ K_L = 2.95 \times 10^{-5} + 1.01 \times 10^{-4} K_S \] \((r^2 = 0.85; P = 0.01)\). 71

Figure 5.1 A reproduction of the thesis outline describing the processes considered in this chapter. An inter-specific comparison of vulnerability to embolism undertaken to explore the possibility that \(E. gomphocephala\) is drought prone compared to co-occurring canopy species. 80

Figure 5.2 A schematic diagram of the pressure collar used to induce a given water potential in stem xylem. A stem is sealed in the chamber with the ends protruding. Air pressure in the chamber is then raised by connecting the collar to a pressure reservoir. The pressure is maintained at a desired level to induce a given xylem water potential. 84

Figure 5.3 The natural (pre-European) geographical distribution of \(E. gomphocephala\) (reproduced from Chapter 2). Overlaid are the regional, 100 year, mean annual rainfall isohyets (at 50 mm intervals). Data supplied by the Department of Conservation and Land Management (2006) and Bureau of Meteorology (2006) (Sheperd 2003). Datum = GDA 94. 86

Figure 5.4 The natural (pre-European) geographical distribution of \(E. marginata\). Overlaid are the regional, 100 year, mean annual rainfall isohyets (at 50 mm intervals). Data supplied by the

Figure 5.5 The natural (pre-European) geographical distribution of Agonis flexuosa. Overlaid are the regional, 100 year, mean annual rainfall isohyets (at 50 mm intervals). Data supplied by the Department of Conservation and Land Management (2006) and Bureau of Meteorology (2006) (Sheperd 2003). Datum = GDA 94. _________ 88

Figure 5.6 The natural (pre-European) geographical distribution of Acacia cyclops. Overlaid are the regional, 100 year, mean annual rainfall isohyets (at 50 mm intervals). Data supplied by the Department of Conservation and Land Management (2006) and Bureau of Meteorology (2006) (Sheperd 2003). Datum = GDA 94. __________________ 89

Figure 5.7 Vulnerability curves, plots of percentage loss hydraulic conductivity (PLC) versus air injection pressure ($P_{m}$), for E. gomphocephala, E. marginata, Agonis flexuosa, and Acacia cyclops. The fitted lines are sigmoidal models; refer to Table 1 for fitting parameters. 91

Figure 5.8 The slope ($a$) of the relationship between percentage loss hydraulic conductivity (PLC) and the air injection pressure ($P_{m}$) (denoted $a$) was negatively correlated with the $P_{m1}$ at PLC = $50\%$ ($\Psi_{50}$). _______________ 92

Figure 5.9 Exponential models described the relationships between $a$ and $\Psi_{50}$ versus the minimum rainfall at which each species occurred on the Swan Coastal Plain. __ __ __ 93

Figure 6.1 The thesis outline highlighting the elements specific to this chapter. These are the rooting properties of roots and their possible association with plant water relations and canopy decline. ________________________ 100

Figure 6.2 Mean ± s.e. ($n = 4$) pre-dawn leaf water potential ($\Psi_{pd}$) for E. gomphocephala saplings (plants 1, 3 and 5) and tress (plants 2, 4 and 6). ___________________________ 107

Figure 6.3 Mean ± s.e. ($n = 6$) percentage loss hydraulic conductivity (PLC) for E. gomphocephala roots from saplings (plants 1, 3, 5) and trees (plants 2, 4, 6). Samples were collected immediately after $\Psi_{pd}$ measurements. ________________________ 108

Figure 6.4 In situ root hydraulic conductivity ($K_{s}$ in situ, kg s$^{-1}$ m$^{-1}$ MPa$^{-1}$) was exponentially related to pre-dawn leaf water potential ($\Psi_{pd}$) in E. gomphocephala saplings (plants 1, 3 and 5) and tress (plants 2, 4 and 6). The exponential model fitted to the data has the equation:

$$K_{s \text{ in situ}} = 3.70 + 0.04e^{0.01}$$(r$^2 = 0.70). ___________________________ 109
Figure 6.5 In situ root hydraulic conductivity, expressed on a per unit length basis \( (L_p) \) was exponentially correlated with \( \Psi_{rd} \) in *Eucalyptus gomphocephala* saplings (plants 1, 3 and 5) and trees (plants 2, 4 and 6). The exponential model describing this correlation has the equation:

\[
L_p = 5.0 \times 10^{-6} + 2.87 \times 10^{-7} e^{331} (r^2 = 0.81)
\]

Figure 6.6 A typical cross section of a *Eucalyptus gomphocephala* root. The image comprises secondary xylem (X), secondary phloem and fibres (SPh), cortex (C) and phellogen (P). Note that measurements of hydraulic conductivity in this study were restricted to the stele (dominated by xylem vessels) and that there was no substantial pith development in sectioned roots.

Figure 6.7 A frequency histogram of xylem vessel diameter (% of total, POT) in *Eucalyptus gomphocephala* roots. Measurements were made on all trees pooled. The fitted Gaussian curve has the following equation: \( \text{POT} = 0.38 + 87.00e^{-536} \) \( (r^2 = 0.81) \).

Figure 6.8 In situ root hydraulic conductivity \( (K_s) \) (A), \( K_s \) max \( (K_{s_{\text{max}}}) \) (B) and percentage loss hydraulic conductivity \( (\text{PLC}) \) (C) were exponentially correlated with the hydraulically weighted mean vessel diameter \( (d) \) for saplings (plants 1, 3 and 5) and trees (plants 2, 4 and 6). The exponential models of A, B and C respectively have the following equations:

\[
K_s \text{ in situ} = 2 + 3815.06e^{-d/3.62} + 3815.06e^{-d/3.62} (r^2 = 0.56).
\]

\[
K_{s_{\text{max}}} = 62.53 - 46.41e^{-d-0.83/5.32} \quad (r^2 = 0.60), \quad K_{s_{\text{max}}} = 44.95 - 31.76e^{-d-0.83/4.43} \quad (r^2 = 0.85).
\]

Figure 6.9 Percentage loss hydraulic conductivity \( (\text{PLC}) \) was positively correlated with maximum hydraulic conductivity \( (K_{s_{\text{max}}}) \) of *Eucalyptus gomphocephala* roots. The fitted linear model has the equation: \( \text{PLC} = 10.23 + 0.52K_{s_{\text{max}}} \) \( (r^2 = 0.82; P = 0.01) \).

Figure 7.1 The thesis outline reproduced here to highlight the elements covered in this chapter. It could be argued that if the current recurrence of *E. gomphocephala* canopy decline is due to the direct effect of reduced available soil water, as a result of climate change, the plant water relations of an individual should relate to the relative visual expression of canopy dieback.

Figure 7.2 A box chart showing that there was no difference in average canopy condition across the three study sites. The horizontal lines in the box denote the 25th, 50th, and 75th percentile values. The error bars denote the 5th and 95th percentile values. The two symbols below the 5th percentile error bar denote the 0th and 1st percentile values. The two symbols above the 95th percentile error bar denote the 99th and 99.9th percentile values.
percentile error bar denote the 99th and 100th percentiles. The square symbol in the box denotes the mean of the column of data.

Figure 7.3 Diameter at breast height was negatively correlated with canopy condition (CC) at each site and across all sites pooled. The poorer the canopy condition, the lower the percentage score. The fitted linear models for graphs A, B, C and D respectively have the equations: 
\[ DBH = 84.88 - 1.1 \times \text{CC} \quad (r^2 = 0.43; \quad P = 0.02), \]
\[ DBH = 139.86 - 1.74 \times \text{CC} \quad (r^2 = 0.36; \quad P = 0.04), \]
\[ DBH = 142.56 - 1.83 \times \text{CC} \quad (r^2 = 0.76; \quad P < 0.001), \]
\[ DBH = 122.72 - 1.57 \times \text{CC} \quad (r^2 = 0.47; \quad P < 0.001). \]

Figure 7.4 Transpiration rate (E) was positively correlated with canopy condition (CC) at site 1 and negatively correlated with CC at site 3. While not significant, a weak positive correlation was also evident between E and CC at site 2. The fitted linear models for graphs A, B and C respectively have the equations: 
\[ E = 0.35 + 0.05 \times \text{CC} \quad (r^2 = 0.45; \quad P = 0.02), \]
\[ E = 0.48 + 0.02 \times \text{CC} \quad (r^2 = 0.15; \quad P = 0.22), \]
\[ E = 2.37 - 0.02 \times \text{CC} \quad (r^2 = 0.40; \quad P = 0.03). \]

Figure 7.5 Theoretical relationship between a physiological property linked to water stress and the time over which the decline event has been witnessed. In this model the initial perturbation (e.g. prolonged water stress) could have invoked a change in some physiological property associated with water stress. However, by rapidly shedding leaves a plant could restabilise the physiological property to within normal limits by altering the root:shoot ratio.

Figure 7.6 The carbon isotope composition of the outer 5 cm of xylem (\( \delta^{13}C_{\text{wood}} \)) was positively correlated with canopy condition. The linear model has the equation: 
\[ \delta^{13}C_{\text{wood}} = -26.12 + 0.1 \times \text{Canopy condition} \quad (r^2 = 0.51, P = 0.01, n = 11). \]

Figure 8.1 This chapter describes the plasticity in leaves of *E. gomphocephala* that develop under partial water stress. The precept for this investigation is the probability that a new cohort of *E. gomphocephala* seedlings will be exposed to less available soil water. The hypothesis is that development under such conditions will induce morphological and physiological plasticity in mature leaves. Elements of the thesis outline covered in this chapter are highlighted.

Figure 8.2 Mean (± s.e.) pre-dawn leaf water potentials (\( \Psi_{pl} \), n = 2 leaves per plant) for *Eucalyptus gomphocephala* seedlings maintained at field capacity (FC) and 50% field capacity (\( P_{50} \)). Plants maintained at FC had a significantly higher \( \Psi_{pl} \) than plants kept at \( P_{50} \) (\( P < 0.05, \) one-way ANOVA).

Figure 8.3 Correlation between stomatal conductance to water vapour (\( g_s \)) and pre-dawn leaf water potential (\( \Psi_{pl} \)) and \( g_s \) and transpiration rate (E) for *Eucalyptus gomphocephala* seedlings.
graphs A and B respectively. The treatments were: T1: watered to field capacity (FC) and 21% of full sun. T2: watered to FC and 7% of full sun, T3 water to 50% FC and 21% of full sun, and T4 watered to 50% FC and 7% of full sun. The exponential fit for A was generated from the equation \( g_{s} = 18.33e^{0.35} \) (P < 0.05). The linear fit for B was generated from \( g_{s} = 1.79 + 84.40E \) (P < 0.001).

Figure 8.4 Correlation between maximum photon yield of PSI I (F\(_{V}/F_{M}\)) and \( \Psi_{ps} \) (A) and F\(_{V}/F_{M}\) and \( g_{s} \) (B) for Eucalyptus gomphocephala seedlings, treatment notation is as per Figure 8.2. The exponential fit for A was generated from the equation \( y = y_{0} + Ae^{\lambda t} \), where \( y \) is F\(_{V}/F_{M}\), \( x \) is \( \Psi_{ps} \), \( y_{0} \) is the offset, \( A \) is the amplitude and \( \lambda \) is a growth constant (P < 0.05). The linear fit for B was generated from \( y = A + Bx \), where \( y \) is F\(_{V}/F_{M}\), \( x \) is \( g_{s} \), \( A \) is the y intercept and \( B \) is the slope (P < 0.001).

Figure 8.5 Plot of specific hydraulic conductivity (K\(_{s}\)) and leaf-area-specific hydraulic conductivity (K\(_{l}\)) versus mean hydraulically weighted vessel diameter (d), graphs A and B respectively, for Eucalyptus gomphocephala seedlings (treatment notation as per Figure 8.2). The fitted linear model of A follows the equation: \( K_{s} = -3.24 + 0.25d \) (r\(^2\) = 0.94; P = 0.03).

Figure 9.1 Relationships between \( A \) and \( p_{g} \) for Eucalyptus gomphocephala seedlings developed on ash-bed or undisturbed (control) soil (A). Photosynthetic operating points (mean ± s.e., \( n = 4 \) for each treatment) are also plotted against fitted exponential functions (B). The arrow in B connects \( p_{g} \) (350 µmol m\(^{-2}\) s\(^{-1}\)) to the photosynthetic operating points.

Figure 9.2 A typical stomatal opening phase in control and ash-bed developed Eucalyptus gomphocephala seedlings. The opening phase is represented by a change in stomatal conductance from a minimum value (the value for stomatal conductance in darkness, \( g_{\text{mm}} \)) to a maximum steady state value after exposure to a PAR of 1000 µmol m\(^{-2}\) s\(^{-1}\). Note that \( g_{s} \) has been normalised as a percentage of its maximum value. The overall treatment difference in \( g_{\text{mm}} \) is presented in Table 9.1.

Figure 9.3 The stomatal closing phase, quantified as the change in \( g_{s} \), with time, in control and ash-bed developed Eucalyptus gomphocephala seedlings. Stomatal closure was induced by darkening leaves. After stabilisation, leaves were absised from plants. Note that \( g_{s} \) has been normalised as a percentage of its maximum value.

Figure 9.4 The relationship between \( g_{s} \) and \( A \) for control (A) and ash-bed (B) developed Eucalyptus gomphocephala seedlings. Note that negative values for \( A \) are associated with dark

---

xvi
respiration \( R_d \) and that \( g_s \) and \( A \) have been normalised as a percentage of their maximum value.

Figure 10.1 A diagrammatic representation of the partitioning of water sources across stage of maturity and season in *E. gomphocephala*. In this figure transition from the summer dry period to the spring (peak groundwater) period is accompanied by an increase in use of surface water in all plants where proximity to groundwater is low and in only seedlings at areas higher in the landscape.

Figure 10.2 Midday stomatal conductance to water vapour \( (g_s) \) was linearly related to \( \Psi_{pd} \) in the field and exponentially related to \( \Psi_{pd} \) in the glasshouse. The fitted line for field data is a linear model: \( g_s = 332.07 + 227.15 \Psi_{pd} \) \((r^2 = 0.58; P < 0.001)\). The fitted line for glasshouse data is an exponential model: \( g_s = 337.13 - 218.09e^{-0.85 \Psi_{pd}} \) \((r^2 = 0.79; P < 0.05)\).
LIST OF TABLES

Table 2.1 Tree species associated with *Eucalyptus gomphocephala* (taken from Gibson et al. (2002)).

Table 5.1 Curve fitting parameters for the sigmoidal fits in Figure 5.7: the slope of the relationship between PLC and the air injection pressure (a) and the value for $P_m$ at PLC = 50% ($\Psi_{s,50}$). Values for $a$ and $\Psi_{s,50}$ are the mean (± s.e., $n = 5$ for each species). Superscript letters indicate homogenous subgroups (one-way ANOVA, Student Newman Klaus Post Hoc analyses: $P < 0.05$, SPSS for Windows version 13.0).

Table 7.1 Multiple linear regression results showing the correlation coefficient ($r^2$) for the top three predictors. The analyses were undertaken from data obtained from each site and all sites pooled. In each row the three * symbols indicate the top three predictors in each analysis. Neither stomatal conductance, leaf $A$ or leaf mass ratio were significant correlates with canopy condition.

Table 8.1 Mean (± s.e.) anatomical characteristics of stomata and rates of stomatal conductance ($g_s$) of *Eucalyptus gomphocephala* seedlings developed under a controlled soil water and light environment (treatment notation as per Figure 8.2). Replication per treatment is as follows: Guard cell length: $n = 120$, aperture length: $n = 120$, stomatal density: $n = 24$, $g_s$, $n = 12$. Different letters indicate significantly different homogenous subgroups $P < 0.05$ (Student-Newman-Keuls Post Hoc analyses).

Table 9.1 Leaf water potential, gas exchange and isotopic properties of leaves developed under control or ash-bed conditions. Values for $A$, $g_s$ and $pi/pa$ represent the photosynthetic operating point (refer to methods for a definition). All values are the mean ± s.e., $n = 8$ for $\Psi_{pi}$, $n = 4$ for $A$, $g_s$, $p_l/p_o$, $R_d$, $g_{min}$, $V_{cmax}$, $J_{max}$ and $\Delta$. Significant differences at the 0.05 level (*) were observed between treatments for $g_{min}$ ($F = 12.053; P = 0.013$) and $R_d$ ($F = 10.920; P = 0.002$).

Table 9.2 Nutrient content of leaves used for gas exchange experiments. Values are mean ± se; $n = 4$ for each. A significant differences at the 0.01 level (**) was found between treatments for manganese ($F = 30.9; P = 0.001$).
LIST OF APPENDICES

Appendix 1 Schematic diagram of the steady state flow meter (SSFM)

Appendix 2 Conversion of neutron moisture meter counts to volumetric water content

Appendix 3 Bounding range errors from the three-component mixing model

Appendix 4 Manuscript
# GLOSSARY AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient partial pressure of CO₂ (pₐ) (µmol mol⁻¹)</td>
<td>The partial pressure of CO₂ at the plant leaf surface</td>
</tr>
<tr>
<td>Aquifer</td>
<td>A body of rock that is sufficiently permeable to conduct groundwater</td>
</tr>
<tr>
<td>Assimilation rate (A) (µmol m⁻² s⁻¹)</td>
<td>The rate of acquisition of CO₂ for photosynthesis</td>
</tr>
<tr>
<td>Bulk density (ρₐ) (g cm⁻³)</td>
<td>The mass of soil for a given volume</td>
</tr>
<tr>
<td>Capillary fringe</td>
<td>The lowest subdivision of the zone of aeration immediately above the water table in which the interstices are filled with water under pressure less than that of the atmosphere, being continuous with the water below the water table but held above it by surface tension</td>
</tr>
<tr>
<td>Carbon isotope discrimination δ¹³C (%)</td>
<td>Generally relates to isotopic fractionation of carbon generated by diffusion through stomata and the enzymatic activity of the C₃ cycle in C₃ plants</td>
</tr>
<tr>
<td>Conductance to water vapour (gₖ) (mmol m⁻² s⁻¹)</td>
<td>Conductance of water that diffuses out of the plant leaf, minus the contribution of the boundary layer</td>
</tr>
<tr>
<td>Critical xylem water potential (Ψₜ,CT) (MPa)</td>
<td>An estimate of the xylem water potential likely to initiate runaway embolism</td>
</tr>
<tr>
<td>Dieback</td>
<td>The rapid and progressive defoliation and death of stems which may eventually result in tree mortality</td>
</tr>
<tr>
<td>Drought avoidance</td>
<td>The ability to avert the stress associated with drought by maintaining hydration or synchronising development with favourable periods</td>
</tr>
<tr>
<td>Drought resistance</td>
<td>The overall ability of a species to survive dry conditions, either though drought avoidance or drought tolerance</td>
</tr>
<tr>
<td>Drought tolerance</td>
<td>The ability to tolerate sustained periods without water</td>
</tr>
<tr>
<td>Ecohydrological niche</td>
<td>The hydrological requirement for a species' survival</td>
</tr>
<tr>
<td>Environmental range</td>
<td>The set of conditions under which a species growth and reproduction can occur</td>
</tr>
<tr>
<td><em>Eucalyptus gomphocephala</em>-dominated woodland</td>
<td>Those plant communities comprising <em>E. gomphocephala</em> as a dominant canopy emergent</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Groundwater</td>
<td>That part of the sub-surface water that is in the zone of saturation, including underground streams</td>
</tr>
<tr>
<td>Huber value ($H$)</td>
<td>The ratio of stem cross sectional area to leaf area</td>
</tr>
<tr>
<td>Hydraulic architecture</td>
<td>The structure of the plant water conducting system</td>
</tr>
<tr>
<td>Hydraulically weighted mean vessel diameter ($d$) ($\mu$m)</td>
<td>An expression for the average vessel diameter scaled to account for the $4^{th}$ power relationship between diameter and flow rate</td>
</tr>
<tr>
<td>Inductively coupled plasma emission spectrometer</td>
<td>Instrument used to measure elemental concentrations</td>
</tr>
<tr>
<td>Leaf intercellular partial pressure of CO$_2$ ($p_i$) ($\mu$mol mol$^{-1}$)</td>
<td>The partial pressure of CO$_2$ available near the site of assimilation, in the intercellular spaces of leaves</td>
</tr>
<tr>
<td>Leaf-area-specific hydraulic conductivity ($K_l$) (kg s$^{-1}$ m$^{-1}$ MPa$^{-1}$)</td>
<td>The mass flow rate of water through an excised stem segment per unit of pressure gradient supporting a known leaf area</td>
</tr>
<tr>
<td>Leaf water potential ($\Psi_{leaf}$) (MPa)</td>
<td>A measure of the free energy of water in leaves relative to the free energy of pure free water</td>
</tr>
<tr>
<td>Mass flow rate ($F$) (kg s$^{-1}$)</td>
<td>The mass flow rate of a solution through a conduit</td>
</tr>
<tr>
<td>Mass spectrometer</td>
<td>Instrument used to measure isotopic ratios</td>
</tr>
<tr>
<td>Native xylem emboli</td>
<td>Air-filled, and hence non-functioning, xylem lumens. Primarily a result of excessive tension associated with plant water stress</td>
</tr>
<tr>
<td>Neutron moisture meter</td>
<td>Instrument used to determine the moisture content of soil</td>
</tr>
<tr>
<td>Photosynthesis system</td>
<td>Instrument, incorporating infra-red gas analysers, used to measure rates of gas exchange from leaves under controlled environmental conditions</td>
</tr>
<tr>
<td>Phenotypic plasticity</td>
<td>The extent to which the appearance of an organism may be modified by expression of a particular genotype in different environments</td>
</tr>
<tr>
<td>Phreatophyte</td>
<td>Plants that have a strong association with saturated soil</td>
</tr>
<tr>
<td>Plant water-use efficiency (WUE)</td>
<td>The amount of carbon fixed through photosynthesis per amount of water lost by transpiration</td>
</tr>
<tr>
<td>(mols mol$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td><strong>Pressure bomb</strong></td>
<td>Instrument used to measure the water potential of leaves</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Productivity (net)</strong></td>
<td>The rate of biomass accumulation by photosynthesis minus the rate of biomass lost by respiration.</td>
</tr>
<tr>
<td><strong>Rhizosphere</strong></td>
<td>The zone around plant roots</td>
</tr>
<tr>
<td><strong>Root zone</strong></td>
<td>The entire area where roots occur</td>
</tr>
<tr>
<td><strong>Soil water</strong></td>
<td>Physiologically available water in soil</td>
</tr>
<tr>
<td><strong>Soil water potential ($\Psi_{soil}$) (MPa)</strong></td>
<td>A measure of the free energy of water in soil relative to the free energy of pure free water</td>
</tr>
<tr>
<td><strong>Specific hydraulic conductivity $K_s$ (kg s$^{-1}$ m$^{-1}$ MPa$^{-1}$)</strong></td>
<td>The hydraulic conductivity of stem xylem normalised to stem cross-sectional area</td>
</tr>
<tr>
<td><strong>Stable isotope fractionation</strong></td>
<td>The process whereby stable isotopes of the same element are discriminated due to differences in size and atomic weight</td>
</tr>
<tr>
<td><strong>Steady-state flow meter</strong></td>
<td>Instrument used to measure flow rates through conduits</td>
</tr>
<tr>
<td><strong>Steady-state porometer</strong></td>
<td>Instrument used to measure rates of gas exchange from leaves</td>
</tr>
<tr>
<td><strong>Transpiration rate (mmol m$^{-2}$ s$^{-1}$)</strong></td>
<td>The flux of water from a leaf</td>
</tr>
<tr>
<td><strong>Turgor loss point (TLP) (MPa)</strong></td>
<td>The water potential at which cell turgor is lost</td>
</tr>
<tr>
<td><strong>Vadose zone</strong></td>
<td>The portion of earth between the land surface and zone of saturation or phreatic zone</td>
</tr>
<tr>
<td><strong>V-PDB</strong></td>
<td>Isotopic standard for carbon</td>
</tr>
<tr>
<td><strong>Volumetric water content ($\theta$) (%)</strong></td>
<td>Soil water content normalised to the bulk density</td>
</tr>
<tr>
<td><strong>V-SMOW</strong></td>
<td>Isotopic standard for hydrogen and oxygen</td>
</tr>
<tr>
<td><strong>Vulnerability curve</strong></td>
<td>Plot of percentage loss hydraulic conductivity versus xylem water potential</td>
</tr>
<tr>
<td><strong>Water Relations</strong></td>
<td>A broad field of plant physiology that encompasses stomatal regulation of transpiration rate and stem-area-specific hydraulic conductivity</td>
</tr>
<tr>
<td><strong>Water Source Partitioning</strong></td>
<td>The use of different water sources by plants</td>
</tr>
<tr>
<td><strong>Water table</strong></td>
<td>The surface between the zone of saturation and the zone of aeration (the vadose zone)</td>
</tr>
<tr>
<td><strong>Water use</strong></td>
<td>The strategies of a plant that optimise productivity within its physiological limitations</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION, RATIONALE AND OBJECTIVES
INTRODUCTION, RATIONALE AND OBJECTIVES

Foreword

The Mediterranean climate of south western Australia has undergone a significant reduction in annual rainfall (Indian Ocean Climate Initiative 2002), perhaps associated with global climate change. The resulting decrease in rainfall recharge of aquifers (Davidson 1995) has been considered a cause of decline in canopy condition of several phreatophytic species of the region (Groom et al. 2000; Zencich et al. 2002). The purpose of this study is to explore the possibility that a similar climate change induced decline in canopy condition has occurred in a large, iconic eucalypt of south western Australia: *Eucalyptus gomphocephala* D. C. (commonly known as Tuart).

This introductory chapter describes the likely effect of a prolonged, climate change induced reduction in annual rainfall on available soil moisture and the associated response of native plant species of south western Australia. Additionally, the chronology of decline in the canopy condition of *E. gomphocephala* (at the Yalgorup National Park study site) is discussed and a rational for experiments undertaken outlined.
1.1 A Linkage between Climate Change and Tree Decline

1.1.1 Climate Change in South Western Australia

Systematic contractions in the geographical distribution of species as a result of climate change will, if migration is impeded, increase the likelihood of extinction (Shoo et al. 2006). Of the changed environmental variables associated with this event, altered rainfall regimes have been suggested to be of most significance to ecosystem dynamics (Weltzin et al. 2003). Widely accepted global circulation models incorporating anthropogenic forcing associated with greenhouse gas emissions (Haughton et al. 2001; IPCC 2001b; IPCC 2007) predict a worldwide increase in rainfall. At a finer scale these same models generally predict an increase in precipitation in the tropics, midlatitudes and high latitudes and a decrease in rainfall in the sublatitudes. The role of altered precipitation on soil moisture represents a central linkage between this element of climate change and ecosystem processes.

Soil scientists have developed good physical models of rainfall interception, infiltration and runoff. However, evapotranspiration and soil water distribution, including the role of hydraulic transport in plants, are much less understood, particularly in relation to different rainfall scenarios. A major constraint is the difficulty in identifying vegetation responses that are clearly linked to long-term shifts in rainfall in natural systems.

Drought-induced dieback of forest has been observed in temperate (Allen and Breshears 1998), tropical (Villalba and Veblen 1998; Suarez et al. 2004), savannah (Fensham and Holman 1999) and Mediterranean ecosystems (Hobbs and Mooney 1995). These dieback events are the visual symptoms of the physiological impact of reduced plant water status. Davis et al. (2002) studying a chaparral community, a northern hemisphere analogue of the vegetation under investigation in this study, showed that prolonged drought-induced branch xylem dysfunction and mortality. In Australia, Rice et al. (2004) observed a similar widespread stem mortality event during an extended drought period in
INTRODUCTION, RATIONALE AND OBJECTIVES

_Eucalyptus crebra_ and _Eucalyptus xanthoclada_, which was attributed to cavitation susceptibility in xylem.

In south western Australia global climate change is thought to have caused a significant (up to 25%) reduction in annual rainfall over the last 30 years (Indian Ocean Climate Initiative 2002). This is consistent with the projections of global scale climate models (Palutikof and Wigley 1996; Haughton et al. 2001; IPCC 2001a; IPCC 2001b; IPCC 2007). Concurrent measures over this period have shown intense El Niño episodes and increasing mean annual maximum atmospheric temperatures. This climatic shift will likely have increased the severity of the annual summer dry season for plants by reducing the yearly contribution of precipitation to soil water\(^1\), reducing rainfall recharge of aquifers and increasing evaporative demand.

1.1.2 Occurrence of Canopy Dieback

Every ecosystem experiences gradual environmental change. Convention holds that such change will invoke a similar gradual transition in the species composition of an ecosystem (Scheffer and Carpenter 2003). However, catastrophic shifts in ecosystem processes can occur abruptly if environmental factors are pushed beyond certain thresholds (Scheffer et al. 2001). If the time frame over which an environmental change has occurred is short there is little opportunity for adaptation or migration, and therefore a higher likelihood that such a change will impact negatively on the ecosystem. Change to the composition and structural complexity of forests due to rapid shifts in climate are an example, with tree decline a frequent and visually conspicuous response.

Canopy dieback\(^2\) is a global phenomenon, with reported occurrences in many different communities (Allen and Breshears 1998; Villalba and Veblen 1998; Fensham and Holman 1999; Lloret et al. 2004; Williams and Cooper 2005; Barrett et al. 2006). In Australia numerous processes have been implicated as the

---

\(^1\) Soil water is defined here as physiologically available water in soil, derived directly from either rainfall or groundwater.

\(^2\) Dieback is defined here as the rapid and progressive defoliation and death of stems which may eventually result in tree mortality.
cause of such events (see Reid and Landsberg (2000) for a comprehensive review). In native Australian eucalypts, some level of canopy dieback is almost ubiquitous; however, the number of eucalypt canopy dieback events seems to have increased in the last 50 years (Kile 1981). While contentious, an escalation in El Niño-induced drought events (Timmerman et al. 1999; Hughes 2003) provides a plausible explanation for this increased frequency. Indeed, such El Niño episodes have been suggested to have broadly influenced the existing composition of Australia’s vegetation (Kershaw et al. 2003).

Despite a well developed understanding of the evolution of Australia’s vegetation under dry conditions, it remains unclear how local eucalypt populations tolerate drought, and whether increased frequency and/or duration of drought episodes will reduce drought tolerance, particularly at the margins of environmental range. All physiological systems fail at some point, including the physiological mechanisms for coping with drought. In south western Australia several species of eucalypt have exhibited the characteristic signs of canopy dieback. In addition to *E. gomphocephala* these include, 1) *E. rudis*, a species associated with riparian and low-lying landscapes, 2) *E. marginata*, a species mostly associated with lateritic soils, and 3) *E. wandoo*, a species that occupies the eastern agricultural belt. All of these species occupy a region of Australia that has been subjected to a rapid and significant decline in annual rainfall.

1.2 A Preliminary Classification of the Eco-Hydrological Niche of *Eucalyptus gomphocephala*

The eco-hydrological niche of a species refers to its environmental range with specific reference to hydrology. Plants in general are capable of exploiting all physical resources available in the biosphere (including groundwater as a water resource). Australian plants are particularly well adapted to exploiting groundwater because of the high probability of seasonal drought or a long-term

---

3 The environmental range of a species is defined as the set of conditions under which its growth and reproduction can occur. Dansereau P (1957) Biogeography: An Ecological Perspective. Ronald Press, New York.
arid climate that keeps upper soil layers very dry (Hatton and Evens 1998). The term phreatophyte was coined to describe the strong association of species with saturated soil (Meinzer 1927; Scott and Le Maitre 1998). Obligate phreatophytes require permanent access to the saturated zone (groundwater) (Smith et al. 1991; Smith et al. 1998; Boulton 2000), while facultative phreatophytes are more opportunistic, being able to survive with roots in drier soil such as the capillary fringe when the water table retreats to beyond their reach, but using the saturated zone when the water table is within reach. Species of the genus *Eucalyptus* are considered opportunistic users of soil water (Bell and Williams 1997). Under the Mediterranean conditions of south western Australia it is considered therefore that, as a probable opportunistic species, *E. gomphocephala* will express a facultative phreatophytic state.

Given that, across locations and stages of maturity, *E. gomphocephala* may access a number of water sources, its capacity for phenotypic plasticity could enable it to survive periodic or chronic tissue water deficit. Changed climatic conditions may alter available soil water directly by decreasing meteoric inputs to the vadose zone or indirectly by inducing a decline in the position of the water table via reduced rainfall recharge of aquifers (Figure 1.1). Variation in plant tissue water relations could reflect access to different soil water sources or the inherent physiological limitations of a given stage of maturity of the species.

There are a number of records of deep root depths in trees from Australian sclerophyll forests similar to those inhabited by *E. gomphocephala*. For example Campion (1926) found *Corymbia calophylla* roots to a depth of 45 m and Dell et al. (1983) discovered *Eucalyptus marginata* roots to a depth of 40 m. In *E. gomphocephala*, roots have been observed to a depth of 30 m (Jasinska et al. 1996). Global studies of mature tree rooting depth have shown consistency, with mean maximum root depth of trees equalling 7.0 ± 1.2 m (Canadell et al. 1996). However, the role of stage of maturity in determining rooting depth has received scant interest. The development of deep roots with maturity in Mediterranean-type habitats may ameliorate the effect of periodic or chronic water deficits, a necessity in some instances for meeting the perennial water requirements of
INTRODUCTION, RATIONALE AND OBJECTIVES

plants (Dawson 1993a). The same process may also allow mature plants to maintain high transpiration rates across seasonal fluctuations and therefore elevated assimilation and growth rates (Dodd and Bell 1993).

Figure 1.1 Diagrammatic representation of the possible difference in *E. gomphocephala* root distribution in relation to landscape position and proximity to the water table (A). This model implies that in some areas a phreatophytic state may exist. Under the scenario of a declining water table (B) phreatophytes may lose contact with the water source.

1.3 Yalgorup National Park: A Centre for *Eucalyptus gomphocephala* Canopy Dieback

Yalgorup National Park (32.90S; 115.69E) incorporates one of the few large continuous populations of *E. gomphocephala* remaining on the Swan Coastal Plain. The area, which consists of 3184 hectares of *E. gomphocephala*-dominated woodland\(^4\), was gazetted for natural heritage in the 1970s. Since this time it has provided a regionally significant recreational area for surrounding residents and visitors.

\(^{4}\) *E. gomphocephala*-dominated woodland is defined here as those plant communities comprising *E. gomphocephala* as a dominant canopy emergent
In the early 1990's considerable public concern was lodged with the Department of Environment and Conservation (DEC), formerly the Department of Conservation and Land Management (CALM), regarding the rapid and spatially conspicuous decline in canopy condition (synonymous with canopy dieback) of *E. gomphocephala* in Yalgorup National Park and its surrounding area (see Figure 1.2 for examples). Canopy dieback in *E. gomphocephala* is not unprecedented (Seddon 1972; Fox and Curry 1980), although it is usually restricted to urban areas (Chilcott 1994). Hence the decline in canopy condition in Yalgorup National Park was initially attributed to natural fluctuations in the larval stage of the Tuart longicorn beetle, the Tuart borer (*Phorocantha impavida*), an insect implicated in the canopy dieback of other *E. gomphocephala* populations. However, the large geographical extent of the Yalgorup National Park infection had not been previously witnessed. Continued decline in canopy condition and a high incidence of mortality of *E. gomphocephala* in this area, which has continued to present day, prompted the suggestion that an underlying factor has predisposed the population to attack from opportunistic sources. Many underlying factors have since been hypothesised, including: 1) altered fire regimes, 2) fungal pathogen infection, 3) altered nutrient regimes, and 4) altered hydrology. This study explores on the water relations properties of *E. gomphocephala* in Yalgorup National Park under the dieback scenario that an altered hydrology has induced or initiated the observed decline.

Any underlying cause of canopy dieback could conceivably increase the likelihood of secondary stresses (such as the Tuart borer infection referenced above). Moreover, it is possible that the relative magnitude of canopy dieback in an individual *E. gomphocephala* tree will be related more to this secondary stress than the suspected underlying climate change-induced perturbation. This prospect could make interpretation of a quantitative assessment of current water

---

5 The larval stage of the Tuart borer (*Phorocantha impavida*) burrows into the meristematic region of trunks and stems where it feeds on photoassimilate and in so doing may girdle plant organs. On approaching a pupae stage the Tuart borer moves deeper into the sapwood of trees, presumably to avoid predators, before emerging as a highly mobile winged adult.

6 *E. gomphocephala* is likely to produce many defence compounds to resist infection from insects and pathogens. The dynamics of resource allocation toward defence compound production under stress in *E. gomphocephala* remains understudied.
stress and associated canopy decline difficult. For this reason it is important to clarify the connectivity between hydrology and climate in Yalgorup National Park in order to emphasise the very real likelihood that climate change has altered available soil water, even if secondary stresses are prominent visual features of the decline in canopy condition.

Figure 1.2 Examples of *E. gomphocephala* canopy decline in Yalgorup National Park. Symptoms range from dieback of terminal branches and associated epicormic resprouting (top two images) to complete mortality (bottom two images).

A comparison of fluctuation in groundwater, derived from an approximate 20 year monitoring program, and the Cumulative Deviation From the Mean (CDFM) of rainfall by Lindsay (2002) showed that groundwater level underlying Yalgorup National park is principally governed by climatic variation (Figure 1.3). This relationship suggests that the regional climatic shift defined by the Indian Ocean Climate Initiative (2002) would alter available soil water by
reducing that water maintained above the zone of saturation and/or by lowering the water table through reduced rainfall recharge.

![Figure 1.3](image)

**Figure 1.3** An example of a comparison of change in groundwater level from a monitoring bore in Yalgorup National Park (line demarked with symbols) and the CDFM rainfall model (unbroken line) over the same period. Note that after an initial training period the bore hydrograph data and rainfall model oscillate (and decline) in synergy. Adapted from Lindsay (2002).

### 1.4 Soil Water and Plant Water Relations

Plants form a bridge in a water potential ($\Psi$) gradient between the soil and the atmosphere. In this sense, the magnitude of the soil water potential ($\Psi_{\text{soil}}$) in the vicinity of the active root zone largely determines the minimum plant water potential ($\Psi_{\text{plant}}$) necessary for water uptake. Changes in soil hydrology, and hence $\Psi_{\text{soil}}$, can invoke physiological responses such as stomatal closure and osmotic adjustment to offset the deleterious effects of plant water stress. However, if these adaptations prove ineffective under excessive soil water deficit, plant health and survival can be threatened. The distribution and accessibility of soil water therefore greatly influences plant growth and community structure, that is, the mixture of plant species present and their growth form. For plants growing together in such natural communities, differential use of water resources, and hence expression of different root
morphologies, has been shown across different growth forms (Caldwell and Richards 1986; Dawson and Ehleringer 1991; Jackson et al. 1995; Canadell et al. 1996) and within similar growth forms (Dawson 1996; Meinzer et al. 1999; Stratton et al. 2000). The expression of a particular root morphology may be an artifact of water availability during development rather than water distribution per se (Dawson and Ehleringer 1991; Ehleringer et al. 1991; Flanagan et al. 1992; Mensforth et al. 1994; Goldstein et al. 1998). For example, when phreatophytic banksias that have developed in close contact with groundwater are separated from this water source as a result of a fall in the water table, severe water stress can be induced (Zencich et al. 2002). The emerging trend from such water use studies is that members of the same species that have developed under a more xeric hydrological regime appear less susceptible to changes in soil water.

Plant water acquisition strategies have been determined in a number of habitats for a range of species using stable isotopes of hydrogen and/or oxygen in water (Ehleringer et al. 1991; Flanagan et al. 1992; Jackson et al. 1995; Dawson 1996; Bleby et al. 1997; Kolb et al. 1997; Alstad et al. 1999; Gebauer and Ehleringer 2000; Stratton et al. 2000; Zencich et al. 2002; Drake and Franks 2003). These studies have shown that spatial and temporal changes in hydrology will influence the type of vegetation that an ecosystem can support and the state of health of such vegetation. The mixture of plant growth forms that occur in natural plant communities typically comprises a range of properties associated with water acquisition and use, such as hydraulic architecture, rooting depth and stomatal function. However, little is known about the response of these properties to changes to the hydrology of the environment they occupy.

Reduced rainfall in southwestern Australia and, specifically, its influence on the underlying hydrology of Yalgorup National Park could be a major factor in the canopy dieback of local *E. gomphocephala* populations. The physiological basis for the dieback could be failure of the plant hydraulic and photosynthetic systems when tissue water deficits exceed critical limits. By studying the water relations of *E. gomphocephala* in response to fluctuations in its hydrological environment

---

*7* Hydraulic architecture is defined as the structure of the plant water conductive system.
insight may be gained into the impact of reduced rainfall on the current and future health of *E. gomphocephala* populations.

### 1.5 Objectives

The overarching goal of this study is to explore the possible linkage between a climate change induced shift in annual rainfall and canopy dieback in *E. gomphocephala* populations in Yalgorup National Park, a region that has suffered extensive canopy dieback. The specific objectives were to address the following research questions:

1) How do the water relations and hydraulic architecture of *E. gomphocephala* vary with proximity to groundwater and season (Chapter 3)?

2) What are the water sources (groundwater, subsurface soil water and surface water) of *E. gomphocephala* and do these sources change with stage of maturity, proximity to groundwater and season (Chapter 4)?

3) Does vulnerability to drought-induced xylem embolism differ between *E. gomphocephala* and co-occurring species (Chapter 5)?

4) Are root anatomical features related to xylem hydraulic properties in *E. gomphocephala* (Chapter 6)?

5) Are plant physiological variables, that is, leaf water potential, leaf transpiration rate, stomatal conductance to water vapour, carbon isotope discrimination of leaves and the leaf mass ratio, related to the degree of canopy dieback in *E. gomphocephala* (Chapter 7)?

6) How do *E. gomphocephala* seedlings respond to development under a suboptimal soil moisture environment (Chapter 8)?

7) How does seedling development on an ash-bed influence leaf physiology (Chapter 9)

8) Is there an association between the canopy dieback in *E. gomphocephala* populations in Yalgorup National Park and changed soil moisture (Chapter 10)?
INTRODUCTION, RATIONALE AND OBJECTIVES

Note that Chapter 9 represents an objective derived from opportunistic access to an experimental fire in Yalgorup National Park (refer to Chapter 9 for a rationale for the subsequent experiment).

Figure 1.4 describes the association between the physiological properties of *E. gomphocephala* and the abiotic environment of Yalgorup national Park. At the same time this diagram shows the linkages across chapters of this thesis.

---

Figure 1.1 A thesis outline (presented as a flow chart) relating aspects of the biotic and abiotic environment of *E. gomphocephala*. In this model climate change would invoke a decline in *E. gomphocephala* canopy condition by its influence on available soil water and associated physiological impact. The relative severity of this shift in available soil water is likely to depend on proximity to groundwater and tree size class (synonymous with stage of maturity). This thesis investigates several aspects of plant water relations, hydraulic architecture and water source partitioning. These are considered in the context of: 1) landscape position (Chapter 3), 2) water sources and stage of maturity (Chapter 4), 3) inter-specific variation (Chapter 5), 4) rooting properties (Chapter 6), 5) canopy condition (Chapter 7), and 6) development under water deficit (Chapter 8).
CHAPTER 2

A DESCRIPTION OF THE STUDY SPECIES AND REGION
2.1 Background

2.1.1 A Description of the Study Species

_Eucalyptus gomphocephala_ D. C., commonly known as ‘Tuart’ from the indigenous Nyoongar term for the species – tooart, is a large evergreen Myrtaceous tree endemic to the Swan Coastal Plain of south western Australia, extending from Jurien Bay (30.2°S) in the north to the Sabina River (33.7°S) near Busselton in the south (Figure 2.1) (Gardiner 1966; Powell 1990). _Eucalyptus gomphocephala_ exhibits a strong degree of endemism, a relatively common feature of the genus _Eucalyptus_. While such endemism is often considered to relate to taxonomic divergence, in _E. gomphocephala_ it is more likely to be coupled to an affinity for a regionally specific soil type.

The natural geographical distribution of _E. gomphocephala_ is strongly associated with local edaphic units underlain by limestone, specifically the Spearwood and Quindalup dune systems (and the Cottesloe and Karrakatta soil units) (Gibson et al. 1994). Indeed, _E. gomphocephala_ is one of the few eucalypts to have adapted to these calcareous alkaline soils. This distribution also includes the largest population centre in south western Australia, Perth, and the allied urban expansion synonymous with most Australian cities. As a result of this close proximity to a relatively high population density, the geographical distribution of _E. gomphocephala_ has been fragmented into a mosaic of isolated populations. Hopkins et al. (1996) estimates that around 65% of _E. gomphocephala_-dominated woodland has been cleared since European settlement, representing an area of approximately 72800 ha.
A DESCRIPTION OF THE STUDY SPECIES AND REGION

Yalgorup National Park; refer to Figure 1.5 for more detail.

Figure 2.1 The geographical distribution of *E. gomphocephala* prior to European settlement. Overlaid are mean annual rainfall isohyets. Data were supplied by the Bureau of Meteorology and the Department of Conservation and Land Management (Sheperd 2003). Datum – GDA 94.
2.1.2 The Price of Clearing

Quantifying the loss of habitat attached to the wholesale clearing and altered disturbance regime of *E. gomphocephala*-dominated woodland is practically impossible. However, the conservation value of existing *E. gomphocephala*-dominated woodland has been assessed. In a study that characterised the diversity of vertebrate fauna in such ecosystems Dell et al. (2002) noted high species richness; specifically 92 species of avifauna, 16 mammals 43 reptiles and seven frogs. The same authors noted that many previously recorded vertebrate fauna of the Swan Coastal Plain have vanished. While direct measures of invertebrate diversity in *E. gomphocephala*-dominated woodland are scant, it is noteworthy that the numerous karst cave systems, derived from the dissolution of soluble rock substrate, underlying such plant communities on the Swan Coastal Plain have been reported to contain the greatest number of animal species ever recorded in aquatic cave habitats (Jasinska et al. 1996), most of which are invertebrates. *Eucalyptus gomphocephala* roots penetrate these caves and, when in contact with the water table, form root mats which provide habitat for stygofauna, defined as fauna that live within groundwater systems. Given that the remnant *E. gomphocephala*-dominated woodland is of high conservation value, it is necessary to understand the historical and current threats to its survival.

The greater area of *E. gomphocephala*-dominated woodland was periodically cleared for agriculture, mining and timber production. *Eucalyptus gomphocephala* trees were harvested commercially until about 1930. *Eucalyptus gomphocephala* timber is dense and resistant to decay, making it desirable for enduring structural purposes such as fence posts and railroad sleepers. Furthermore, *E. gomphocephala*-dominated woodland has been displaced in some areas for plantation forestry, particularly maritime pine (*Pinus pinaster*). The current high rate of residential expansion within the natural distribution of *E. gomphocephala* has seen further reductions in the geographical area and species composition of *E. gomphocephala*-dominated woodland.
2.1.3 Plant Community Structure

_Eucalyptus gomphocephala_ occurs as a dominant medium to tall tree in tall woodland, as a smaller tree in heath and also as a mallee in some locations. _Eucalyptus gomphocephala_ co-occurs with 13 tree species (Gibson et al. 2002) (Table 1) and overall is associated with 575 vascular plants, 72 % of which are native (Keighery et al. 2002). These taxa comprise 9 major families.

Coates et al. (2002) suggest that _E. gomphocephala_ shows little morphological variation throughout its range apart from the aforementioned mallee form, which occurs on the Quindalup dune system, and an unusual form with a red as opposed to yellow heartwood occurring on the Swan River. This apparent low variation has been characterised, strictly speaking, as gross morphology and therefore does not account for less obvious indications of morphological plasticity such as xylem architecture or leaf phenology.

Table 2.1 Tree species associated with _Eucalyptus gomphocephala_ (taken from Gibson et al. (2002)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agonis flexuosa</em></td>
<td>MYRTACEAE</td>
</tr>
<tr>
<td><em>Allocasuarina fraseriana</em></td>
<td>CASUARINACEAE</td>
</tr>
<tr>
<td><em>Banksia attenuata</em></td>
<td>PROTEACEAE</td>
</tr>
<tr>
<td><em>Banksia grandis</em></td>
<td>PROTEACEAE</td>
</tr>
<tr>
<td><em>Banksia littoralis</em></td>
<td>PROTEACEAE</td>
</tr>
<tr>
<td><em>Banksia menziesii</em></td>
<td>PROTEACEAE</td>
</tr>
<tr>
<td><em>Banksia prionotes</em></td>
<td>PROTEACEAE</td>
</tr>
<tr>
<td><em>Callitris preissii</em></td>
<td>CUPRESSACEAE</td>
</tr>
<tr>
<td><em>Corymbia calophylla</em></td>
<td>MYRTACEAE</td>
</tr>
<tr>
<td><em>Eucalyptus marginata</em></td>
<td>MYRTACEAE</td>
</tr>
<tr>
<td><em>Eucalyptus rudis</em></td>
<td>MYRTACEAE</td>
</tr>
<tr>
<td><em>Melaleuca rhapsiphylla</em></td>
<td>MYRTACEAE</td>
</tr>
<tr>
<td><em>Nyutsia floribunda</em></td>
<td>LORANTHACEAE</td>
</tr>
</tbody>
</table>
2.2 Climatic Trends in the Study Area

As part of the Swan Coastal Plain, Yalgorup National Park is considered to have a Mediterranean-type climate with hot dry summers and cool wet winters (Gentilli 1972). For the study area, the hottest and driest conditions are experienced from December to February (Figure 2.2). While the mean annual rainfall for Yalgorup National Park is 875 mm, only 4% falls during this summer period when mean maximum air temperature is 29°C (Australian Bureau of Meteorology, 2006). Air temperature and vapour pressure deficit can exceed 39°C and 4 kPa respectively during the summer dry period. While the distribution of *E. gomphocephala* spans a rainfall gradient of approximately 700 mm to 850 mm (Figure 2.1), the major canopy dieback event is located toward the higher end of this rainfall zone. This precludes use of trees growing in marginal regions of the geographical distribution of *E. gomphocephala* since major canopy dieback is not evident at such locations.

![Figure 2.2 Mean monthly rainfall and average maximum monthly temperature in the vicinity of the study (Mandurah 32.52°S; 115.75°E) as a representative of the overall climatic pattern of southwestern Australia. Data represent the mean of 117 years (Australian Bureau of Meteorology, 2006).](image-url)
Prior to the mid 1970’s, south western Australia was known for its reliable climate. This would typically comprise a wet winter that would replenish reservoirs, superficial aquifers and wetlands after the annual summer drying phase. In the mid 1970’s south western Australia shifted abruptly into a drier phase (Figure 2.3) (Indian Ocean Climate Initiative 2002). The declining trend in rainfall in south western Australia, depicted graphically in Figure 2.3, can be described by a significant convergent linear model (Annual rainfall (mm) = 2901.27 - 1.06 × year; P = 0.001). However, to illustrate the sharp drop in rainfall, horizontal lines representing the mean of annual rainfall for the period 1876-1975 and 1976-2002 have been included in Figure 2.3.

At some locations in south western Australia, the magnitude of this climatic shift is in the order of a 25% decrease in winter rainfall (Indian Ocean Climate Initiative 2002). It is disturbing that this shift, coinciding with a rising mean maximum atmospheric temperature (Figure 2.4), imitates the increase in atmospheric pressure and decrease in rainfall predicted by global climate change. However, regional scale research suggests that multi-decadal changes in climate of the magnitude experienced in the 1970’s could be at least partly natural (Cai and Shi 2005; Cai and Cowwan 2006). While the exact physical origin of, what could be described as, the ‘stepped-change’ in rainfall remains to be determined with certainty, Indian Ocean Climate Initiative (2002) quantitatively related it to large scale changes in atmospheric circulation. The implications of this drying phase are likely to be particularly detrimental to phreatophytic ecosystems, reliant on very wet years for surface water and groundwater recharge.
Figure 2.3  The average annual rainfall for Perth (32.92°S; 115.87°E) has shown a stepped-change since the mid 1970’s. In graph A horizontal lines depicting the average rainfall between the period 1876-1975 and 1976-2002 are included. Perth 1, Perth 2 and Perth Airport represent relocations of Perth’s monitoring station. In graph B data for Perth 1 and Perth 2 are reproduced as a percentage deviation of the mean annual rainfall over the period 1876-1975 and overlaid with a 10 year running mean. Data obtained from the Australian Bureau of Meteorology.
Figure 2.4 Mean maximum annual temperature for Perth (32.92°S; 115.87°E) over the period 1876-2003. Monitoring stations are as described in Figure 2.3. Data supplied by the Australian Bureau of Meteorology.

2.3 Hydrology of Yalgorup National Park

2.3.1 Defining Below Ground Wet Bodies in Yalgorup National Park

To avoid confusion between the terms water table, capillary fringe, groundwater and aquifer each will be defined here in preparation for reference throughout this thesis (Figure 2.5). Terminology is that adopted by the American Geological Institute (Bates and Jackson 1984):

1) Groundwater: That part of the sub-surface water that is in the zone of saturation, including underground streams.

2) Aquifer: A body of rock or sand that is sufficiently permeable to conduct groundwater. Usually regarded as yielding economically significant quantities of water to wells and springs.
3) Water table: The surface between the zone of saturation and the zone of aeration.
4) Capillary Fringe: The lowest subdivision of the zone of aeration immediately above the water table in which the interstices are filled with water under pressure less than that of the atmosphere, being continuous with the water below the water table but held above it by surface tension.

While a large reduction in rainfall is not universal across the entire south west of Australia, the declining trend is obvious. This trend will, at some point, be paralleled by a general decline in groundwater (often reflected by a vertical draw-down in the position of the water table) and water in the unsaturated zone. An exploration into the linkage between possible changes in groundwater accessibility and canopy dieback in Yalgorup National Park requires an understanding of the local geology and hydrology.

![Subsurface Profile of Yalgorup National Park](image)

Figure 2.5 A schematic diagram of the subsurface profile of Yalgorup National Park representing the below ground wet bodies that occur.

Yalgorup National Park is part of a well preserved coastal geological feature with origins in the late Pleistocene. Yalgorup National Park is situated on the Spearwood Dune System, comprising the Yalgorup Plain, which consists of well-drained sediments of Pleistocene to Holocene origin at the surface, superimposed on a range of fossiliferous limestones, aeolian limestones and quartz sand units (Semeniuk 1997). At the eastern margin a ridge of aeolian limestone and quartz is present and to the west a higher Holocene coastal aeolian ridge or Holocene estuary deposit occurs.
A DESCRIPTION OF THE STUDY SPECIES AND REGION

(Semeniuk 1997). A series of three parallel lake systems is present in Yalgorup National Park (Figure 2.6). These are the swales of ancient dune systems (derived from beach ridge progradation and karst formation) and, in addition to direct accession, are supplied by the inflow of groundwater. No outflow occurs from these regional groundwater sinks, which are hypersaline as a result of evaporation from their surface and interactions with underlying bodies of hypersaline groundwater (Figure 2.7). The freshwater groundwater underlying the park is, in effect, a series of superficial aquifers of varying depths that are underlain by hypersaline groundwater. The interface between the fresh and hypersaline groundwater is well defined and comprises a thin mixed zone (Commander 1988). The water table generally resides close to sea level except in late summer. The superficial aquifer formations are hydraulically connected, effectively creating an unconfined aquifer stretching westward from the Darling Scarp to the coast (Deeny, 1989). Of the three flow systems (incorporated into superficial formations) identified in the region, only Myalup discharges directly into the Yalgorup National Park Lakes.
Figure 2.6 Remnant *E. gomphocephala* (Tuart) woodland in Yalgorup National Park. The park is comprised of three parallel lake systems. These lakes form groundwater sinks for the regional freshwater superficial aquifers. They are made hypersaline by evaporation from their surface and interactions with underlying hypersaline groundwater. The three sites indicated are presented here in preparation for reference below and in subsequent chapters. Datum – GDA 94.
A description of the study species and region

Figure 2.7  A diagrammatic representation of the groundwater flow systems in Yalgorup National Park. Rainfall in the catchment contributes to fresh groundwater flow into lakes which are subsequently transformed into a hypersaline state due to evaporation from their surface and interaction with underlying hypersaline groundwater. The vertical scale (in metres) portrays the approximate distance from sea level to various hydrological features of Yalgorup National Park. Adapted from Commander (1988). An estimate of the distribution of *E. gomphicephala* in this system is indicated by the images of trees.

The calcareous origin of soils and rocks of Yalgorup National Park create a highly alkaline substrate. Figure 2.8B shows such high alkalinitities in three pH profiles from soil cores obtained from Yalgorup National Park (sites are those indicated in Figure 2.6). Referring forward to Chapter 3, these sites were also used for periodic measurement of plant water relations properties during this study. The same soil profiles had low electrical conductivities (although groundwater electrical conductivity was substantially higher than soil) (Figure 2.8A).
A DESCRIPTION OF THE STUDY SPECIES AND REGION

The typical stratigraphy of soil profiles underlying Yalgorup National Park are depicted in Figure 2.9. The profiles represent the sites described in Figure 2.6. These can be broadly described as deep sand inter-dispersed with limestone fragments, which (through the action of percolating rainfall) have also formed solid limestone in some layers.

Groundwater samples were collected from purged peizometers. Soil cores were obtained with a push-core drill rig. Samples (groundwater and soil) were refrigerated prior to analysis in the laboratory. Soil sub-samples (n = 3 per depth increment) were taken at periodic intervals, diluted in distilled water (5 parts water; 1 part soil) and agitated for 10 minutes. Steady-state pH and conductivity of the soil and groundwater were then measured with a pH and conductivity meter. This methodology was adopted from Dewis J. Freitas F (1970) Physical and chemical methods of soil and water analysis. FAO soils bulletin, Rome.

Figure 2.8 Vertical profiles of soil conductivity (A) and pH (B) from cores collected during summer 2004 from Yalgorup National Park. Depth to groundwater from the natural surface was 2,30 m, 8,04 m and 21,73 m for sites 1, 2, and 3 respectively. The vertical lines dissociated from data points represent the values for groundwater at the respective sites. The inset in A incorporates both groundwater and soil electrical conductivity (note the change of scale). The differing salinity of soil and groundwater is typical of a system where leached salts from soil accompany groundwater recharge.
Figure 2.9  A description of the soil profiles of three sites in Yalgorup National Park. The arrows indicate the approximate position of the water table.
Because of the connectivity between the superficial aquifers and the lakes of Yalgorup National park, changes in the position of the water table are likely to be reflected by lake water levels. The monitored water levels in Lake Clifton, for example, exhibited a drop that coincided with a 0.8 m fall in the local water table between 1991 and 1994 (Longman and Keighery 2002). Despite an apparent hydrological linkage with climate, no studies have investigated the possibility that native plant species of Yalgorup National Park are experiencing deleterious water stress as a result of reduced rainfall recharge.

2.4 Summary

*Eucalyptus gomphocephala*-dominated woodland is of high conservation value. The stepped reduction in rainfall across south western Australia could provoke water stress and potentially mortality in *E. gomphocephala* trees by reducing available water in the vadose zone and/or by lowering water tables. Underlying Yalgorup National Park, the focus of this study and a centre for *E. gomphocephala* canopy dieback, a series of superficial freshwater aquifers occur. The vertical movement of the water table in this system is tightly coupled to rainfall. Hence a plant species that: 1) is unable to endure the increasingly severe summer drought conditions of the vadose zone, and 2) fails to deploy roots into a receding capillary fringe, would be likely to show symptoms of drought-induced canopy dieback.
CHAPTER 3

DROUGHT TRAITS OF *EUCALYPTUS GOMPHOCEPHALA*
ACROSS THE LANDSCAPE OF YALGORUP NATIONAL PARK

Elements of this chapter are reproduced in Plant, Cell and Environment as Franks, Drake and Froend in acknowledgment of Peter Franks' interpretation of the data. See appendix 4 for an abstract for this manuscript.
Summary

The hydrological environment of *Eucalyptus gomphocephala*, while well characterised from a hydrogeological perspective, remains understudied in the context of the traits that underpin a tolerance to tissue dehydration. This chapter focuses on this knowledge gap in order to explore the possible mechanisms of drought intolerance in this species. Over two successive wet-dry seasonal cycles three *E. gomphocephala* populations, located at various positions in the landscape, exhibited a seasonal oscillation in pre-dawn and midday leaf water potential ($\Psi_{pd}$ and $\Psi_{md}$ respectively), estimated leaf transpiration rate ($E^*$) and stomatal conductance to water vapour ($g_s$). The amplitude of this oscillation depended on the proximity of the water table from the natural surface. This dependency was reflected by the ability of trees growing low in the landscape to express a lower turgor loss point ($\Psi_{TL}$) and leaf water use-efficiency (quantified as a higher leaf carbon isotope discrimination, $\Delta_{\text{leaf}}$). Over the study period native leaf water potential did not fall below $\Psi_{TL}$ but it is likely that substantial xylem embolisms occurred during the hot dry conditions of summer. Using landscape position as a proxy for eco-hydrological niche, it is concluded that the current hydrological habitat of Yalgorup National Park is conducive to the survival of *E. gomphocephala*. 
Figure 3.1 The thesis outline highlighting the relevant processes covered in this chapter. Root zone water potential is determined partly by proximity to groundwater. Proximity to groundwater varies spatially due to topography and geology, and temporally due to seasonal rainfall and climatic variability. Root zone water potential, hydraulic conductance and transpiration rate are all integral to canopy water potential, which ultimately determines canopy condition. This chapter quantifies and integrates these characteristics in order to assess the impact of future climate scenarios on E. gomphocephala populations in Yalgorup National Park.
3.1 Introduction

In the last 30 years annual rainfall in south western Australia has fallen approximately 25% below the previous 100 year annual mean (Indian Ocean Climate Initiative 2002). This reduced rainfall recharge has caused water tables to recede in a number of superficial aquifers (Davidson 1995). Such changes in hydrology have been implicated in canopy dieback and tree mortality in populations of several phreatophytic tree species of south western Australia (Groom et al. 2000; Zencich et al. 2002). Once the water table recedes below the roots of phreatophytic species, effectively decoupling them from the water saturated zone, substantial reductions in tree water potential ($\Psi_{\text{plant}}$) are likely. This will enhance the likelihood of cavitation, a break in liquid continuity in the xylem water column due to excessive tension (Zimmerman 1983), with an ensuing loss of xylem hydraulic conductivity due to subsequent formation of air embolisms in the xylem conduits (Tyree and Sperry 1989). Excessive cavitation and xylem embolisms may ultimately cause plant mortality due to failure of the xylem to maintain adequate water to the canopy (Zimmerman 1983; Tyree and Sperry 1988; Davis et al. 2002). Resistance to cavitation is therefore an important aspect of drought tolerance (Tyree and Sperry 1989) or, in this case, the capacity to withstand drought in combination with a possible change in groundwater hydrology due to reduced rainfall. Failure of drought resistance systems, comprising aspects of both drought avoidance and drought tolerance, at the species level could cause a compositional shift in plant community structure.

Studies of water acquisition by plants, as revealed by analyses of natural variation in stable isotopes of hydrogen and/or oxygen, suggest that community structure is dependent, in part, on soil or landscape hydrology (Ehleringer et al. 1991; Flanagan et al. 1992; Jackson et al. 1995; Dawson 1996; Stratton et al. 2000). In seasonally dry environments tolerance to drought will typically comprise a range of physiological properties associated with rooting depth, hydraulic architecture (conductivity, embolism susceptibility) and stomatal regulation of transpiration rate. There has been increased focus on the function and coordination of these properties within and between species (Brodribb and Feild 2000; Clearwater and Meinzer 2001; Meinzer
DROUGHT TRAITS OF *E. GOMPHOCEPHALA*

2002; Brodribb et al. 2003; Bucci et al. 2004; Domec et al. 2004; Meinzer et al. 2004; Domec et al. 2006b; Renninger et al. 2007) but few studies have considered the significance of these properties and their variability under contrasting hydrologies. An intuitive example of the importance of hydrology in dry habitats would be that an individual developed in close proximity to groundwater expressing a high transpiration rate would prioritise hydraulic efficiency over a more conservative architecture since the severity of seasonality will be buffered at such locations.

This chapter examines the interaction between landscape hydrology and water stress in three *Eucalyptus gomphocephala* populations in Yalgorup National Park, with a view to gain insight into the role of water stress in currently observed canopy dieback. The populations studied occur on a superficial aquifer system that is considered to be tightly coupled to rainfall (Lindsay 2002). In light of the stepped change in rainfall since the 1970’s, it is possible that the combination of reduced soil water in the unsaturated zone and a drop in the water table may have caused a breach to the limits of drought tolerance in *E. gomphocephala*. The hypothesis here is that the seasonal change in plant traits that confer exposure to tissue water stress will vary with landscape position. The objective of this chapter is to study this plasticity in drought tolerance to help define the eco-hydrological niche of *E. gomphocephala* in Yalgorup National Park.

### 3.2 Material and Methods

#### 3.2.1 Field Sites and Plant Material

Three sites from within Yalgorup National Park were selected with varying average depths to the water table (Site 1: 2.30 ± 0.09 m, Site 2: 8.04 ± 0.02 m and Site 3: 21.73 ± 0.03 m, n = 16 for each site over a 20 month period). The three sites were in the boundaries of Yalgorup National Park and were within a distance of 20 km of one another (refer to Figure 2.6 for exact locations). Twelve individual *E. gomphocephala* trees from each site were monitored periodically for water relations properties over two successive wet-dry seasonal cycles.
3.2.2 Leaf Water Potential

Pre-dawn (04:00-06:30 local standard time) and midday (11:30-14:00 local standard time) leaf water potentials ($\Psi_{pd}$ and $\Psi_{mm}$ respectively) were determined approximately monthly with a Scholander–type pressure chamber (model 3005, Soil Moisture Equipment, Santa Barbara, California) on each tree ($n = 12$ trees per site) (Scholander et al. 1964). Sources of error associated with measurement of water potential via the pressure chamber technique, as outlined in Richie and Hinkley (1975), were minimised. Measurements were undertaken on the same day to reduce the potential for time lags (Goldstein et al. 1998). Leaf samples, obtained from the upper canopy, were kept in snap-lock plastic bags (petiole protruding) throughout the procedure to minimize transpirational water loss during measurement. Access to the upper canopy required use of an extension ladder and long-handled secateurs in some cases. Subsequent physiological measurements were obtained from material in the same region of the canopy.

3.2.3 Leaf Gas Exchange

Stomatal conductance to water vapour ($g_s$, mmol m$^{-2}$ s$^{-1}$) and an estimate of transpiration rate ($E^*$, mmol m$^{-2}$ s$^{-1}$) were measured approximately monthly from each individual tree ($n = 12$ trees per site) under natural conditions at midday (11:30-14:00 local standard time) from sun-lit leaves with a steady-state null balance porometer (model Li 1600, Li-cor Inc Lincoln Nebraska). Measurements were undertaken on the same day as leaf water potential determination using the abaxial surface of leaves (adaxial surfaces had much lower stomatal conductances). Because the steady-state porometer measures total leaf conductance ($g_l$), a correction for boundary layer conductance ($g_b$) was undertaken. Boundary layer conductance ($g_b$), which was kept constant in the leaf chamber at 0.067 m s$^{-1}$, was converted to molar units via:

$$g_b\ (\text{mmol m}^{-2}\ \text{s}^{-1}) = \frac{g_{s}^{*}}{RT} \tag{3.1}$$
where \( \rho \) (Pa) is the air density, \( g_b^* \) is the boundary layer conductance (in m s\(^{-1}\)), \( R \) is the gas constant and \( T \) is the temperature (°K). Stomatal conductance to water vapour was then calculated as:

\[
g_s (\text{mmol m}^{-2} \text{s}^{-1}) = \frac{g_b g_i}{g_b - g_i}
\]  

(3.2)

3.2.4 Carbon Isotope Discrimination

Newly expanded canopy leaves were collected from each tree during early summer (2004) for determination of carbon isotope composition (\( n = 5 \) leaves for each of the 12 sampled trees of each site). Leaves were oven dried at 60°C for 48 hours and finely ground with a ball mill. \(^{13}\)C to \(^{12}\)C ratios were measured by means of a continuous flow mass spectrometer (PDZ Europa Model 20-20 Cheshire, UK). The carbon isotope composition of leaves (\( \delta^{13}\text{C}_{\text{leaf}} \)) was calculated as:

\[
\delta^{13}\text{C}_{\text{leaf}}(\%o) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

(3.3)

where, \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the \(^{13}\)C/\(^{12}\)C ratios of the leaf sample and the V-PDB standard, respectively. \( \delta^{13}\text{C}_{\text{leaf}} \) was then converted to leaf carbon isotope discrimination (\( \Delta \)) according to Farquhar and Richards (1984):

\[
\Delta_{\text{leaf}}(\%o) = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{leaf}}}{1 + \delta^{13}\text{C}_{\text{leaf}}}
\]

(3.4)

where \( \delta^{13}\text{C}_{\text{air}} \) was taken as 7.8‰. The advantage of using \( \Delta \) rather than \( \delta^{13}\text{C} \) is that it incorporates the isotopic composition of both the source (the atmosphere) and the product (leaf biomass) (Farquhar and Richards 1984).

3.2.5 Pressure Volume Curves

Pressure volume curves were constructed from leaves collected from each tree (\( n = 12 \) trees for each site) according to Tyree and Hammel (1972). Leaves were sampled
prior to sunrise during early summer, when $\Psi_{\text{p}}$ was high. From the subsequent plots of the inverse of water potential ($1/\Psi$) and relative water content (RWC), the osmotic pressure at full turgor ($\pi_i$) and the water potential at the point of turgor loss ($\Psi_{\text{TL}}$) were determined. Specifically, the osmotic potential at full turgor was calculated as the y-intercept of linear models fitted to the linear component of the relationship between $1/\Psi$ and RWC for each leaf. The turgor loss point was taken as the water potential just prior to where the linear model departed from the actual data set of each leaf. Refer forward to the Results section for actual pressure volume curves.

3.2.6 Xylem Hydraulic Properties

Prior to sunrise intact branches approximately (0.3 - 0.4 m in length) were collected from the same 12 individuals of each site that were sampled for leaf water potential and leaf gas exchange properties. Collection took place during winter and early spring to ensure that branches were close to full hydration. Branches were double bagged, humidified and immediately transported to the laboratory.

Vulnerability to xylem embolism was determined as the relationship between percentage loss hydraulic conductivity (PLC) and xylem water potential ($\Psi_x$) (Sperry and Tyree 1988). Briefly, branches were removed from bags and allowed to dehydrate under laboratory conditions ($20 \pm 3^\circ\text{C}; 50 \pm 10\% \text{ RH}$) to induce a given $\Psi_x$ (Sperry et al. 1987). The time frame for this process was 0 to 5 days. Twelve hours prior to the determination of $\Psi_x$ and PLC, each branch was sealed in a plastic bag to equilibrate water potential ($\Psi$) across all organs (leaves, petioles and stems). The xylem water potential of a given branch was then determined from measurements of leaf water potential ($n = 3$ leaves for each branch) with a Scholander-type pressure chamber. A stem segment was then excised under distilled water for subsequent measurement of PLC. Segments were at least 10% longer than the longest measured vessel and 0.005 - 0.01 m in diameter. Vessel length was measured by passing gaseous nitrogen, at a pressure of 5 kPa, through a stem and cutting sections back from the distal end until gas flow was detected. PLC was determined with a steady state flow meter (SSFM) filled with a perfusion solution, 0.01 M KCl and degassed double distilled water (Zimmerman 1978):
DROUGHT TRAITS OF *E. GOMPHOCEPHALA*

\[
\text{PLC} = 100 \left( \frac{k_{\text{max}} - k_h}{k_{\text{max}}} \right) \quad (3.5)
\]

where, \( k_h \) is the stem hydraulic conductivity \((\text{kg}_{\text{water}} \, \text{s}^{-1} \, \text{m}^{-2} \, \text{MPa}^{-1})\) at \( \Psi_x \) and \( k_{\text{max}} \) is the maximum stem hydraulic conductivity after flushing segments with the perfusion solution for 15 minutes at 100 kPa. Fifteen minutes of flushing was deemed appropriate since further flushing did not increase hydraulic conductivity. Briefly, the SSFM was similar to that used by Feild et al. (2001), incorporating the pressure drop across a section Peek™ tubing \((\text{length} = 0.407 \, \text{m}, \text{inside diameter} = 1.96 \times 10^{-7} \, \text{m}^2)\) with a known conductance connected in series with the excised stem segment.

"Vulnerability curves" were generated for each species as plots of PLC versus \( \Psi_x \). Data were then fitted with exponential-sigmoidal functions:

\[
\text{PLC} = \frac{100}{1 + \exp\left[a(\Psi_x - b)\right]} \quad (3.6)
\]

where \( a \) is the gradient of a linear transformation and \( b \) is \( \Psi_x \) where PLC = 50 (Pammenter and Vanderwilligen 1998).

Using the same apparatus as described above, hydraulic conductivity of stem xylem, normalised to stem cross-sectional area \((K_s; \text{in kg s}^{-1} \, \text{m}^{-1} \, \text{MPa}^{-1})\), was measured in stem segments according to the general principle of Zimmerman (1978). \( K_s \) is defined by:

\[
K_s = J_v \times \frac{\Delta l}{\Delta P} \quad (3.7)
\]

where \( J_v \) \((\text{kg}_{\text{water}} \, \text{s}^{-1} \, \text{m}^{-2} \, \text{stem})\) is the mass flux density of the perfusion solution \((0.01 \, \text{M KCl in degassed, double distilled water)}\), \( \Delta l \) is the length of the stem segment \((\text{m})\) and \( \Delta P \) is the water pressure difference along the length of stem segment \((\text{MPa})\). Instantaneous branch \( K_s \) (incorporating the effects of any native embolism) was monitored in trees on a monthly basis throughout seasonal wet and dry cycles. Measurements were obtained from material sampled pre-dawn to assess maximum daily \( K_s \). Prior to sunrise intact branches \((\text{approximately} \, 0.3 - 0.4 \, \text{m in length})\) were
collected from the same 12 trees at each site \((n = 12)\) that were sampled for leaf water potential and leaf gas exchange properties. Sampled branches were double-bagged in plastic and returned to the laboratory where they were re-cut to their final length \((0.05 - 0.17 \text{ m})\) while immersed in distilled degassed water. Segments were at least 10% longer than the longest measured vessel and \(0.005 - 0.01 \text{ m}\) in diameter.

### 3.3 Results

Both \(\Psi_{pd}\) and \(\Psi_{md}\) varied with season, exhibiting more negative values with the onset of dry conditions (where less precipitation occurred) in both 2004 and 2005 (Figure 3.2). The lowest value for \(\Psi_{pd}\) was recorded toward the end of the 2004 dry season and was -0.76 MPa, -1.11 MPa and -1.24 MPa for Site 1 (2.30 m to groundwater), Site 2 (8.04 m to groundwater) and Site 3 (21.73 m to groundwater) respectively. Where the water table was closest to the natural surface (Site 1) the amplitude of the seasonal fluctuation in \(\Psi_{pd}\) was less apparent than sites where the water table was deeper in the soil profile. This can be quantified as the difference between the maximum and minimum \(\Psi_{pd}\) over the study period (demarked by the arrows in Figure 3.2). For sites 1, 2 and 3 respectively this difference was 0.46 MPa, 0.88 MPa and 0.87 MPa).
Drought Traits of *E. gomphocephala*

![Graphs showing leaf water potentials (ψ) at different times and sites](image)

Figure 3.2 Mean (± s.e., *n* = 12) pre-dawn and midday leaf water potentials (ψ*ᵣ* and ψ*ᵣ* respectively) for *Eucalyptus gomphocephala* growing at different depths to the water table. Distances from the natural surface to the water table for sites 1, 2 and 3 respectively were 2.30 m, 8.01 m and 21.73 m. Also shown is monthly rainfall for the same location over the study period and the monthly long-term average. Arrows indicate the range of pre-dawn leaf water potentials observed over the study period.

Stomatal conductance to water vapour was positively correlated, in an exponential form, with *E* (Figure 3.3). This means that as *E* increased *gₛ* reached a maximum value (at approximately 320 mmol m⁻² s⁻¹). At low values of *gₛ* and *E*, as stomatal apertures opened estimated transpiration rates increased, but at higher values an increase in *E* did not correspond to an increase in *gₛ*. Given that the boundary layer...
conductance was constant across measurements, this correlation is likely to depict the competing dependencies of $E^*$ on $g_s$ and $g_s$ on $E^*$. The lowest values for $E^*$ and $g_s$ were confined to Site 2 and Site 3. Stomatal conductance to water vapour was also positively correlated with $\Psi_{pd}$ (Figure 3.4A), (linear regression, $r^2 = 0.57$, $P < 0.001$). Stomatal conductance to water vapour was only weakly correlated with $\Psi_{md}$ (linear regression, $r^2 = 0.23$, $P < 0.001$; Figure 3.4B).

![Stomatal conductance to water vapour](image)

**Figure 3.3** Plot of midday estimated transpiration rate ($E^*$) versus midday stomatal conductance to water vapour ($g_s$) for *Eucalyptus gomphocephala* ($n = 12$ for each point). The fitted line is an exponential function:

$$g_s = 321.05 - 233.11 \times e^{-\left(\frac{E^* - 1.21}{2.07}\right)}$$

($r^2 = 0.44$, $P < 0.05$). Sites 1, 2 and 3 are as in Figure 3.2.
The pressure volume curves generated from leaves collected at each site are presented in Figure 3.5. Interpretation of these curves (refer to the Material and Methods section for the procedure) revealed that across sites \( \pi_i \) was positively dependent on \( \Psi_{TL} \) (linear model, \( r^2 = 0.75 \), \( P < 0.0001 \); Figure 3.6).
Figure 3.5  Pressure volume curves, plots of $1/\Psi$ versus the relative water content (RWC), for leaves collected from the 12 individuals of each site. The fitted linear models (refer to methodology for fitting procedure) have the following equations: $1/\Psi = 0.04 - 0.52 \text{RWC}$, $1/\Psi = 0.19 - 0.69 \text{RWC}$ and $1/\Psi = 0.15 - 0.55 \text{RWC}$ for graphs A ($r^2 = 0.69; P < 0.001$), B ($r^2 = 0.71; P < 0.001$) and C ($r^2 = 0.73; P < 0.001$) respectively. Refer to Figure 3.2 for respective depths to groundwater at each site.
A significant convergent linear correlation was observed between $\Psi_{\text{TL}}$ and the average groundwater depth of each site ($r^2 = 0.82$, $P < 0.0001$; Figure 3.7A) but at no point did $\Psi_{\text{leaf}}$ fall below $\Psi_{\text{TL}}$ (refer to Figure 3.2). Similarly, $\Delta_{\text{leaf}}$ was dependent on groundwater depth (linear model, $r^2 = 0.31$, $P < 0.001$; Figure 3.7B).
Both \( \Psi_B \) (A) and \( \Delta_x \) (B) were correlated with mean depth to groundwater in a convergent linear manner. The linear fit for A has the equation: 
\[
\Psi_B = -2.30 - 0.03 \times \text{Depth to groundwater (m)} \quad (r^2 = 0.82, \ P < 0.0001). 
\]
The linear fit for B has the equation: 
\[
\Delta_x = 21.88 - 0.11 \times \text{Depth to groundwater (m)} \quad (r^2 = 0.31, \ P < 0.001). 
\]

Vulnerability curves for each site (Figure 3.8) showed a similar pattern, with \( \Psi_x \) at 50% PLC (parameter \( b \) in Equation 3.6) differing by only 0.11 MPa across sites. Actual values for \( b \) were -2.23 MPa, -2.13 MPa and -2.29 MPa for sites 1, 2 and 3 respectively. Parameter \( a \), related to the slope of the relationship between PLC and \( \Psi_x \) was also relatively uniform across sites. Actual values for \( a \) were 0.69, 0.72 and
0.70 for sites 1, 2 and 3 respectively. At no time during the study did $\Psi_{pd}$ drop below $PLC_{50}$ (refer to Figure 3.2).

Stem hydraulic conductivity did not obviously fluctuate in synchrony with season at any site and was not correlated with either $\Psi_{pd}$ or $\Psi_{md}$. The mean $K_s$ ($kg\ s^{-1}\ m^{-1}\ MPa^{-1}$) for site 1, site 2 and site 3 respectively was $0.78 \pm 0.03$, $0.67 \pm 0.02$ and $0.71 \pm 0.03$.

A significant ($P < 0.05$) divergent linear correlation was observed between the $\Psi_{md}$ and $\Psi_{pd}$ at each site (Figure 3.9). The slope of the linear model for Site 1 was greater than that at Site 2 and Site 3. This means that the same change in $\Psi_{pd}$ would provoke a larger change in $\Psi_{md}$ at Site 1 compared to Site 2 and Site 3.
DROUGHT TRAITS OF *E. GOMPHOCEPHALA*

Figure 3.8 Vulnerability curves, plots of percentage loss hydraulic conductivity (PLC) versus xylem water potential ($\Psi_x$), for *Eucalyptus gomphocephala* growing at different depths to groundwater (refer to Figure 3.2 for respective depths). Fitted lines are exponential-sigmoidal models (Sammelner and Vanderwilligen 1998) with the equation:

$$PLC = \frac{100}{[1 + \exp\{0.67(\Psi_x + 2.23)\}]}$$  \( \left( r^2 = 0.80 \right) \) for Site 1,

$$PLC = \frac{100}{[1 + \exp\{0.72(\Psi_x + 2.13)\}]}$$  \( \left( r^2 = 0.75 \right) \) for Site 2 and

$$PLC = \frac{100}{[1 + \exp\{0.70(\Psi_x + 2.29)\}]}$$  \( \left( r^2 = 0.75 \right) \) for Site 3.
Plots of the midday leaf water potential ($\Psi_m$) versus pre-dawn leaf water potential ($\Psi_{pd}$) for *Eucalyptus gomphocephala* growing at different depths to groundwater (refer to Figure 1 for respective depths). Fitted lines are linear models: $\Psi_m = -0.62 + 1.27 \Psi_{pd}$ ($r^2 = 0.26$, $P < 0.03$), $\Psi_m = -0.68 + 0.98 \Psi_{pd}$ ($r^2 = 0.75$, $P < 0.01$) and $\Psi_m = -0.91 + 0.88 \Psi_{pd}$ ($r^2 = 0.71$, $P < 0.01$). The dashed line in each graph represents the 1:1 ratio.

Figure 3.9
3.4 Discussion

At any given location *Eucalyptus gomphocephala* exhibits a classical seasonal oscillation in $\Psi_{\text{leaf}}$, $g$, and $E^*$. However, the amplitude of this seasonal oscillation was smaller where groundwater was close to the natural soil surface. This suggests that a shallow water table can offset the severity of the annual summer dry period in this species, an observation consistent with the behavior of a facultative phreatophyte. Traits that become embedded over a leaf developmental timeframe, $\Psi_{\text{TL}}$ and $\Delta$, seemed to parallel the instantaneous measures of water stress, stomatal aperture and leaf water vapour flux. By contrast, characteristics of vulnerability to xylem embolism, which are likely to be influenced by more long term conditions, were not linked to access to seasonally permanent water.

Pre-dawn and midday leaf water potentials reached their minimum value between February and April of both monitored years of this study, coinciding with an extended period where little precipitation occurred. In the pre-dawn phase leaf water potential ($\Psi_{\text{leaf}}$) is assumed to closely parallel the soil water potential ($\Psi_{\text{soil}}$) in the active rhizosphere (however see Donovan et al. (1999) and Donovan et al. (2001) for exceptions). The fluctuation in $\Psi_{\text{pd}}$ in this study is likely therefore to reflect the magnitude of available water in soil at a given time, an association observed in other eucalypts (Landsberg and Wylie 1983; Crombie et al. 1988; Zubrinich et al. 2000; Thomas and Eamus 2002). Since a seasonally-induced oscillation in $\Psi_{\text{pd}}$, and hence $\Psi_{\text{soil}}$, was observed at each site, landscape position, and therefore proximity to permanent soil water (groundwater), did not completely offset the effect of dry conditions. The alternative possibility is that, by tightly regulating stomatal aperture, individuals were able to express a higher and more constant $\Psi_{\text{mf}}$, and thus increase the likelihood of nighttime hydraulic recharge. However, this is unlikely given the positive correlation between $g$, and $\Psi_{\text{mf}}$.

Despite this similar temporal (seasonal) trend in $\Psi_{\text{pd}}$ across sites, the amplitude of the oscillation was less pronounced where the water table was in close proximity to the natural surface. Indeed, the highest pre-dawn leaf water potentials were observed at
site 1 followed by site 2 and site 3 respectively. Easy access to seasonally permanent water, such as that provided at site 1 in this study, would seem to create a favourable soil moisture environment (or ecohydrological state) for *E. gomphocephala*. These differences in the soil moisture environment could invoke a degree of adaptive plasticity in *E. gomphocephala*. Cutler et al. (1977), for example, showed that water stress during development can lead to the formation of leaves with high stomatal densities and Alder et al. (1996) observed a change in root xylem anatomy with transition across a soil moisture gradient. Such anatomical differences would clearly impart changed physiological expression of leaf gas exchange and xylem hydraulic properties of mature plants.

The correlation between *g*<sub>s</sub> and *E*<sup>*</sup> provides insight into the mechanism of stomatal regulation of transpiration rate under field conditions for *E. gomphocephala*. During favourable moist conditions *E. gomphocephala* at all sites expressed higher *g*<sub>s</sub> and *E*<sup>*</sup>. However, on exposure to the annual dry season plants at site 2 and site 3 curtailed substantial transpirational water loss by reducing stomatal conductance whereas plants at site 1 were able to maintain substantially higher rates of leaf gas exchange. The correlation between *g*<sub>s</sub> and *Ψ*<sub>soil</sub> in *E. gomphocephala* implies that *Ψ*<sub>soil</sub> limits the possible expression of *g*<sub>s</sub> at any given time. This effect could be due to: (1) stomatal regulation of transpiration rate as a mechanism to minimise xylem dysfunction from excessive cavitation (Meinzer and Grantz 1990; Jones and Sutherland 1991), or (2) modification of transpiration rate as a result of cavitation (Jones and Sutherland 1991; Sperry and Pockman 1993). While it is difficult to distinguish between the specific forms with certainty, the weight of evidence implies that stomata are responding passively to seasonality, particularly where soil moisture is not buffered by a shallow water table.

Midday leaf water potential is mainly a function of transpiration rate, *Ψ*<sub>soil</sub> and the hydraulic conductance across the soil to leaf pathway. It could be argued that the poor correlation between *g*<sub>s</sub> and *Ψ*<sub>md</sub> was due to the effect of microclimatic variables, such as vapour pressure deficit and wind speed, on leaf transpiration rate, and hence *g*<sub>s</sub>. Alternatively, *Ψ*<sub>md</sub> and *g*<sub>s</sub> could become decoupled within a site due to differences in the hydraulic capacitance of individuals. Phillips et al. (2003), for example,
showed that water storage in xylem contributes a greater proportion of daily water use in large trees and old forests. If such a disparity developed in *E. gomphocephala*, larger individuals could offset the daily drawdown in $\Psi_{md}$ by drawing on this store of water.

In crop species $\Delta_{leaf}$ is generally assumed to be a surrogate for plant water-use efficiency (WUE) (Farquhar and Richards 1984). Olbrich et al. (1993), however, caution the generality of this relationship in trees because of the complexity of variation in carbon allocation and leaf-to-air vapour pressure difference, confounding factors that need to be acknowledged in this study. Nevertheless, several studies on eucalypts have examined the relationship between intrinsic WUE and the climate of native habitats. Anderson et al. (1996), for example, found that for a given rainfall, phreatophytic eucalypts clearly separated from non-phreatophytes by expressing a lower WUE. The carbon isotope discrimination results of this study imply that *E. gomphocephala* is a facultative phreatophyte (or opportunist), exploiting seasonally permanent soil water when in close proximity to the natural surface, an advantage that subsequently allowed leaves to operate with a high intercellular to ambient partial pressure of CO$_2$. The associated high $\Psi_{TL}$ in the same plants implies that a low probability of excessive water stress also reduced the necessity to osmotically adjust to possible tissue water deficits. This follows the reported pattern of response to contrasting soil moisture environments in other Eucalyptus species (for example, Mielke et al. (2000)). It should be noted, however, that adjustments in WUE (changed $\Delta_{leaf}$) and $\Psi_{TL}$ under different soil moisture regimes, while representing the plasticity of leaf phenotype in *E. gomphocephala*, do not account for whole plant morphological adjustments such as the capacity of plants to maintain functional contact with permanent pools of soil water through root development.

From the correlation between $g_s$ and $\Psi_{pd}$ it would appear low soil water potentials (less than -0.8 MPa) would invoke very low stomatal conductances. Such conditions were predominantly experienced where the water table was located at greater depths (sites 2 and 3). This pattern of disparate soil water environment (across sites) was apparent over the two successive dry seasons of the study, which represented a developmental time frame for leaves and small branches. Because of this it would be
expected that contrasting susceptibilities to drought-induced xylem embolism in twigs would be observed across sites. Across taxa, considerable variation has been observed in such hydraulic properties (Tyree and Dixon 1986; Tyree and Ewers 1991; Patino et al. 1995; Tyree and Ewers 1996) but few studies have examined the capacity for intra-specific variation across differing moisture environments. Intuitively it would be anticipated that phreatophyte growth in close proximity to the water table would lead to the development of xylem conduits with a relatively high susceptibility to loss of xylem function with exposure to water stress. This is because the perennially moist conditions of such areas should favour the production of efficient xylem conduits with a low safety margin for loss of xylem function. The initial interpretation of vulnerability data suggested that this was not the case. Xylem water potentials at 50% PLC were similar across sites, as was the apparent range of water potentials over which cavitation events occurred. Hence across sites it would appear that critical thresholds for loss of xylem function in twigs occur at similar xylem tensions and cavitation events occur across a similar range of water potentials. Pammenter and Vanderwilligen (1998) suggests that these parameters are associated with key anatomical features of pit complexes. Their similarity across sites in this study would indicate that pit complex anatomy is an embedded trait in *E. gomphocephala* twigs that does not vary under contrasting developmental soil water regimes, although detailed microscopic analyses would be required to confirm this proposition.

The slopes of the linear models fitted to plots of $\Psi_{\text{mat}}$ versus $\Psi_{\text{pd}}$ imply that, for a given change in $\Psi_{\text{pd}}$, *E. gomphocephala* trees growing in close proximity to the water table would experience a more negative $\Psi_{\text{leaf}}$ at midday. Under a scenario of universal, landscape-wide, soil water deficit, *E. gomphocephala* trees growing in close proximity to the water table would therefore reach tissue water potentials that would breach $\Psi_{\text{TL}}$ and induce significant xylem embolisms prior to trees growing higher in the landscape. In the field, however, these conditions were not met at any site and indeed the proximity of *in situ* tissue water potential to $\Psi_{\text{TL}}$ was greatest at site 1.
3.5 Conclusions

Seasonality in water relations properties was expressed at each ecohydrological niche. However, development under a given ecohydrology influenced the magnitude of the observed seasonal oscillation. Specifically, *E. gomphocephala* growing in close proximity to the water table maintained proportionally higher rates of stomatal conductance during the dry season and on a leaf developmental timeframe expressed a higher $\Psi_{TL}$ and were less water-use efficient. While vulnerability curves derived from distal canopy stems showed a similar pattern across sites, the trajectory of the relationship between $\Psi_{mid}$ and $\Psi_{pd}$ implies that if the same change in $\Psi_{soil}$ occurred across sites, trees growing low in the landscape would be most likely to experience drought-induced dieback. In the field, however, neither midday nor pre-dawn leaf water potentials breached $\Psi_{TL}$ or were consistently in the range to promote extensive xylem embolisms in *E. gomphocephala*, suggesting that the safety margin for this species is compatible with its current habitat.
CHAPTER 4

SPATIAL AND TEMPORAL PARTITIONING OF WATER SOURCES ACROSS STAGE OF MATURITY IN *EUCALYPTUS GOMPHOCEPHALALA*
Summary

The relative allocation of roots for resource acquisition across stages of maturity and proximity to groundwater is an important but challenging topic in habitats subjected to reduced rainfall recharge. It is important because an understanding of such allocation in a changing habitat will help land managers predict impacts. In this chapter natural variation in stable isotopes of hydrogen were exploited to estimate the relative contribution of a given water source in *E. gomphocephala* across stage of maturity and season and at different proximities to groundwater. This chapter also explores the linkage between use of a given water source and canopy physiological processes. Coupled together this information builds on the knowledgebase developed in Chapter 3. Across stages of maturity and location, *E. gomphocephala* is an opportunist, able to express a phreatophytic state when conditions are favourable. Despite this, canopy processes suggest that, at a given site, seedlings were more stressed than saplings or trees. Large trees growing high in the landscape were surprisingly less stressed than other individuals. It is argued that this observation could reflect the advantage of a long term adaptation to dry conditions that are now experienced generally across the landscape as a result of a stepped reduction in annual rainfall. Figure 4.1 highlights aspects of the thesis outlined and discussed in this chapter.
PARTITIONING OF WATER SOURCES

Climate change

Rainfall recharge  Vadose zone moisture

Size class

Available soil water  Proximity to groundwater

Transpiration rate  Hydraulic architecture  Canopy water potential  Water source  Rooting properties

Canopy condition

Figure 1.1 The thesis outline highlighting the relevant processes covered in this chapter. The role of transition to maturity, quantified by size class, and proximity to groundwater are assessed in terms of the spatial and temporal partitioning of water sources. Differences in water source strategies, and accompanying distinction in rooting properties, are associated with plant water relations and canopy hydraulic architecture.
4.1 Introduction

Since the allocation of resources to roots is usually at the expense of shoot biomass (Caldwell and Richards 1986; Caldwell 1987) the net carbon gained by preferential allocation to below ground biomass is often lower than with equal allocation to roots and shoots. The distribution and availability of water in soil will greatly influence the relative investment in such root biomass. This influence extends to the temporal dimension since water reliability rather than its distribution per se may govern rooting strategies (Dawson and Ehleringer 1991; Ehleringer et al. 1991; Flanagan et al. 1992; Thorburn and Walker 1994; Goldstein et al. 1998; Drake and Franks 2003). The term water source partitioning was coined to describe the spatial and temporal separation of water sources in plant communities and has been recognised in a range of habitats such as tropical (Jackson et al. 1995; Meinzer et al. 1999; Drake and Franks 2003), arid (Ehleringer et al. 1991; Flanagan et al. 1992; Gebauer and Ehleringer 2000) and Mediterranean (Zencich et al. 2002). Such studies have shown that while root distribution profiles often reveal a great abundance of root biomass near the soil surface of forest communities (Habib and Lafolie 1991), taproots may penetrate into deep soil horizons to access a perennially stable water supply (Burgess et al. 1998; Jackson et al. 1999).

Generally the ability of a plant to access and maintain contact with deep soil water reserves (groundwater) depends on: 1) the depth to the water table, 2) the soil characteristics that establish limits to root penetration, and 3) the intrinsic ability of a plant to develop deep roots (Scott and Le Maitre 1998). The Mediterranean-type climate of south western Australia should favour the development of species with deep taproots that offset the effects of the annual summer dry season and allow for higher annual rates of carbon gain. Indeed, studies of phreatophytic Banksia species in the region have shown use of groundwater in summer, which required penetration of taproots deep into the soil profile (Dodd and Bell 1993; Dawson and Pate 1996; Zencich et al. 2002). Groom et al. (2000) highlighted that use of groundwater by such phreatophytic banksias can be essential for survival. To date, however, no studies of south western Australian vegetation have characterised the generality of this mode of
PARTITIONING OF WATER SOURCES

water use by investigating the seasonal shift in water sources in phreatophytes of families other than Proteaceae.

The transition in acquisition strategies with maturation has been characterised by Feild and Dawson (1998) studying the morphosis from epiphytic, hemiepiphytic to arborescent state in *Didmopanax pittieri*. While this dramatic change in the deployment of root architecture is generally not reflected in the vegetation of south western Australia, maturation in some species will conceivably involve a transition from a mainly vadophytic to phreatophytic state, depending on proximity to an accessible aquifer. The importance of understanding water source partitioning during this transition has been accentuated by a recent lowering of water tables in the underlying superficial aquifers of south western Australia (Davidson 1995) and a decline in winter rainfall by up to 25% (Indian Ocean Climate Initiative 2002). Over the same period there have been an increasing number of canopy dieback events in large tree species such as *E. gomphocephala*. This chapter examines the water sources of *E. gomphocephala* to explore generality of seasonal water use properties in phreatophytes of south western Australia and the strategies employed by this species in particular across stages of maturity and for a given ecohydrological niche. The hypothesis of this chapter is that partitioning of water sources will be reflected in both stage of maturity and season. The objectives were: 1) to determine the water sources of *E. gomphocephala* across the seasonal transition from summer to winter, and 2) to determine whether a given water use strategy is reflected in aspects of plant water relations. In the previous chapter it was found that different ecohydrological niches elicited contrasts in water relations and xylem hydraulic properties. This chapter builds upon these observations by investigating the water sources of a given ecohydrological niche. Coupled together this information will contribute to a landscape level conceptual model of *E. gomphocephala* water use, providing insight into the role of water stress in the past decline of the species in Yalgorup National Park.
4.2 Material and Methods

4.2.1 The Study Area

Two study sites were selected within Yalgorup National Park (32.90S; 115.69E) in south western Australia. The sites, each of which consisted of a 50 m × 50 m plot, were located within a radius of 15 km of one another (in Chapter 3 these were defined as site 1 and site 2). Annual average rainfall for the area is 875 mm, with only 4% falling during summer when the average maximum temperature is 29°C. Most canopy species occupying this Mediterranean-type habitat (Gentilli 1972) are from the families Myrtaceae and Proteaceae. Renowned for their capacity for deploying an extensive root system, species from these families would readily extend roots deep into the sandy soil profile (derived from the Spearwood dune system (McArthur and Bettenay 1960)).

The sites contrasted in average proximity to the water table (2.30 m and 8.04 m below the natural surface for site 1 and site 2 respectively). The study species, *E. gomphocephala*, was a canopy dominant at both sites. Moreover, a full complement of stage of maturity characterised by diameter at breast height (DBH) was represented at each site. These were: seedlings (DBH = 0.02 m – 0.05 m), saplings (DBH = 0.07 m – 0.29 m) and mature trees (DBH = 0.38 m – 1.70 m). Sampling took place in February 2004, July 2004 and October 2004 representing the transition from the summer dry period to the winter wet period. While the outward expression of the canopy decline syndrome, i.e. canopy dieback, was evident at each site, only plants of greater than 50% of maximum health were studied. At each site 12 individuals (four seedlings, four saplings and four trees) were sampled at each measurement period, unless otherwise stated.

4.2.2 Soil, Groundwater and Stem Samples

Neutron moisture probe access tubes were installed at each site prior to the initiation of the study to a depth beyond the water table. Soil moisture was
PARTITIONING OF WATER SOURCES

monitored at each sampling period with a neutron moisture probe. Soil cores, adjacent to the neutron moisture probe access tubes, were obtained with a push core pneumatic drilling rig. Soil cores were subdivided into definable stratified layers and volumetric water content ($\theta$) determined according to Dewis and Freitas (1970):

$$\theta = \frac{w \rho_b}{\rho} \cdot 100 \%,$$  \hspace{1cm} \text{(4.1)}

where $w$ = soil water content (grams water per gram dry weight of soil; g g$^{-1}$), $\rho_b$ = the bulk density of soil (g cm$^{-3}$) and $\rho$ = the density of water (taken as 1 g cm$^{-3}$). Soil dry weight was determined by oven drying soil at 105°C for 48 hours.

At each sampling period additional soil samples were collected at intervals in the soil profile, sealed in 125 ml vials and frozen in preparation for extraction of water for $\delta^2$H determination. The relationship between neutron moisture meter counts and volumetric water content was obtained for each stratified layer so that the output of the neutron moisture probe could be converted to volumetric water content. Groundwater depth was monitored from a piezometer located at each site (adjacent to the neutron moisture meter access tube) approximately monthly with a depth probe. Groundwater samples were obtained from each piezometer in February, July and October with a bailer after first purging three well volumes. Groundwater samples were sealed in centrifuge tubes and frozen.

Non-photosynthetic stem samples ($n = 6$ plants per size class and site) were collected from terminal branches at each sampling period. Stems were wrapped in plastic film, sealed in centrifuge tubes and frozen prior to extraction of water for $\delta^2$H determination. While it is generally accepted that uptake of water by plant roots does not cause isotopic fractionation (Barnes and Allison 1983), Lin and Sternberg (1993) revealed that in some species ultrafiltration by salt exclusion membranes can deplete the $^2$H signal in roots by up to 10%. However, the protocol of Thorburn et al. (1993b) showed that such fractionation is unlikely in Eucalyptus trees, implying that stem samples provide a good
representation of the isotopic composition of water in the upper parts of the non-transpiring canopy. A cautionary note, however, is the possibility of isotopic contamination of xylem water in stems with downward-moving phloem; although the significance of such contamination has not been shown.

4.2.3 $\delta^2$H Determination

Water was extracted from soil and non-photosynthetic stems by cryogenic vacuum distillation (Dawson 1993b). Isotopic ratios of hydrogen ($^{2}$H/$^{1}$H) were then measured from extracted water (soil water, stem xylem water and groundwater) with a continuous flow mass spectrometer (PDZ Europa Model 20-20 Cheshire, England). The data produced were normalised following Coplen (1988) and expressed relative to the V-SMOW standard (Gonfiantini 1978):

$$\delta^2H = \left( \frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 1000 \ (%),$$

(4.2)

where $R_{sample}$ and $R_{standard}$ are the hydrogen isotopic ratios of the water sample and the V-SMOW sample respectively.

A three component water source mixing model was applied to the $\delta^2$H data to predict the likely contribution of a water source to that present in plant xylem (White et al. 1985; Thorburn and Walker 1994; Zencich et al. 2002) (refer to Appendix 3 for potential errors associated with this technique). The three potential water sources were considered to be surface soil water, subsurface water and groundwater. Surface soil water (the $\delta^2$H of soil water obtained between 0.0 m – 0.5 m below the natural surface at site 1 and 0.0 m – 1.0 m at site 2) was considered most susceptible to isotopic instability due to evaporation and infiltration based on observation in soil volumetric water content. Subsurface water (the $\delta^2$H of soil water obtained between 0.5 m – 2.3 m below the natural surface at site 1 and 1.0 m – 8.0 m below the natural surface at site 2), while not saturated, was less subject to short term fluctuation due to evaporation and infiltration. Groundwater was the hydrogen isotopic signal derived from water collected from piezometers. The values for $\delta^2$H of stem xylem water at a
given sampling period and individual plant were incorporated into the model and
an average proportion of water source used determined for each stage of
maturity.

4.2.4 Leaf Water Potential

Pre-dawn (04:30-06:00 local standard time) leaf water potential ($\Psi_{pd}$) was
determined during February, July and October from each individual ($n = 12$ for
each site) using a Scholander-type pressure chamber (Soilmoisture Equipment
Corp, Model 3005, Santa Barbara, California) (Scholander et al. 1964). Leaves
were sampled from the upper canopy of each size class. For trees this meant use
of long-handled secateurs and an extension ladder. Leaf samples were kept in
snap-lock plastic bags (petiole protruding) throughout the procedure to minimize
transpirational water loss during measurement (Richie and Hinkley 1975).

4.2.5 Chlorophyll Fluorescence

The midday (11:30-13:30 local standard time) maximum photon yield of
photosystem II ($F_v/F_M$) was periodically (monthly) determined for canopy leaves
of each individual plant ($n = 12$ per site) with a pulse modulated fluorescence
probe (Model OS1-FL, Opti Sciences Inc, Tyngsboro MA).

4.2.6 Hydraulic Conductivity

Maximum vessel lengths were determined by passing nitrogen gas at a pressure
of 5 kPa through a length of stem and cutting sections back from the distal end
until gas flow was detected (by dipping the end in distilled water and watching
for small bubbles). Initial stem lengths were 1.0 m for trees and absolute
maximum vessel length was less than 0.02 m. Stem-area-specific hydraulic
conductivity ($K_S$) and leaf-area-specific hydraulic conductivity ($K_L$) were
determined for stems of a greater length than the longest measured vessel and
with a diameter of 0.003 m – 0.01 m. Leaf-area-specific hydraulic conductivity
was measured using a steady state flow meter (SSFM), similar to that described
by Feild et al. (2001). The SSFM consisted of a length of peek™ resistance tubing (with a known relationship between pressure and flow rate) ordered in series with the petiole. Intact terminal branches were periodically (monthly) collected between February 2004 to October 2004 from each individual \((n = 12\) for each site) and immediately transported to a laboratory. Branches were collected prior to sunrise to ensure that minimum native embolisms were present. In the laboratory leaves were removed and stems re-cut under distilled water. Prior to placing the stems in the SSFM the ends were trimmed with a razor blade (while immersed in distilled water). The SSFM was filled with a perfusion solution consisting of 0.01 M KCl and degassed double distilled water with a pH of 6.6, filtered to 0.11 µm. The pressure difference across the stem was maintained to less than 5 kPa during measurements. Leaf-area-specific hydraulic conductivity, defined here as the mass flow movement of water through the excised stem segment per unit of pressure gradient per unit of leaf area, was determined as:

\[
K_L = K_S \times H \quad \text{(kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}),
\]

where \(K_S\) = the xylem-area-specific hydraulic conductivity \((\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1})\) and \(H\) = the Huber value, the ratio of stem cross sectional area \(A_x\) \((\text{m}^2)\) to leaf area \(A_L\) \((\text{m}^2)\) receiving water from that stem. Leaf area was measured with an area meter (model Delta-T Type W DIGC-2, Delta T Devices, Cambridge UK) and \(A_x\) was measured accurately with digital callipers. Xylem cross sectional area was considered to relate very closely to stem cross sectional area in the small diameter stems of this study. Although some native embolisms were likely even in the wet season, this study sought only to measure existing, natural hydraulic conductivities under the given environmental conditions. Thus maximum potential hydraulic conductivity was not measured.
4.3 Results

The proximity of the water table to the natural surface was dependent on rainfall recharge at both sites (Figure 4.2). There was only a small lag period between the start of the annual wet period and the upward migration of the water table at each site. Site 1 showed the greatest vertical movement in the position of the water table with transition to the wet season.

Plots of soil depth versus volumetric water content (Figure 4.3A and 4.3C) showed an obvious increase in the saturated zone. As with groundwater depth measurements, the vertical migration of this saturated zone was greatest at site 1. Site 1 also showed the greatest temporal increase, with transition to the wet season, in vadose zone volumetric water content. At both sites the inputs of rainfall were apparent as a rise in volumetric water content throughout most of the soil profile. The least affected zone was the surface soil layer (0.0 m – 0.5 m for site 1 and 0.0 m – 1.0 m for site 2) where the effects of evaporation were still apparent in the wet season.
At site 1 the $\delta^2$H of soil water was, with the exception of the surface soil layer, similar between February and July (Figure 4.3B). The October sampling period yielded a different pattern in the subsurface layers, with an enriched zone of soil water apparent between 0.5 m and 1.5 m. A similar pattern of isotopic instability was apparent in the surface soil layer of site 2 (Figure 4.3D). Moreover, transition to the wetter conditions of July provoked a general enrichment of soil water throughout the soil profile. Further enrichment was apparent in the surface

Figure 4.2: Groundwater depth from the natural surface at site 1 (A) and site 2 (B) and daily rainfall for the area over the same period (C). Note that both water tables responded rapidly to rainfall recharge but that the magnitude of this response was greater at site 1.
and subsurface soil layers in October. At both sites the $\delta^2\text{H}$ of groundwater remained relatively stable over the study period and was close to the $\delta^2\text{H}$ value of the deepest soil water obtained.

![Figure 4.3](image)

Figure 4.3 Plots of soil depth versus volumetric water content ($\theta$) (A and C) and soil water $\delta^2\text{H}$ (B and D) for the periods February, July and October. The vertical shaded bars of B and D represent the mean ± s.e. ($n = 3$) of groundwater $\delta^2\text{H}$ from samples collected in February, July and October.
Results of the three component mixing model from samples collected at site 1 (Figure 4.4) suggest that use of surface soil water increased across all stages of maturity with the transition from dry to wet season, although the most apparent increase was in seedlings. This switch in water source was primarily at the expense of subsurface soil water. Use of groundwater peaked in July in all stages of maturity before decreasing in October. Over the same period pre-dawn leaf water potential increased toward zero in all stages of maturity (Figure 4.4). A similar switch in water source was evident in seedlings at site 2 (Figure 4.5). However, the same peak in groundwater use during July in seedlings was not apparent. In more mature individuals (sapling and trees) this trend did not recur. Instead the proportional use of each water source was relatively static except for a slight increase in groundwater use in July. Over the same period pre-dawn leaf water potential increased toward zero in each stage of maturity, although the greatest increase was in seedlings (Figure 4.5).

The mean maximum yield of photosystem II (PSII) was positively correlated (via a linear model) with the mean Huber value ($H$) across stage of maturity and site (Figure 4.6). Within a site the transition to maturity tended to promote an increase in both $F_v/F_M$ and the Huber value and a general increase in $F_v/F_M$ and the Huber value was apparent at site 2. Mean leaf-area-specific hydraulic conductivity ($K_L$) and specific hydraulic conductivity ($K_S$) were positively correlated and also trended toward an increase with transition to maturity (Figure 4.7), but there was no measurable by-site difference.
Figure 4.4  Proportion of water source used with transition from the summer dry season (February) to winter wet season (July) and spring (October) for seedlings (A) saplings (C) and mature trees (E) at site 1 ($n = 6$ for each stage of maturity). Predawn leaf water potential ($\Psi_{ps}$) over the same period for seedlings (B), saplings (D) and mature trees (F) are also shown ($n = 4$ for each stage of maturity).
Figure 1.5 Proportion of water source used with transition from the summer dry season (February) to winter wet season (July) and spring (October) for seedlings (A) saplings (C) and mature trees (E) at site 2 (n = 6 for each stage of maturity). Pre-dawn leaf water potential (Ψₚ) over the same period for seedlings (B), saplings (D) and mature trees (F) are also shown (n = 4 for each stage of maturity).
Figure 4.6  Correlation between the maximum yield of photosystem II \( (F_v/F_M) \) and the Huber value \( (H) \) for data collected over the study period. The linear fit follows the equation: \( F_v/F_M = 0.78 + 435.78H \) (\( r^2 = 0.81; P < 0.02 \)). Seedlings - ■, saplings - ● and trees - ▲. Closed symbols are plants from site 1, open symbols are plants from site 2. Each point is the mean ± s.e. \( (n = 36 \text{ for each}) \).
PARTITIONING OF WATER SOURCES

Figure 4.7 Leaf-area-specific hydraulic conductivity ($K_L$) was positively correlated with stem-area-specific hydraulic conductivity ($K_S$) in *E. gymnosperma* stems. Each point represents the mean (± s.e., $n = 36$); closed symbols are data from samples collected at site 1, open symbols are data from samples collected at site 2. The data corresponds to branches collected over the study period and therefore *in situ* $K_L$ and $K_S$ integrated across the dry to wet seasonal transition. The fitted linear model has the equation: $K_L = 2.95 \times 10^{-5} + 1.01 \times 10^{-4} K_S$ ($r^2 = 0.85$, $P < 0.01$).

4.4 Discussion

At both sites, hydrology (quantified as proximity of the water table to the natural surface) and soil moisture in the unsaturated zone were strongly associated with rainfall. Over the period of measurement the contribution of rainfall induced a 1.3 m and 0.27 m rise in the position of the water table at sites 1 and 2
PARTITIONING OF WATER SOURCES

respectively (Figure 4.2). The lag phase between the initiation of the annual wet season in early April and a deviation in the position of the water table was small, occurring within the same month. This is consistent with the results of Lindsay (2002), who observed a long term linkage between climate and hydrology in the same region, and the fact that the sandy soils of the region are highly conductive (Sharma and Hughes 1985). The accentuated seasonal shift in the position of the water table at site 1 compared to site 2 is typical of a shallow water table. This is because a short path length between the natural surface and the zone of saturation maintains a high degree of connectivity between rainfall recharge and evapotranspiration. This 'by site' contrast (as reflected by a difference in water table response to seasonality) highlights the role of groundwater as a conditioning factor for plant species (Klijn and Witte 1999). At site 1, where the volume of rhizosphere is constrained because of a close proximity to saturated soil, the substantial temporal migration of the water table over a season will likely induce considerable adaptation in either the active zone of root water uptake or rooting morphology. With transition to the wet season this adaptation could take the form of a rapid deployment of root biomass in newly developed favourable hydrological niches or extensive aerenchyma formation in roots persisting in the saturated zone. Aerenchyma, typically formed by the collapse and lysis of mature cortical cells of roots, enhances aerobic respiration and root function in water logged situations (Kawase 1981).

The lowest soil moistures observed in the dry season, represented by the January sampling period, were due to the combination of evaporation and transpiration (Figure 4.3A and 4.3C). With transition from dry to wet season, the inputs of rainfall, coupled with an upward migration of the water table and capillary fringe, promoted a general increase in soil moisture across the rhizosphere profile of each site (Figure 4.3A and 4.3C). Preferential flow, greater than average flow, through the soil profile can enable these two processes to occur simultaneously. The change in soil moisture content from dry to wet season was most apparent at site 1. However, the soil moisture content of this site in the dry season was higher than site 2. This latter observation would suggest that soil water in the vadose zone, while temporally variable, is still a viable water source during the dry season at site 1. Indeed, a high usage of subsurface soil water during the dry
season was apparent at this site (see below for a discussion on partitioning of water sources).

The different water sources available to plants, although usually interfacing with one another, are subject to different physiological and physical processes and can therefore be physically and chemically distinct. The two processes that dominate the δ²H of soil water: precipitation, which adds meteoric water of a certain δ²H, and evaporation of water from soil, which results in the ²H enrichment of this water (Barnes and Allison 1988), strongly influenced the relative expression of δ²H in the soil profiles of this study (Figure 4.3B and Figure 4.3C). Evaporative enrichment of ²H was evident during the February and October sampling periods at site 1 and mainly during the October sampling period at site 2. Surprisingly, wet season precipitation provided an enriched water source, the signature of which was deposited in the soil profile during July. Such wet season inputs are typically a depleted source of deuterium but, in Western Australian streams, it has been shown that isotopic relaxation periods between significant rainfall events can cause a return toward a more enriched state (Turner and Macpherson 1990). This same process may occur in soil. The δ²H signal of groundwater remained relatively stable over the study period (mean = -20.25 ± 0.41 %o and -18.97 ± 0.91 %o for sites 1 and 2 respectively), which is typically a wet season weighted average expression of meteoric water (Barnes and Allison 1983).

The three major sources of water examined in this study were: 1) surface water, 2) subsurface water, and 3) groundwater. Plant access to these sources of water reflects the manner in which biomass has been allocated to root architecture and may influence the method by which a plant uses water. At both sites and across all stages of maturity pre-dawn leaf water potential responded to the seasonal transition, increasing toward zero (Fig 4.4B, 4.4D, 4.4F, 4.5B, 4.5D and 4.5F), although the magnitude of this shift was greater at site 2. This exemplifies the general relationship between season and plant water status in this species. The transition to the wet season could have influenced plant water status by directly increasing soil moisture in the vadose zone and/or by raising the position of the water table and capillary fringe to within the region of active roots.
The application of component-type mixing models to describe plant water source uptake, such as that used in this study, must acknowledge that discrete end-members have been used to define an essentially gradational natural system (Cook and O'Grady 2006). Nevertheless, the bounding range errors observed in this study (refer to Appendix 3) would suggest confidence in this technique on this occasion. At site 1 all stages of maturity increased their surface water use with transition to the wet season (Figure 4.4). Of the stages of maturity, seedlings showed the greatest use of surface water. Groundwater use at this same site increased in July but decreased in October. This would imply that both direct meteoric inputs and the vertical migration of the water table improved plant water status in July, but rainwater-derived surface water was more important in October. These observations are consistent with the likelihood that roots of *E. gomphocephala* seedlings are most abundant in the upper region of the soil profile, but also confer a degree of dimorphism in more mature plants. The presence of functionally dimorphic root systems purely for water absorption are more likely to occur if the seasonal recharge of water in the upper soil profile is reliable, since the energy expenditure of root turnover is high (Ehleringer 1995). Where such seasonal recharge is unreliable, maintaining a functionally dimorphic root system may be primarily to assist in structural support and nutrient and oxygen supply. In this study wet season rainfall recharge is considered reliable. However, with a climate change induced reduction in winter rainfall it could be argued that the advantage of maintaining a functionally dimorphic root system will diminish if its only function is to acquire water.

At site 2 *E. gomphocephala* seedlings also increased their use of surface water with the transition to the wet season, but a similar trend did not recur in the saplings or trees (Figure 4.5). Instead, these more mature plants slightly increased their use of groundwater, but overall maintained a similar proportional use of surface water, subsurface water and groundwater. Such opportunistic use of soil water with seasonal transience is not atypical of a seasonally dry or saline habitat, having been observed in other eucalypts (Thorburn et al. 1993a; Thorburn and Walker 1994; Akeroyd et al. 1998), *Melaleuca halmaturorum* (Mensforth and Walker 1996) and banksias (Zencich et al. 2002).
PARTITIONING OF WATER SOURCES

In studies of various phreatophytic banksias in south western Australia, a change in water source is usually reflected in an increase in groundwater use during summer when soil moisture in the vadose zone is lowest (Dodd and Bell 1993; Dawson and Pate 1996; Zencich et al. 2002). It would seem that the eucalypt species of this study operates in a slightly different fashion. A variety of water sources are used in the summer dry period and, if possible, groundwater is opportunistically accessed during the wet period. Presumably, this is because upward vertical migration of the water table by rainfall recharge brings this source to within the active root zone. While this opportunistic strategy enabled all stages of maturity to improve water status rapidly with the onset of wet conditions, the relative expenditure (across stages of maturity) of maintaining dimorphic roots in seasonally dry soil layers could be reflected in canopy physiology.

The relationship between the maximum photon yield of PSII and the Huber value (Figure 4.6) provides information on how an investment in water supply capacity (for a given leaf area) influences the performance of the photosynthetic apparatus in *E. gomphocephala*. Generally a greater sapwood area for a given leaf area was associated with a high $F_v/F_M$. The trend for a given site was that large mature plants exploited this had a higher $F_v/F_M$. This observation implies that conservative deployment of leaf area for a given sapwood area promotes enhanced photosynthetic capacity in this species. While the relative imperative of this investment was driven by stage of maturity, a close proximity to saturated soil did not appear influential. There are two possible explanations for this: 1) plants at site 1 could have a greater investment in total leaf area which could have offset the disadvantage of a low $F_v/F_M$, or 2) the small volume of rhizosphere at site 1 could have impacted on photosynthetic capacity.

The role of stage of maturity in the expression of the Huber value at a given site (Figure 4.6) was consistent with the pre-dawn leaf water potential which an individual experienced. For a given site, seedlings had a somewhat lower $\Psi_{pd}$, Huber value and $F_v/F_M$. The most likely explanation for this trend is that seedling roots occupy the region of the rhizosphere most susceptible to low soil
PARTITIONING OF WATER SOURCES

It remains unclear what the carbon cost is for trees that support less leaves for a given sapwood area (reflected by a high Huber value) but it is of significance that most canopy dieback in this species occurs in such large trees (refer forward to Chapter 7 for an examination of tree size and canopy condition).

For a given transpiration rate and hydraulic conductance an increase in the length of the hydraulic flow path will be accompanied by a proportional increase in the water potential gradient between the soil and leaf, invoking a greater probability of xylem dysfunction. Since leaf gas exchange properties have been shown to be coupled to leaf specific hydraulic conductivity (Sperry et al. 1993; Sperry and Pockman 1993; Brodribb and Feild 2000), it is likely that large trees, with a concomitant long hydraulic flow path, will express conservative gas exchange properties and reduced carbon gain in the upper canopy. Such compensation has been implicated in limiting tree height (Koch et al. 2004) and forest productivity (Hubbard et al. 1999; Schafer et al. 2000). In this study such compensation for tree size in leaf processes, quantified as $F_v/F_M$, was not evident. In fact it was observed that $F_v/F_M$ increased with an increasing hydraulic flow path (associated also with transition to maturity and greater depth to groundwater). Similarly, for a given site the apparent increase in $K_s$ and $K_L$ with stage of maturity (Figure 4.7) is at variance with the generally reported decline in this parameter with tree size (Meinzer and Grantz 1990; Saliendra et al. 1995; Delzon et al. 2004). An explanation for this is that the range of tree sizes in this study was not large enough to invoke such compensation and indeed a progression toward maturity could have been favourable, on a leaf area basis, because of a greater occupation of habitat by what could be considered an opportunistic root system.

4.5 Conclusions

Seedlings examined in this study maintained a greater proportion of functional roots in the upper, seasonally dry, soil profile which was demonstrated by a large increase in surface water use with transition to the wet season. On a per site basis, this investment in shallow roots that function mainly during favourable wet
One explanation is that the consequence of maintaining these shallow roots is reduced photosynthetic and hydraulic efficiency in the above ground canopy. A low $K_s$ in seedlings could represent a microstructural modification to xylem tissue, for example narrower xylem vessels, to reduce susceptibility to embolisms. Despite this adaptation seedlings still seemed to be under more stress than larger trees. This could mean that: 1) the anatomical modification was insufficient to prevent deleterious water potentials at midday, or 2) leaf microclimatic conditions around seedlings resulted in hotter leaves imposing stress to the photosynthetic system.

The results of this chapter confirm that, across stages of maturity and location, *E. gomphocephala* is an opportunist, able to express a phreatophytic state when conditions are favourable. Despite this general similarity in behaviour, subtle differences in root deployment for water uptake were reflected in seedling, sapling and tree canopy processes. While it remains unclear whether large trees situated in areas where the depth to groundwater is great are expressing more or less canopy dieback, the observation that such trees had a high Huber value and $F_v/F_M$ warrants further investigation. This observation is in conflict with a typical, soil water limited, system where large trees high in the landscape usually express greatest water stress. It could be that adaptation to dryer soil profiles present high in the landscape has enabled these trees to resist the impact of a general shift to a drying environment.
CHAPTER 5

VULNERABILITY TO XYLEM EMBOLISM IN
EUCALYPTUS GOMPHOCEPHALA AND ITS CO-
OCCURRING CANOPY SPECIES OF YALGORUP
NATIONAL PARK
Summary

This chapter is an interspecific study of vulnerability to water stress-induced xylem embolisms in *Eucalyptus gomphocephala* and several co-occurring canopy species in Yalgorup National Park. Drawing upon these physiological comparisons, the pre-European geographical distribution on the Swan Coastal Plain of the studied species is examined in regard to the role of drought tolerance and species distribution. Vulnerability to xylem embolisms did vary across the small but diverse group of species in this study. However, there was no relationship between this variability and the minimum rainfall at which an individual species occurs. A rationale for this is discussed in terms of growth rate and plant organ longevity. Figure 5.1 highlights the elements of the thesis outline that are covered in this chapter.
Figure 5.1 A reproduction of the thesis outline describing the processes considered in this chapter. An inter-specific comparison of vulnerability to embolism undertaken to explore the possibility that *E. goyderi* is drought prone compared to co-occurring canopy species.
5.1 Introduction

An important aspect of drought resistance in plants is the avoidance of loss of hydraulic conductivity of xylem due to air embolisms (Tyree and Sperry 1989). Air embolisms form as a result of the expansion of a small air bubble that has been drawn into the xylem water column, which is under tension (Zimmerman 1983; Tyree and Sperry 1989). The explosive expansion of the water bubble under tension is known as cavitation. The higher the tension in the xylem water column, the higher the chance of cavitation and air embolism. Once embolised a xylem conduit will be non-functional, therefore reducing conductance of the hydraulic flow path (Sperry and Tyree 1988; Tyree and Sperry 1989). Vulnerability to embolism is typically quantified by vulnerability curves (refer also to Chapter 3). These are plots of percentage loss of hydraulic conductivity $K$ over a range of xylem water tensions, quantified as negative water potentials ($\Psi$) (Sperry et al. 1987).

The process of refilling embolised xylem conduits, particularly under tension, remains a topic of intense research effort (Tyree et al. 1999; Cochard et al. 2000; Hacke and Sperry 2003), and is theorised to involve migration of osmotically active solutes from live tissue around xylem. This process aside, a low susceptibility to cavitation has been suggested to engender a species with a high resistance to drought (Pockman and Sperry 2000). However, this somewhat narrow assessment of resistance to drought needs to be coupled to an appraisal of gross morphology such as rooting depth.

To reiterate from previous chapters, reduced rainfall in south western Australia (Indian Ocean Climate Initiative 2002) has been suggested as a possible mechanism for the decline of several native tree species. Most of these species are large canopy emergents from the genus Eucalyptus and are iconic elements of south western Australian forests. Members of this genus are renowned as opportunistic scavengers of soil water (as reported in Chapter 4), capable of exploiting deep groundwater pools (Bell and Williams 1997), but are also considered to have clearly defined geographical distributions associated with
local environmental conditions such as soil type and rainfall (Hughes et al. 1996). Under a scenario where reduced rainfall has compounded the effect of the annual summer dry season in southwestern Australia, by either directly reducing soil moisture or lowering water tables, species formerly adapted to more mesic conditions will be disadvantaged. Indeed, since woody plants may operate close to the point of catastrophic xylem dysfunction (Tyree and Sperry 1988), such conditions could invoke the decline of susceptible species by increasing the likelihood of xylem embolism formation.

As mentioned previously, *Eucalyptus gomphocephala* D. C. is a species showing the characteristic signs of decline, reduced canopy condition and mortality, particularly in the vicinity of Yalgorup National Park (32.90S; 115.69E), while co-occurring species of this same area appear largely unaffected. To better understand the hypothesised linkage between the *E. gomphocephala* canopy decline event and reduced rainfall, this chapter builds on the knowledgebase developed in the thesis thus far by conducting an interspecific comparison of vulnerability to cavitation. In addition to *E. gomphocephala*, the species assessed for vulnerability to cavitation comprised two further members of the family Myrtaceae (*Eucalyptus marginata* Sm. and *Agonis flexuosa* (Wild. Sweet)) and one from the family Fabaceae (*Acacia cyclops* G. Don). This small but locally diverse group of plants represents canopy species that currently co-occur with *E. gomphocephala* in Yalgorup National Park. To test the likelihood that differences in vulnerability to embolism influence the distribution of these species, an assessment of the natural (pre-European) geographical distribution on the Swan Coastal Plain and mean annual rainfall of the region was also undertaken.

5.2 Material and Methods

5.2.1 Plant Material and Field Site

The three species selected for this study, in addition to *E. gomphocephala*, are canopy co-dominants in Yalgorup National Park that are not showing the
characteristic signs of a canopy decline syndrome. All sampling took place from mature individuals during spring 2005. All samples were collected from within Yalgorup National Park (32.90S; 115.69E) early in the morning and immediately transported to the laboratory.

5.2.2 Vulnerability Curves

Vulnerability to xylem embolism was determined as the relationship between percentage loss hydraulic conductivity (PLC) and xylem water potential ($\Psi_x$) (Sperry and Tyree 1988). Stems were initially 35 – 40 cm long but were trimmed with a razor blade under distilled water in the laboratory to 25 - 30 cm long, representing a length longer than the measured vessel size of each species. Cavitation events were induced by the air injection principle (Cochard et al. 1992; Sperry and Saliendra 1994), which, unlike the classical method of Chapter 3, allowed for replication of PLC at a given $\Psi_x$. Air embolisms can be simulated by use of positive pressures. This principle raises the pressure in the air phase of stems to create a pressure gradient across the air water interface of xylem tissue. Use of positive pressure has involved whole stems in pressure chambers (Tyree et al. 1984) or parts of stems using pressure collars (Cochard et al. 1992; Sperry and Saliendra 1994), the protocol used in this study.

Briefly, a stem segment was inserted inside a pressure collar (Figure 5.2) and the ends attached to an apparatus designed to measure flow rates through stems (see Chapter 3). This comprised of a section of Peek™ resistance tubing ordered in series with the stem segment. The measured relationship between the pressure drop across the Peek™ tubing (which was maintained to less than 5 kPa during measurements) and mass flow rate ($Q$, kg s⁻¹) permitted determination of stem flow rate (Feild et al. 2001).
Figure 5.2  A schematic diagram of the pressure collar used to induce a given water potential in stem xylem. A stem is sealed in the chamber with the ends protruding. Air pressure in the chamber is then raised by connecting the collar to a pressure reservoir. The pressure is maintained at a desired level to induce a given xylem water potential.

An initial maximum measurement of hydraulic conductivity $k_h$ ($k_{\text{max}}$, kg(water) s$^{-1}$ m$^{-2}$ (xylem) MPa$^{-1}$) was made after flushing the stem with a filtered (0.11 µm) degassed perfusion solution (1 mM KCl in double distilled water). Air was then injected into the collar to a desired pressure, referred to here as the air injection pressure ($P_{\text{inj}}$, MPa), and maintained for 15 minutes. The air injection pressure is equivalent to the water potential of xylem multiplied by negative one. The pressure in the collar was then slowly released and $k_h$ remeasured. This procedure was repeated to progressively higher pressures and the relationship between percentage loss hydraulic conductivity (PLC) and $\Psi_x$ determined:

$$\text{PLC} = 100 \left( \frac{k_{\text{max}} - k_h}{k_{\text{max}}} \right)$$

(5.1)

A dose response sigmoidal model was fitted to plots of PLC versus the air injection pressure:

$$\text{PLC} = \frac{100}{1 + \left( \frac{\log \left( 1 - \frac{P_{\text{inj}}}{P_{\text{inj, max}}} \right)}{b} \right)}$$

(5.2)
where $a$ is the maximum gradient of the curve and $\log b$ is $P_{mj}$ where $\text{PLC} = 50$ (denoted here as $\Psi_{x50}$). The exponential-sigmoidal fit of Pammenter and Vanderwilligen (1998) was not used because it was a poor description of the relationship between PLC and $P_{mj}$ in this case.

5.3 Results

The pre-European natural geographical distribution on the Swan Coastal Plain of the four species appears to correspond with rainfall distribution (Figures 5.3 to 5.6; rainfall data from records for the last 100 years). Of the four species, $A. cyclops$ occupies drier regions than the remaining three species, suggesting that this species is the most tolerant of low rainfall.
Figure 5.3 The natural (pre-European) geographical distribution of *E. gomphoecephala* (reproduced from Chapter 2). Overlaid are the regional, 100 year, mean annual rainfall isohyets (at 50 mm intervals). Data supplied by the Department of Conservation and Land Management (2006) and Bureau of Meteorology (2006) (Sheperd 2003). Datum – GDA 94.
Figure 5.4. The natural (pre-European) geographical distribution of *E. marginata*. Overlaid are the regional, 100 year, mean annual rainfall isolyets (at 50 mm intervals). Data supplied by the Department of Conservation and Land Management (2006) and Bureau of Meteorology (2006) (Sheperd 2003). Datum - GDA 94.
Figure 5.5  The natural (pre-European) geographical distribution of *Agonis flexuosa*. Overlaid are the regional, 100 year, mean annual rainfall isolyets (at 50 mm intervals). Data supplied by the Department of Conservation and Land Management (2006) and Bureau of Meteorology (2006) (Sheperd 2003). Datum - GDA 94.
Vulnerability curves (Figure 5.7) suggest that the Myrtaceous species have a similar cavitation response with progressive exposure to tension in xylem,
becoming fully embolised when the air injection pressure exceeded 6.0 MPa (equivalent to a xylem water potential of \(-6.0 \text{ MPa}\)). *Acacia cyclops*, on the other hand, became fully embolised at the much lower air injection pressure of between 2 and 3 MPa. Comparing the slope \((a)\) and the value for \(\Psi'_{x,50}\) for each individual stem yielded a convergent linear correlation (Figure 5.8) described as: 

\[ a = 2.88 - 1.05 \Psi'_{x,50} \]  

\((r^2 = 0.99, P < 0.01)\). In other words, a greater slope was associated with a lower \(\Psi'_{x,50}\). Statistical analysis showed that both \(a\) and \(\Psi'_{x,50}\) for *A. cyclops* was significantly different from the remaining species (1-way ANOVA, Table 5.1).

Both \(a\) and \(\Psi'_{x,50}\) were exponentially correlated with the minimum rainfall at which a species occurred (Figure 5.9). For \(a\) versus minimum rainfall the correlation was described as: 

\[ a = 0.40 + 1.55 e^{\left(-\frac{\text{min rainfall}-650}{3122}\right)} \]

and for \(\Psi'_{x,50}\) versus minimum rainfall the correlation was described as:

\[ \Psi'_{x,50} = 2.41 - 1.51 e^{\left(-\frac{\text{min rainfall}-650}{3788}\right)} \]  

\((r^2 = 0.99\) and for the relationship between \(a\) and minimum rainfall and \(r^2 = 0.99\) for the relationship between \(\Psi'_{x,50}\) and minimum rainfall respectively). This would mean that the capacity to occupy a low rainfall region is associated with a high \(a\) and low \(\Psi'_{x,50}\).
VULNERABILITY TO DROUGHT IN CO-OCCURRING SPECIES

Figure 5.7 Vulnerability curves, plots of percentage loss hydraulic conductivity (PLC) versus air injection pressure ($P_{in}$), for *E. gomphocephala*, *E. marginata*, *Agonis flexuosa*, and *Acacia cyclops*. The fitted lines are sigmoidal models; refer to Table I for fitting parameters.
Table 5.1  Curve fitting parameters for the sigmoidal fits in Figure 5.7: the slope of the relationship between PLC and the air injection pressure (a) and the value for $P_x$ at PLC = 50% ($\Psi_{x50}$). Values for a and $\Psi_{x50}$ are the mean (± s.e., n = 5 for each species). Superscript letters indicate homogenous subgroups (one-way ANOVA, Student Newman Keuls Post Hoc analyses; $P < 0.05$, SPSS for Windows version 13.0).

<table>
<thead>
<tr>
<th>Species</th>
<th>a</th>
<th>$\Psi_{x50}$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. marginata</td>
<td>0.40 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A. flexuosa</td>
<td>0.45 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.36 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>E. gomphocephala</td>
<td>0.74 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.98 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>A. cyclops</td>
<td>1.95 ± 0.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.91 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Figure 5.8  The slope (a) of the relationship between percentage loss hydraulic conductivity (PLC) and the air injection pressure ($P_x$) (denoted a) was negatively correlated with the $P_x$ at PLC = 50% ($\Psi_{x50}$).
VULNERABILITY TO DROUGHT IN CO-OCCURRING SPECIES

Figure 5.9 Exponential models described the relationships between $a$ and $\Psi_{50}$ versus the minimum rainfall at which each species occurred on the Swan Coastal Plain.
5.4 Discussion

Differences in the capacity of plants to resist cavitation with increasing water deficit have been suggested to be closely associated with tolerance of drought and therefore to explain patterns of species distribution in relation to rainfall (Pockman and Sperry 2000). Where a change in rainfall has induced a decline in available soil water, such as in south western Australia, the benefits of this could be significant. In this way the regional climatic shift witnessed in the last 30 years may influence the composition and structural complexity of vegetation communities by either directly reducing soil moisture and/or by lowering water tables, favouring species that can withstand long periods of water deficit.

The above rationale should be viewed with some caution, however. While rainfall is perhaps the strongest single limiting factor in plant survival, the environmental range of a species may be influenced also by other factors, such as soil type, nutrient availability and competition. Nevertheless, differences were observed in the natural geographical distribution of the study species that could imply a contrasting vulnerability to drought, as a result of occurrence in dissimilar ranges of rainfall. Specifically, given that *A. cyclops* occurs in drier regions than *E. gomphocephala, E. marginata* and *A. flexuosa*, a reasonable inference would be that this species is more adept at resisting drought, by avoiding cavitation, than the latter three species.

However, on the basis of an interpretation of the relationship between PLC and the air injection pressure (Figure 5.6), susceptibility to xylem embolism (as estimated from the arbitrary value of $\Psi_x$ at PLC = 50%), and hence vulnerability to drought, would decrease in order of *A. cyclops, E. gomphocephala, A. flexuosa*, and *E. marginata* (Table 5.1). Similarly the slope of the relationship between PLC and $-\Psi_x$ decreased in the same order. Under the assumptions of Pammenter and Vanderwilligen (1998), the slope of the relationship between PLC and the air injection pressure is related to the distribution of maximum pit pore size per vessel and $\Psi_{x50}$ PLC is associated with the mean of the largest pit pore per vessel. The correlation between these parameters in this study (Figure
5.7) would imply that they are interdependent. A large average pit pore per vessel tended to associate with a distribution of these complexes that maximised the rate of cavitation exchange across vessels over a water potential threshold. It could be argued that the arbitrary utilisation of $\Psi_{x,50}$ as a comparison of cavitation susceptibility masks the true impact of drought on xylem integrity because it assumes that this inflexion point is representative of catastrophic xylem dysfunction. Moreover, plants that regulate transpiration rate such that isohydry is achieved cannot be directly compared to anisohydric species on the basis of $\Psi_{x,50}$. Perhaps a more meaningful representation of cavitation susceptibility is a maximum slope of the relationship between PLC and $P_{\text{inj}}$, which describes the range of water potentials over which xylem embolisms occur. A species that experiences cavitation events across a large range of water potentials may be described as expressing substantial native embolisms. Congruent with this argument is the observation that a high $a$ (analogous to a small range of water potentials over which cavitation occurs) was related to species that occurred in low rainfall zones in this study (Figure 5.8A). The plasticity of phenotype could extend to xylem hydraulic properties in *E. gomphocephala* and the three additional species of this study. Hence it could be argued that assumptions based on vulnerability curves generated from species occurring in Yalgorup National Park do not account for the possible variability expressed across an environmental range.

Under the scenario that *E. gomphocephala* canopy dieback in Yalgorup National Park is related to reduced rainfall and an associated increase in the severity of the annual dry season, it could be argued that this species should express a heightened vulnerability to xylem embolism compared to co-occurring species that are not showing the symptoms of a decline syndrome. The results of this study, however, do not support this argument, with species not showing the outward visual expression of canopy decline comprising stems that are more vulnerable to drought induced xylem embolisms. Further study capturing *in situ* water relations patterns (that is, the range of water potentials and gas exchange properties expressed across seasons) in a range of species would clarify this uncertainty. In a preliminary study of the response of phreatophytic *Banksia*
species on the Swan Coastal Plain to reduced water availability, for example, Froend and Drake (2006) used this suggested methodology to help predict the survival of a species under periods of prolonged exposure to water deficit. The same study yielded a pattern of the relationship between PLC and $\Psi$, that proved compatible with in situ measurements, tracking leaf water potential to the point of mortality under an artificially declining hydrology. In this current study a similar exercise could potentially confirm the validity of ranking species according to $\Psi_{x50}$ or the slope of the relationship between PLC and the air injection pressure.

The protocol outlined above would need to acknowledge the mode of the effect of xylem embolisms on plants, which can take two forms: 1) plants can modify transpiration rate in an effort to reduce the formation of xylem embolisms, or 2) transpiration rate can be modified as a result of embolism formation (Jones and Sutherland 1991). Actively regulating transpiration rate by stomatal control is likely to be a favourable strategy in plants with a slow turnover in biomass, low productivity or in plants that express isohydry (Tardieu and Simonneau 1998). Whereas plants that rapidly turn over biomass, expressing high productivity, may maintain high rates of leaf gas exchange at the expense of a greater frequency of cavitation events. Such differences in longevity and functional imperatives typify the segmentation hypothesis that Zimmerman (1983) used to describe likely contrasts in susceptibility to cavitation across plant organs. In this study the relatively slow growing members of the family Myrtaceae could be expected to have a greater resistance to cavitation because of the greater longevity of organs. Acacia cyclops, on the other hand, is a fast growing species that is likely to rapidly turn over biomass.

5.5 Conclusions

Eucalyptus gomphocephala does not present drought intolerance that differs from its co-occurring canopy species. The differences that were observed between the co-occurring species of Yalgorup National Park could relate to their
respective relative growth rate and organ longevity. Further study of these parameters could yield a greater understanding of the role of extended periods of drought on the health and species composition of *E. gomphocephala*-dominated communities.
CHAPTER 6

HYDRAULIC PROPERTIES OF \textit{Eucalyptus gomphocephala} ROOTS
Summary

This chapter focuses on the hydraulic and anatomical characteristics of *Eucalyptus gomphocephala* roots growing in Yalgorup National Park. Under the scenario that an altered hydrology, as a result of climate change, has induced a decline in this species, it could be expected that current hydraulic and anatomical properties of roots are incompatible with the edaphic conditions of Yalgorup National Park. Percentage loss of hydraulic conductivity (PLC) was significant even under ideal growth conditions. This could support the notion that the optimal ecohydrological niche of *E. gomphocephala* has been lost and an ensuing loss in root hydraulic conductivity has been experienced. However, because PLC and $\Psi_{pd}$ were not correlated, the expression of native embolisms may be high in this species even under such ideal conditions. With greater investment in an anatomy that maximises hydraulic conductivity, roots appear to experience more embolisms in the field. This is despite the current view that vulnerability to embolism is only partially coupled to aspects of xylem anatomy that dictate hydraulic efficiency. The processes explored in this chapter are highlighted in a reproduction of the thesis outline (Figure 6.1).
Figure 6.1 The thesis outline highlighting the elements specific to this chapter. These are the rooting properties of roots and their possible association with plant water relations and canopy decline.
6.1 Introduction

Root architecture is related to the capacity of a plant to acquire soil resources. The arrangement and functional attributes of roots in soil often govern plant processes that are directly linked to the transpiration stream. One reason for this is that the transport pathway between soil and root xylem prescribes the greatest resistance to liquid water flow in the soil-plant-atmosphere continuum, particularly in dry soil. In this way the distribution of water in soil may influence the xylem hydraulic properties of an entire plant since a hydraulic limitation will occur as the soil-to-root water potential approaches and moves beyond a critical threshold (Tyree and Sperry 1988; Hacke and Sauter 1996; Sperry et al. 1998; Nardini and Pitt 1999; Pinol and Sala 2000; Hacke and Sperry 2001; Martinez-Vilalta et al. 2002). Root systems therefore represent a highly responsive feature of the catenary model of water movement in plants (van den Honert 1948) because they interface with steep water potential gradients that may exceed the inherent hydraulic limits of xylem conduits. An inter-specific adaptive response to hydraulic limits in xeric habitats includes the development of an extensive root system (Canadell et al. 1996; Jackson et al. 1996; Jackson et al. 2000). Such systems allow for greater exploration and subsequent exploitation of soil resources and improve the likelihood of maintaining a water potential gradient across the soil root interface that does not exceed critical thresholds for loss of xylem function.

Zencich et al. (2002) have shown differential access and use of soil water (groundwater and surface water) in *Banksia*-dominated communities on the Swan Coastal Plain of south-western Australia. In these systems banksias occupy a similar ecohydrological niche to the species under investigation here. The inference that can be drawn from Zencich et al. (2002) is that individuals display significant morphological plasticity in the root zone, and that conditions during development determine the magnitude of the response of individuals (and hence the expression of this plasticity) to subsequent changes in the distribution of soil water. The capacity for root systems to respond to changes to soil water is likely to play a significant role in drought tolerance (Saliendra and Meinzer 1992; Meinzer et al. 1999; Moreira et al. 2003).
Many species are thought to operate close to the point of detrimental loss of xylem function (Tyree and Sperry 1988). This is due, in part, to the trade-off between the advantages of a high hydraulic conductivity provided by an efficient hydraulic flow system versus the disadvantage of the same system experiencing a greater likelihood of xylem embolisms. One theoretical explanation for this trade-off, and a practical linkage to the air-seeding hypothesis, is that large xylem conduits, while representing an efficient flow path for water (flow is proportional to conduit diameter in the quadratic dimension), are likely to expose a proportionally greater number of large pits to water potential gradients that could invoke cavitation events (Hargrave et al. 1994). On the other hand, experimental evidence has shown that pit membrane properties *per se*, which should determine drought induced cavitation (Choat et al. 2002), are only partially coupled to conduit diameter (Hacke and Sperry 2001; Domec et al. 2006a). In the process of freezing-induced cavitation, wider conduits are considered more vulnerable (Ewers 1985; Davis et al. 1999), but this is because wider conduits contain more air in solution for a given length. In the context of water deficit, it could be argued that favouring a safe or efficient xylem hydraulic strategy will associate with the ecohydrological niche of a species. For example, access to seasonally permanent soil water could favour the expression of high hydraulic conductivities and, a yet to be accorded, modification to xylem anatomy.

*Eucalyptus gomphocephala* is known to possess an extensive root system and occupies a region where access to permanent (and often deep) soil water is likely to be advantageous (refer to Chapter 4 for a detailed description of water source strategies in *E. gomphocephala*). This is because the severity of the annual summer dry season induces very dry conditions in the vadose zone. With an extensive root system to access seasonally permanent water, *E. gomphocephala* should possess a root xylem anatomy comprised of large diameter conduits designed to transport water efficiently. A recent reduction in annual rainfall recharge in this region (Indian Ocean Climate Initiative 2002) coupled with the declining canopy condition of *E. gomphocephala* could be interpreted to mean that the rooting properties of this species are no longer compatible with its environment. This incompatibility could take the form of a poor ability to respond to changes in the position of the water table accompanied by extensive
cavitation of xylem conduits forced to experience the very low water potentials of the vadose zone. With this proposition in mind, the objective of this chapter is to undertake an investigation of the functional root architecture of *E. gomphocephala*. Specifically, the aim of this chapter is to characterise the trade-off in safety versus efficiency in *E. gomphocephala* roots and to correlate variation in xylem anatomy with *in situ* root hydraulic properties. If, as theorised, *E. gomphocephala* possesses an efficient root xylem anatomy, the loss of the species' ecohydrological niche should be reflected as compromised *in situ* root hydraulic properties.

### 6.2 Material and Methods

#### 6.2.1 Study Area and Sampling Design

The study was conducted in Yalgorup National Park (32.90S; 115.69E), south-western Australia, comprising one of the last populations of *E. gomphocephala* that has not been subjected to fragmentation resulting from urbanisation. As previously mentioned, *E. gomphocephala* in Yalgorup National Park have suffered canopy decline since the early to mid 1990’s. The climate of the region is described as Mediterranean, with cool wet winters and hot dry summers. During the last 25 years winter rainfall in this region was approximately 25% below the previous 25 year average (Indian Ocean Climate Initiative 2002). The substrate of Yalgorup National Park consists of well draining sediments inter-dispersed with unconsolidated limestone overlying limestone bedrock. The underlying hydrology of the area is dominated by a number of shallow superficial aquifers that drain internally into a series of hypersaline lakes, the swales of ancient dune systems (Commander 1988).

Six randomly selected trees, from within Yalgorup National Park (three saplings and three small trees) were partially excavated with an air spade (2000 Series Concept Engineering, PA, USA) to expose proximal regions of lateral roots. While all trees showed signs of decline, they were observed to have maintained greater than 50% of their total canopy. Sapling diameters at breast height (DBH)
ranged from 9.8 cm to 14.0 cm. Small tree DBHs ranged from 18.0 cm to 22.0 cm. Care was taken to ensure that fine roots were left intact during excavation (except where this material was collected) and that the surrounding soil was subjected only to necessary compaction for access. Sampling took place during November, coinciding with the end of the annual wet period, and hence when root biomass would be greatest.

6.2.2 Leaf Water Potential

Pre-dawn (04:00 – 05:30 local standard time) leaf water potential ($\Psi_{pd}$) was determined for each tree after the partial excavation process was completed ($n = 4$ for each plant) with a Scholander-type pressure chamber (model SKPM 1405, Skye Instruments, London UK) (Scholander et al. 1964). Leaf samples were kept in snap-lock plastic bags throughout the procedure to minimise transpirational water loss (Richie and Hinkley 1975).

6.2.3 Root Hydraulic Properties

Root hydraulic properties were determined for secondary roots (originating from laterals) of each tree ($n = 6$) using a steady state flow meter (SSFM), similar to that described by Feild et al. (2001) (and as discussed in earlier chapters), and a perfusion solution consisting of 0.01 M KCl and degassed double distilled water with a pH of 6.6, filtered to 0.11 µm. Root samples were collected at pre-dawn (immediately after $\Psi_{pd}$ determination) to ensure they were at maximum in situ hydraulic capacitance and therefore likely to express minimum native embolisms. Roots were double bagged and immediately transferred to a field laboratory for subsequent measurement of xylem-area-specific root hydraulic conductivity ($K_s$) and percentage loss hydraulic conductivity (PLC). Sampled roots were initially greater than 0.25 m but were later recut and trimmed with a razor blade while immersed in distilled water to a range of 0.043 m – 0.087 m representing a length at least 10% greater than the longest measured vessel. Prior to insertion in the SSFM, tissues external to the vascular cambium (phloem, phloem fibres, cortex and periderm) were carefully removed from the ends of the
HYDRAULIC PROPERTIES OF ROOTS

Root segment so that measurements were made on the xylem only. Root segment diameters ranged from 0.026 m to 0.008 m after this procedure. Xylem-area-specific root hydraulic conductivity is defined here as the mass flow movement of water through the excised root segment per unit of pressure gradient:

\[ K_x = J_v \times \Delta l / \Delta P \]  \hspace{1cm} (kg m\(^{-1}\) s\(^{-1}\) MPa\(^{-1}\)),  \hspace{1cm} (6.1)

where \( J_v \) (kg\(_{(\text{water})}\) s\(^{-1}\) m\(^{-2}\) (root xylem)) is the mass flux density of the perfusion solution (0.01 M KCl in degassed, double distilled water), \( \Delta l \) is the length of the root segment (m) and \( \Delta P \) is the water pressure difference across the length of root segment (MPa). Xylem area was assumed to closely parallel stele cross sectional area (measured accurately with digital callipers).

Percent loss hydraulic conductivity (PLC) of roots was determined from the difference between an initial measurement of hydraulic conductivity \( k_i \) (kg\(_{(\text{water})}\) s\(^{-1}\) m\(^{-2}\) (xylem) MPa\(^{-1}\)) and a maximum value \( k_{\text{max}} \) measured after roots had been flushed with the perfusion solution at high pressure (100 kPa for 15 minutes):

\[ PLC = 100(k_{\text{max}} - k_i) / k_{\text{max}} \]  \hspace{1cm} (6.2)

In addition, maximum \( K_x \) (denoted here as \( K_{x_{\text{max}}} \)) was determined from equation 6.1 where \( J_{\text{max}} \) (the maximum mass flux density) was substituted for \( J_v \). Xylem-area-specific root hydraulic conductivity of stems measured in their native state is denoted as \( K_x_{\text{in situ}} \) to distinguish it from \( K_{x_{\text{max}}} \). \( K_x_{\text{in situ}} \) was also expressed on a per unit root length basis (L\(_p\), m\(^2\) s\(^{-1}\) MPa\(^{-1}\)):

\[ L_{P_i} = \frac{Q}{\Delta P} \times \frac{1}{\Delta l} \]  \hspace{1cm} (m\(^2\) s\(^{-1}\) MPa\(^{-1}\)),  \hspace{1cm} (6.3)

where \( Q \) (m\(^3\) (water) s\(^{-1}\)) is the volume flux of water through the root.
6.2.4 Root Anatomy

Selected root segments, adjacent to those used for hydraulic conductivity measurements, were fixed in FAA then stored in 70% ethanol. Segments were rehydrated by rinsing under tap water then immersing them in distilled water for one hour. Root samples were then embedded in paraffin wax and transverse sections cut to 10 µm with a rotary microtome (Leica model RM 2125, Leica Microsystems, Wetzlar, Germany). Sections were then positioned on slides that were dipped in 2% gelatin immediately prior to mounting. Slides were then placed in a coplin jar with filter paper soaked in formaldehyde to allow vapour fixation (of section to gelatin). The coplin jar was covered with a lid and the section allowed to dry at room temperature for 12 hours. Sections were then stained in 0.1% aqueous toluidine blue, examined under a compound light microscope and images captured with a digital camera. Xylem vessel diameters ($n = 50$ randomly distributed vessels for each sample) were then measured in Adobe Photoshop version 5.5 using the measured relationship between a graticule scale and image pixel size. Since conduit radius and flow rate are related in the quadratic dimension (Hagen-Poiseuille law) the hydraulically weighted mean vessel diameter ($d$) of Sperry et al. (1994) was used:

$$d = 2 \left( \frac{\sum r^5}{\sum r^4} \right) \quad (\mu m), \quad (6.4)$$

where $r$ is the radius of a xylem vessel.

6.3 Results

Pre-dawn leaf water potentials ranged from $-0.5$ to $-0.2$ MPa (Figure 6.2). A one-way ANOVA indicated that there was no significant difference in $\Psi_{pd}$ between saplings and trees ($P > 0.05$). A similar analysis revealed that there was no difference between individual plants ($P > 0.05$).
Percentage loss hydraulic conductivity did not differ on the basis of size of individual (one-way ANOVA; $P = 0.427; F = 0.646$, Figure 6.3); however there was a significant difference between individual plants (one-way ANOVA; $P = 0.003; F = 4.692$).

Figure 6.2 Mean ± s.e. ($n = 4$) pre-dawn leaf water potential ($\Psi_{pd}$) for *E. gomphocephala* saplings (plants 1, 3 and 5) and trees (plants 2, 4 and 6).
Figure 6.3  Mean ± s.e. (n = 6) percentage loss hydraulic conductivity (PLC) for *E. guamphoeplada* roots from saplings (plants 1, 3, 5) and trees (plants 2, 4, 6). Samples were collected immediately after $\Psi_0$ measurements.

PLC of roots was not linearly related to pre-dawn leaf water potential ($P = 0.88$ for PLC). However, $K_s$ *in situ* was exponentially correlated to $\Psi_{pd}$ (Figure 6.4). Within this correlation there was no apparent grouping between saplings and trees.
HYDRAULIC PROPERTIES OF ROOTS

In situ root hydraulic conductivity ($K_{s\text{\_in situ}}$, kg s$^{-1}$ m$^{-1}$ MPa$^{-1}$) was exponentially related to pre-dawn leaf water potential ($\Psi_{pd}$) in *Eucalyptus globulus* saplings (plants 1, 3 and 5) and trees (plants 2, 4 and 6). The exponential model fitted to the data has the equation: 

$$K_{s\text{\_in situ}} = 3.70 + 0.04e^{0.01(\Psi_{pd} - 0.70)}.$$ 

Similarly, when *in situ* root hydraulic conductivity was expressed on a per unit length basis ($L_{ph}$, m$^2$ s$^{-1}$ MPa$^{-1}$) an exponential correlation with $\Psi_{pd}$ emerged (Figure 6.5). Once again there was no discernable difference between saplings and trees.
HYDRAULIC PROPERTIES OF ROOTS

Figure 6.5  *In situ* root hydraulic conductivity, expressed on a per unit length basis ($L_p$) was exponentially correlated with $\Psi_{pd}$ in *Eucalyptus gomphocephala* saplings (plants 1, 3 and 5) and trees (plants 2, 4 and 6). The exponential model describing this correlation has the equation:

$$L_p = 5.0 \times 10^{-6} + 2.87 \times 10^{-7} e^{0.31 \psi_{pd}} (r^2 = 0.81).$$

In each of the sectioned roots little to no pith was apparent but there was a noticeable trend of smaller vessel diameters in older wood (Figure 6.6). Vessel diameters extended from 20 to 190 µm, each stem being comprised of a wide range of diameters. A frequency distribution of vessel diameter of all sampled roots (Figure 6.7) indicated that the majority of vessel diameters were in the order of 75-80 µm.
Figure 6.6 A typical cross section of a *Eucalyptus gomphocephala* root. The image comprises secondary xylem (X), secondary phloem and fibres (SPh), cortex (C) and phellogen (P). Note that measurements of hydraulic conductivity in this study were restricted to the stele (dominated by xylem vessels) and that there was no substantial pith development in sectioned roots.
HYDRAULIC PROPERTIES OF ROOTS

In situ root hydraulic conductivity \( (K_{S_{\text{in situ}}}) \) was exponentially related to hydraulically weighted mean vessel diameter \( (d) \) with \( d \) approaching infinity at 0 \( K_{S_{\text{in situ}}} \) (Figure 6.8A). Maximum root hydraulic conductivity \( (K_{S_{\text{max}}}) \) was also exponentially correlated with \( d \) but in this case \( d \) approached infinity at a \( K_{S_{\text{max}}} \) of 62.50 kg s\(^{-1}\) m\(^{-1}\) MPa\(^{-1}\) (Figure 6.8B). Similarly, percentage loss hydraulic conductivity (PLC) was exponentially related to \( d \), with \( d \) approaching infinity at a PLC 44.95 (Figure 6.8C).

\[ \text{POT} = 0.38 + 87.00e^{-\frac{(\text{vessel diameter}-12.32)}{5.36}} \]
HYDRAULIC PROPERTIES OF ROOTS

Figure 6.8  In situ root hydraulic conductivity ($K_s$ (in situ), A), $K_{\text{max}}$ ($K_s$ (max), B) and percentage loss hydraulic conductivity (PLC, C) were exponentially correlated with the hydraulically weighted mean vessel diameter ($d$) for saplings (plants 1, 3 and 5) and trees (plants 2, 4 and 6). The exponential models of A, B and C respectively have the following equations:

$$K_{\text{in situ}} = 2 + 3815.06e^{-\left(\frac{d}{3.62}\right)} + 3815.06e^{-\left(\frac{d}{7.62}\right)} \quad (r^2 = 0.56),$$

$$K_{\text{max}} = 62.53 - 46.41e^{-\left(\frac{d-80.83}{5.32}\right)} \quad (r^2 = 0.60),$$

$$K_{\text{max}} = 44.95 - 31.76e^{-\left(\frac{d-80.83}{4.43}\right)} \quad (r^2 = 0.85).$$
Percentage loss hydraulic conductivity was positively correlated with the maximum hydraulic conductivity of roots (Figure 6.9).

\[ \text{PLC} = 10.23 + 0.52K_{s_{\max}} \] (\( r^2 = 0.82; P = 0.01 \)).
HYDRAULIC PROPERTIES OF ROOTS

6.4 Discussion

All individuals exhibited a relatively high $\Psi_{pd}$ at the time of sampling (mean $= -0.30 \pm 0.02$ MPa). By sampling root material simultaneously with these measurements, native hydraulic properties should represent the most favourable diurnal condition, assuming maximum hydraulic recharge of plant xylem occurred in the pre-dawn phase. While susceptibility to xylem embolism in *E. gomphocephala* roots remains to be investigated in more detail, the range of $\Psi_{pd}$ recorded in this study seems unlikely to constitute catastrophic tensions for loss of xylem function. However, any disparity between individuals could relate to the availability of soil water, since $\Psi_{pd}$ is often assumed to closely parallel the soil water potential of the active rhizosphere (however see Donovan et al. (1999) for exceptions). With progression through the dry season this disparity would likely become exaggerated depending on the relative change in depth to seasonally permanent soil water underlying an individual and/or the extent of root systems which could be classified as disparate between saplings and trees.

Despite the high pre-dawn leaf water potentials of this study, substantial partial embolism of roots was observed in each individual (mean $= 32.53 \pm 2.99\%$). As mentioned, the pre-dawn sampling regime should mean that the PLC presented in this study reflects a minimum diurnal value. This would seem to suggest that, under the dry conditions of summer, *E. gomphocephala* roots will operate within the range of water potentials that could induce substantial xylem failure (Tyree and Sperry 1988). Alder et al. (1996) propose that partial xylem embolisms, such as that observed here, are not necessarily detrimental to plants but rather provide a regulatory mechanism (in addition to stomatal control of transpiration rate) during unfavourably dry conditions. The suggested mechanism of this control is by a negative feedback loop reducing the probability of a break in the hydraulic continuum by constraining volume flux within the xylem. Alternatively, this observation could be evidence that significant cavitation events in *E. gomphocephala* roots have occurred due to reduced rainfall recharge and subsequent decoupling of roots from permanent soil water. A similar result
HYDRAULIC PROPERTIES OF ROOTS

would be observed if roots had not recovered from previous periods of water stress.

A number of studies have indicated that roots can adapt, developmentally, to water deficit by producing a highly suberised interface between living tissue and the rhizosphere (see Steudle (2000) for a review). Such an adaptation would lower the overall root hydraulic conductivity but simultaneously minimise root water loss. While the results of this study did show a range of specific hydraulic conductivity in roots, the nature of measurements did not account for radial water movement. Hence the variation in $K_S$ in situ is more likely to relate to partial loss of xylem function induced by embolisms or the influence of developmental water stress on xylem conduit anatomy.

While the range of pre-dawn leaf water potentials in individual plants was low, the weak correlation between $K_S$ in situ and $L_p$ with $\Psi_{pd}$ implies that hydraulic recharge of plants overnight does indeed dictate the native hydraulic efficiency of *E. gomphocephala* roots. Shimizu et al. (2005) showed a similar pattern in four late successional species. However, the small range of $\Psi_{pd}$ in this study limits the inferences that can be drawn about the role of soil hydrology on the native root hydraulic properties of *E. gomphocephala*.

The data of this study, while preliminary, suggest that in this species roots with a smaller average hydraulically weighted mean vessel diameter express a greater native specific root hydraulic conductivity, lower maximum hydraulic conductivity and lower percentage loss hydraulic conductivity. This is similar to the results of Taneda and Tateno (2005) who observed a greater PLC in species with a larger hydraulically weighted mean vessel diameter. However the intra-specific correlations of this study are unlikely to be generally applicable across seasons. Rather, it is likely that dry conditions would cause a systematic offset in both correlations. That is, for any given $d$, $K_S$ in situ would be lower and PLC would be higher under drier conditions compared to those of this study. These correlations also describe the benefit of a conservative xylem anatomy in roots of this species since, in the native state, roots with smaller vessel diameters are
more hydraulically efficient. Under conditions of declining rainfall, such as that experienced by *E. gomphocephala*, it is important to understand the extent to which root systems can evolve a more conservative anatomy to maintain hydraulic efficiency.

The trade-off between hydraulic efficiency and safety alluded to above is depicted in the correlation between PLC and $K_s$ max. This correlation implies that the downside to developing xylem conduits presenting an efficient hydraulic flow path, due partly to a large conduit diameter, is a greater probability of embolism formation under native conditions. The relationship between xylem vessel diameter, and hence hydraulic conductivity, and vulnerability to freezing-induced embolisms is well established (Ewers 1985; Davis et al. 1999), but the same relationship is generally much weaker where drought induces xylem embolisms. The strength of the relationship between PLC and $K_s$ max in this study could indicate that the coupling of pit membrane properties to drought-induced xylem embolisms is weak compared to the influence of vessel diameter in *E. gomphocephala* roots. Tyree et al. (1994) suggest that the influence of drought recurrence on the anatomy of water transport efficiency is strong enough to be of evolutionary significance to plants. However, the observation that roots with a greater potential hydraulic conductivity are more prone to embolise under native conditions in this study could indicate that a conservative hydraulic anatomy is not a cost-effective means of drought adaptation in *E. gomphocephala*.

### 6.5 Conclusions

The observation that root PLC was significant even under the relatively ideal conditions of this study could support the notion that the optimal ecohydrological niche of *E. gomphocephala* has been lost and an ensuing loss in root hydraulic conductivity has been experienced. It is cautioned, however, that a poor association between PLC and $\Psi_{pd}$ could mean that the expression of native embolisms is high in this species even under ideal conditions. With greater
investment in $K_s_{max}$ roots appear to be encumbered by proportionally more embolisms in the field. This is despite the current view that vulnerability to embolism is only partially coupled to aspects of xylem anatomy that dictate hydraulic efficiency. While evidence of hydraulic efficiency in roots of *E. gomphocephala* was observed, it would appear that the current habitat of the species favours a more conservative strategy.
CHAPTER 7

PHYSIOLOGICAL INDICATORS OF CANOPY CONDITION
IN EUCALYPTUS GOMPHOCEPHALA
Summary

This chapter tests the possibility that present day *Eucalyptus gomphocephala* canopy condition is linked to dry season plant physiological traits that are associated with acute water stress. Using a multiple linear regression approach, it became evident that current dry season physiological traits are not strongly associated with current canopy condition in *E. gomphocephala*. The best correlates with canopy condition were diameter at breast height (DBH) and estimated transpiration rate ($E^*$), although the relationship between $E^*$ was both divergent and convergent depending on site conditions. While this implies that dry season physiological traits and canopy condition are only weakly coupled, a past reduction in rainfall, an associated decrease in aquifer recharge could have triggered the canopy dieback event that has become most visually conspicuous at present. The specific processes covered in this chapter are depicted in a reproduction of the thesis outline (Figure 7.1).
Figure 7.1 The thesis outline reproduced here to highlight the elements covered in this chapter. It could be argued that if the current recurrence of *E. grandis* canopy decline is due to the direct effect of reduced available soil water, as a result of climate change, the plant water relations of an individual should relate to the relative visual expression of canopy dieback.
7.1 Introduction

The debate about the underlying cause of tree decline syndromes in Australian eucalypts remains ongoing (see Jurskis (2005) for a recent review). Of the abiotic elements considered, climate change has a broad influence across the Australian continent. Extreme climatic conditions, of which extended periods of drought are an example, are predicted to increase under recently developed climate change scenarios (IPCC 2001a). This is an important consideration because an assessment of average change in rainfall (at for example 10 year time intervals) can mask the true extreme drought conditions faced by ecosystems. In Mediterranean habitats, such as south western Australia, this decline in rainfall will be severe (Palutikof and Wigley 1996; Haughton et al. 2001; Indian Ocean Climate Initiative 2002) hence understanding future change in the periodicity of drought events represents an important challenge for climatologists and water resource managers. Moreover, the accompanying effect of an increased probability of drought on forest ecosystems in south western Australia remains unclear and a topic that requires a coordinated research effort.

Drought-induced dieback of forest has been observed in temperate (Allen and Breshears 1998), tropical (Villalba and Veblen 1998; Suarez et al. 2004), savanna (Fensham and Holman 1999) and Mediterranean (Hobbs and Mooney 1995) ecosystems. These dieback events can be described as the outward visual expression of the physiological impact of reduced plant water status (or water stress). The physiological impact of water stress resulting in branch, and ultimately tree, dieback will often take the form of xylem cavitation, a break in liquid continuity in the xylem water column, due to excessive tension (Zimmerman 1983) followed by the formation of embolisms in the xylem conduits. Prior to complete failure of the plant hydraulic system, tissue water potential will typically decline to accommodate water uptake under a drying soil environment. Due to the functional interdependence of stomata and the plant hydraulic system, plants experiencing water stress will also exhibit changes linked to leaf gas exchange.
Measurement of instantaneous water stress indicators in plants and their coupling to a quantitative expression of canopy condition will contribute to general causal models of canopy decline syndromes. It would be expected, for example, that greater exposure to water stress (and its relative reflection in physiological properties) will also be manifested in the visual expression of a canopy decline syndrome. On the other hand it should be acknowledged that, where the water stress perturbation has initiated secondary stresses, the magnitude of canopy dieback (which is an integration of long term conditions) will possibly become disconnected from instantaneous physiological properties.

The *Eucalyptus gomphocephala* tree decline syndrome in Yalgorup National Park (south western Australia) represents a possible example of drought-induced canopy dieback. The event, which is considered to have been initiated in the early 1990s and remains ongoing, has seen extensive reductions in canopy condition and mortality in concert with reduced rainfall as a result of climate change (Indian Ocean Climate Initiative 2002). If the hypothesis that this event is due to excessive water stress is true, water relations traits could correlate with the magnitude of the outward visual expression of the canopy decline syndrome. With this hypothesis in mind the objectives of this chapter are to determine: 1) what contribution water relations traits make to the prediction of canopy condition, and 2) what water relations traits are the best predictors of canopy condition.

### 7.2 Material and Methods

#### 7.2.1 Study Sites

Three sites from within Yalgorup National Park (32.90S; 115.69E) were selected with varying average depths to the water table (Site 1: 2.30 ± 0.09 m, Site 2: 8.04 ± 0.02 m and Site 3: 21.73 ± 0.03 m, *n* = 16 for each site over a 20 month period). The three sites, see Chapter 3 for a detailed description, were within a radius of 20 km. Contrasting the canopy decline syndrome across a range of
groundwater depths allows assessment of the effect of landscape hydrology on physiological parameters associated with canopy dieback (since the severity of seasonality in rainfall and evaporation is buffered where the water table is located close to the natural surface (see Chapter 3). From each site 12 randomly selected individual trees of a range of size (quantified as diameter at breast height, DBH) were tagged for measurement of canopy dieback and dry season traits.

7.2.2 Quantifying Canopy Dieback

Canopy dieback was quantified by an instantaneous estimate of canopy condition. This was achieved by estimating three elements of canopy condition: canopy completeness, proportion of live branches and amount of epicormic growth (Grimes 1987). Each element was assessed visually by three observers and the rankings were converted to an average percentage of maximum canopy condition. Hence, a full healthy canopy scored 100%, and a completely dead canopy scored 0%. It should be acknowledged that this expression of canopy condition is a temporal integration of canopy change in an individual.

7.2.3 Physiological Indicators

Dry season plant physiological traits that are possible indicators of stress were measured during February 2004. These were: 1) pre-dawn leaf water potential ($\Psi_{\text{pd}}$), 2) midday leaf water potential ($\Psi_{\text{md}}$), 3) the difference between pre-dawn and midday leaf water potential ($\Delta\Psi$), 4) estimated transpiration rate ($E^*$), 5) stomatal conductance to water vapour ($g_s$), 6) the carbon isotope discrimination of leaves ($\Delta$), and 7) the leaf mass ratio (LMR). All measurements were undertaken on the same day. Additionally, stem diameter at breast height (DBH) was tested as a possible predictor based on casual observations that larger trees tended to express a greater degree of canopy dieback. Dry season measurements were chosen because this period represents the greatest annual exposure to water stress and therefore conditions most likely to induce physiological symptoms of acute water stress.
PHYSIOLOGICAL INDICATORS OF CANOPY CONDITION

7.2.3.1 Leaf Water Potential

Pre-dawn (05:00 - 06:30 local standard time) and midday (11:30 – 14:00 local standard time) leaf water potential ($\Psi_{\text{pd}}$ and $\Psi_{\text{md}}$ respectively, MPa) were measured with a Scholander-type pressure chamber (Soilmoisture Equipment Corp, Model 3005, Santa Barbara, California) (Scholander et al. 1964). Leaves were sampled from the upper canopy of each size class. For trees this meant use of long-handled secateurs and an extension ladder, a technique employed for subsequent measures of other physiological traits from the same individuals (excluding those measures of stomatal conductance ($g_s$) and estimated transpiration rate ($E^*$) which were taken on intact leaves). Leaf samples were kept in snap-lock plastic bags (petiole protruding) throughout the procedure to minimize transpirational water loss during measurement (Richie and Hinkley 1975). The approximation of the water potential gradient between the soil and leaf was quantified as $\Delta\Psi$ (MPa).

7.2.3.2 Stomatal Conductance and Transpiration Rate

Stomatal conductance to water vapour ($g_s$, mmol m$^{-2}$ s$^{-1}$) and an estimate of transpiration rate ($E^*$, mmol m$^{-2}$ s$^{-1}$) were measured in February 2004 from each individual under natural sun lit conditions at midday (11:30-14:00 local standard time) with a steady-state porometer (model Li 1600, Li-cor Inc Lincoln Nebraska). As in Chapter 3, a correction for boundary layer conductance ($g_b$) was undertaken (refer to section 3.2.3 for details).

Fluctuation in estimated leaf transpiration rate and stomatal conductance to water vapour could be linked to the capability of an individual to maintain tissue hydration. If tissue dehydration is the basal cause of *E. gomphocephala* canopy decline, $E^*$ and $g_s$ could associate with a visual expression of this decline.
7.2.3.3 Carbon Isotope Discrimination

Farquhar et al. (1982) showed that the carbon isotope composition of C₃ plants is a reliable indicator of long-term intercellular CO₂ partial pressure ($p_i$). Specifically, Farquhar et al. (1982) demonstrated that during photosynthesis, discrimination against $^{13}$C is proportional to $p_i$. In this sense carbon isotope measurements made at a single time represent the integration of internal and external events that influenced carbon acquisition during the lifespan of the plant organ sampled (Goldstein et al. 1998). Water use efficiency (WUE, the ratio of biomass accumulated relative to water loss) is, under the assumption of constant internal and external vapour pressure, inversely proportional to the ratio of intercellular to ambient partial pressure of CO₂ ($p_i/p_a$) and hence $^{13}$C discrimination (Farquhar et al. 1989). Water stress tends to reduce stomatal conductance and hence $p_i$, thereby increasing WUE and decreasing $^{13}$C discrimination. By measuring the carbon isotope ratio ($^{13}$C/$^{12}$C) in leaf tissue a time-integrated measure of $^{13}$C discrimination during photosynthesis can be obtained and used to infer average $p_i$ and WUE during leaf development. Measurement of $^{13}$C discrimination in *E. gomphocephala* trees therefore enabled assessment of water-stress-induced changes in WUE across the population.

Newly expanded canopy leaves were collected from each individual during February (2004) for determination of carbon isotope composition ($n = 5$ leaves for each of the 12 sampled trees of each site). Leaves were oven dried at 60°C for 48 hours and finely ground with a ball mill. $^{13}$C to $^{12}$C ratios were measured by means of a continuous flow mass spectrometer (PDZ Europa Model 20-20 Cheshire, UK). The carbon isotope discrimination of leaves ($\delta^{13}$Cleaf) was calculated as:

$$\delta^{13}\text{Cleaf}(\text{‰}) = (\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1) \times 1000,$$

(7.1)

where, $R_{\text{sample}}$ and $R_{\text{standard}}$ are the $^{13}$C/$^{12}$C ratios of the leaf sample and the V-PDB standard, respectively. $\delta^{13}$Cleaf was then converted to relative leaf carbon isotope discrimination ($\Delta$) according to Farquhar and Richards (1984):


\[
\Delta_{\text{inc}}(\%o) = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{leaf}}}{1 + \delta^{13}\text{C}_{\text{leaf}}},
\]

(7.2)

where \(\delta^{13}\text{C}_{\text{air}}\) was taken as 7.8\%. The advantage of using \(\Delta\) rather than \(\delta^{13}\text{C}\) is that it incorporates the isotopic composition of both the source (the atmosphere) and the product (leaf biomass) (Farquhar and Richards 1984).

1.2.3.4 Leaf Mass Per Unit Leaf Area

Leaf mass per unit leaf area (LMA), the ratio of leaf dry weight (g) to leaf surface area (mm\(^2\)), was calculated for leaves collected in February 2004. Newly expanded leaves were collected from canopy branches. Leaf area was measured with an area meter (model Delta-T Type WDIGC-2, Delta T Devices, Cambridge UK) before being oven dried at 70°C and weighed. Leaf mass per unit leaf area is influenced by any environmental variation that alters the balance between assimilate production and utilisation (Dijkstra 1989). In this study LMA was used to explore the possibility that water stress has altered this balance in \(E. gomphocephala\), although it should be acknowledged that other environmental perturbation such as CO\(_2\) and temperature can also influence this balance (Acock et al. 1979; Leadley and Reynolds 1989).

7.2.4 Statistical Analyses

Multiple linear regression (MLR) models were used to examine what were the best predictors (independent variables) of canopy dieback (SPSS for Windows Version 13.0). The models were applied to data collected from each site and data from all three sites pooled. Three MLR models used were: 1) a simultaneous MLR, 2) a stepwise MLR and 3) a hierarchical MLR. The basis for use of these three models was: 1) to determine the predicting power of all independent variables combined, 2) to eliminate redundant independent variables, and 3) to rank the independent variables in order of the best predictors of the variation in canopy dieback. The MLR models took the form of:
\[ \hat{Y} = \alpha + \beta_1 \chi_{iji} + \beta_2 \chi_{ij2i} + \beta_3 \chi_{ij3i} + \ldots + \beta_f \chi_{ijfi}, \]  

(7.3)

where \( \hat{Y} \) is the independent variable, \( Y \) is the dependent variable, \( \alpha \) is the y-intercept, \( \beta \) is the regression coefficient of an independent variable, and \( \chi_{iji} \) is the \( j \)th observation of the independent variable.

While there was scope for some independent variables to express multicollinearity (Zar 1996), examination of the Condition Index and Eigen Values generated by the statistical analysis software (SPSS for Windows version 13.0) indicated that it was unlikely in the data interpreted. Scatter plots of standardised residuals versus standardised predictors were consistent with the assumption of linearity. Mahalanobis distance values were examined to ensure that there were no multivariate outliers among the independent variables (Coakes and Steed 2001).

7.3 Results

There was no difference in average canopy condition across the three study sites (one-way ANOVA, \( P > 0.05 \), Figure 7.2).

DBH was the only measured variable that was a ubiquitous correlate with canopy condition (\( P < 0.05 \), Figure 7.3). A stepwise regression indicated that DBH alone could explain 47\% of the variation in the data from all sites pooled (Figure 7.2D). Using the top three variables in a hierarchical fashion did not yield a large improvement in prediction power (increasing to 48\% Table 7.1).

On a by site basis DBH was not always the salient predictor of canopy condition. At site 1 a simultaneous regression indicated that all variables together explained 68\% of the variation, and a stepwise regression showed that \( E^* \) was the best predictor of canopy condition (Figure 7.4A) followed by DBH (Figure 7.3A). Further analysis via hierarchical regression indicated that the independent variable \( E^* \) explained 45\% of the variation and that the relationship between \( E \)
and canopy condition was divergent (Figure 7.4A). At site 2 all variables together explained 72% of the variation (simultaneous regression). A stepwise regression indicated that only DBH satisfied the criteria (P < 0.05, Figure 7.2B) and a hierarchical regression showed that this variable alone could explain only 36% of the variation (Table 7.1). While not significant, a similar divergent trend between $E^*$ and canopy condition was evident (Figure 7.4B). At site 3 all variables combined explained 94% of the variation (simultaneous regression) and both DBH (Figure 7.3C) and $E^*$ (Figure 7.4C) satisfied the confidence criteria (P < 0.05, stepwise regression). Indeed these two variables together explained 88% of the variation (hierarchical regression, Table 7.1). At no site were the variables $g_s$, $\Delta$leaf or LMA able to explain, with confidence, the instantaneous canopy condition of an individual. With the small sample sizes, statistical power was admittedly limited such that only very highly correlated variables could be detected.

![Box chart](image)

Figure 7.2 A box chart showing that there was no difference in average canopy condition across the three study sites. The horizontal lines in the box denote the 25th, 50th, and 75th percentile values. The error bars denote the 5th and 95th percentile values. The two symbols below the 5th percentile error bar denote the 0th and 1st percentile values. The two symbols above the 95th percentile error bar denote...
the 99th and 100th percentiles. The square symbol in the box denotes the mean of the column of data.

Figure 7.5 Diameter at breast height was negatively correlated with canopy condition (CC) at each site and across all sites pooled. The poorer the canopy condition, the lower the percentage score. The fitted linear models for graphs A, B, C and D respectively have the equations: 

- DBH = 84.88 - 1.10CC ($r^2 = 0.43; P < 0.02$),
- DBH = 139.86 - 1.74CC ($r^2 = 0.36; P < 0.01$),
- DBH = 142.56 - 1.83CC ($r^2 = 0.76; P < 0.001$),
- DBH = 122.72 - 1.57CC ($r^2 = 0.47; P < 0.001$).
Table 7.1 Multiple linear regression results showing the correlation coefficient ($r^2$) for the top three predictors. The analyses were undertaken from data obtained from each site and all sites pooled. In each row the three * symbols indicate the top three predictors in each analysis. Neither stomatal conductance, leaf $\Delta$ or leaf mass ratio were significant correlates with canopy condition.

<table>
<thead>
<tr>
<th>Site</th>
<th>Groundwater depth (m)</th>
<th>$r^2$</th>
<th>DBH</th>
<th>$E^*$</th>
<th>Pre-dawn $\Psi$</th>
<th>Midday $\Psi$</th>
<th>Stomatal Conductance</th>
<th>Leaf $\Delta$</th>
<th>Leaf Mass Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.30</td>
<td>0.54</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8.04</td>
<td>0.58</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>21.73</td>
<td>0.88</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>All Sites</td>
<td>0.48</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>
Figure 7.4 Estimated transpiration rate ($E^*$) was positively correlated with canopy condition (CC) at site 1 and negatively correlated with CC at site 3. While not significant, a weak positive correlation was also evident between $E^*$ and CC at site 2. The fitted linear models for graphs A, B and C respectively have the equations: $E = 0.35 + 0.05CC$ ($r^2 = 0.15; P = 0.02$); $E = 0.48 + 0.02CC$ ($r^2 = 0.15; P = 0.22$); $E = 2.37 - 0.02CC$ ($r^2 = 0.10; P = 0.03$).
7.4 Discussion

The consistent correlation at each site between DBH and canopy condition is evidence that large mature *E. gomphocephala* trees are experiencing the most severe stress associated with the current decline syndrome in Yalgorup National Park. Age related tree declines are strongly associated with forest maturation (Ryan et al. 2004; Mencuccini et al. 2005; Ryan et al. 2006; Vanderklein et al. 2007) (and see Assmann (1970) for a comprehensive review). Typically, this phenomenon has been attributed to the compounding effects of a decline in primary production and an increase in the respiratory costs associated with stems. While older trees have been shown to express reduced photosynthetic rates (Yoder et al. 1994) and older stands have been observed to have lower transpiration rates as a result of reduced sapwood area (Roberts et al. 2001), the apparent decline in processes linked to net primary production is not considered a universal cause of age-related tree decline (Ryan et al. 1997). Binkley et al. (2002) outline an alternative hypothesis derived from competition related changes in stand structure and the resource use by individuals. While this study may support the general precept of these hypotheses, the localised nature of the current severe decline syndrome to Yalgorup National Park would seem to preclude natural age-related shifts in stand dynamics as a universal causal agent for canopy dieback in *E. gomphocephala* populations.

Dry season physiological traits were, overall, poor predictors of canopy condition. While the rationale for use of these traits as correlates of canopy condition is well grounded, contrasts in the scales of time over which the physiological measurements were taken and the initiation of the decline syndrome could have obscured the true causal agent. For example, a past deleterious shift in soil water could have initiated a cascade of events culminating in canopy decline and mortality. However, by functionally scaling leaf area to root area this water stress may not manifest in the instantaneous physiological properties of this study that were taken some 10-15 years after the initial perturbation (Figure 7.5). In this way a reduced leaf area could minimise water loss (at the whole plant scale) (Nemani and Running 1989). This theoretical explanation highlights the importance of monitoring traits associated
with drought during exceptional periods, which will dictate the survivability of long lived tree species (Davidson and Reid 1989).

![Diagram of physiological property](image)

**Figure 7.5** Theoretical relationship between a physiological property linked to water stress and the time over which the decline event has been witnessed. In this model the initial perturbation (e.g. prolonged water stress) could have invoked a change in some physiological property associated with water stress. However, by rapidly shedding leaves a plant could restabilise the physiological property to within normal limits by altering the root:shoot ratio.

An alternative possibility for the overall poor correlation between physiological properties and the magnitude of canopy dieback is that individuals that survived the initial event are those less sensitive to water stress. Suarez et al. (2004) suggest that individual trees with variable growth are prone to mortality from drought stress. This would leave only drought resilient individuals in the remaining population, which could mask any attempt to correlate current canopy condition with dry season physiological traits. It should be noted that individual trees in this study did express reduced canopy condition but under the above paradigm only the most severely affected individuals would die.
Similarly, Williams and Ehleringer (1996) suggest that drought can, in some individuals, invoke the development of deep roots to access groundwater and the capillary fringe. While canopy dieback would still be evident as a result of the initial exposure to drought, the water status under the above scenarios would not necessarily correlate with the magnitude of canopy dieback. Landsberg and Wylie (1983) showed a similar poor correlation between water relations traits (specifically $\Psi_r$) and tree health, the suggested reason being that secondary factors, such as insect and pathogen infection (as opposed to water stress), were more closely linked to the visual expression of canopy decline.

Excluding DBH, only transpiration rate satisfied the criteria for prediction ($P < 0.05$); and this criteria was only met at site 1 and site 3. The correlation between $E^*$ and canopy condition was steep and positive at site 1, less steep and non-significant at site 2 and negative at site 3. Remembering that this site transition represents an increase in depth to groundwater from the natural surface, this change could provide insight into the advantage of adapting stomatal control for a given soil hydration. Maintaining a high $E^*$ was only related to a greater canopy condition where groundwater was easily accessed. Interpretation of this data also means that a high $E^*$ was related to larger trees (data omitted but refer to Chapter 4), suggesting that, as with Martinez-Vilalta et al. (2007), the hydraulic limitation in larger trees at this site is negligible.

A capability of regulating transpiration rate across a range of hydrologies, such that stomatal conductance optimises carbon uptake while reducing the risk of tissue dehydration, would be expected to translate to the time integrated, long-term, expression of $p_v/p_a$ ($\Delta_{carf}$), a process that could mitigate against drought-induced defoliation. However, the carbon isotope discrimination of leaves was not, within a site or in pooled data, a good predictor of canopy condition. This would suggest that canopy condition is not related to a leaf lifespan-integrated signal of plant WUE. Perhaps use of carbon isotope discrimination data derived from a more time integrated source, such as wood, would provide more insight. A preliminary assessment of $\delta^{13}C$ of wood from the outer 5 cm of trunk xylem yielded a significant positive correlation at site 3 (Figure 7.6). Further study
across other sites would provide more certainty. In support of the relationship between $E^*$ and canopy condition at this site, it would seem that a high time integrated WUE in wood favours good canopy condition, although a small sample size warrants cautious interpretation.

The carbon isotope composition of the outer 5 cm of xylem ($\delta^{13}$C$_{\text{wood}}$) was positively correlated with canopy condition. The linear model has the equation: $\delta^{13}$C$_{\text{wood}} = -26.12 + 0.01 \cdot \text{Canopy condition}$ ($r = 0.51$, $p < 0.01$, $n = 11$).

Pre-dawn leaf water potential, while not a significant correlate, was a trait that improved the predicting capability of the regression model at all sites. This gives credibility to both transpiration rate and pre-dawn leaf water potential as future useful physiological traits (in a more extensive, longer term study) for predicting canopy decline in *E. gomphocephala*. 
7.5 Conclusions

The results of this study indicate that current dry season physiological traits are not strongly associated with the current canopy condition in *E. gomphocephala*, although they may influence canopy condition (i.e. their effects may be manifested in the condition of the canopy) after a time lag. Canopy condition was most strongly correlated with DBH and estimated transpiration rate, although the relationship between estimated transpiration rate and canopy condition was either divergent or convergent depending on site conditions. This does not fully support the hypothesis that reduced rainfall is directly responsible for the current canopy decline event in Yalgorup Nation Park. However, this interpretation should be viewed with caution. A past reduction in rainfall, and an associated decrease in aquifer recharge, could have triggered the canopy dieback event that has become most visually conspicuous at present.
CHAPTER 8

ONTOGENY OF *EUCALYPTUS GOMPHOCEPHALA* LEAVES
Summary

The recent decline in annual rainfall across the natural distribution of *Eucalyptus gomphocephala* will likely reduce the soil moisture available for seedling development. Seedlings of *E. gomphocephala* typically develop at high densities on ash-beds, so it is possible that both light and soil water are crucial factors in seedling establishment. This chapter tests the hypothesis that development under different soil water and light regimes will influence mature leaf anatomy and physiology. The results of this study show that *E. gomphocephala* seedlings are capable of considerable morphological plasticity with long-term exposure to such changed environments. Specifically, *E. gomphocephala* seedlings were capable of: 1) manipulating leaf epidermal anatomy which was linked to the range of gas exchange properties expressed at leaf maturity, and 2) maintain a constant leaf-area-specific hydraulic conductivity across growth conditions. While the photon yield of PSII was impacted by growth at 50% of field capacity (*FC*), it is believed that this was mainly due to stomatal-limited gas exchange. Growth under 50% of *FC* was more significant than shade to morphological development and physiology of leaves at maturity. Figure 8.1, a reproduction of the thesis outline, describes the elements discussed in this chapter.
This chapter describes the plasticity in leaves of *E. gomphocephala* that develop under partial water stress. The precept for this investigation is the probability that a new cohort of *E. gomphocephala* seedlings will be exposed to less available soil water. The hypothesis is that development under such conditions will induce morphological and physiological plasticity in mature leaves. Elements of the thesis outline covered in this chapter are highlighted.
8.1 Introduction

An ongoing research interest in south western Australia is the association between a regional decline in annual rainfall (Indian Ocean Climate Initiative 2002) and the health of endemic vegetation. *Eucalyptus gomphocephala* D. C. has shown signs of canopy dieback over the period of declining rainfall but evidence of a causal linkage between this dieback event and water stress remains elusive. Another aspect of reduced rainfall is that any new cohort of *E. gomphocephala* seedlings will develop in a drier soil medium. It remains uncertain how this growth form will adapt to such conditions but the outcome could be significant to the regeneration of the species.

A number of studies have investigated the response of eucalypts to water deficit, particularly those species of economic importance (Hatton et al. 1998; Morris et al. 1998; Mielke et al. 2000; Ngugi et al. 2003). These studies have shown that sympatric eucalypt species respond to drought by reducing transpirational water loss via stomatal closure and, after a prolonged soil water deficit, adjust osmotically. However, few studies have examined the response of eucalypts to a developmental water stress in order to determine their capacity for plasticity in changing environments.

Leaf epidermal morphology (Cutler et al. 1977) and functional attributes (Spence et al. 1986; Franks and Farquhar 2001) commonly vary according to developmental hydration. Leaves that are grown under mild water stress (a water stress that will modulate normal development but allow for cell expansion) develop stomata with relatively small guard cells and high spatial densities. Such developmental changes in stomatal structure and arrangement have been attributed to the plant growth regulator abscisic acid (ABA) (Bradford et al. 1983; Franks and Farquhar 2001; Aasamaa et al. 2002), which is commonly found in plant tissue at higher concentrations when tissue is partially desiccated. The concentration of ABA in primordial leaves mediates a developmental synergy between water availability in soil and gas exchange at maturity since the size and density of stomatal apertures will largely control the range of leaf transpirational flux. An additional environmental variable known to affect
stomatal development is light quality. Excessive blue light, for example, has been shown to reduce stomatal initiation in primordial leaves of soybean (Liu-Gitz et al. 2000) and hence the stomatal density in mature leaves.

Franks and Farquhar (1999) provided a theoretical basis for a correlation between hydraulic capacity, photosynthetic capacity and stomatal conductance, and recent studies are providing evidence in support of this (Brodribb and Feild 2000; Comstock 2000). Such a linkage should extend to morphological adaptation with development under contrasting soil moistures (Alder et al. 1996). For example, a plant grown under water deficit may express a low stomatal conductance, partly due to the size and density of stomata, and a low hydraulic conductivity due to the development of xylem conduits with a small diameter.

An investigation into such properties in *E. gomphocephala* leaves developed under reduced soil water and light is important to understand how the species will recruit under future changed environments. Water and light play a significant role in early *E. gomphocephala* development because seedlings of this species, like many eucalypts, recruit at high densities on ash beds. The hypothesis here is that exposure to suboptimal conditions in a controlled setting will influence the traits of mature leaves. The objectives of this chapter are to: 1) determine whether different soil water and light environments during *E. gomphocephala* seedling development influence leaf level anatomy and physiology at maturity, and 2) resolve whether light or water is more relevant to early *E. gomphocephala* development.

### 8.2 Material and Methods

#### 8.2.1 Plant Material and Growth Conditions

Twenty-four *E. gomphocephala* seedlings were germinated from seed, which was sourced from within Yalgorup National Park, and allowed to develop in a standard potting medium (70:30 sand:humus) in a plant growth facility. They were well watered and exposed to a day/night temperature of 24/15°C until the
third or fourth leaf had fully expanded. Seedlings were then transferred to the following treatments (n = 6 for each): T1, watered to field capacity (FC) and exposed to 21% of full sun, T2, watered to FC and exposed to 7% of full sun, T3, watered to 50% FC and exposed to 21% of full sun, and T4, watered to 50% FC and exposed to 7% of full sun. Day/night temperatures were maintained at 24/15°C for all treatments. Fifty percent field capacity was controlled by maintaining pots at a desired weight. This was dependent upon the physical properties of the soil (that is, its water holding capacity) and the actual volume of soil in each pot. Field capacity was calculated for a soil sub-sample, with a volume of $1.29 \times 10^{-4}$ m$^3$, as:

$$FC = \frac{W_{sat} - W_{dry}}{V_{dry}} \text{ (kg}_{\text{water}}/\text{m}^3_{\text{dry soil}}),$$

(8.1)

where: $W_{sat} =$ weight of saturated soil (kg), $W_{dry} =$ weight of oven-dried soil (kg) and $V_{dry} =$ volume of oven-dried soil (m$^3$). $W_{sat}$ was measured after soaking and allowing soil to free-drain for 12 hours. The weight of soil + pot at 50% FC (denoted $P_{50}$) was calculated as:

$$P_{50} = \frac{V_{pot}(0.5FC + W_{dry})}{V_{dry}} + W_{pot} \text{ (kg)},$$

(8.2)

where: $V_{pot} =$ volume of dry soil with which pots were filled (m$^3$), $W_{pot} =$ weight of empty pot (kg). Pots were weighed approximately every 4 days and (if required) the appropriate weight of water was added to bring pots back to the target weight ($P_{50}$). The small increase in total pot mass due to growing seedlings was considered negligible in this experiment. The physiological state of water in plants kept at FC or $P_{50}$ was quantified as pre-dawn leaf water potential ($\Psi_{pd}$) measured with a Scholander-type pressure chamber ($n = 2$ leaves per plant) (model 3000 Soil Moisture Equipment, Santa Barbara, California) (Scholander et al. 1964). It is acknowledged that an unequal distribution of water will arise from the surface application of water (to maintain 50% field capacity), but the intention of this treatment is to mimic processes of partial soil hydraulic recharge that would occur in the field.
Developmental light conditions were altered by growing plants under white (the ambient conditions of the plant growth facility) or white and green shade cloth and each light environment quantified as 21% of full sun and 7% of full sun respectively using a quantum meter (model LI 250A, Li-Cor Inc. Lincoln, Nebraska). The low light environment was imposed to mimic the conditions likely to be experienced by seedlings developing at high densities on ash beds. When seedlings had developed fully expanded leaves under the conditions described above they were assessed for physiological and morphological properties. Because the focus of this chapter was to implement a controlled microcosm-type study, no attempt was made to bring the \( P_{50} \) treatment back to field capacity or the 7% full sun treatment to 21% full sun (so that only developmentally induced effects were evident). Hence the traits expressed by plants grown at \( P_{50} \) and 7% full sun should mimic those of plants growing at high densities in dry soil.

### 8.2.2 Leaf Blade Physiology

Stomatal conductance to water vapour (\( g_s, \text{mmol m}^{-2} \text{s}^{-1} \)) and an estimate of transpiration rate (\( E^*, \text{mmol m}^{-2} \text{s}^{-1} \)) were measured at midday (11:30 – 13:30 local standard time) with a steady-state porometer (model Li 1600, Li-cor Inc Lincoln Nebraska). As in Chapter 3 and Chapter 7, a correction for boundary layer conductance (\( g_b \)) was undertaken (refer to section 3.2.3 for details). Measurements were made on the abaxial surface of leaves (\( n = 2 \) leaves per plant) because prior measurements showed little gas exchange from adaxial surfaces.

On the same day as leaf gas exchange measurements the midday maximum photon yield of PSII (\( F_V/F_M \)) was determined for the same leaves that were assessed for gas exchange properties (\( n = 2 \) leaves per plant) with a pulse modulated fluorescence probe (Model OS1-FL, Opti Sciences Inc, Tyngsboro Massachusetts).
8.2.3 Petiole Hydraulic Properties

To understand how leaf blade traits scale with supporting petiole physiology under contrasting developmental conditions, this chapter also investigated the xylem hydraulic properties of *E. gomphocephala* petioles. Xylem-area-specific hydraulic conductivity \( K_s \) and leaf-area-specific hydraulic conductivity \( K_L \) were determined for one of the two petioles of the leaves sampled for gas exchange and fluorescence properties using a steady state flow meter (SSFM), similar to that described by Feild et al. (2001). Synoptically, the SSFM consisted of a length of Peek™ resistance tubing (with a known relationship between pressure and flow rate) connected in series with the petiole. Petioles (+ leaf blades) were collected prior to sunrise to ensure that minimum native embolisms were present. Petioles were cut and maintained in distilled water prior to measurement in the SSFM. Prior to placing the petiole in the SSFM the leaf blade was removed and the ends trimmed with a razor blade (while immersed in distilled water). The length of petioles at the time of measurement ranged from 0.011 m to 0.029 m (mean = 0.016 m). Petiole diameter ranged from 0.0010 m to 0.0017 m (mean = 0.0013 m). The SSFM was filled with a perfusion solution consisting of 0.01 M KCl and degassed double distilled water with a pH of 6.6, filtered to 0.11 µm. Xylem-area-specific hydraulic conductivity, defined here as the mass flow movement of water through the excised petiole segment per unit of pressure gradient per unit of xylem cross sectional area, was determined as:

\[
K_s = J_y \times \frac{\Delta l}{\Delta P} \quad \text{(kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}), \quad (8.3)
\]

where \( J_y \) is the mass flux density \([\text{kg water} \text{ m}^{-2} \text{ (xylem)} \text{ s}^{-1}]\), \( \Delta l \) is the length of the petiole segment (m) and \( \Delta P \) is the water pressure difference across the length of the petiole (MPa). Xylem cross sectional area \( (A_x) \) was determined microscopically (see below for sectioning details). Leaf blade area \( (A_L) \) (assumed to represent transpirational surface area) was measured with an area meter (model Delta-T Type WDIGC-2, Delta T Devices, Cambridge UK) and \( K_L \) calculated as:
\[ K_L = K_S \times \frac{A_x}{A_l} \]  \hspace{2cm} (kg m\(^{-3}\) s\(^{-1}\) MPa\(^{-1}\)), \hspace{1cm} (8.4)

8.2.4 Microscopy

After taking hydraulic measurements petioles were transferred to 70% ethanol until sectioned. Immediately prior to sectioning petioles were rehydrated by rinsing under tap water then immersing in distilled water for one hour. Pieces of petiole were then embedded in paraffin wax and transverse sections cut to 20 µm with a rotary microtome. Sections were stained in 0.1% aqueous toluidine blue for one minute, rinsed in distilled water then mounted on slides with Permount. Slides were then viewed under a compound light microscope and images captured with a digital camera. Xylem vessel diameter \((n = 50\) for each sample) and total xylem area were then measured digitally (Image J software version 1.34s) using the measured relationship between image pixel size and a graticule scale. Since conduit radius and flow rate are related in the quadratic dimension (Hagen-Poiseuille law) the hydraulically weighted mean vessel diameter \((d)\) of Sperry et al. (1994) was used:

\[ d = 2 \frac{\sum r^5}{\sum r^4} \]  \hspace{1cm} (µm), \hspace{1cm} (8.5)

where \(r\) is the radius of a xylem vessel.

Epidermal impressions were obtained from the abaxial surface of each blade of the leaf supplied by the sampled petioles using nail polish. Peels were then mounted on microscope slides in distilled water for examination under a compound light microscope. Images were then captured with a digital camera. Stomatal complex dimensions (stomatal pore length and total stomatal length, ie. length of guard cells; \(n = 20\) randomly selected complexes for each leaf and 120 complexes for each treatment) and densities (stomatal complexes/mm\(^2\); \(n = 4\) randomly selected fields of view per leaf and \(n = 24\) fields of view per treatment) were then calculated using a similar approach as xylem vessel anatomy analysis.
8.2.5 Statistical Analyses

Where appropriate means of treatments were compared with a one-way or two-way ANOVA at the 0.05 level of significance. For each ANOVA a test for homogeneity of variance suggested that the assumption of the analysis had not been violated.

8.3 Results

Seedlings maintained at 50% $FC$ had a significantly lower $\Psi_{pd}$ than those maintained at $FC$ (one-way ANOVA, $P < 0.001$, $F = 67.40$; Figure 8.2). The mean difference in $\Psi_{pd}$ across these differing watering regimes was 0.5 MPa.

![Figure 8.2](image)  
Mean (± s.e.) pre-dawn leaf water potentials ($\Psi_{pd}$; $n = 2$ leaves per plant) for *Eucalyptus gomphocephala* seedlings maintained at field capacity ($FC$) and 50% field capacity ($P_{50}$). Plants maintained at $FC$ had a significantly higher $\Psi_{pd}$ than plants kept at $P_{50}$ ($P < 0.05$, one-way ANOVA).
Stomatal conductance to water vapour was correlated with $\Psi_{pd}$ (Figure 8.3A). Higher rates of $g_s$ equated to higher pre-dawn leaf water potentials. An exponential function was used to describe the association between $g_s$ and $\Psi_{pd}$, meaning that as $g_s$ approached zero changes in $\Psi_{pd}$ had little effect on the size of stomatal pores. Within this correlation treatment groups clearly separated into FC (high $g_s$ and $\Psi_{pd}$) and 50% FC (low $g_s$ and low $\Psi_{pd}$). A similar correlation was evident between $g_s$ and $E^*$ (Figure 8.3B) but on this occasion a linear function described the association well. Once again treatments separated clearly into FC (high $g_s$ and $E$) and 50% FC (low $g_s$ and $E$).

The photon yield of PSII was exponentially correlated with $\Psi_{pd}$ (Figure 8.4A). As with the correlation between $g_s$ and $\Psi_{pd}$ there was, within this relationship, an apparent separation by treatments with FC plants showing a high $F_v/F_M$ and $\Psi_{pd}$ and 50% FC plants exhibiting a low $F_v/F_M$ and $\Psi_{pd}$. $F_v/F_M$ was also correlated to $g_s$ (in a positive linear fashion) and a similar separation by treatments was apparent (Figure 8.4B).
Figure 8.3  Correlation between stomatal conductance to water vapour ($g_w$) and pre-dawn leaf water potential ($\Psi_{pd}$) and $g_w$ and transpiration rate ($E^*$) for *Eucalyptus gomphocephala* seedlings, graphs A and B respectively. The treatments were: T1: watered to field capacity (FC) and 21% of full sun, T2: watered to FC and 7% of full sun, T3 water to 50% FC and 21% of full sun, and T4 watered to 50% FC and 7% of full sun. The exponential fit for A was generated from the equation $g_w = 18.33 e^{0.35 \Psi_{pd}}$ ($P < 0.05$). The linear fit for B was generated from $g_w = 1.79 + 84.40 E^* (P < 0.001)$.
Development at 50% FC and 7% of full sun resulted in a significant reduction in the length of stomata (\( P < 0.001, F = 295.77 \) for water and \( P < 0.001, F = 43.17 \) for light; two-way ANOVA, Table 1) and a significant interaction between soil water and light was found (\( P = 0.01, F = 6.74 \); two-way ANOVA, Table 1). While overall, plants grown at 50% FC had smaller stomatal lengths, for a given soil water status, plants grown at 7% of full sun had smaller stomatal lengths than plants grown at 21% of full sun. Stomatal pore length was also smaller in plants grown at 50% FC and 7% of full sun (\( P < 0.001, F = 108.66 \); two-way ANOVA, Table 1).
ANOVA for water and $P < 0.001$; $F = 21.09$ for light, Table 1) and a significant interaction between soil water and light was observed ($P < 0.001$, $F = 47.92$). However, while this interaction showed a reduction in the mean pore length in 7% of full sun plants grown at $FC$ (as with stomatal length), at 50% $FC$ mean pore length did not differ between developmental light treatments. Development under 50% $FC$ ($P < 0.001$, $F = 20.901$; two-way ANOVA, Table 1) and 21% of full sun ($P < 0.001$, $F = 20.901$; two-way ANOVA, Table 1) resulted in a significant increase in stomatal density, but no interaction between light and soil water was apparent ($P > 0.05$ for each). Stomatal conductance to water vapour was significantly lower in plants grown at 50% $FC$ ($P < 0.001$, $F = 304.97$; two-way ANOVA, Table 1) but light had no effect on $gs$ and no interaction between soil water and light was evident ($P > 0.05$ for each).

Table 8.1  
<table>
<thead>
<tr>
<th>Guard cell length (µm)</th>
<th>Pore length (µm)</th>
<th>Stomatal density (mm⁻²)</th>
<th>$gs$ (mmol m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 19.02 ± 0.29A</td>
<td>13.61 ± 0.34A</td>
<td>334.02 ± 23.58AB</td>
<td>332.25 ± 17.53A</td>
</tr>
<tr>
<td>T2 16.62 ± 0.17B</td>
<td>12.61 ± 0.40B</td>
<td>209.83 ± 15.05A</td>
<td>310.33 ± 16.62A</td>
</tr>
<tr>
<td>T3 13.84 ± 0.34C</td>
<td>10.45 ± 0.39C</td>
<td>411.54 ± 25.43C</td>
<td>53.62 ± 13.84B</td>
</tr>
<tr>
<td>T4 12.80 ± 0.21D</td>
<td>10.87 ± 0.37C</td>
<td>334.02 ± 21.85AB</td>
<td>82.80 ± 12.80B</td>
</tr>
</tbody>
</table>

Mean specific hydraulic conductivity was positively correlated with hydraulically weighted vessel diameter (Figure 8.5A) although note that considerable variation exists within each treatment. Leaf-area-specific hydraulic conductivity and $d$ did not show such a correlation (Fig 8.5B). Indeed, there was no apparent difference in $K_L$ across scales of $d$ and therefore treatments.
Figure 8.5  Plot of specific hydraulic conductivity ($K_s$) and leaf-area-specific hydraulic conductivity ($K_L$) versus mean hydraulically weighted vessel diameter ($d$), graphs A and B respectively, for *Eucalyptus gomphoecephala* seedlings (treatment notation as per Figure 8.2). The fitted linear model of A follows the equation: $K_s = -3.24 + 0.25d$ ($r^2 = 0.94; P = 0.03$).
ONTOGENY OF *E. GOMPHOCEPHALA* LEAVES

### 8.4 Discussion

The effectiveness of controlling available soil moisture as FC or $P_{50}$ was confirmed by the significant difference in $\Psi_{pd}$ between the two conditions (Figure 8.2). Since leaf initiation and expansion continued in the $P_{50}$ treatment, the affect of the controlled water stress treatment would have been experienced over a leaf developmental time frame.

Stomatal conductance to water vapour was constrained under $P_{50}$. This effect is represented by the correlation between $g_s$ and $\Psi_{pd}$ across all treatments (Figure 8.3A) and is a reflection of how transpirational water loss is controlled by *E. gomphocephala* under differing soil water availabilities (Figure 8.3B). The interaction between $\Psi_{pd}$ (taken here to be closely associated with soil water potential, $\Psi_{soil}$) and $g_s$ became most evident when $\Psi_{pd}$ fell below -0.40 MPa. Indeed, a small shift in $\Psi_{pd}$ from -0.25 MPa to -0.40 MPa invoked a 79% down regulation in stomatal conductance (declining to 68 mmol m$^{-2}$ s$^{-1}$ from 321 mmol m$^{-2}$ s$^{-1}$). Based on the trajectory of the correlation between $g_s$ and $\Psi_{pd}$, long term soil moisture of less than 50% of field capacity would create conditions that would significantly limit gas exchange in leaves of *E. gomphocephala* seedlings, impacting on carbon uptake and leaf expansion.

The water stress-induced shift in stomatal conductance observed in this experiment would typically comprise three interrelated mechanisms: 1) stomatal closure mediated by a sensitivity to transpiration rate (Lange et al. 1971; Mott and Parkhurst 1991) and soil water potential (Blackman and Davies 1985), 2) a decrease in hydraulic conductivity across the soil to leaf flow path via partial xylem embolism (Tyree and Sperry 1988) or changed xylem conduit anatomy (Sperry and Saliendra 1994), and 3) a change in leaf epidermal anatomy (stomatal density and shape) (Bradford et al. 1983). In this chapter the focus has been on the behaviour of plants under native conditions. Hence no attempt was made to normalise ABA concentrations in leaves across treatments so that only developmentally-induced treatment differences were expressed. However, it is
necessary that long-term changes (xylem and epidermal anatomy), and short-term (stomatal closure and xylem embolism) changes are seen as distinct.

Selected vessel characteristics (their tangential diameter and element length) have been shown to increase in *Eucalyptus grandis* and the hybrid *E. grandis × camaldulensis* in well watered compared to a water deficit treatment (February et al. 1995). The same study showed that this relationship is not always consistent, with the hybrid *E. grandis × nitens* showing no such increase vessel diameter or vessel length. Similarly, in this study no water stress-induced change in the xylem-area-specific hydraulic conductivity of petioles or hydraulically weighted vessel diameter was observed, although these parameters were weakly correlated (Figure 8.5A). Specific hydraulic conductivity and hydraulically weighted mean vessel diameters appeared more affected by developmental irradiance than available water in this study.

Development under 21% of full sun reduced xylem-area-specific hydraulic conductivity and hydraulically-weighted mean vessel diameter in *E. gomphocephala* leaves. Since plants of this treatment expressed a range of transpiration rates, this reduction in $K_s$ could not have been driven by adaptation to higher transpiration rates. Vulnerability to cavitation has been shown to acclimate to light in *Fagus sylvatica* (Cochard et al. 1999), but unlike the results presented here, the light-induced cavitation response in *F. sylvatica* was not paralleled by changes in xylem hydraulic conductivity or vessel diameter. When scaled to transpirational surface area, hydraulic properties of *E. gomphocephala* leaf xylem were maintained constant across treatments. This is comparable to the stem hydraulic data of Feild et al. (2001) for sun and shade plants of the primitive angiosperm *Amborella trichopoda* which showed little to no difference in leaf-area-specific hydraulic conductivity. To clarify the adaptive hydraulic capacity of *E. gomphocephala* seedlings, a more detailed analysis of vessel structure, including pit complex anatomy, should be undertaken. This is particularly pertinent because of the poor interspecific relationship between hydraulic properties and xylem conduit diameter, given that pit complex anatomy is somewhat detached from xylem conduit diameter (Hacke and Sperry 2001).
The observation of a decrease in stomatal pore length (due to an overall reduction in stomatal dimension) and an increase in stomatal density with development under water stress (Table 1) is consistent with the findings of Bradford et al. (1983) who used elevated ABA concentrations to invoke a developmental stress response in tomato which comprised qualitatively similar anatomical changes. The reduction in the size of stomatal pore lengths in *E. gomphocephala* seedlings grown at 50% FC provides evidence of an anatomical adaptation to long-term water stress. Clearly this would compromise the likelihood of expressing the full range of stomatal conductance such as that exhibited by seedlings grown at FC. Indeed, it could be argued that this anatomical adjustment is partially reflected in the conservative gas exchange properties of *E. gomphocephala* leaves developed at *P*₅₀.

The average values of maximum PSII photon yield (Fᵥ/Fₐ) of *E. gomphocephala* seedlings that were developed under a controlled water stress were perturbed compared to seedlings kept at field capacity. Evidence from the literature suggests that, usually, water stress does not directly influence the primary events of photosynthesis (for example, Genty et al. (1987)). Indeed, given the correlation between Fᵥ/Fₐ and Ψₑ and *g*ₛ (Figure 8.4), it is suspected that any decrease in photosynthetic electron transport in this study was due to stomatal-limited leaf gas exchange. Under more severe water stress the mechanism of drought-induced photosynthetic down-regulation could be more direct (Sharkey and Seemann 1989; Flexas et al. 1999) such as the theorised effect of ABA on carbon fixation via reduced Rubisco activity.

In this study both irradiance and plant water status were controlled. As such it is necessary to appreciate that water stress and irradiance interact strongly to impact on the photosynthetic apparatus (Björkman and Powles 1984). A slightly higher Fᵥ/Fₐ in shade-developed compared to well lit and well watered plants was noted, but this same difference was not observed in plants grown under a controlled water stress. This indicates that, in this case, water stress (specifically its effect on stomatal control of leaf gas exchange) was of greater significance to
photosynthetic processes than photoinhibition-induced disruption to electron transport.

8.5 Conclusions

Under conditions where *E. gomphocephala* seedlings develop at high densities, such as recruitment after fire, the ability to adapt to a variable light and soil water environment would be advantageous. The results of this study show that *E. gomphocephala* seedlings are capable of considerable morphological plasticity with long-term exposure to such changed environments. Specifically, *E. gomphocephala* seedlings were capable of: 1) altering leaf epidermal morphology to regulate the range of gas exchange properties expressed at maturity, and 2) maintaining a constant leaf-area-specific hydraulic conductivity across growth conditions. While the photon yield of PSII was impacted by growth at 50% FC, it is believed that this was mainly due to stomatal-limited gas exchange. Growth under 50% of FC was more significant than shade to morphological development and physiology of leaves at maturity. These findings suggest that contest for water in mass recruitment events would supersede competition for light in this species.
CHAPTER 9

PHYSIOLOGICAL CHANGE IN *EUCALYPTUS GOMPHOCEPHALA* IN RESPONSE TO FIRE-DRIVEN NUTRIENT CYCLING
Summary

Fire has helped shape global ecosystems for millions of years; its presence in some instances maintaining the structure and function of fire-prone plant communities (Bond and Keeley 2005). Its use by Aborigines to re-shape the Australian continent has engendered different plant communities with a dependency on a distinct fire cycle (that is, its frequency and intensity) (Bowman 1998). Replication of these fire cycles by land managers has proven difficult because of poor knowledge on the precise fire frequency and intensity that will perpetuate a given community type (Ward et al. 2001).

Plants of Mediterranean-type ecosystems can, in the context of fire, be ascribed the categories: 1) seeders, or 2) resprouters (Trabaud 1981). On exposure to fire, seeders regenerate from seed and resprouters regenerate from underground organs. These broad categories are associated with physiological and morphological strategies that maximise regeneration after fire (Pausas 1999). In addition to the direct destruction of aboveground biomass and the subsequent strategies employed by seeders and resprouters, fire can also alter the biogeochemical composition of the rhizosphere (see Certini (2005) for a recent review). Fire is, for example, considered a salient element in soil nutrient cycling and plant productivity across a range of ecosystems (Redmann 1978; Knapp 1985; Reich et al. 1990; Elliott and Vose 1993).

The role of fire in *E. gomphocephala*-dominated woodland has been a topic of much research interest. Loss of traditional fire-stick farming with European settlement in the natural geographical range of *E. gomphocephala* has left land managers the formidable task of conserving biodiversity in communities adapted to regular fire cycles whilst mitigating against loss of property or human life. The recruitment of a new *E. gomphocephala* cohort is strongly associated with fire and in particular ash-beds and the health of mature *E. gomphocephala* trees could be linked to nutrient cycling driven by regular fire events.

A shift in the disturbance regime, formerly maintained by traditional fire-stick farming, has been postulated as a causal element of the *E. gomphocephala*
canopy dieback event in Yalgorup National Park. The role of fire in nutrient cycling in both developing and mature *E. gomphocephala* remains understudied. However, as part of the wider investigation into the *E. gomphocephala* canopy dieback event, an opportunity arose to study these processes in a *E. gomphocephala*-dominated woodland in Yalgorup National Park.

In this study the steady-state gas exchange properties of *E. gomphocephala* seedlings that were developed on ash-beds were compared to those developed on undisturbed soil. Development on undisturbed soil resulted in an elevated stomatal conductance in darkness and less manganese in leaves. It is postulated that a deficiency in Mn perturbed the oxidisation of water during photosynthesis which impaired energy dependent stomatal closure. Nutrient cycling, driven by fire, is therefore important for stomata of *E. gomphocephala* to integrate environmental stimuli.
9.1 Introduction

The photosynthetic capacity of leaves is dependent on nutritional content, primarily nitrogen, phosphorous and potassium (Longstreth and Nobel 1980). This effect is usually attributed to the relative capacity of plants to synthesise proteins comprising the C3 cycle and chloroplast electron transport chain. However, deficiencies in key nutrients can also constrain the capability for stomata to respond to environmental stimuli via a perturbed protein mediated signal pathway (Assmann 1993). This latter aspect has received little attention generally and no studies have investigated the possible linkage between a fire-induced nutritional pulse and regulation of transpiration rate in fire adapted species.

On exposure to fire, soil properties can express short-term, long-term or permanent change depending on the intensity and frequency of fires and climatic conditions (Certini 2005). These changed properties can be ascribed the following categories: 1) physical and mineralogical, 2) chemical, and 3) biological. The altered nutritional status of soils in response to fire and the subsequent effect of this alteration on plant productivity has been a topic of much research (Pryor 1963; Loneragon and Loneragon 1964; Humphreys and Lambert 1965; Redmann 1978; Knapp 1985; Reich et al. 1990; Elliott and Vose 1993). Some nutrients, such as nitrogen (N), can be lost from the soil to the atmosphere through combustion (Mroz et al. 1980) while others, primarily cations, can be deposited to the soil, as ash, as a result of fire (Boerner 1982). This compositional shift in soil nutrients, the so called ‘ash-bed’ effect, increases short term plant productivity in many ecosystems (Reich et al. 1990; Grogan et al. 2000) but has been suggested to lower productivity in the long term (Ojima et al. 1994; Thornley and Cannell 2004) although in Australian forests this latter process has not been demonstrated (Attiwill et al. 1996; Neary et al. 1999). The mode of the short term effect is believed to be an increase in per unit leaf area photosynthetic capacity due to the biochemical advantage provided by the soil nutritional pulse (Oechel and Hastings 1983; Gilbert et al. 2003; McCarron and Knapp 2003; Clemente et al. 2005). To date, however, no studies have
investigated the possibility that fire may also cause changed stomatal regulation of leaf gas exchange.

Swanborough et al. (2003) suggest that the water-use efficiency (WUE, the ratio of carbon uptake relative to water loss), as evidenced from carbon isotope discrimination, of plants in south western Australia is influenced by fire-mediated nutrient pulses. In an ecological sense, this linkage could be credited to the long term exposure of such plants to regular fire cycles. The recruitment of *Eucalyptus gomphocephala* is strongly associated with fire and in particular ash-beds. As a result it is suspected that the advantage of development on ash-beds will be reflected in both photosynthetic capacity and the ability of stomata to respond to environmental stimuli. With this hypothesis in mind the objective of this chapter is to assess the steady-state leaf gas exchange properties of ash-bed and non ash-bed developed *E. gomphocephala* and associate these properties with foliar nutrient content.

### 9.2 Material and Methods

#### 9.2.1 Experimental Design and Plant Material

In spring 2004 an ash-bed was artificially generated by burning a pile of logs and branches that had accumulated via litter fall from the forest on-site, which included debris from a natural population of *E. gomphocephala*. Combustion was complete within several hours with a layer of grey ash and charcoal deposited on the soil surface. Four weeks after burning, eight *E. gomphocephala* seedlings were planted in the ash-bed and eight additional *E. gomphocephala* seedlings were planted at a distance of 2 m from the ash-bed (controls) in an undisturbed plot. Seedlings were approximately five months old and had grown in potting mix under glasshouse conditions. The ash-bed and control plots were situated in a natural canopy gap and planting distances (50 cm apart) were equivalent in both plots. Seedlings were allowed to develop for 12 months under natural conditions and from the pool of eight individuals in each treatment four were randomly selected for subsequent measurements over a four week period.
RESPONSE TO FIRE

This sampling period represented the end of the annual wet period and hence conditions of maximum annual soil moisture content.

9.2.2 Leaf Water Potential

Pre-dawn (05:30-0630 local standard time) leaf water potential ($\Psi_{pd}$) was determined for each plant ($n = 2$ leaves for each) with a Schollander-type pressure chamber (model 3005, Soil Moisture Equipment, Santa Barbra, California) (Scholander et al. 1964) in accordance with methods to reduce the potential for errors (Richie and Hinkley 1975).

9.2.3 Leaf Gas Exchange

Leaf gas exchange properties were measured in situ on clear sunny days with an open-flow portable photosynthesis system (Model Li 6400, Li-cor Inc, Lincoln, Nebraska) on one leaf per plant ($n = 4$ plants per treatment). All experiments were initiated early in the morning (07:30 – 08:30) and were concluded within the natural daylight photoperiod. Measurements were made on fully expanded leaves (three or four leaves back from a branch apex) from a north facing aspect. Leaves were less than 6 months old and therefore developed under the treatments described above. Throughout experiments the ambient partial pressure of CO$_2$ was maintained at 350 µmol mol$^{-1}$ (except for relationships between assimilation rate and intercellular partial pressure of CO$_2$), leaf temperature set at 20°C and leaf-to-air vapour pressure difference ($D$) regulated to 1 kPa. Minimum steady-state stomatal conductance to water vapour ($g_{min}$) and dark respiration rate ($R_d$) were determined with zero leaf irradiance; the duration of zero illumination was approximately 20 minutes. A stomatal opening phase, comprising the transition from $g_{min}$ to a maximum steady-state stomatal conductance ($g_s$), was then recorded by exposing leaves to a photosynthetically active radiation (PAR) of 1000 µmol m$^{-2}$ s$^{-1}$ (remaining chamber variables kept constant). This took approximately 120 minutes. At this point, defined here as the photosynthetic operating point, the steady-state assimilation rate ($A$), and ratio of intercellular to ambient partial pressure of CO$_2$ ($p_i/p_a$) were also recorded. Also at this point the
relationship between $A$ and $p_i$ was obtained (see below). PAR was then returned to zero and the subsequent decline in stomatal conductance to a minimum steady-state value documented. The leaf was then excised from the plant and any further decline in stomatal conductance recorded. Data were logged every 60 seconds during all transient opening and closing phases.

When the photosynthetic operating point had been reached, the relationship between $A$ and $p_i$ was obtained by manipulating $p_a$ over the range 50 µmol mol$^{-1}$ to 2000 µmol mol$^{-1}$ and described empirically by rectangular hyperbolas (Olsson and Leverenz 1994):

$$A \, (\mu\text{mol m}^{-2} \text{ s}^{-1}) = \frac{CE \times p_i \times A_{\text{max}}}{CE \times p_i + A_{\text{max}}} - r$$

where $CE$ is the carboxylation efficiency, $A_{\text{max}}$ is the assimilation rate at saturating $CO_2$ and $r$ is the combination of light and dark respiratory processes. Initial estimates of $CE$ were taken as the slope of linear regression models fitted to plots of $A$ and $p_i$ where $p_i$ was low (< 250 µmol mol$^{-1}$). Initial estimates of $r$ were taken as the y-intercept of the same models. The assimilation rate at saturating $CO_2$ was estimated from the largest value for $A$. Rectangular hyperbolas were then fitted to the data using an iterative least squares fit approach.

From the relationship between $A$ and $p_i$ the maximum rate of carboxylation ($V_{C_{\text{max}}}$) and light saturated rate of electron transport ($J_{\text{max}}$) were estimated according to von Caemmerer and Farquhar (1981) and Harley et al. (1992) (Photosyn Assistant Software 1.1.2, Dundee Scientific, Dundee UK).

9.2.4 Carbon Isotope Discrimination

On completion of gas exchange measurements each leaf was collected and separated into two sections: 1) for determination of carbon isotope discrimination, and 2) for analysis of leaf nutrient content. Leaf material
collected for carbon isotope discrimination was oven dried at 60°C for 48 hours and finely ground with a ball mill. $^{13}$C to $^{12}$C ratios were measured by means of a continuous flow mass spectrometer (PDZ Europa Model 20-20 Cheshire, England). The carbon isotope composition of leaves ($\delta^{13}$C$_{leaf}$) was calculated as:

$$\delta^{13}$C$_{leaf}(\%) = \left( \frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000$$

(9.2)

where, $R_{sample}$ and $R_{standard}$ are the $^{13}$C/$^{12}$C ratios of the leaf sample and the V-PDB standard respectively. $\delta^{13}$C$_{leaf}$ was then converted to leaf carbon isotope discrimination ($\Delta$) according to Farquhar and Richards (1984)

$$\Delta_{leaf}(\%) = \frac{\delta^{13}$C$_{air} - \delta^{13}$C$_{leaf}}{1 + \delta^{13}$C$_{leaf}}$$

(9.3)

where $\delta^{13}$C$_{air}$ was taken as 7.8%. The advantage of using $\Delta$ rather than $\delta^{13}$C is that it incorporates the isotopic composition of both the source (the atmosphere) and the product (leaf biomass) (Farquhar and Richards 1984).

9.2.5 Leaf Nutrients

Sections of leaves (the remaining material after sub-sampling for carbon isotope determination) were oven dried at 60°C for 48 hours and finely ground to a powder using a ball mill. The ground material was then analysed for phosphorus, calcium, magnesium and manganese content using inductively coupled plasma-emission spectrometry (ICP, Model 700-ES, Varian, Palo Alto, California). Total carbon and nitrogen content were determined via mass spectrometry (PDZ Europa Model 20-20 Cheshire, England).

9.2.6 Statistical Analyses

Where appropriate means were compared across treatments using t-tests at the 0.05 level of significance (SPSS for Windows version 13.0). Tests for homogeneity of variance indicated that the assumptions of an ANOVA were not
violated. It should be acknowledged that there was no true replication of treatments in this experiment; hence it could be expected that only large differences would be identified.

9.3 Results

Overall, photosynthetic capacity was not different between control and ash-bed developed plants. This is represented by the similarity in the relationship between \( A \) and \( p_i \) across treatments (Figure 9.1A) and is defined by rectangular hyperbolas:

\[
A = \frac{1.28 \times p_i \times 51.51}{1.28 \times p_i + 51.51} - 28.53, \quad r^2 = 0.95 \quad \text{for the control treatment}
\]

and

\[
A = \frac{0.64 \times p_i \times 44.91}{0.64 \times p_i + 44.91} - 19.12, \quad r^2 = 0.95 \quad \text{for the ash-bed treatment.}
\]

Similarly, the steady-state operating points, defined as the assimilation rate and intercellular partial pressure of \( CO_2 \) at \( p_a = 350 \ \mu mol \ mol^{-1}, D = 1 \ kPa, PAR = 1000 \ \mu mol \ m^{-2} \ \text{s}^{-1} \) and leaf temperature = 20°C, did not differ between control and ash-bed treatments (Figure 9.1B). The arrow in Figure 9.1B connecting \( p_a \) with the photosynthetic operating points shows, visually, the similarity in the maximum steady-state stomatal conductance across treatments (\( g_s \) is proportional to the slope of the line connecting \( p_i \) and \( p_a \)).
The stomatal opening phase, characterised as a change in stomatal conductance with time exposed to a PAR of 1000 µmol m$^{-2}$ s$^{-1}$, showed considerable difference across treatments. For a direct comparison the example of this difference (Figure 9.2, comprising an individual plant from each treatment) has been normalised by expressing stomatal conductance as a percentage of the maximum $g_s$ measured. The key difference between treatments was the...
minimum stomatal conductance ($g_{min}$), that is, the steady-state stomatal conductance measured prior to illuminating the leaf chamber. The minimum stomatal conductance was, on average, six times higher in control compared to ash-bed developed plants (Table 9.1). The overall time for stomatal opening did not differ between treatments. However, because $g_{min}$ was lower in ash-bed developed plants, the rate of stomatal opening (that is, the change in stomatal conductance with time) in this group was longer than in controls.

![Graph](https://via.placeholder.com/150)

Figure 9.2 A typical stomatal opening phase in control and ash-bed developed *Eucalyptus gunnii* seedlings. The opening phase is represented by a change in stomatal conductance from a minimum value (the value for stomatal conductance in darkness, $g_{min}$) to a maximum steady state value after exposure to a PAR of 1000 µmol m$^{-2}$ s$^{-1}$. Note that $g$ has been normalised as a percentage of its maximum value. The overall treatment difference in $g_{ss}$ is presented in Table 9.1.
Table 9.1  Leaf water potential, gas exchange and isotopic properties of leaves developed under control or ash-bed conditions. Values for \( \Delta \), \( g_\text{s} \) and \( p_\text{i}/p_\text{s} \) represent the photosynthetic operating point (refer to methods for a definition). All values are the mean ± s.e.; \( n = 8 \) for \( \Psi_\text{pd} \); \( n = 4 \) for \( \Delta \), \( g_\text{s} \), \( p_\text{i}/p_\text{s} \), \( R_\text{d} \), \( g_\text{min} \), \( V_\text{cmax} \), \( J_\text{max} \) and \( \Delta \). Significant differences at the 0.05 level (*) were observed between treatments for \( g_\text{min} \) (F = 12.053; P = 0.013) and \( R_\text{d} \) (F = 10.920; P = 0.002).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ash-bed</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Psi_\text{pd} ) (MPa)</td>
<td>-0.15 ± 0.03</td>
<td>-0.22 ± 0.02</td>
</tr>
<tr>
<td>( A ) (µmol m(^{-2}) s(^{-1}))</td>
<td>18.25 ± 0.64</td>
<td>17.35 ± 0.52</td>
</tr>
<tr>
<td>( g_\text{s} ) (mol m(^{-2}) s(^{-1}))</td>
<td>0.62 ± 0.09</td>
<td>0.45 ± 0.06</td>
</tr>
<tr>
<td>( p_\text{i}/p_\text{s} )</td>
<td>0.80 ± 0.01</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td>( R_\text{d} ) (µmol m(^{-2}) s(^{-1}))</td>
<td>1.27 ± 0.07</td>
<td>1.55 ± 0.05*</td>
</tr>
<tr>
<td>( g_\text{min} ) (mol m(^{-2}) s(^{-1}))</td>
<td>0.31 ± 0.07</td>
<td>0.05 ± 0.01*</td>
</tr>
<tr>
<td>( V_\text{cmax} ) (µmol m(^{-2}) s(^{-1}))</td>
<td>43.68 ± 1.58</td>
<td>45.20 ± 2.49</td>
</tr>
<tr>
<td>( J_\text{max} ) (µmol m(^{-2}) s(^{-1}))</td>
<td>121.75 ± 5.74</td>
<td>129.75 ± 5.78</td>
</tr>
<tr>
<td>( \Delta ) (%)</td>
<td>22.17 ± 0.62</td>
<td>22.25 ± 0.13</td>
</tr>
</tbody>
</table>

On exposure to darkness the stomatal closing phase also showed distinction across treatments. Ash-bed developed plants reached a lower stomatal conductance compared to controls. As with the stomatal opening phase, the example of stomatal closure (Figure 9.3, comprising an individual from each treatment) was normalised as a percentage of maximum stomatal conductance for comparison. After leaf abscission both treatments reached a similar stomatal conductance. As with the stomatal opening phase, the response time (the change in stomatal conductance with time) between leaf abscission and minimum steady-state stomatal conductance was longer in ash-bed compared to control plants.
The stomatal closing phase, quantified as the change in $g$ with time, in control and ash-bed developed *Eucalyptus gomphocephala* seedlings. Stomatal closure was induced by darkening leaves. After stabilisation, leaves were abscised from plants. Note that $g$ has been normalised as a percentage of its maximum value.

In another form the difference in stomatal response to environmental stimuli can be represented by the relationship between $g_s$ and $A$ (normalised as the percentage of maximum $g_s$ and $A$) during stomatal opening (Figure 9.4). In ash-bed developed plants $g_s$ and $A$ were well defined by an exponential model across all ranges of $A$, whereas for control plants the exponential increase in $A$ with increasing $g_s$ was most marked only when $A$ reached approximately 70% of its maximum. The exponential models describing these relationships are:

$$g_s = 33.23 + 2.68e^{\frac{A}{3250}} \quad (r^2 = 0.74)$$
for controls and

$$g_s = 4.49e^{\frac{A}{1111}} \quad (r^2 = 0.92)$$
for ash-bed developed plants.
In addition to differences in $g_{\text{min}}$ across treatments, $R_a$ was significantly lower in control compared to ash-bed developed plants (Table 9.1). While there was an apparent trend of greater calcium, magnesium, manganese, nitrogen and carbon in ash-bed developed plants, a t-test indicated that only Mn was significantly higher in ash-bed compared to control plants (Table 9.2). With the small sample
sizes, statistical power was admittedly limited such that only very large differences were likely to have been detected.

Table 9.2 Nutrient content of leaves used for gas exchange experiments. Values are mean ± se; n = 4 for each. A significant differences at the 0.01 level (**) was found between treatments for manganese ($F = 30.9; P = 0.001$).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control</th>
<th>Ash-bed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorous (%)</td>
<td>0.071 ± 0.007</td>
<td>0.084 ± 0.007</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.299 ± 0.278</td>
<td>2.375 ± 0.445</td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.136 ± 0.044</td>
<td>0.203 ± 0.030</td>
</tr>
<tr>
<td>Manganese (mg kg$^{-1}$)</td>
<td>35.900 ± 7.821</td>
<td>110.450 ± 10.895**</td>
</tr>
<tr>
<td>Total nitrogen (%)</td>
<td>1.760 ± 0.029</td>
<td>1.845 ± 0.051</td>
</tr>
<tr>
<td>Total Carbon (%)</td>
<td>47.437 ± 0.616</td>
<td>45.286 ± 0.770</td>
</tr>
</tbody>
</table>

9.4 Discussion

Since $\Psi_{pl}$ was not significantly different between treatments (Table 9.1), any variation in the gas exchange properties of *E. gomphocephala* was likely to be due to development under control or ash-bed conditions rather than short term differences in soil moisture, although differences in $\Psi_{pl}$ on a leaf developmental time frame cannot be excluded. Moreover, because seedlings were introduced to an ash-bed, the confounding effect of resprouting after fire was removed. While resprouts can show a different photosynthetic capacity with development on ash-beds, *per se* (for example Oechel and Hastings (1983)), in some species a reduced shoot mass in itself will alter water and nutrient availability in the canopy (Wellington 1984; Landsberg 1990; Fleck et al. 1996; Crombie 1997; Knox and Clarke 2005).

The most influential effect of ash-bed development on leaf gas exchange under the conditions of this experiment was likely to have been an enhanced photosynthetic capacity due to a soil nutrient-meditated increase in Rubisco
levels and greater capacity for RuBP regeneration. The across treatment similarity in the relationship between \( A \) and \( p_i \) (Figure 9.1A) suggest that a nutritional pulse during leaf development on an ash-bed did not provide such a biochemical photosynthetic advantage over control plants. This was conferred as a non significant difference in \( V_{c_{\text{max}}} \) and \( J_{\text{max}} \) (Table 9.1).

A literature search has suggested that measurement of steady-state rates of photosynthesis and intercellular partial pressure of \( \text{CO}_2 \) in a controlled ash-bed experiment has not been previously attempted. The results presented here are therefore the first attempt to provide a realistic comparison of any photosynthetic advantage gained by development on an ash-bed under typical environmental conditions. The similarity in the photosynthetic operating point across treatments suggests that development on an ash-bed does not provide an advantage to leaf area based productivity in \( E. \) gomphocephala, although seedling height, crown and stem diameter were significantly greater on ash-beds (Archibald, pers. Comm.). One explanation could be that the deposition of nutrients associated with an ash-bed promoted greater leaf biomass.

This is unlike the results of Sala et al. (2005), working on \( P\. \) ponderosa, who observed higher \textit{in situ} photosynthetic rates in newly developed needles after fire. Such a difference is usually attributed to fire-induced increases in soil inorganic nitrogen pools (primarily \( \text{NH}_4^+ \)) and indeed a slight (but non significant) increase in total leaf nitrogen between ash-bed and control treatments was observed in this study. However, the duration of the influence of this pulse is likely to be short lived because of the rapid turnover rate of the nitrogen cycle and the subduction of the highly mobile pool of inorganic nitrogen due to leaching (Stock and Lewis 1986). Moreover, Warren et al. (2000), working on \( E. \) diversicolor, another endemic eucalypt of south western Australia, provide evidence that maximum useable amounts of nitrogen can be reached in this genus, a conclusion that cannot be discounted here. Additionally, the nitrogen status of seedlings developing on ash-beds may not necessarily be superior where high temperatures have volatilised large quantities of nitrogen in ash and soil and/or where recolonisation of mychorrhizal fungi is delayed (Launonen et al. 1999). Thus, even if any advantage was provided to the
seedlings in the ash-bed by the greater level of available nutrients, this may have been offset by the more rapid mycorrhizal infection of the seedlings in the control soils.

Both treatments operated with the same $p/p_a$ (Figure 9.1B) and had similar water-use efficiencies. This latter observation was conferred as a non-significant difference in $\Delta_{\text{leaf}}$ (Table 9.1) across treatments. In resprouting leaves Fleck et al. (1996) observed a lower $\Delta_{\text{leaf}}$ in Quercus ilex; however, results in the literature do not show a similar trend in plants with a strong association between ash-beds and seedling regeneration.

The stomatal opening phase, characterised by plots of $g_s$ verses time (Figure 9.2), showed distinctive differences between control and ash-bed plants. The minimum steady-state stomatal conductance ($g_{\text{min}}$), that $g_s$ observed in darkness, was approximately six times higher in control plants. This meant that, over the course of a typical daily opening phase, the range of stomatal aperture expressed by control plants was much narrower than ash-bed developed plants and is more consistent with the minimum stomatal conductance of humid tropical species (Körner 1995). This suggests that the responsiveness of E. gomphocephala stomata to various environmental stimuli will be perturbed if developed on a relatively undisturbed soil, at least until mycorrhizal associations develop (refer forward to a discussion on the linkage between nutrient availability and stomatal regulation). The control of stomatal conductance by change to stomatal aperture is the principal mechanism whereby transpirational flux is governed. Therefore, if this mode of stomatal operation persisted under conditions of unfavourable soil moisture and high evaporative demand, control plants would likely experience detrimental water stress prior to ash-bed developed plants.

The interpretation of the results thus far has centred on the likely benefit of a fire-mediated nutrient pulse to E. gomphocephala seedlings. However, fire has also been shown to alter the composition of soil mycoflora (Baar et al. 1999). In addition, certain fungal pathogens have been shown to illicit physiological change to leaves. Guimarães and Stotz (2004) found that oxalic acid evolved...
from the phytopathogenic fungi, *Sclerotinia sclerotiorum*, for example, evoked stomatal opening at night by inhibiting ABA-induced stomatal closure and promoting the accumulation of osmotically active molecules that cause stomatal opening. While such an effect cannot be excluded in this experiment, no studies have yet identified a soil-borne fungus that duplicates the properties of *S. sclerotiorum*. Overall, knowledge of allelochemicals (released by microbes and plants) and their influence on stomatal opening is incomplete.

The differing relationships between $A$ and $g_s$ across treatments (Figure 9.4) provide insight into the assimilatory capacity of plants at any given $g_s$. For plants that developed on ash-beds the relationship between $A$ and $g_s$ held across all ranges of $g_s$, but for controls this relationship was only apparent after $g_s$ had achieved approximately 70% of maximum. Surprisingly this difference did not invoke a time integrated change in $p/p_a$ (as correlated with $\Delta_{\text{icar}}$) suggesting that: 1) this difference is a transient feature on a leaf developmental time frame, or 2) on an integrated time frame operational (maximum) gas exchange rates are more important to WUE.

The contrasting dynamic of stomatal closure across treatments (Figure 9.3) reveals information about the effect of ash-bed development on regulation of stomatal volume flux. As with the stomatal opening phase, the minimum stomatal conductance in darkness was higher in control compared to ash-bed developed plants. After leaf abscission, however, both treatments showed a further decrease in stomatal conductance, reaching a value likely to mainly represent cuticular conductance. This indicates that the differing capacities for the response of stomata to changed environmental variables, in this case light, was a function of guard cell volume change (mediated by solute flux) because plants of both treatments had the ability to close stomata via hydromechanical means (upon loss of leaf turgor).

Regulation of stomatal aperture involves turgor changes in guard cells. Increased solute accumulation increases guard cell turgor prompting stomatal opening and solute efflux causes a decrease in guard cell turgor and stomatal closure
RESPONSE TO FIRE

MacRobbie 1981). Potassium ions are considered the primary solute in the osmoregulation of guard cells. Selectively permeable ion channels, located in the guard cell plasmalemma, allow the accumulation and release of potassium ions (Schroeder et al. 1984). Both inward and outward rectifying potassium ion permeable voltage-gated channels have been elucidated. The outward rectifying channels of guard cells work by membrane depolarisation (Schroeder et al. 1986). While this process could be passive, the stomatal closing response to darkness (Karlsson and Schwartz 1988) and ABA (Weyers et al. 1982) are partially repressed by hypoxia and metabolic inhibitors. Hence stomatal closure is likely to be driven by metabolic membrane depolarisation. This means that a constant source of ATP is required for active control of stomatal closure.

Change in stomatal aperture, driven by guard cell turgor fluctuations, results from an integrated interpretation of plant water status, CO₂ concentration, light levels, plant growth regulator stimuli and other environmental variables. For stomata to optimise leaf gas exchange they should keep $p_i$ constant as long as light intensity remains constant. If light intensity decreases stomata should close to induce a decrease in $p_i$. When plants of this study were not developed on an ash-bed, stomata failed to interpret darkness and modify guard cell turgor accordingly, resulting in an elevated $g_{\text{min}}$ compared to ash-bed developed plants. It is conceivable that energetic-dependent stomatal closure was suppressed in these plants because they were deprived of key nutrients needed for photosynthetic energy capture.

The only leaf nutrient that was significantly reduced in control plants of this study was Mn, although control leaves showed no external symptoms of such a deficiency. This is consistent with a fire-induced increase in total soil Mn content and its readily reducible oxide forms (Gonzales Parra et al. 1996). Manganese has been suggested to play an important role in the distribution of eucalypts in Australia (Hill et al. 2001) with some species (known collectively as the symphyomyrts, which includes *E. gomphocephala*) regarded as avid accumulators. Manganese is best known for its role in splitting water during photosynthesis (Marschner 1986) but is also essential for the activation of numerous enzymes. One theoretical explanation for the failure of control plants
to completely respond to darkness is that a Mn deficiency interrupted the oxidisation of water during photosynthesis, which subsequently diminished the reduction of CO$_2$ and production of cellular ATP, and this impaired metabolic membrane depolarisation of guard cells.

Alternatively, a Mn deficiency could impair the regulation of stomatal aperture through its involvement in the expression or activity of gated membrane water channels (aquaporines). The hydraulic conductivity of plasma membranes, including those of guard cells, is largely determined by the presence and activity of aquaporins (Kjellbom et al. 1999). Aquaporins are therefore essential for large cellular volume fluxes and the regulation of water flow across membranes (Maurel 1997). Research has shown that aquaporin activity is regulated by cations, specifically Ca$^{2+}$ (Gerbeau et al. 2002; Alleva et al. 2006). It is conceivable that Mn has a similar regulatory role and its deficiency could, in control plants of this study, have disrupted guard cell hydraulic conductivity and stomatal aperture regulation.

9.5 Conclusions

Ash-bed deposition, as a result of fire, is generally considered to enhance plant productivity through a nutrient-mediated increase in photosynthetic capacity. In this study a new role for ash-bed deposition has been observed. Rather than improve steady-state photosynthetic rate, development on an ash-bed enabled *E. gomphocephala* seedlings to fully integrate environmental stimuli and modify stomatal conductance accordingly.
CHAPTER 10

GENERAL DISCUSSION AND CONCLUSIONS
10.1 Objective 1: How do the water relations properties and hydraulic architecture of *E. gomphocephala* differ with proximity to groundwater and season (Chapter 3)?

The results of Chapter 3 confirm that seasonality is expressed in the plant water relations properties across a range of landscape positions (2.30 m, 8.04 m and 21.73 m depth to groundwater from the natural surface) in *E. gomphocephala*. The magnitude of this seasonal oscillation was, however, different at various landscape positions. In this way a close proximity to groundwater could be said to buffer the severity of the annual summer dry season in *E. gomphocephala*. Specific hydraulic conductivity of stems (*K_s*) under native conditions was relatively constant throughout the study and at all sites the leaf hydrodynamic pressure gradient (the difference between pre-dawn and midday leaf water potential, \(\Psi_{pd}\) and \(\Psi_{md}\) respectively, -\(\Delta\Psi\)) remained relatively constant. This consistency, referred to as isohydrodynamic, is discussed in detail in a manuscript, reproduced in Appendix 4. Briefly, the maintenance of a constant leaf hydrodynamic pressure gradient is well defined within the parameters of a hydromechanical stomatal control model, but only when plant hydraulic conductance is positively dependent on transpiration rate.

Stomatal conductance to water vapour (*g_s*) was well correlated with \(\Psi_{pd}\) across sites suggesting a coupling between stomatal conductance and soil water potential (\(\Psi_{soil}\)). The lowest \(g_s\) and \(\Psi_{pd}\) were experienced where the water table was deepest. Close proximity to groundwater thus enabled trees to maintain greater tissue hydration and operate with wider stomatal apertures. Such buffering of the annual dry season does not necessarily imply a greater dependency on groundwater. Instead, the relative proximity to groundwater (and its associated capillary fringe) from the natural surface influences the amount of moisture in the root zone of *E. gomphocephala* in the dry season. A more extensive investigation into the water sources of *E. gomphocephala* was undertaken in Chapter 4.
Vulnerability curves obtained from distal canopy branches showed a similar pattern across landscape positions, suggesting that phenotypic plasticity was not expressed in the xylem anatomy of small branches in this species. However, the carbon isotope discrimination of leaves ($\Delta_{\text{car}}$) and the turgor loss point (TLP) decreased with increasing depth to groundwater, implying that access to soil water influences leaf phenotypes in this species.

At no site or sampling period (over two successive wet-dry seasonal cycles) was the turgor loss point breached, suggesting that the plasticity of leaves is in synchrony with the current environment of the species.

10.2 Objective 2: What are the water sources of *E. gomphocephala* and do these sources differ with stage of maturity, proximity to groundwater and season (Chapter 4)?

In Chapter 4 site 1 and site 2, as described in Chapter 3, proved conducive to determination of water sources through stable isotope analyses. Over the period of measurement, which comprised the transition from a summer dry season to a winter wet season, the position of groundwater in the soil profile proved to be tightly coupled to rainfall. This connectivity tended to be accentuated at site 1 because of the short path length between the natural surface and the water table.

Soil moisture, quantified as volumetric water content, showed a marked seasonality in the vertical representation of the vadose zone and also highlighted the vertical migration of the water table and capillary fringe with rainfall inputs. The moisture content at site 1, while showing the greatest temporal variability, was generally higher than site 2 emphasising the effectiveness of a close proximity to groundwater to buffer the severity of the summer dry season.

Results of the three-component mixing model (presented diagrammatically in Figure 10.1) suggest that *E. gomphocephala* is an opportunist, taking advantage of rainfall inputs during the favourable wet season by deriving much of its xylem...
water from surface soil layers (0.0-0.5 m for site 1 and 0.0-1.0 m for site 2). This switch from a range of water sources in summer to principally surface soil water in spring was mainly observed at site 1 and in seedlings at site 2. Maintaining a root system capable of exploiting surface soil water (derived from recent rainfall) in seedlings would seem advantageous because of the excessive costs of deploying deep roots in plants comprising a limited canopy. Moreover, a similar capability in more mature plants at site 1 would seem compatible with the small volume of rhizosphere available at this location which would likely constitute an increase in competition for below ground resources.

As an opportunist, it could be argued that both stage of maturity (or size class), proximity to groundwater and season have a bearing on the water source used by *E. gomphocephala*. However, despite the apparent differences in water sources, all plants increased their $\Psi_{p\downarrow}$ with the onset of the winter wet period. For a given site the correlation between the maximum yield of PSII ($F_v/F_M$) and the Huber value ($H$) implies that large trees exploited a conservative investment in leaf area for a given sapwood area to maximise $F_v/F_M$. Nevertheless, proximity to groundwater, and hence access to a rhizosphere with a differential buffering capacity from the annual dry season, did not have a bearing on $F_v/F_M$ or $H$. 
Figure 10.1 A diagrammatic representation of the partitioning of water sources across stage of maturity and season in *E. gomphocephala*. In this figure transition from the summer dry period to the spring (peak groundwater) period is accompanied by an increase in use of surface water in all plants where proximity to groundwater is low and in only seedlings at areas higher in the landscape. The figure is based on data from Figures 4.4 and 4.5.
10.3 Objective 3: Does vulnerability to drought-induced xylem embolism differ between *E. gomphocephala* and co-occurring canopy species (Chapter 5)?

Comparison across the entire geographical ranges of four co-occurring species of Yalgorup National Park suggested that resistance to drought should contrast (Chapter 5). This assessment was based on the minimum rainfall at which a species occurs naturally. It should be acknowledged, however, that this methodology fails to fully capture the environmental range of a species which will be driven by many interdependent environmental factors.

Vulnerability curves revealed that the three Myrtaceous species of this study have a similar cavitation response with exposure to tension in xylem. *Acacia cyclops*, however, became fully embolised much earlier than the three remaining species. This would suggest that *A. cyclops* is highly susceptible to drought. However, this species occupies regions of low rainfall (compared to the other species investigated).

The slope of the relationship between percentage loss hydraulic conductivity (PLC) and the air injection pressure was correlated negatively with the air injection pressure that induced a PLC of 50% (refer to Figure 5.8). While the sampled group of plants is small, this correlation implies interdependency between pit pore size and the pit pore distribution that determines cavitation exchange between vessels. In Chapter 5 it is argued that, in this case, a more meaningful expression of vulnerability to embolisms is the slope of the relationship between PLC and $\Psi_\infty$. A species with a low slope would experience cavitation events across a large range of water potentials and could be expected, therefore, to express substantial native embolisms. Indeed a high slope was associated with species that occurred naturally in low rainfall zones.

Of the four species assessed only *E. gomphocephala* shows evidence of a decline syndrome in Yalgorup National Park. Ranking of species by the minimum rainfall of their natural geographical distribution or vulnerability to xylem
embolism did not yield a result that would place *E. gomphocephala* as the most susceptible species to drought. Further work that emulates drought conditions is required to test the supposition of water stress susceptibility in the species outlined in this chapter.

10.4 Objective 4: Are root anatomical features related to xylem hydraulic properties in *E. gomphocephala* (Chapter 6)?

The results of Chapter 6, while somewhat preliminary, imply that in *E. gomphocephala* roots comprising a small average hydraulically weighted vessel diameter (*d*) express a greater native specific hydraulic conductivity and lower percentage loss hydraulic conductivity. Combined, these findings suggest that, under the current conditions of Yalgorup National Park, a root anatomy that favours hydraulic safety over efficiency is a favourable adaptation. Similarly the observation that roots with a greater maximum hydraulic efficiency experience a greater difference between maximum and *in situ* hydraulic conductivity suggests that a more conservative root hydraulic architecture would be advantageous under the current conditions experienced in Yalgorup National Park.

Maximum hydraulic conductivity of roots was of a magnitude to indicate that *E. gomphocephala* can deploy an efficient root system when saturated soil is available. The observation of a high proportion of native embolisms in roots, even at the opportune time of measurement in this study, implies that this species regularly experiences environmental conditions that could be construed as damaging to the hydraulic integrity of root systems. Where declining rainfall may be altering the available water for uptake by plants it is important to understand the phenotypic plasticity of, in this case, root xylem anatomy and its associated effect on hydraulic properties for a given species. This chapter has provided preliminary insight into such plasticity in *E. gomphocephala* and sets a subsequent research direction for future investigation into *E. gomphocephala* decline.
10.5 **Objective 5: Are current plant water relations properties related to the degree of canopy dieback in *E. gomphocephala* (Chapter 7)?**

In this chapter it became apparent that, across the three sites under investigation, large *E. gomphocephala* trees are experiencing the most severe outward expression of the canopy decline syndrome (canopy dieback). This type of correlation has typically been defined by forest maturation models incorporating a combination of a decline in primary production and an increase in respiratory costs associated with stems. The alternative hypothesis, involving changes in competition and stand structure with maturity, are also considered in chapter 7. However, the locally defined *E. gomphocephala* decline syndrome (within Yalgorup National Park) would likely conflict with both hypotheses that describe the more general concept of age-related decline of forests. To frame this conflict as a question: Why is the same pattern of canopy decline not expressed in all mature stands of *E. gomphocephala*?

Of the dry season physiological traits assessed only instantaneous estimated transpiration rate ($E^*$) related, with any confidence, to canopy condition. Moreover, this correlation was not uniform across sites but tended to depend on proximity to groundwater. Specifically, the efficacy of a close proximity to groundwater to buffer the severity of the annual dry season appeared to enable *E. gomphocephala* trees with a relatively intact canopy to express a higher $E^*$. Where this advantage was not available a strategy of conservative expression of $E^*$ was associated with a better canopy condition.

Overall, dry season physiological traits were poor predictors of *E. gomphocephala* canopy condition. However, excluding water stress as an underlying cause of the current decline syndrome, centred on Yalgorup National Park, on this basis alone should be done so with caution. Two theoretical explanations that retain the integrity of a drought-induced canopy decline scenario but also provide a basis for the poor correlations between dry season physiological traits and canopy condition are outlined in Chapter 7. These are: 1) the temporal separation from the initial event and physiological measurements could preclude an interpretable correlation with current instantaneous dry season
physiological traits, and 2) through attrition, the retention of resilient individuals could mask the undying effect of water stress.

10.6 Objective 6: How do *E. gomphocephala* seedlings respond to development under a suboptimal soil moisture environment (Chapter 8)?

Chapter 8 examined the role of contrasting growth conditions on *E. gomphocephala* mature leaf physiology. Under a developmental water deficit *E. gomphocephala* seedlings were capable of manipulating leaf epidermal morphology (increasing the density of stomata and reducing their anatomical dimensions) and expressing conservative leaf gas exchange properties. Because this experiment sought to implement conditions likely to be experienced in the field it is difficult to attribute conservative leaf gas exchange properties to changed stomatal anatomy.

The relationship between $g_s$ and $\Psi_{pd}$ observed in this microcosm-type experiment needs to be interpreted with caution. Figure 10.2 depicts this relationship in concert with that obtained from the field-based data of Chapter 3. Whereas a linear relationship holds across all values of $\Psi_{pd}$ for field grown plants, the relationship for glasshouse plants is exponential. An explanation could be that the root zone of potted glasshouse plants dries out more quickly, or by a larger amount, than field grown plants due to a much smaller soil volume. Despite this it could be argued that a similar plasticity in leaf gas exchange and epidermal development in the field, as observed in the glasshouse, would permit carbon uptake (and leaf expansion) where soil moisture was 50% of field capacity. The results of this study could be further expanded to determine the developmental response of *E. gomphocephala* under more severe conditions.

While specific hydraulic conductivity of petioles ($K_s$) was weakly related to the average hydraulically weighted mean vessel diameter ($d$), $K_s$ appeared to be effected more by developmental irradiance than water availability. Surprisingly this affect meant that seedlings that developed under 2% of full sun exhibited a
higher $K_S$. The consistency of leaf-area-specific hydraulic conductivity ($K_L$) across treatments would imply that leaf area scales according to the hydraulic efficiency of the supplying petiole.

![Graph](image.png)

**Figure 10.2**  Midday stomatal conductance to water vapour ($g_s$) was linearly related to $\Psi_{pd}$ in the field and exponentially related to $\Psi_{pd}$ in the glasshouse. The fitted line for field data is a linear model: $g_s = 332.07 + 227.15\Psi_{pd}$ ($r^2 = 0.58; P < 0.001$). The fitted line for glasshouse data is an exponential model: $g_s = 337.13 - 218.09e^{\left(\frac{\Psi_{pd} = 0.95}{0.85}\right)}$ ($r^2 = 0.79; P < 0.05$).

The results of Chapter 8 also suggest that the controlled water stress invoked a down regulation of maximum yield of PSII ($F_v/F_m$). The correlations between
Fv/FM and Ψpd and Fv/FM and midday gs strongly imply that this down regulation was as a result of stomatal-limited gas exchange.

Combined, the results of Chapter 8 provide evidence for ontogeny in the leaves of *E. gomphocephala* seedlings developed under contrasting light and soil water regimes. Of the two growth conditions applied, a developmental water stress provoked the greatest physiological response in leaves. This response would be of most significance to a newly developing *E. gomphocephala* cohort exposed to conditions of increased water stress as a result of reduced annual rainfall.

**10.7 Objective 7: How does development on ash-beds influence leaf-area-specific properties of *E. gomphocephala* (Chapter 9)?**

Photosynthetic capacity is typically superior in vegetation developed after exposure to fire. This increase is considered to be mediated by: 1) increasing nutrient sequestration from ash for leaf protein synthesis, or 2) altering water and nutrient availability through reduced shoot mass. Chapter 9 focussed on the former process by developing seedlings of *E. gomphocephala*, a species known to be advantaged by fire, on an ash-bed exposed or undisturbed (control) soil.

Surprisingly photosynthetic capacity, quantified as steady state assimilation rate (*A*), was similar across treatments, as was the maximum steady state stomatal conductance to water vapour (*g*$_s$), the maximum rate of carboxylation (*Vc*$_{\text{max}}$) and the light saturated rate of electron transport (*J*$_{\text{max}}$). Differences were observed, however, in the dynamic of the stomatal opening phase. Specifically, minimum *g*$_s$ (that *g*$_s$ measured in darkness, *g*$_{\text{min}}$) was six times lower in ash-bed developed plants compared to controls. Leaves of ash-bed developed plants also had a greater concentration of several cation species, primarily Mn. It was hypothesised from these results that development on control soils, lacking the advantages provided by an ash-bed, deprives *E. gomphocephala* seedlings the key elements required by stomata to fully respond to environmental stimuli.
Further study examining the role of cations during leaf development in this species would confirm this supposition.

\subsection{10.8 Conclusions}

\textit{Eucalyptus gomphocephala} is an opportunist, capable of exploiting a range of water sources in the rhizosphere depending on temporal and spatial patterns of soil water distribution. The species is also adept at modifying its phenotype when it develops under contrasting soil water environments, a conclusion drawn from both field and glasshouse experiments. These two properties, no doubt, contributed to the observation that breach of the turgor loss point was not experienced at any field location in Yalgorup National Park. \textit{Eucalyptus gomphocephala} can also be described as isohydrodynamic, able to maintain a consistent hydrodynamic pressure gradient across seasons. While these conclusions, alone, do not preclude water stress as an underlying cause of the species’ decline, they do imply that the current ecohydrological niche of Yalgorup National Park is compatible for its survival.

Coupled to the conclusions above is the observation that vulnerability curves of \textit{E. gomphocephala} stems are not atypical of co-occurring canopy species that are not expressing symptoms of a canopy decline syndrome. Additionally, there was no statistical evidence to link dry season water relations traits with the magnitude of canopy decline in an individual tree. However, caution should be exercised when interpreting this poor correlation because of the temporal separation from the initiation of the canopy decline syndrome and the water relations measurements. Moreover, the discovery of a relatively high proportion of native embolisms in roots, even under ideal conditions, warrants further scrutiny of the below ground biomass in future diagnostic studies.

The opportunistic access to an ash-bed experiment revealed the possibility that fire, as a form of disturbance, can liberate cation species that are important for normal stomatal function. The role fire, the liberation of cations and interactions with mycoflora is a topic that requires further investigation.
Integrated, the results of this study do not support the hypothesised linkage between a climate change-induced shift in annual rainfall and the decline syndrome of *E. gomphocephala* in Yalgorup National Park. Rather, the study revealed that the species is capable of substantial phenotypic plasticity when faced with contrasting soil water regimes, a capacity that has contributed to compatibility with the current seasonally dry habitat of Yalgorup National Park.
REFERENCES


REFERENCES


191
REFERENCES


Chilcott C (1994) The decline of Tuart (Eucalyptus gomphocephala) health in the Kwinana remnant bushland, Perth WA. Proceedings of the National Greening Australia Conference, pp 241-243


Coates DJ, Keighery GJ, Broadhurst L (2002) Genetic and morphological variation, and the mating systems in Tuart. In: Keighery BJ, Longman VM (eds) Tuart (Eucalyptus gomphocephala) and Tuart Communities. Perth Branch of the Wildflower Society of Western Australia, Nedlands, pp 89-107


Dawson TE (1996) Determining water use by trees and forests from isotopic, energy balance and transpiration analyses - the roles of tree size and hydraulic lift. Tree Physiology 16:263-272


consequences of variation in growth rate and productivity of higher plants. SPB Academic Publishing, The Hague, pp 125-140


Gardiner CA (1966) Eucalypts of Western Australia. Western Australian Department of Agriculture, Perth


Gentilli J (1972) Australian Climatic Patterns. Nelson, Melbourne


Conservation and Land Management and the Conservation Council of Western Australia (Inc.)


Hacke UG, Sperry JS (2003) Limits to xylem refilling under negative pressure in *Laurus nobilis* and *Acer negundo*. Plant, Cell & Environment 26:303-311


REFERENCES

Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge


Humphreys FR, Lambert MJ (1965) An examination of a forest site which has exhibited the ash-bed effect. Australian Journal of Soil Research 3:81-94

Indian Ocean Climate Initiative (2002) Climate variability and change in the south-west. Department of the Environment: Water and Catchment Protection (and others in Partnership), Perth


REFERENCES

Jurskis V (2005) Eucalypt decline in Australia, and a general concept of tree
inhibitors, ATPase inhibitors and a potassium-channel blocker on
stomatal opening and closing in isolated epidermis of Commelina
communis L. Plant, Cell and Environment 11:165-172
Kawase M (1981) Anatomical and morphological adaptation of plants to
waterlogging. HortScience 16:30-34
of Tuart and the community with which it lives. In: Keighery BJ,
Longman VM (eds) Tuart (Eucalyptus gomphoecephala) and Tuart
Communities. Perth Branch Wildflower Society of Western Australia
(Inc.), Nedlands
term climatic variability on the Australian continent. Freshwater Biology
48:1274-1283
Kile GA, Ohmart CP (eds) Eucalypt dieback in forest and woodlands.
CSIRO, Melbourne, pp 13-26
Aquaporins and water homeostasis in plants. Trends in Plant Science
4:308-314
plant ecology. Hydrogeology Journal 7:65-77
Knapp AK (1985) Effect of fire on the ecophysiology of Andropogon gerardii
and Panicum virgatum in a tallgrass prairie. Ecology 66:1309-1320
Knox KJE, Clarke PJ (2005) Nutrient availability induces contrasting allocation
and starch formation in resprouting and obligate seeding shrubs.
Functional Ecology 19:690-698
Nature 428
Kolb TE, Hart SC, Amundson R (1997) Boxelder water sources and physiology
at perennial and ephemeral stream sites in Arizona. Tree Physiology
17:151-160
Körner C (1995) Leaf diffusive conductances in the major vegetation types of the
globe. In: Schulz E-D, Caldwell MM (eds) Ecophysiology of
Photosynthesis. Springer-Verlag, New York, pp 463-490
Lange OL, Losch R, Schulz E-D, Kappen L (1971) Responses of stomata to
changes in humidity. Planta 100:76-86
Launonen TM, Ashton DH, Keane PJ (1999) The effect of regeneration burns on
the growth, nutrient acquisition and mycorrhizae of Eucalyptus regnans
F. Muell. (mountain ash) seedlings. Plant and Soil 210:273-283
Leadley PW, Reynolds JF (1989) Effect of carbon dioxide enrichment on
development of the first six main stem leaves in soybean. American
Journal of Botany 76:1551-1555

198
REFERENCES


Lindsay RP (2002) Climate and land use impacts on groundwater levels in the Lake Clifton and Lake Preston area. School of Applied Geology. Curtin University of Technology, Perth, p 46


Loneragan OW, Loneragan JF (1964) Ashbed and nutrients in the growth of seedlings of karri (Eucalyptus diversicolor F. v. M). Journal of the Royal Society of Western Australia 47:75-80

Longman VM, Keighery BJ (2002) Tuart issues. In: Keighery BJ, Longman VM (eds) Tuart (Eucalyptus gomphocephala) and Tuart Communities. Perth Branch of the Wildflower Society of Western Australia, Nedlands


McArthur WM, Bettenay E (1960) Development and distribution of soils of the Swan Coastal Plain, Western Australia. CSIRO Australia


REFERENCES

REFERENCES


Pryor LD (1963) Ash bed growth response as a key to plantation establishment on poor sites. Australian Forestry 27:48-51


REFERENCES

Scholander PF, Hammel HT, Hemmingsen EA, Bradstreet ED (1964) Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proceedings of the National Academy of Science 52:119-125
Seddon G (1972) Sense of Place. University of Western Australia Press, Perth
REFERENCES


REFERENCES


Thorburn PJ, Walker GR (1994) Variation in stream water uptake by *Eucalyptus camaldulensis* with differing access to stream water. Oecologia 100:293-301


REFERENCES

Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Plant Physiology 88:574-580


REFERENCES

Appendix 1

Schematic diagram of the steady state flow meter (SSFM)

Key

= omni directional valve

= pressure transducer (connected to 100x voltage amplifier and voltmeter)

= Compression fitting

= Peek resistance tubing

This schematic diagram depicts the instrument described in Chapter 3, Chapter 4, Chapter 5, Chapter 6 and Chapter 8, which was used to measure hydraulic properties of stems and roots.
A linear model described the relationship between neutron moisture meter (NMM) counts and volumetric water content ($\theta$) across sites. The fitted line has the equation: $\text{NMM} = 27.75 + 6.95\theta$ ($r^2 = 0.57$, $P < 0.001$). This model was subsequently used to normalise NNM (counts) to $\theta$ in Chapter 4. Note that the variation implied in this figure is consistent with a variable deep profile.
Appendix 3

Bounding range errors from the three-component mixing model

The three-component mixing model used in Chapter 4 derives bounding range errors associated with variability in the data set. To demonstrate typical variability in the data presented in Chapter 4, an example of these errors are given for seedlings from site 1 (see figure below). The full detail of the three-component mixing model is given in Zencich et al 2002.

Bounding range errors for seedling xylem water sampled at site 1 during February 2004 (A) and October 2004 (B). Variability in this case was most marked during the summer dry period, represented by February 2004.
Appendix 4

Manuscript

Anisohydric but isohydrodynamic: Seasonally constant plant water potential gradient explained by a stomatal control mechanism incorporating variable plant hydraulic conductance and a root-shoot signal.

P. J. Franks¹, P. L. Drake², and R. H. Froend²

¹ School of Tropical Biology, James Cook University, PO Box 6811, Cairns, Queensland 4870, Australia.

² Centre for Ecosystem Management, School of Natural Sciences, Edith Cowan University, 100 Joondalup Drive, Joondalup, WA 6027, Australia

Corresponding author: P. J. Franks
Phone: +61 7 40421237
Fax: +61 7 40421284
E-mail: peter.franks@jcu.edu.au

Running title: Constant plant water potential gradient
Isohydric and anisohydric regulation of plant water status has been observed over several decades of field, glasshouse and laboratory studies, yet the functional significance and mechanism of both remain obscure. We studied the seasonal trends in plant water status and hydraulic properties in a natural stand of *Eucalyptus gomphocephala* through cycles of varying environmental moisture (rainfall, groundwater depth, evaporative demand) in order to test for isohydry and to provide physiological information for the mechanistic interpretation of seasonal trends in plant water status. Over a 16-month period of monitoring, spanning two summers, midday leaf water potential correlated with pre-dawn leaf water potential, which was correlated with water table depth below ground level, which in turn was correlated with total monthly rainfall. *Eucalyptus gomphocephala* was therefore not isohydric. Despite strong stomatal down-regulation of transpiration rate in response to increasing evaporative demand, this was insufficient to prevent midday leaf water potential from falling to levels below -2.0 MPa in the driest month, well into the region likely to induce significant xylem air embolisms. However, even though midday leaf water potential varied by over 1.2 MPa across seasons, the hydrodynamic plant water potential gradient (\(\Delta \Psi_{\text{plant}}\)), inferred as the difference between pre-dawn and midday leaf water potential, was relatively constant across seasons, averaging about 0.6 MPa. This unusual pattern of hydraulic regulation, referred to here as isohydrodynamic, is predicted by a hydromechanical stomatal control model, but only when plant hydraulic conductance is dependent on transpiration rate. We observed a correlation between midday transpiration rate and whole-plant hydraulic conductance that was consistent with this requirement, although conditions did not allow dependence of one on the other to be established. The accuracy of the model is improved slightly with the addition of a root-shoot signal allowing guard cell osmotic pressure to decline in response to soil water potential. The implications of the observed pattern of hydraulic regulation are discussed in the context of mechanistic requirements in the stomatal control system, and its possible function in related physiological processes.

Key words: isohydric, anisohydric, stomata, xylem, hydraulic, water relations