A comparative study of the conducting tissue of evergreen and deciduous trees of the Northern Territory

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Declaration

I hereby declare that the work herein is the results of my own investigations and all references to idea and work of other researchers have been specifically acknowledged. The work embodied in this thesis has not previously been accepted in substance for any degree and is not currently submitted in candidature for any other degree.

Signed:

Date:
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Abstract

The climate of the top end of the Northern Territory is dominated by alternating wet and dry seasons. Trees of the Northern Territory have developed a variety of responses to this seasonal climate, including a range of leaf phenologies (evergreen, semi-deciduous, brevi-deciduous and deciduous). These phenological groups differ in their anatomical and physiological characteristics.

This study investigated the relationship between vessel anatomy and susceptibility to drought induced xylem cavitations. Xylem vessel characteristics were compared among deciduous and evergreen species growing in seasonally dry forests near Darwin. Vessel diameter, vessel length, vessel element length, vessel density and pit membrane pore diameters were measured in six evergreen and six deciduous species and one coniferous species. This study also examined the linkage between hydraulic conductance, vulnerability to xylem embolism and vessel diameter in deciduous and evergreen species. Deciduous species had a greater proportion of vessels with large diameters than did evergreen species.

The mean vessel diameters in (branches, petioles and mid-ribs) were larger in deciduous species (50 µm ± 1.7, 28 µm ± 1.56, 28 µm ± 2.1) than evergreen species (32 µm ± 0.6, 18 µm ± 1.2, 18 µm ± 1.1). The mean pit membrane pores diameter in deciduous species (0.49 µm± 0.34) was larger than in evergreen species (0.24.µm ± 0.15). The average hydraulic conductivity in branch of deciduous species (0.03 g m s⁻¹ MPa⁻¹) was larger than in evergreen species (0.01 g m s⁻¹ MPa⁻¹).

These results support the conclusion that since deciduous species only have to conduct water during the wet season, their large xylem vessels with large pit membrane pores, which confer relatively larger hydraulic conductivity do not expose the canopy to an unacceptably high risk of xylem cavitation. By contrast, evergreen trees must transport water during more extreme dry season conditions, when they would potentially be more susceptible to xylem embolism. To protect against this, they have developed vessels with generally smaller diameters and with small pit membrane pores or vestured pits.
# Table of Contents

Declaration ........................................................................................................................... ii

Acknowledgements ........................................................................................................ iii

Abstract ............................................................................................................................ iv

Table of Contents ............................................................................................................. v

List of Figures ................................................................................................................ ix

List of Tables .................................................................................................................. xii

List of Plates .................................................................................................................... xiv

List of Abbreviations ....................................................................................................... xvi

Chapter 1: General Introduction ..................................................................................... 1

1.1 Savannas ..................................................................................................................... 1

1.1.1 Northern Territory climate ................................................................................... 2

1.1.2 Northern Territory vegetation ............................................................................. 3

1.1.3 Physiological differences between evergreen and deciduous species .......... 4

1.2 Hydraulic conductance ............................................................................................... 6

1.2.1 Embolism ............................................................................................................... 7

1.3 Xylem anatomy .......................................................................................................... 8

1.3.1 Difference in xylem anatomy between evergreen and deciduous species .... 14
Chapter 2: Light microscopical studies of conducting tissues

2.1 Introduction

2.1.1 Xylem

2.1.2 Phloem

2.1.3 Aims

2.2 Materials and Methods

2.2.1 Study sites and species

2.2.3 Plant materials

2.2.4 Vessel element length and diameter

2.2.5 Phloem anatomy

2.2.6 Distributions of xylem vessel lengths of branch wood

2.2.7 Statistical analysis
Chapter 2: Results

2.3 Results

2.3.1 Comparison of maceration and sectioning methods

2.3.2 Vessel diameters as a function of branch diameter in different plant parts

2.3.3 Vessel dimensions in evergreen and deciduous species

2.3.4 Leaf anatomy

2.3.5 Phloem cross-sectional area in seedlings

2.4 Discussion

2.4.1 Sectioning method vs maceration method

2.4.2 Xylem anatomy

2.4.3 Phloem

Chapter 3: Seasonal changes in hydraulic conductance and xylem vulnerability of deciduous and evergreen species

3.1 Introduction

3.2 Materials and Methods

3.2.1 Study site and species

3.2.2 Hydraulic conductance

3.2.3 Xylem vessel measurement

3.2.4 Vulnerability curves

3.2.5 Statistical analyses
3.3 Results .......................................................................................................................... 71

3.3.1 Seasonal patterns of hydraulic characteristics ................................................. 71

3.3.2 Xylem anatomy and percentage loss in conductivity ............................ 79

3.3.3 Vulnerability curves ......................................................................................... 79

3.4 Discussion ............................................................................................................. 83

Chapter 4: Scanning Electron Microscopy studies of xylem pit membrane pores......................................................................................................................... 86

4.1 Introduction ............................................................................................................. 86

4.2 Materials and Methods .......................................................................................... 89

4.2.1 Study sites and species .................................................................................... 89

4.2.2 Plant materials ................................................................................................. 91

4.2.3 Statistical analysis ......................................................................................... 91

4.3 Results .................................................................................................................... 91

4.4 Discussion ............................................................................................................. 101

Chapter 5 General Discussion and Conclusion ..................................................... 104

References ................................................................................................................. 111
List of Figures

Fig. 1.1. Diagram of a typical dicot plant. Transverse section of leaf, stem and root with xylem and phloem cells. (Inspiration for this diagram from “Biology of Plants”, 5th ed. Raven P., Evert R., Eichhorn S. 1992) ...............................................................11

Fig. 1.2. Diagram of (a) surface view and transverse section of bordered pit with torus in the middle (b) surface view and transverse section of simple pit (Inspiration for this diagram from “Anatomy of seed plants”, Katherine Easu, 1960) ...............12

Fig. 2.1. The apparatus used for paint infusion measurements. .................................35

Fig. 2.2. Comparison of vessel diameter using maceration and sectioning methods. Vessel diameter was smaller when determined by the sectioning than the maceration method. Full species names are given in 2.2.1. Vertical bars represent standard error. ..................................................................................................................40

Fig. 2.3. Mean vessel diameters (measured using the maceration method) in three different size classes of branch for evergreen and deciduous species. Vessel diameters were larger in deciduous species than evergreen species. Vertical bars represent standard error. ..................................................................................................................40

Fig. 2.4. Comparison of mean vessel and tracheid diameters in midribs, petioles and branches of evergreen, deciduous and conifer species. Vertical bars represent standard error. ..................................................................................................................41

Fig. 2.5. Mean vessel element length in branches of different diameter of evergreen and deciduous species. Vessel element length is longer in narrower branches than in wider diameter branches. Vertical bars represent standard error ...............41

Fig. 2.6. Frequency of vessels of different lengths, as determined by the paint infusion method, in tree species from different phenological guilds, □ evergreen, □ semi-deciduous and □ deciduous species. Vertical bars represent standard error. ..................................................................................................................42

Fig. 2.6(Contd). Frequency of vessels of different lengths, as determined by the paint infusion method, in tree species from different phenological guilds, □ evergreen, □ semi-deciduous and □ deciduous species. Vertical bars represent standard error. ..................................................................................................................43

Fig. 2.7. Mean vessel density and mean vessel diameter for evergreen and deciduous species. There was a negative correlation between vessel diameter and vessel density ($r^2 = 0.86, P <0.002$) ..................................................................................................................51
Fig. 2.8. Mean vessel diameter in evergreen and deciduous species and tracheid diameter in conifer species. Full species names are given in section 2.2.2. Vertical bars represent standard error.

Fig. 2.9. Changes in mean (a) leaf dry weight, (b) root dry weight and (c) phloem cross sectional area within monthly seedlings age. Symbols: (●) E. tetrodonta, (○) C. polysciada, (▼) M. viridiflora and (∇) T. ferdinandiana. The bars indicate standard error.

Fig. 2.10. Relationship between whole plant RGR and seedling ages (days) (♣). E. tetrodonta, (○). M. viridiflora, (▼). C. polysciada, (∇). T. ferdinandiana.

Fig. 2.11. Relationship between stem diameter and log root dry weight (a) E. tetrodonta, (b) M. viridiflora, (c) C. polysciada, and (d) T. ferdinandiana symbols (●). Month 1, (○). Month 2, (▼). Month 3, (∇). Month 4, (■). Month 5.

Fig. 2.12 Relationship between stem diameter and log dry leaf weight (a) E. tetrodonta, (b) M. viridiflora, (c) C. polysciada, and (d) T. ferdinandiana symbols (●). Month 1, (○). Month 2, (▼). Month 3, (∇). Month 4, (■). Month 5.

Fig. 2.13. Relationship between stem diameter and xylem cross-sectional area (a) E. tetrodonta, (b) M. viridiflora, (c) C. polysciada, and (d) T. ferdinandiana symbols (●). Month 1, (○). Month 2, (▼). Month 3, (∇). Month 4, (■). Month 5.

Fig. 2.14. Relationship between stem diameter and phloem cross sectional area (a) E. tetrodonta, (b) M. viridiflora, (c) C. polysciada, and (d) T. ferdinandiana symbols (●). Month 1, (○). Month 2, (▼). Month 3, (∇). Month 4, (■). Month 5.

Fig. 2.15. Relationship between stem diameter and log dry root weight (a) E. tetrodonta, (b) M. viridiflora, (c) C. polysciada, and (d) T. ferdinandiana symbols (●). Month 1, (○). Month 2, (▼). Month 3, (∇). Month 4, (■). Month 5.

Fig. 3.1. Schematic representation of the apparatus for measuring hydraulic conductance (A). vacuum pump; (B). guage; (C). vacuum chamber; (D). branch; (E). pipette; (F). three-way valve; (G) compressed air tank with regulator; (H). Reservoir. Line connecting is a pressure hose.

Fig. 3.2. Percentage loss of hydraulic conductance increased between February (late wet season) and September (late dry season) in (a) four evergreen species and (b) one semi-deciduous species and one deciduous species.

Fig. 3.3. The relationship between vessel diameter and percent loss of hydraulic conductance in (a) February, (b) May, (c) July and (d) September.
Fig. 3.4. Percentage loss of hydraulic conductance and in February (wet season), May, July and September (dry season). Vertical bars represent standard error.

Fig. 3.5. The relationship between percentage loss of hydraulic conductance due to xylem embolism and water potential in terminal branches of laboratory dry segments of evergreen, semi-deciduous and deciduous species sampled in 2001 back-transformed from equation (1). (inset: linear Regression of water potential and % loss of hydraulic conductance).

Fig. 4.1. Mean pit membrane pores diameter in evergreen and deciduous species. Full species names are given in Table 2.1. Vertical bars represent standard error.

Fig. 4.2. Mean pit membrane pore diameter in evergreen species and deciduous species, showing average pit membrane pore diameter in deciduous was wider than that of evergreen species. Vertical bars represent standard error. (P<0.05)

Fig. 4.3. The relationship between the mean vessel diameter and pit membrane pore diameter in evergreen (●) and deciduous (○) species.

Fig. 4.4. The relationship between pit membrane pore diameter and xylem water potential at 25% loss of hydraulic conductance from the vulnerability curves presented in chapter 3.
List of Tables

Table 2.1. Family, phenology and common name of species chosen for examination of xylem (a) and phloem (b) anatomy..............................................................27

Table 2.2. Tissue processing methodology described the stages of dehydration of samples (branch and petioles) prior to resin infiltration. ........................................31

Table 2.3. Tissue processing methodology describing the stages of dehydration of leaf samples. ...........................................................................................................31

Table 2.4. Examples of a calculation of vessel length in *E. tetrodonta* branch. ......36

Table 2.5. Vessel diameters measured by the maceration method observed in branches of different diameter in evergreen species and deciduous species ..........38

Table 2.6. Mean vessel diameter and length and vessel density (± 1 SE) for deciduous and evergreen species and the result of ANOVA comparing .................50

Table 2.7. Initial mean stem diameter at one month age seedling, shoot and root dry weight for 5 specimens in each species (Standard error are also shown). ....54

Table 3.1 (a). Branch dimension, % loss of conductance due to embolism and conductance and conductivity of terminal branches of evergreen, semi-deciduous and deciduous species in February (2001). .................................................................73

Table 3.1 (b). Branch dimension, % loss of conductance due to embolism and conductance and conductivity of terminal branches of evergreen, semi-deciduous and deciduous species in May (2001)...............................................................74

Table 3.1 (c). Branch dimension, % loss of conductance due to embolism and conductance and conductivity of terminal branches of evergreen, semi-deciduous and deciduous species in July (2001). ...............................................................75

Table 3.1 (d). Branch dimension, % loss of conductance due to embolism and conductance and conductivity of terminal branches of evergreen, semi-deciduous and deciduous species in September (2001)........................................................76

Table 3.2. Hydraulic conductance and conductivity for 4 evergreen, 1 semi-deciduous and 1 deciduous tree species from the NT of Australia measured during the late wet season and the early, mid and late dry season. Also shown are the hydraulic conductivities exposed per unit branch transverse area, per unit leaf area, the percentage loss of conductance and average vessel diameter. ........................77
Table 3.4. Values of coefficients a and b from exponential sigmoidal equation \( \ln \left( \frac{100}{\text{PLC} - 1} \right) = a\psi - ab \) used to describe the vulnerability curves presented in Fig. 3.4. "b" represents the \( \psi \) at which 50% hydraulic conductance was lost (ie. PLC=50) ..................................................................................................................................................81

Table 5.1. Summary of properties of evergreen and deciduous trees in seasonally dry tropics........................................................................................................................................................................109
List of Plates

Plate 1.1. Simple and bordered pit in longitudinal section of branch (a) simple pit in vessel of *Croton arnhemicus* with pit membrane pores, (b) bordered pit in tracheid of *Callitris intratropica*, showed the torus and fibrils network of the margo .................................13

Plate 2.1. Cross section of *E. tetrodonta* vascular bundle (a) bi-collateral bundles with xylem is associated with outer phloem and inner phloem, (b) close up of inner phloem showing companion cells and sieve plates.........................................................32

Plate 2.2. Vessels of *E. tetrodonta* showing green paint stains from paint infusion method. (a) The basal end at the point of paint injection; at (b) 2 cm; (c) 4 cm; (d) 6 cm; (e) 16 cm and (f) 18 cm distance from paint injection point.................................44

Plate 2.3. Tracheids and vessels shown using the maceration method (a) tracheids in *C. intratropica*, (b) vessel elements in *E. tetrodonta* and (c) vessel elements in *B. megaphyllus* (scale bar = 50 µm)....................................................................................................................45

Plate 2.4. Transverse section of lamina showing epidermis, stomata and vascular bundles. (a) *C. intratropica*, (b) *P. falcate* and (c) *T. ferdinandiana* (scale bar = 50 µm).................................................................................................................................................................................................................46

Plate 2.5. Transverse section of midrib showing vascular bundles (a) *E. miniata*, (b) *A. dimidiata* and (c) *P. careya* (scale bar = 50 µm). .................................................................................................................................47

Plate 2.6. Transverse section of petiole showing xylem cells (a) tracheid cells in *C. intratropica* (coniferous), (b) vessel cells in *A. auriculiformis*, and (c) *B. megaphyllus* (scale bar = 50 µm)..........................................................................................................................................................................................................................48

Plate 2.7. Transverse section of branch showing xylem cells (a) tracheids cells in *C. intratropica*, (b) vessels cells in *M. viridiflora* and (c) *B. megaphyllus* (scale bar = 50 µm). .........................................................................................................................................................................................................................................49

Plate 4.1. Scanning Electron micrographs tracheary elements pitting morphology in branch (a) longitudinal section of *B. megaphyllus* vessel members with spiral, helical and pitted wall thickening, (b) longitudinal section of *C. polysciada* vessel member with vestured pits on vessel walls. .........................................................................................................................87

Plate 4.2. SEM photographs of the variation in bordered pits (a) bordered pits with non-vestured pits on the vessel wall in *T. ferdinandiana* (b) with torus at the centre of tracheid cell wall of *C. intratropica* (c) with vestured pits on the vessel wall of *A. dimidiata* ..................................................................................................................................................................................90
Plate 4.3. Scanning Electron Micrographs of (a) visible damage to the membrane caused by knife while sectioning in *E. miniata* (b) pit membrane completely removed while preparing samples and an unobscured view of the pit aperture (arrow) .................................................................................................................................93

Plate 4.4. Scanning Electron micrographs of pit membrane pores (a) pit membrane pores (arrow) on pit membrane of *C. arnhemicus*, (b) *B. megaphyllus* ........................................94

Plate 4.5 (a) SEM photographs of vestured pits on the lateral wall of vessels from longitudinal sections of branches of Mimosaceae (a) *A. auriculiformis*, (b) *A. dimidiata*, and Proteaceae (c) *P. falcata* ..........................................................................................................................95

Plate 4.5(b) SEM photographs of vestured pits on the lateral wall of vessels from longitudinal sections of branches of Myrtaceae family (d) *E. tetrodonta*, (e) *E. miniata*, (f) *M. viridiflora* and (g) *C. polysciada* ..........................................................................................................................96

Plate 4.6. SEM photographs of vestured pits on the vessel wall (a) *E. tetrodonta* vessel with simple perforation plate (x1100, scale bar 10 µm), (b) vestured pits of *E. tetrodonta*, (x3500, scale bar 5 µm) (c) *E. miniata* vessel with simple perforation plate (x1100, scale bar 10 µm), (d). vestured pits of *E. miniata* (x3500, scale bar 5 µm). ........................................................................................................................................97

Plate 4.7. Scanning Electron micrographs of border pits in tracheid of *C. intratropica* (a) tracheids showing uniform bordered pits with pit apertures (small circles in the centre of the border regions) (b) torus can be seen in the centre of the membrane. The surrounding margo has thick radial strands and an interconnecting network of thinner strands.................................................................................................................................98
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>cm</td>
<td>Centimetre</td>
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<tr>
<td>°C</td>
<td>Degree Celsius</td>
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<tr>
<td>DBH</td>
<td>Diameter at Breast Height</td>
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<tr>
<td>dP</td>
<td>Pressure (kPa)</td>
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<td>dQ</td>
<td>mass flow (g⁻¹)</td>
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<tr>
<td>FAA</td>
<td>Formalin 2: acetic acid 2: Ethyl alcohol 50: Distilled water 46</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>k</td>
<td>Hydraulic conductance (g kPa⁻¹s⁻¹)</td>
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<tr>
<td>kₙ</td>
<td>Hydraulic conductivity (g m kPa⁻¹s⁻¹)</td>
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<tr>
<td>LAVPD</td>
<td>Leaf-to-Air Vapour Pressure Deficits</td>
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<td>min</td>
<td>Minute</td>
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<td>mm</td>
<td>Milimetre</td>
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<tr>
<td>µm</td>
<td>Micron meter</td>
</tr>
<tr>
<td>RGR</td>
<td>Relative Growth Rate (mm mm⁻¹ day⁻¹)</td>
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<tr>
<td>s.e</td>
<td>Standard error</td>
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<tr>
<td>SEM</td>
<td>Scanning Electron Microscope</td>
</tr>
<tr>
<td>SLA</td>
<td>Specific leaf area (leaf area/leaf dry weight; m² Kg⁻¹)</td>
</tr>
<tr>
<td>VPD</td>
<td>Vapour Pressure Deficit (KPa)</td>
</tr>
<tr>
<td>Ψpd</td>
<td>Pre-dawn leaf water potential (MPa)</td>
</tr>
<tr>
<td>Ψw</td>
<td>Xylem pressure potential (MPa)</td>
</tr>
</tbody>
</table>
Chapter 1: General Introduction

1.1 Savannas

Savannas are extensive ecosystems worldwide, characterised by a discontinuous tree over-storey and a seasonally continuous grass under-storey (Scholes and Archer 1997). They cover areas of tropical and sub-tropical Africa, Australia, South America and Southeast Asia. Savannas are important for several reasons, including their conservation, cultural, and economic value. They support an increasing number of people globally and have a significant role to play in regional water balances and global carbon cycles. As such, they are becoming more important globally as well as to the Australian national economy (Holmes 1996, Johnson et al. 1999).

The climate in regions where savannas are found is distinctly seasonal, having marked wet and dry seasons (Anderson 1996). Seasonal drought is a common feature of savannas worldwide, although the length and severity of the drought period varies (Murphy and Lugo 1986, Cook et al. 1998). Drought generally reduces growth rates of plants, reduces leaf stomatal conductance, decreases hydraulic conductance and affects leaf, flower and fruit phenology (Hsaio 1976, Sperry et al. 1998, Hacke et al. 2000).

The impact of seasonal rainfall on soil water availability and atmospheric water content is a major determinant of savanna structure (Williams et al. 1996). In addition to distinctly seasonal water availability, other factors such as nutrient availability, herbivory and fire also contribute to determining savanna structure and function (Bowman 1996, Scholes and Walker 1993, Williams et al. 1996).

Savannas cover approximately 25% of the Australian continent. Tropical savanna ecosystems are distributed across Northern Australia, where rainfall varies between 500 mm/yr to >1800 mm/yr (Myers et al. 1997; Williams et al. 1997). North Australian savannas are generally dominated by evergreen species (*Eucalyptus* spp) in the upper storey; broadleaf deciduous or partly deciduous trees and shrubs in the mid-storey, and annual and perennial grasses in the understory (Williams et al. 1997, Brock 1993). In contrast, the tropical dry forests of Venezuela consist predominantly of deciduous species, with a few evergreen species also present (Sobrado 1991). The seasonally dry forests of Costa Rica are dominated by a mix
of semi-deciduous and deciduous species (Medina 1982), while African savannas are dominated by deciduous species (Chidumayo 1990). Finally, the Llanos savannas of South America are dominated by evergreen species (Sarmiento et al. 1985).

1.1.1 Northern Territory climate

The northern part of the Northern Territory, NT, (the “Top-End”) is monsoonal, with two distinct seasons, the wet and the dry. The dry season, which officially spans May to September, is characterised by relatively cool nights, dry weather and south-easterly winds. The wet season lasts from October to April. The early wet season is characterised by increasing night temperature, humidity and shower or thunderstorm activity. This period is often called the “build-up”. The onset of the monsoon most often occurs over the northern Top-End around late December when a deep trough of low pressure either forms or moves over land. Wind turns west north-westerly and cloudy, rainy days with squally showers result.

Darwin’s mean annual rainfall is 1669 mm, 95% of which falls in the wet season from November to April (Commonwealth Bureau of Meteorology, Australia, 2000). Day-time temperatures are high year around, with mean maximum and mean minimum temperatures of 33.2°C and 25.4°C in the wet season and 30.4°C and 19.3°C in the dry season (Commonwealth Bureau of Meteorology, Australia 2000).

VPD is lowest in the wet season (January and February) and highest during the dry season (June - August) (Duff et al. 1997, Prior et al. 199a). VPD ranges between 0.5 - 2.5 kPa in the dry season and 0.1 - 0.8 kPa during the wet season (Duff et al. 1997). Vapour pressure deficit plays a large part in determining stomatal conductance and carbon assimilation in savannas trees species (Berryman et al. 1994, Cole 1994, Eamus et al. 1995, Myers et al. 1997).
1.1.2 Northern Territory vegetation

Leaf phenology is an important aspect of the ecophysiology of tropical trees species since many physiological characteristics show correlations with phenological patterns (Duff et al. 1997, Prior et al. 1999a, Myers et al. 1997, Eamus and Prichard 1998, Eamus 1999, Eamus and Prior 2001). Pan-tropical flora is an important component of savanna vegetation in the NT; in the Alligator River region, 61-75% of the genera found in Eucalypt open forests and woodlands are of pan-tropical region (Taylor and Dunlop 1985). Eucalypt open forests and woodlands are amongst the most species-rich communities in the Northern Territory (Taylor and Dunlop 1985).

Evergreen species retain the majority of their leaves all year but lose some leaves during the dry season. In Northern Territory evergreen species, for example E. tetrodonta and E. miniata, leaf longevity is less than one year even though their canopy is maintained at relatively high levels throughout the year. This is indicated by the dominance of new, expanded leaves in December and the absence by this time, of most foliage known to have been present during the dry season (Williams et al. 1997, O'Grady et al. 1999). This compares with southern Eucalypt species, which have a leaf lifespan of typically 2-3 years. Evergreen species retain a full canopy and continue to transpire throughout the year (Williams et al. 1997, O'Grady et al. 2000). This ability to retain canopy throughout the year might be made possible by a root system that accesses deep layers of soil (Sobrado 1986, Cook et al. 1998).

Semi-deciduous species have been defined as those that lose not less than 50% of their leaves every year (Williams et al. 1997). In contrast, deciduous species are defined as those that lose all leaves during the early to mid-dry season, and remain leafless for a least one month (Williams et al. 1997). This may be to avoid drought stress; by shedding leaves, these trees reduce tree water use (Williams et al. 1997). For both evergreen and deciduous species, declines in stomatal conductance occur as VPD increases, and this prevents excessive dehydration of foliage. The impact of VPD on stomatal conductance and assimilation varies amongst Australian savanna trees species. Prior et al. (1997b) found that temperature, independently of VPD, also had a large impact on stomatal conductance and assimilation by Eucalyptus tetrodonta.
1.1.3 **Physiological differences between evergreen and deciduous species**

The highly seasonal climate of savannas has an important influence on ecophysiological traits. The effects of seasonality on soil and atmospheric water content, carbon assimilation, stomatal conductance and leaf water potential have been studied for many dominant species of savannas near Darwin (the main city in the NT) (Cole 1994, Fordyce *et al.* 1995, Eamus and Cole 1997, Duff *et al.* 1997, Myers *et al.* 1997, Prior *et al.* 1997a, Eamus 1999). Evergreen and deciduous species respond differently to these climatic factors due to their different phenology.

Assimilation rates of leaves of savanna species are higher in the wet season than in the dry season (Eamus and Cole 1997, Prior *et al.* 1997a, Sobrado 1996). Eamus *et al.* (1999) showed there was a dry season assimilation decline of 20% in the two dominant evergreen Eucalypts (*E. miniata* and *E. tetrodonta*), while semi-deciduous species demonstrated a decline of 25-75% at the same site.

Assimilation rates during the dry season are higher in the morning than in the afternoon (Sobrado 1996, Eamus and Cole 1997, Prior *et al.* 1997a). Assimilation rates declined in response to a decline in stomatal conductance resulting from higher afternoon leaf-to-air vapour pressure deficits (LAVPD) (Eamus and Cole 1997, Eamus *et al.* 1999). Prior *et al.* (1997b) also found that assimilation rates were lower in the afternoon regardless of season in *E. tetrodonta* saplings, suggesting that saplings are more susceptible to diurnal water deficits than deeper rooted mature trees.

On a leaf dry weight basis, deciduous trees in tropical savannas in the Northern Territory have higher photosynthetic rates than evergreen trees (Eamus *et al.* 1999), (Prior *et al.* 2003). Since deciduous trees cannot photosynthesise for at least one month, because of leaf loss, they need a higher assimilation rate to recover the cost of leaf construction and maintenance costs over a shorter leaf lifespan (Eamus and Prichard 1998). Similarly, semi-deciduous species in a Venezuelan forest have higher rates of photosynthesis per unit leaf mass than evergreen species (Medina and Francisco 1994).

In savannas, the major determinants of stomatal conductance are soil and atmospheric water content and temperature (Eamus and Cole 1997, Prior *et al.*
Stomatal conductance of evergreen and deciduous species are typically higher during the wet season than the dry season, and in the morning compared to the afternoon, especially in the dry season (Eamus and Cole 1997, Prior et al. 1997a). Stomata of evergreen species were more sensitive to increased LAVPD than stomata of deciduous species (Myers et al. 1997).

Pre-dawn leaf water potential ($\psi_{pd}$) of woody species of savannas of northern Australia is higher in the wet season and declines as the dry season progresses (Duff et al. 1997, Williams et al. 1997, O'Grady et al. 1999). In a study of eight species of the north Australian savanna, pre-dawn leaf water potential of mature, pre-dawn leaf water potential of mature trees (10-15 m tall) varied between $-0.02$ MPa in the wet season and $-1.5$ MPa in the dry season (Duff et al. 1997). For smaller trees, the difference in $\psi_{pd}$ was larger, such that dry season minima were as low as $-2.5$ MPa (Prior et al. 1997a).

Two evergreen species ($E. miniata$ and $E. tetrodonta$) maintained higher $\psi_{pd}$ during the dry season than two of the semi-deciduous species ($Erythrophleum chlorostachys$ and $Xanthostemon paradoxus$) (Duff et al. 1997). This supports the hypothesis of Sobrado (1996) that evergreen species possess deeper root systems, and are therefore able to supply more water to their leaves during the dry season. In contrast, the other semi-deciduous species ($Corymbia polysciada$) (formerly referred to as $Eucalyptus clavigera$) maintained higher $\psi_{pd}$ through the year than both evergreen species and the other two semi-deciduous species (Duff, et al. 1997).

The leaves of evergreen species ($E. tetrodonta$ and $E. miniata$) and semi-deciduous species ($C. polysciada$) were significantly more sclerophyllous than those of two deciduous species ($Planchonia careya$ and $Terminalia ferdinandiana$) (Myers et al. 1997). “Sclerophyllous” means hard-leaved and is related to several anatomical features such as abundance of sclerenchyma, thick cell walls and cuticles, and thick leaves (Medina et al. 1990).

Cost : benefit analyses of deciduous and evergreen species of tropical forests have determined in Venezuela (Sobrado 1991) and the Northern Territory (Eamus and Prichard 1998, Eamus et al. 1999). These studies have compared the cost of construction and maintenance, as well as the photosynthetic rates, of leaves of...
evergreen and deciduous species. In a north Australian savanna, maximum leaf construction costs (per unit mass) were higher for evergreen than deciduous species, although deciduous species invested more nitrogen into shorter-lived leaves to maximise carbon assimilation over the short leaf life-span (Eamus and Prichard 1998). Evergreen leaves must be tougher to allow them to live longer and, thus, benefit from a longer payback interval (the period to repay construction costs by fixing carbon). However, these results are in contrast to results from studies in other savannas where leaves of deciduous species had higher construction costs than evergreens (Williams et al. 1989, Sobrado 1991). This difference is probably because Australian savannas are dominated by Eucalypt species, which are more sclerophyllous and have a higher oil content than evergreen species elsewhere in the tropics (Eamus 1999).

It has been suggested that deciduous species have a higher leaf nitrogen content when compare with evergreens to support a large assimilation rate (per unit leaf mass) each day of the wet season and their short-lived leaves fix large amounts of carbon for a relatively short time, up to six months of each year. On a mass basis, evergreen species do not invest as much nitrogen into their leaves, and assimilation rates are consequently lower, but they fix carbon for the whole year (Eamus 1999).

### 1.2 Hydraulic conductance

The ways in which water moves through conducting vessels of plants has consequences for transpiration, assimilation, growth and mineral transport. The hydraulic properties of the vascular system of a plant determine the relation between transpiration flux and the difference between soil and leaf water potential (Tyree and Ewers 1991).

Hydraulic conductance ($k$, with unit of $g \text{ kPa}^{-1} \text{s}^{-1}$) is defined as the flow rate of water per unit pressure difference (Reich and Hinckley 1989). Hydraulic conductance is calculated from the slope of the relationship between change in mass flow rate ($dQ$, with unit of $g \text{ s}^{-1}$) and change in pressure ($dP$, with unit of kPa). Hydraulic conductivity ($k_h$, with unit of $g \text{ m kPa}^{-1} \text{s}^{-1}$) is calculated as the flow rate through the stem per pressure gradient (Sperry and Pockman 1993).

Lower hydraulic conductance means a lower volume flow rate of water for the same driving force: that is, the difference in water potential between the root and the leaf. Shoot hydraulic conductivity may decrease in response to water stress. This
decrease is a result of interruption of the water column in the vessels by the formation of xylem emboli or modification of the size of the xylem vessels (Lovisolo and Schubert 1998).

A decrease of xylem conductivity due to vessel embolism can directly reduce water flow to the shoot (Schultz and Matthews 1988) and at the same time can induce stomatal closure, thereby minimising the development of further embolism and limiting transpiration (Sperry 1986, Sperry and Pockman 1993). Whole tree hydraulic conductance correlates with stomatal conductance (Reich and Hinckley 1989), transpiration (Zhang et al. 1999) and leaf water potential (Franks et al. 1995).

1.2.1 Embolism

Transpiration at the leaf surface pulls liquid water from the soil, through the plant, and into the atmosphere as a water continuum (Van den Honert 1948). Xylem conduits are prone to embolism because xylem sap is under negative pressure (tension) as it moves through the xylem towards the leaves, typically between –1 and –2 MPa (Tyree and Sperry 1989). Water in the xylem contains dissolved gases and as the tension in water increases, there is an increased tendency for the dissolved gases to move into the vapour phase, forming a gas bubble. The gas bubble inside a conduit causes disruption because it expands, as gas cannot resist tensile forces. If the expansion of gas bubbles is high enough, this will cause cavitation or an air blockage that breaks the continuity of the water column (Tyree and Sperry 1989).

Embolism is often expressed as percentage loss in hydraulic conductance due to air embolism (Williams et al. 1997, Prior and Eamus 2000). Cavitation in plants can result from water stress, and each species has a characteristic “vulnerability curve”, which is a plot of the percent loss k (hydraulic conductance) in stems versus the xylem pressure potential (Ψ_w), required to induce the loss (Sperry et al. 1987).

Embolism of conducting vessels may also cause a decrease in whole tree hydraulic conductance over the course of a day and ultimately cause declines in photosynthesis and growth (Sperry 1986, Sperry and Pockman 1993). Embolism has been cited as the cause of decreased hydraulic conductance during drought over long periods of time (Sobrado 1997). It was thought that refilling of embolised
vessels did not occur in large trees, and consequently diurnal changes in the percentages of embolised vessels were small. However, there is increasing evidence that emboli may refill overnight (Salleo et al. 1996, Zwieniecki and Holbrook 1998, Tyree et al. 1999).

Vulnerability to cavitation is larger in tropical than temperate trees, a characteristic that probably results from the requirement of tropical trees to support large transpiration rates (Machado and Tyree 1994). An embolised conduit does not conduct water, and if not repaired, imposes restrictions on transpiration and hence stomatal conductance and photosynthetic rates. Because emboli reduce stem hydraulic conductivity and block the main water transport pathway of water to the leaves, they may cause dehydration and death of the leaves (Tyree and Sperry 1989).

Some evidence supports the view that large conduits are more vulnerable to embolism than small ones (Tyree and Dixon 1986, Salleo and Lo Gullo 1986; Sperry and Tyree 1990, Hargrave et al. 1994), but it may be accepted that the diameter of the pit membrane pore determines a conduit’s vulnerability to drought-induced cavitation (Tyree and Sperry 1989). There is strong evidence that water stress-induced cavitation is caused by air-seeding at pores in the pit membranes (Sperry and Tyree 1990, Sperry and Saliendra 1994). Within a species, there tends to be a correlation between conduit size and pit membrane pore size, but the relationship often breaks down between species (Tyree and Sperry 1989).

Plants have a limited capacity to reverse emboli. In order to dissolve air in embolised conduits, xylem pressure must be positive or close to atmospheric pressure (Tyree and Sperry 1989). However, Zwieniecki and Holbrook (1998) found evidence of embolised vessels refilling overnight in three tree species of temperate deciduous forest, while similar refilling has not been found to occur in young laurel plants (Salleo et al. 1996).

1.3 Xylem anatomy

Basic xylem anatomy has been thoroughly reviewed by Esau (1965). The xylem of the plant has three principle functions: transport, storage and mechanical support. Xylem is composed of tracheary elements, fibres and xylem parenchyma.
Tracheary elements are the most highly specialised cells of the xylem; their function is to conduct water. They consist of elongated cells and are non-living at maturity. They have lignified walls with secondary thickening and a variety of pits, allowing water to move freely through their lumens. Tracheary elements are composed of vessels and tracheids.

A vessel is a series of vessel elements stacked end-to-end. These form long continuous tubes through which water can move readily. Vessel elements have one or more perforation plates on their end walls, and sometimes also on a side wall. The perforation plates are simple or multi-perforate. Multi-perforate plates are scalariform if the perforations are elongated and arranged parallel to each other. The simple plates have only one opening and a narrow rim of end-wall (Fig.1.1). Vessels can range from less than 1 mm to many metres in length in different species (Zimmermann and Jeje 1981, Ewers et al. 1990).

The tracheid is a more primitive type of cell than a vessel. Tracheids originate from single cells, and are pointed at both ends and imperforate. In tracheids, the passage of water from cell to cell occurs mainly through pit pairs, in which the pit membranes are highly permeable to water and dissolved substances. Tracheids are present in all groups of vascular plants. However, gymnosperms only have tracheids for conducting water. The tracheids length ranges between 2 mm and 6 mm (Panshin and Zeeuw 1980).

Vessels are more efficient at conducting water than tracheids because water can flow from vessel to vessel through the perforation. However, they are more susceptible to embolism. Water flow from tracheid to tracheid occurs through the membranes of the pit pairs. Although the porous pit membranes offer relatively little resistance to the flow of water across them, they isolate even the smallest of air bubbles, protecting the water column from embolism (Zimmermann 1983, Tyree and Sperry 1989, Cochard et al. 1992, Lancashire and Ennos 2002). Sometimes tracheids may form air bubbles, but the water column is not blocked because alternate pathway through non-air blocked tracheids is formed.

Simple and bordered pits are encountered in the secondary walls of vessels and tracheids. They allow water to move laterally between adjacent cells. Each pit has a complementary pit, exactly opposite it in the wall of the neighbouring cell. These two neighbouring cells are called a pit-pair. The cavity formed by the break in the
secondary wall is called the pit cavity. The pit membrane is composed of the primary cell walls and middle lamella, and separates the two pit cavities of the pit pair. This membrane is not a lipo-protein membrane, as found in living cells, but rather a middle lamella with a very thin layer of primary wall on each side. Xylem pit membranes are not metabolically active. The opening of the pit on the inner side of the cell wall is called a pit aperture (Fig. 1.2).

Bordered pits are more complicated in their structure and are variously shaped. The bordered pits of conifers are large, and are raised above the level of the rest of the secondary wall. The pit membrane is also a modified structure, composed of radial microfibrils with a central thickened area called a “torus”. The thinner part of the pit membrane around the torus is called a “margo”. When the torus is in the central position of the pit chamber, sap may pass from one cell to the next through the margo and pit apertures (Plate. 1.3 b). If the torus is deflected against either aperture of the pit-pair, then flow of sap is obstructed (Esau 1965, Meylan and Butterfield 1972). Therefore the bordered pit can act as a valve.

The diameters of pit pores of conifers range from 20 to 4000 nm (Siau 1984). In dicotyledons, the membrane pores of vessels are much smaller than in tracheids of conifers and the pore ranges in size from 5 to 170 nm in diameter (Siau 1984). In some dicotyledons, thin, simple, or branched sculpturings are present on the secondary wall that forms the pit chamber or around the pit aperture. Such pits are called “vestured pits” (Fahn 1982).

Fibres are long cells with secondary, commonly lignified walls. The walls vary in thickness but are usually thicker than that of tracheids in the same wood. They are mainly concerned with the strengthening of the plant body. Xylem parenchyma cells have secondary thickening and are lignified. They store starch, oils, and many other crystalline substances (non-protoplasmic products) (Esau 1965).
Fig.1.1. Diagram of a typical dicot plant. Transverse section of leaf, stem and root with xylem and phloem cells. (Inspiration for this diagram from “Biology of Plants”, 5th ed. Raven P., Evert R., Eichhorn S. 1992)
Fig. 1.2. Diagram of (a) surface view and transverse section of bordered pit with torus in the middle (b) surface view and transverse section of simple pit (Inspiration for this diagram from "Anatomy of seed plants", Katherine Easu, 1960).
Plate 1.1. Simple and bordered pit in longitudinal section of branch. (a) simple pit in vessel of *Croton arnhemicus* with pit membrane pores, (b) bordered pit in tracheid of *Callitris intratropica*, showed the torus and fibrils network of the margo.
1.3.1 Difference in xylem anatomy between evergreen and deciduous species

Xylem anatomy of evergreen trees differs markedly from that of deciduous species. Differences can be found in vessel element length, vessel length, vessel diameter, pit membrane pore diameter and wood anatomy (Carlquist 1975, Fahn et al. 1986, Tyree and Ewers 1991, Zimmermann 1983). Although most work has examined differences between evergreen and winter deciduous species (i.e. in the Northern hemisphere), such studies have also been conducted in the tropics. In a Venezuelan dry forest, average vessel diameter of several deciduous species was larger than that of several evergreen species and vessel elements were longer in deciduous than evergreen species. Vessel pit sizes were also larger in deciduous species than evergreen species (Lindorf 1994). In deciduous forests of Mexico, vessel diameter in deciduous vines was greater than that of evergreen vines (Gartner et al. 1990). These trends are similar to those found for the evergreen and winter deciduous groups in the Northern Hemisphere (Gorsuch et al. 2001, Davis, et al. 1999, Hacke et al. 2000).

The wood of trees has been classified as "ring-porous" or "diffuse-porous". Ring-porous wood consists of "early wood", with large, long vessels that alternate with the smaller vessels of the latewood. In diffuse-porous wood, vessels are much more uniform in diameter (Esau 1965). Ring-porous trees tend to have much wider vessels than diffuse-porous trees (Zimmermann and Jeje 1981). Differences in wood anatomy between ring-porous, diffuse-porous and coniferous trees affect their hydraulic characteristics. In general, the xylem of a conifer, with its numerous small tracheids, has a relatively low conductivity, but is less vulnerable to embolism than xylem of angiosperm trees (Tyree and Ewers 1991, Wang et al. 1992). Similarly, the narrow and short vessels typical of diffuse-porous wood have low conductivity and are less vulnerable to embolism, than the wide and long vessels of early wood in ring-porous species (Greenidge 1952).

In temperate trees of the Northern Hemisphere, deciduous trees tend to be ring-porous, and evergreen trees tend to be diffuse-porous. However, in the tropical and temperate areas of Australia this model is inadequate, as the vessels of *Eucalyptus* species are markedly wider in earlywood than in late wood (Carlquist 1988), producing a ring-porous wood despite these species being evergreen. Maximum
length of vessels often exceeds 1 m in various *Eucalyptus* species (Franks *et al.* 1995), including *E. tetrodonta* (Prior, unpublished data), an evergreen dominant tree found over large areas of the savannas in the Northern Australia. By contrast maximum vessel lengths in diffuse-porous species found in America are generally less than 60 cm, with most vessels being less than 10 cm long (Zimmermann and Jeje 1981).

1.3.2 Phloem anatomy

Long distance translocation of sucrose and other metabolites occurs in the phloem in higher plants. Phloem is associated with xylem in the vascular system. Like xylem, phloem consists of several types of cells. Phloem may be classified as primary or secondary phloem. The primary phloem is derived from procambium and the secondary phloem originates in vascular cambium (Esau 1977).

The development and structure of phloem and xylem are similar. Phloem is a complex tissue, composed of several different cell types, including sieve elements, companion cells, parenchyma, fibers and sclereids (Esau 1965, Cutter 1978). The main conducting cells of the phloem are the sieve elements, which are distinguished into two types, namely, sieve cells and sieve-tube members. Sieve cells are single, elongated cells with specialised sieve areas in their lateral, and sometimes in their terminal walls. Sieve-tube members are joined end-to-end into sieve tubes and with sieve areas in or near an end wall.

The part of the wall bearing the sieve areas with large pores is called a sieve plate. Connecting cytoplasm passes through the sieve plate pores from one sieve tube element to another (Fig.1.1). Both sieve cells and sieve tube members are conducting cells in plants (Esau 1965, Cutter 1978) however they differ in that sieve tube members have sieve plates and sieve cells do not (Cutter 1978). The sieve cell is a more primitive type of cell than the sieve tube member. Sieve cells are the only type of conducting cell in most seedless vascular plants and gymnosperms, whereas only sieve-tube elements occur in angiosperms (Esau 1965).

Companion cells originate from the same mother cells as the sieve-tube member. These are parenchymatous cells and are smaller in diameter than sieve-tube elements. Phloem fibres are elongated with thick lignified secondary walls with simple or bordered pits. Their function is supporting and sometimes storage.
Parenchyma cells have primary cell walls with primary pit fields. Parenchyma cells may store and translocate substances (Esau 1965).

1.4 Functional relationships of conducting tissue

1.4.1 Xylem vessel length

Vessel length and diameter are different in different species and in different parts of the plant. Longer vessels confer greater conductive efficiency and shorter vessels confer greater safety (less vulnerability to embolism) to the conductive process (Zimmermann 1978). Longer vessels are, in general, correlated with wide vessel diameter (Zimmermann and Jeje 1981). Increased vessel diameter increases efficiency of water conduction but decreases resistance to embolism (Zimmermann 1983).

Vessel element length is largest in trees, intermediate in shrubs, and least in sub-shrubs (Carlquist 1966, Wallace 1986). Different vessel element lengths are found in various parts of the plant. Vessel elements tend to be shorter in branches than the main stem (Iqbal and Ghouse 1983), and tend to be wider and longer in roots than in stems (Carlquist 1978a).

Longer vessel elements have been correlated with more mesic habitats (Carlquist 1966, 1975). Correlations between vessel element lengths may be traced to environmental factors such as water availability and temperature (Baas 1973). Vessel elements are longer and narrower in Western Australian and Southern Californian trees species than the Venezuelan species (Carlquist 1977, Lindorf 1994). This may be because the Venezuelan species possess less xeromorphic wood than those of the sand heaths of Western Australia and Southern Californian (Lindorf 1994). Also environmental conditions are not as extreme in Venezuela, where the dry season is shorter and the temperature is lower.

1.4.2 Xylem vessel diameter

The ability of a xylem vessel to conduct water is dependent on its diameter. Vessel diameter is probably the most important anatomical variable in angiosperm wood because hydraulic conductivity is proportional to the radius (xylem diameter) raised
to the fourth power (Poiseuille’s law). Thus a doubling of the diameter confers a 16 fold increase in conductivity (Zimmermann 1978, 1983).

Vessel diameter increases from roots to the canopy branches (Zimmermann and Potter 1982, Gartner 1995, Ewers et al. 1997, Hacke et al. 2000). Wide vessel diameter contributes to greater conductive efficiency (Carlquist 1975, Bass 1976, Zimmermann 1978). However narrow vessels, should not be regarded as “conductive inefficient” because they are less prone to embolisms than wide ones (Zimmermann and Brown 1971, Ellmore and Ewers 1985; Ewers 1985). Wide vessels are also less capable of recovery from embolism than narrow ones (Ewers 1985).

In arid zones, plants may experience large negative pressures in the xylem and therefore may be at high risk of embolism or cavitation, thereby blocking conducting ability. The frequent appearance in the xylem of numerous vessels with narrow, short elements and pit membrane pores with small diameters contributes to “conductive safety” (Baas and Carlquist 1985, Carlquist 1966, 1977, 1980, Zimmermann 1983). Similarly, tracheids have been associated with greater conductive safety (Carlquist 1984a).

The resistance to water flow of simple perforation plates is lower than that of scalariform plates (Ellerby and Ennos 1998). However, Sperry (1986) suggested that scalariform plates may enhance recovery of vessels after cavitation. As water potential rises towards zero at night, in plant with scalariform plates, a meniscus forms across the vessel lumen, dividing a single water bubble into a chain of small bubbles which presumably dissolve more quickly.

1.4.3 Pit membrane

A pit membrane is a thin, porous depression in the wall where secondary wall material is lacking. According to the air-seeding hypothesis (Zimmermann 1983), the spread of water stress-induced embolisms are controlled by the size of the pores on pit membranes that connect adjacent conduits (Zimmermann 1983, Sperry and Tyree 1988, Tyree and Sperry 1989, Jarbeau et al. 1995). The smaller the pit membrane pores, the lower the water potential required to induce embolism (Sperry and Tyree 1988, Cochard et al. 1992).
A vestured pit is a bordered pit with the pit cavity wholly or partially lined with projections from the secondary wall. Vestured pits and vesturing are characteristic of the families of Myrtales (Vilet and Baas 1984). Vesturing is present in wood from arid zones, such as in *Eucalyptus* species and many of the Myrtaceae family (Zweypfenning 1978). Scurfield et al. (1970) have shown that the vestures in *Eucalyptus regnans* are not outgrowths of the cell wall, but are deposits left as accretions produced by the protoplasts when the cells die.

Zweypfenning (1978) discusses the possible physiological significance of vestured pits and gives reasons for believing that they may be important in preventing air-embolism in angiosperm vessels. Vestured pits prevent excessive deflection and rupture of the pit membranes during pressure drops due to emboli (Carlquist 1982, 1983, 1988). Vesturing may increase the surface area in vessels and tracheids, thereby increasing bonding of water and may prevent formation of emboli. Vesturing may also aid in resorption of emboli once they form (Carlquist 1988).

### 1.5 The correlation of root biomass and phloem cross-sectional area of phloem

The allocation of biomass, nitrogen and sapwood within a tree seriously impacts on the physiology, growth and distribution of tree species. According to the pipe model theory (Shinozaki et al. 1964), leaf area is correlated with cross sectional sapwood area (Rogers and Hinckley 1979, Waring et al. 1980; Waring et al. 1982). The pipe model can be used in some cases to predict the canopy leaf weight or leaf area from stem cross-sectional area, and it is of some value in terms of understanding tree growth and resource allocation (Long et al. 1981, Waring et al. 1982). The cross-sectional area of xylem in a branch is generally proportional to the leaf area of that branch (Long et al. 1981). Grier and Waring (1974) discovered a strong linear relationship between total foliar weight and cross sectional area of sapwood at breast height. There is also a significant positive correlation between total root biomass and DBH, above ground biomass and total leaf area (Eamus et al. 2002). As root biomass increases, the need for carbon for respiration increases and therefore the cross-sectional area of phloem may increase. Carbon compounds produced by photosynthesis in the leaves are distributed through the plant by translocation in the phloem. However, the scaling relationship between phloem cross sectional area, root biomass and leaf area have not been investigated either within or between species.
1.6 General aims of this thesis

This project aims to examine anatomical traits in the conducting tissues of deciduous and evergreen species in tropical savanna woodlands of the Northern Territory, Australia. This information will provide a more complete understanding of the vegetation of these complex ecosystems. In particular, this study compares the root, stem, branch and leaf anatomy of deciduous and evergreen species of north Australian savannas. In addition, this project will investigate anatomical differences among seasons and across phenological groups, for seven evergreen species: (Acacia auriculiformis, Acacia dimidiata, Callitris intratropica, Eucalyptus miniata, Eucalyptus tetrodonta, Melaleuca viridiflora, and Persoonia falcate), and five deciduous species: (Brachychiton megaphyllus, Cochlospermum fraseri, Croton arnhemicus, Corymbia polyscada, Planchonia careya, and Terminalia fedinandiana). These species were chosen as they represent the two extreme phenologies present in the tropical woodlands of north Australia and they are all commonly occurring species. Trees were sampled from sites where a range of physiological studies had been previously conducted (Duff et al. 1997, Eamus and Cole 1997, Myers et al. 1997, Prior et al. 1997ab, Williams et al. 1997, Eamus and Prichard 1998, Eamus et al. 1999; O’Grady et al. 1999, O’Grady et al. 2000, Prior and Eamus 2000; Eamus and Prior 2001, Prior et al. 2003).

This thesis addresses the following hypotheses:

Evergreen and deciduous species have systematic differences in xylem anatomy.

Vulnerability of stem and branch segments to embolism is determined by pit membrane diameter.

Vulnerability of stem and branch segments to embolism is correlated with xylem diameter.

The amount of functional xylem in a stem varies seasonally.

There is a correlation between the amount of root biomass and phloem cross sectional area in stems of seedlings both within and between species.

The structure of the thesis is described briefly below:
Chapter Two examines the comparative xylem dimensions of different tree species. Vessel length and diameter were measured using sectioning and maceration methods. Vessel length distributions were measured using the paint infusion method.

Chapter Three examines branch hydraulic conductance and xylem embolism. The correlation between vulnerability to embolism and pit membrane pore diameter is examined.

Chapter Four presents Scanning Electron Microscopy studies of xylem pit pores.

Chapter Five summaries the findings of the study.
Chapter 2: Light microscopical studies of conducting tissues

2.1 Introduction

The major feature of the climate in the monsoonal tropics of northern Australia is seasonal drought (Taylor and Dunlop 1985). Trees growing in the seasonally-dry tropics must either avoid drought by shedding leaves (deciduous) or tolerate it (evergreen). Australian savannas are generally dominated by evergreen species in the upper-storey, deciduous or partly deciduous species in the mid-storey and grass in the under-storey (Bowman et al. 1988).

In north Australian savannas, deciduous species lose all of their leaves every year for one to three months or longer when the dry season is particularly long (William et al. 1997), and thus avoid the large transpiration demand imposed by the dry season. Evergreen species, by contrast, maintain transpiration through the dry season with up to 90% of the full wet season canopy (Williams et al. 1997). Mature evergreen trees maintain high leaf water potentials and high rates of assimilation, transpiration and leaf flushing during the dry season (Myers et al. 1997, Williams et al. 1997, Myers et al. 1998, O'Grady et al. 1999, Eamus et al. 1999).

2.1.1 Xylem

Both evergreen and deciduous habits are successful in the savanna of the NT since representatives of both guilds are common in the wet-dry tropical savannas of Australia. It is likely that differences in leaf phenology and water relation of evergreen and deciduous species are reflected in other aspects of ecophysiology, including features of their hydraulic architecture, such as xylem anatomy.

The ease with which water moves through conducting tissues of plants has consequences for transpiration, carbon assimilation, growth and transport of minerals (Tyree and Ewers 1991). Water transport occurs in the xylem as a result of evaporation at the leaf’s surface. The long-distance flow path of a plant is typically composed of many xylem conduits. Xylem conduits differ in length (Zimmermann and Brown 1971, Zimmermann and Jeje 1981, Zimmermann 1983, Ewers and Fisher 1989, Ewers et al. 1990) and diameter in branches, stems and roots of individual trees, and between trees of the same species. Xylem conduits consist of
tracheids in conifers and vessels and tracheids in angiosperms. The tracheids generally have a smaller diameter than vessels.

Wide xylem vessels are very efficient at transporting water (high flow rate) but are generally more susceptible to embolism (break in the xylem sap the formation of ‘bubbles’ in an individual conduit leading to cavitation), compared to narrow vessels. There is a trade-off between efficiency and security (resistance of the xylem against embolism formation) of the water transport system (Tyree and Sperry 1989, Tyree et al. 1994).

Drought-induced water stress can cause emboli to form in vessels and tracheids (Tyree and Sperry 1989). Xylem conduits are prone to embolism because xylem sap is under tension, typically between -1 MPa and -2 MPa during daylight hours (Tyree and Sperry 1989). Xylem vessels that become blocked by emboli have reduced hydraulic conductance and lower canopy productivity (Tyree and Ewers 1991, Tyree et al. 1992, Sperry et al. 1994). Emboli may occur as a result of drought or freezing, but the mechanisms are different for each stress factor (Sperry and Pockman 1993). In this thesis, the emphasis is on drought-induced embolism.

Resistance to emboli can occur at the leaf level because stomatal control can prevent the development of sufficient tension that leads to breaks in the water column (Sperry et al. 1994). Alternatively, resistance to emboli can occur at the xylem level through variation in dimension, because both large conduits (Zimmermann 1978), and large pit membrane pores (Sperry et al. 1994) are positively correlated with increased embolism formation and therefore narrow pores and conduits result in increased resistance to emboli.

2.1.2 Phloem

Carbon compounds produced by photosynthesis in the leaves are distributed within the plant through phloem translocation. The major compound translocated in the phloem is the disaccharide sucrose (Zimmermann and Brown 1971). Sucrose is the osmotically dominant solute in sieve tube, or phloem sap of nearly all plants, but in some plant families it is supplemented by raffinose and sugar alcohols as well as oligosaccharides (Zimmermann and Ziegler 1975).
As root biomass increases, the amount of sucrose required by root respiration increases. Therefore, the amount of phloem required to supply the additional sucrose for respiration might be expected to increase because sucrose concentration can not continue to increase without limit, due to the effect on sap viscosity. The concentration of sucrose in the phloem sap *Eucalyptus* species, *Melaleuca* species and *Terminalia* species is 10-30%, 2-10% and 10-20% respectively (Zimmermann and Ziegler 1975).

Plants are able to co-ordinate the growth of their organs to maintain and a functional balance between the biomass invested in shoots and in roots. The relative allocation of biomass to different plant organs depends on species, ontogeny and on the environment experienced by the plant (Poorter and Nagel 2000). Relative growth rate (RGR) is the most useful indicator of growth rate for plants (Hunt 1990). Species growing in nutrient-poor conditions tend to have lower RGR than species growing in nutrient-rich habitats (Poorter and De Jong 1999). This is because species from nutrient-poor conditions allocate more resources to nutrient capture leading to increased root growth and lower above-ground RGR.

Plant species with different leaf attributes (for examples, specific leaf area, leaf longevity) frequently occupy different habitats. For example, leaves from dry monsoon forests had significantly higher light-saturated assimilation rates, and chlorophyll and P contents than leaves from eucalypt open forest, mixed woodland and *Melaleuca* swamp (Prior *et al.* 2003). Several hypotheses have been proposed to explain the advantage of having a longer or shorter leaf life span (Aert 1995, Eamus and Prichard 1998). Most studies have found that deciduous species show higher maximum potential growth rates, larger specific leaf area (SLA) and higher photosynthetic rates than evergreen species (Reich *et al.* 1992). This has been shown in both temperate environments (Castro-Díez *et al.* 2000) and in the seasonally-dry tropics near Darwin (Prior *et al.* 2003). The greater RGRs of deciduous species have been considered important for forest production efficiency (Reich *et al.* 1992). However, evergreen trees, such as conifers and eucalypts, may be better able to grow in harsh and infertile areas (Chabot and Hicks 1982).

The allometric relationship between stem basal or sapwood area and whole tree leaf area is well known (Waring *et al.* 1982). Diameter at breast height (DBH) is highly correlated with other plant variables such as above-and below-ground biomass, total leaf area, total root biomass and leaf biomass. Allometric relationships between
DBH and biomass have been derived for a number of north Australian savanna species (O’Grady et al. 1999, 2000, Werner and Murphy 2001, Eamus et al. 2002). There are very strong relationships amongst DBH and sapwood area and leaf area in mountain ash *Eucalyptus regnans*, *E. tetrodonta* and *E. miniata* (Hatton et al. 1995, Vertessy et al. 1995, O’Grady et al. 1999, Chen et al. 2002). Tree stem diameter changes in response to cambial growth, and changes in water content and water tension. Thus, as stem diameter increases, the xylem and phloem cross sectional area would also be expected to increase. Given that leaf area, fine root mass and phloem transport capacity are all strongly correlated with DBH, it is hypothesised that root biomass could be estimated from phloem cross sectional area. This is useful as phloem cross sectional area is easier to measure than root biomass.

### 2.1.3 Aims

The aims of the work presented in this chapter were to:

1) Investigate whether evergreen, deciduous and coniferous species of north Australian savannas have systematic differences in xylem anatomy. Specifically, in this chapter, I report measurements of:

   - Vessel diameter as determined using both maceration and sectioning.
   - Vessel element length as determined using a maceration method.
   - Vessel length distribution as determined using a paint infusion method.
   - Vessel density as determined using sectioning.

2) Examine the relationship among stem diameter, leaf dry weight and root dry weight, phloem cross sectional area and xylem cross-sectional area.

3) Examine the relationship between root biomass and phloem cross sectional area in seedlings of evergreen and deciduous species.
2.2 Materials and Methods

2.2.1 Study sites and species

(a) Xylem anatomy

Samples were collected from Berrimah, Lee Point and Charles Darwin National Park, near Darwin in the Northern Territory of Australia. The vegetation was open forest (*sensu* Specht 1981) dominated by *E. tetrodonta* and *E. miniata* in the over-storey, with low woody deciduous and semi-deciduous species, such as *T. ferdinandiana*, *P. careya*, *C. fraseri* and *B. megaphyllus*, in the mid-storey, and grasses in the under-storey. The climate of the region is monsoonal, with a distinct wet season (November-March) and a distinct dry season (April-October) (Chapter 1).

Seven evergreen, six deciduous or semi-deciduous and one coniferous species, listed in Table 2.1, were selected for study of xylem anatomy. These represented the most common woody species at the sites.

(b) Phloem anatomy

Samples were grown in a shade house at Charles Darwin University. Two evergreen species (*E. tetrodonta* and *M. viridiflora*), one semi-deciduous (*C. polycladá*) and one deciduous (*T. ferdinandiana*) species were selected for study of phloem anatomy. The evergreen and semi-deciduous species were from the Myrtaceae family and the deciduous species was from the Combretaceae family. Species were chosen because of their prevalence in the vegetation of Northern Territory savannas.

2.2.2 Plant materials

(a) Xylem anatomy

Samples were collected from three different trees of each species at Berrimah, Lee Point and Charles Darwin National Park. From each tree sampled, three terminal branches, three petioles and three fully expanded leaves were collected for determination of vessel element diameter and vessel element length. These
specimens were preserved immediately in a solution of FAA (Formalin 2 : Acetic acid 2 : Ethyl alcohol 50 : Distilled water 46) for later examination. To determine the distribution of vessel lengths, terminal branch segments were selected in a diameter range of about 2 to 8 mm.

(b) Phloem anatomy

Seeds of the four species (*E. tetrodonta, M. viridiflora, C. polysciada* and *T. ferdinandiana*) were sown at the same time (November 2000) in plastic trays with a soil of sand and peat (3:1). The seeds were bought from a local seeds supplier. After germination, 35 seedlings of each species were transplanted to 6 litre pots contain the same soil mix as used for germination. The seedlings were grown in a shade house at the Charles Darwin University. They were irrigated daily and fertilised regularly with slow release fertiliser (Osmocote). Five randomly selected plants were harvested each month for five months from December 2000 to April 2001. Due to germination problems for *T. ferdinandiana*, seed were re-germinated in March 2001 and growth examined for the following five months.

At each harvest, whole plants were separated from the pot and the roots were washed with water to remove all traces of soil. Subsequently, stem diameter was measured with callipers, and roots, stems and leaves were separated and their fresh weights were obtained. Leaf petioles were included in the leaf samples. Leaf and root samples were oven dried at 70°C for 48 hours to obtain their dry weight.
Table 2.1. Family, phenology and common name of species chosen for examination of xylem (a) and phloem (b) anatomy.

<table>
<thead>
<tr>
<th>Species Name</th>
<th>Family</th>
<th>Phenology</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia auriculiformis</em> A.Cunn. Ex Benth.</td>
<td>Mimosaceae</td>
<td>Evergreen</td>
<td>Black Wattle</td>
</tr>
<tr>
<td><em>Acacia dimidiata</em> Benth.</td>
<td>Mimosaceae</td>
<td>Evergreen</td>
<td>-</td>
</tr>
<tr>
<td><em>Eucalyptus miniata</em> A.Cunn. ex Schauer</td>
<td>Myrtaceae</td>
<td>Evergreen</td>
<td>Woollybutt</td>
</tr>
<tr>
<td><em>Eucalyptus tetrodonta</em> F. Muell.</td>
<td>Myrtaceae</td>
<td>Evergreen</td>
<td>Stringybark</td>
</tr>
<tr>
<td><em>Melaleuca viridiflora</em> Sol. ex Gaertn.</td>
<td>Myrtaceae</td>
<td>Evergreen</td>
<td>Paperbark</td>
</tr>
<tr>
<td><em>Persoonia falcata</em> R. Br.</td>
<td>Proteaceae</td>
<td>Evergreen</td>
<td>-</td>
</tr>
<tr>
<td><em>Callitris intratropica</em> R.T. Backer</td>
<td>Cupressaceae</td>
<td>Evergreen</td>
<td>Cypress pine</td>
</tr>
<tr>
<td><em>Corymbia polysciada</em> F. Muell.</td>
<td>Myrtaceae</td>
<td>Semi-deciduous</td>
<td>-</td>
</tr>
<tr>
<td><em>Brachychiton megaphyllus</em> Guymer</td>
<td>Sterculiaceae</td>
<td>Deciduous</td>
<td>Red flowered kurrajong</td>
</tr>
<tr>
<td><em>Cochlospermum fraseri</em> Planch.</td>
<td>Bixaceae</td>
<td>Deciduous</td>
<td>Kapok bush</td>
</tr>
<tr>
<td><em>Croton arnhemicus</em> Mull. Arg.</td>
<td>Euphorbiaceae</td>
<td>Deciduous</td>
<td>-</td>
</tr>
<tr>
<td><em>Planchonia careya</em> (F. Muell.) Kunth</td>
<td>Lecythidaceae</td>
<td>Deciduous</td>
<td>Cocky apple</td>
</tr>
<tr>
<td><em>Terminalia ferdinandiana</em> Exell</td>
<td>Combretaceae</td>
<td>Deciduous</td>
<td>Billy goat plum</td>
</tr>
</tbody>
</table>
2.2.3 Vessel element length and diameter

Vessel diameters were measured by both sectioning and maceration methods. However, vessel element length can only be measured using the maceration method. A 1 cm length of each sample was fixed in FAA solution for at least 48 hours prior to use for maceration or sectioning.

(a) Maceration

Branch samples were grouped into three categories of branch diameter for study: (a) 1-3 mm (small), (b) 4-6 mm (medium) and (c) 7-10 mm (large). Branch specimens were prepared using Schultze’s method (Prakash 1986) in which 1 mm lengths of pieces of branch from each group were submerged in concentrated nitric acid to which a few crystals of potassium chlorate were added. The test tubes were then heated in a water bath until the plant material turned white. This took approximately 30 minutes. At this point, a small sample was tested for ease of separation after washing in water. If the cells separated easily, heating was stopped and the specimens were washed thoroughly in water whilst teasing the material apart with a probe, and then centrifuged. The macerations were stained with safranin and mounted with Canada balsam on a glass microscope slide and covered with a cover slip. Measurements of vessel elements were made directly from these slides using a light microscope (Olympus, Japan) at magnifications of 10X, 20X and 40X.

Vessel diameters were measured from transverse sections at the widest part of the vessel lumen and did not include the cell wall. Vessel element lengths were measured including the vessel tails. Twenty vessel samples were measured per section and three sections were viewed per branch, giving sixty vessel samples per species for each branch size category.

(b) Sectioning

Tissue processing branch and petiole samples

Branch and petiole samples that had been stored in FAA solution were placed in a LYNX (Leica, UK) tissue processor and dehydrated in a graded ethanol series. The dehydration times and temperatures are described in Table 2.2.
**Resin infiltration**

The dehydrated samples were removed from the tissue processor and placed into labelled small bottles. Samples were then infiltrated with a mixture of Spurr low viscosity resin and acetone (25:75) for 3 hours. Subsequently the mixture was removed and replaced with a resin/acetone mix (50:50). After a further three hours the mixture was removed and replaced by a 75:25 resin/acetone mix and left overnight. In the final stage of resin infiltration, the mixture was replaced by 100% resin. This was repeated three times at intervals of 3 hours. Finally, the specimens were placed in a labelled silicone rubber mold (BOJAK mold, Polysciences Warrington, PA, USA). The mold was filled with resin until the specimens were completely covered. These molds were then placed in a polymerisation oven at 70°C and left overnight. The mold was removed from the oven and left to cool at room temperature and the resin blocks were gently removed. The blocks were sectioned using a Nova Ultratome (LKB, Bromma, Sweden) utilizing a glass knife. Longitudinal and cross sections 0.5 – 2 µm thick were obtained. Thin sections were scooped onto glass slides and dried on a warming plate. When dry the samples were soaked in a solution of 0.05% toluidine blue in distilled water to remove resin from the samples and then stained with safranin and fast green by the (Johansen 1940) method.

**Tissue processing Leaf samples**

Leaf samples stored in FAA solution were placed in a Shandon Southern (Fisher 266MP, England) tissue processor and dehydrated with the times and temperatures described in Table 2.3.

**Sectioning leaf samples**

Dehydrated samples were removed from the tissue processor and placed into paraffin wax and embedded in a slotted cassette receptacle in a paraffin wax bath. Specimens were subsequently removed from the bath and cooled on an ice plate until the wax had hardened, followed by overnight storage in a freezer. The hardened wax blocks were then sectioned 5 to 8 µm thick with a microtome (Spencer 820, USA). Sections were placed on slides and stained with safranin and fast green according to the method of (Johansen 1940).
All sections were examined under a light microscope, and images were captured with a digital camera (DP 11, Olympus, Japan). All measurements were determined using NIH Image 1.23 image analysis software (National Institutes of Health, USA). When a vessel cell was not circular in shape, the maximum and minimum diameter were averaged.

The sectioning method is considered more useful for calculating the theoretical hydraulic conductance (k) of a stem (Gibson et al. 1984). Using a transverse view of a section, the number of vessels per square mm (vessel density) and the diameter of each vessel lumen were determined.

2.2.4 Phloem anatomy

Twenty five stems from each species were cut to 5 mm lengths and fixed in FAA solution for at least two weeks. The stems were dehydrated in an ethanol series as described in Table 2.2 and embedded in spurr resin. The samples were sectioned in transverse section with a sliding microtome (10 - 30 µm thick) and stained with safranin. Images were photographed from a digital camera attached to a light microscope (DP 11, Olympus, Japan). Each image was imported into NIH Image 1.25 software to calculate the area of phloem. The vascular bundles of species in the Myrtaceae family (E. tetrodonta, C. polysciada and M. viridiflora) have bicollateral bundles (ie. the xylem is associated with phloem on both its outer and inner surfaces) (plate 2.1). If the samples were too large in cross sectional area to view a suitable resolution to clearly identify the phloem, the sample area was divided into equal sections (halves or quarters) and one section measured. The result was then multiplied by the number of sections to provide the average cross-sectional area of phloem in the sample.
Table 2.2. Tissue processing methodology described the stages of dehydration of samples (branch and petioles) prior to resin infiltration.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Concentration</th>
<th>Temp (°C)</th>
<th>Time (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>50%</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>60%</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>70%</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>80%</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>90%</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>95%</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>100%</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Ethanol : Acetone</td>
<td>75% : 25%</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol : Acetone</td>
<td>50% : 50%</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol : Acetone</td>
<td>25% : 75%</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Acetone</td>
<td>100%</td>
<td>20</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2.3. Tissue processing methodology describing the stages of dehydration of leaf samples.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Concentration</th>
<th>Temp (°C)</th>
<th>Time (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>70%</td>
<td>40</td>
<td>0.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>80%</td>
<td>40</td>
<td>0.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>95%</td>
<td>40</td>
<td>1.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>100%</td>
<td>40</td>
<td>1.5</td>
</tr>
<tr>
<td>Xylene</td>
<td>100%</td>
<td>40</td>
<td>1.5</td>
</tr>
<tr>
<td>Paraffin</td>
<td>100%</td>
<td>58</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Plate 2.1. Cross section of *E. tetrodonta* vascular bundle (a) bi-collateral bundles with xylem is associated with outer phloem and inner phloem, (b) close up of inner phloem showing companion cells and sieve plates.
2.2.5 Distributions of xylem vessel lengths of branch wood

The latex paint infusion method (Ewers and Fisher 1989) was used to determine the distribution of vessel lengths in branches of *A. auriculiformis*, *A. dimidiata*, *E. miniata*, *E. tetrodonta*, *C. polysciada* and *P. careya*. That were within a size range of 20-30 cm in length and 3-7 cm in diameter. The paint infusion method is based on the principle that paint particles can infuse into vessels and perforation plates, but cannot pass through the pit membranes, and thus, only cut vessels will contain paint.

The longest terminal branches were selected for this study. Stems were cut from the plant and the cut proximal end was immediately recut under water and kept submerged in water, and transported back to the laboratory. The cut end was recut, and the leaves excised from petioles, with a fresh razor blade under water. Within 2 hours the proximal end was trimmed with a sharp razor blade and clear vinyl tubing was tightly fitted around it. The stem was then placed in a vacuum chamber (Fig. 2.1). A brief pressured infiltration with distilled water was performed for 20 minutes at 175 kPa to remove embolisms that may have been present. A dilute latex solution (see below) was then sucked into the stem by applying a vacuum to the distal end of the branch.

The latex solution consisted of green latex paint (Dulux, Brunswick Green gloss, water based, product no. 420-33797) with distilled water at 100:1 diluted and then filtered through Whatman #1 filter paper. The filter paper prevented particles with a diameter larger than 5 µm passing through. Subsequent filtering with a Millipore filter (0.2 µm) demonstrated that all pigment particles were larger than 0.2 µm, and thus too large to pass through pit membranes. The latex solution was fed into the proximal end of the stem segment with a pressure difference of 87 kPa (vacuum). The solution was allowed to pass through the stem until flow completely stopped, which in some cases took up to 1 to 3 days.

Pigment-saturated stem segments were cut into uniform 20 mm lengths, and stored in a vertical position with the surface on which vessel counts were made facing down on a glass surface. Within the next 24 hours the basal (proximal) end of each stem surface was then smoothed with a sharp razor blade, and photographed. The number of paint-filled vessels was counted in each segment. Vessels were counted
as paint filled even if only partially filled with latex paint. Vessel length distribution was calculated as shown in Appendix, Table 2.4 and described as follows.
Fig. 2.1. The apparatus used for paint infusion measurements.
Table 2.4. Examples of a calculation of vessel length in *E. tetrodonta* branch.

<table>
<thead>
<tr>
<th>Distance (mm)</th>
<th>Raw vessel count</th>
<th>1st Difference</th>
<th>2nd Difference</th>
<th>Steps to zero</th>
<th>No. vessels</th>
<th>Corr. vessel No.(f)</th>
<th>Length class</th>
<th>% length class</th>
<th>Mid-point (x)</th>
<th>xf</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>293</td>
<td>121</td>
<td>72</td>
<td>1</td>
<td>72</td>
<td>72</td>
<td>0 to 20</td>
<td>24.6%</td>
<td>10</td>
<td>720</td>
</tr>
<tr>
<td>20</td>
<td>172</td>
<td>49</td>
<td>8</td>
<td>2</td>
<td>16</td>
<td>16</td>
<td>20 to 40</td>
<td>5.5%</td>
<td>30</td>
<td>480</td>
</tr>
<tr>
<td>40</td>
<td>123</td>
<td>41</td>
<td>17</td>
<td>3</td>
<td>51</td>
<td>51</td>
<td>40 to 60</td>
<td>17.4%</td>
<td>50</td>
<td>2550</td>
</tr>
<tr>
<td>60</td>
<td>82</td>
<td>24</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>20</td>
<td>60 to 80</td>
<td>6.8%</td>
<td>70</td>
<td>1400</td>
</tr>
<tr>
<td>80</td>
<td>58</td>
<td>19</td>
<td>-4</td>
<td>5</td>
<td>-2</td>
<td>35</td>
<td>80 to 100</td>
<td>11.9%</td>
<td>90</td>
<td>3150</td>
</tr>
<tr>
<td>100</td>
<td>39</td>
<td>23</td>
<td>15</td>
<td>6</td>
<td>90</td>
<td>35</td>
<td>100 to 120</td>
<td>11.9%</td>
<td>110</td>
<td>3850</td>
</tr>
<tr>
<td>120</td>
<td>16</td>
<td>8</td>
<td>2</td>
<td>7</td>
<td>14</td>
<td>14</td>
<td>120 to 140</td>
<td>4.8%</td>
<td>130</td>
<td>1820</td>
</tr>
<tr>
<td>140</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>40</td>
<td>40</td>
<td>140 to 160</td>
<td>13.7%</td>
<td>150</td>
<td>6000</td>
</tr>
<tr>
<td>160</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>160 to 180</td>
<td>0.0%</td>
<td>170</td>
<td>0</td>
</tr>
<tr>
<td>180</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>180 to 200</td>
<td>3.4%</td>
<td>190</td>
<td>1900</td>
</tr>
<tr>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>200 to</td>
<td>0.0%</td>
<td>210</td>
<td>0</td>
</tr>
</tbody>
</table>

Mean vessel length = $\frac{xf}{f}$

74.64
The raw vessel count at each increment represents the number of vessels continuous from the basal end of the stem. The difference between the raw vessel counts of a segment and the next distal segment represents the number of vessel ends in the segment. Assuming a random distribution of vessels ends in the stem, the difference between the numbers of vessel ends in a segment and the number of vessel ends in the next distal segment represents the rate of linear increase for vessels of this length class. This difference multiplied by the number of increments from the base (steps to zero) gives the number of vessels of the length class (see Appendix and Table 2.4 for a sample calculation). This number can then be expressed as a percentage of the paint-filled vessels at the zero point. The sum of the calculated numbers of vessels in each size class should equal the raw vessel count at the zero point (Ewers and Fisher 1989).

2.2.6 Statistical analysis

*Xylem anatomy*

For each parameter, means and standard errors were calculated for each species and for each phenology. Differences among (i) species, (ii) plant parts (petioles, midrib and branch), (iii) branches size classes and (iv) between the two methods (maceration and sectioning) in vessel element length, vessel diameter, and vessel length were analysed using ANOVA (Statistica, StatSoft Inc., Tulsa USA, 1999). Data were log transformed to stabilise variance, where required.

Mean vessel length (three branches for each species) was compared using one factor ANOVA, after log transforming the data to correct for the fact that distribution of vessel lengths was skewed to the longer vessels. The correlation between vessel diameter and pit membrane pore diameter was determined.

*Phloem anatomy*

Regression analysis was used to test for significance in the relation between species. For dry root and dry shoot weight, a logarithmic transformation was used in order to fit a straight line.

Mean RGRs based on whole fresh weight of plants were calculated separately for all four species (*E. tetrodonta, M. viridiflora, C. polysciada* and *T. ferdinandiana*)
RGR = (ln weight 2 - ln weight 1)/(day 2 - day 1)

2.3 Results

2.3.1 Comparison of maceration and sectioning methods

In both deciduous and evergreen species, mean vessel diameters were 4% larger (p<0.08) using the maceration method than the sectioning method (Fig. 2.2). However, in the coniferous species, the mean tracheid diameters were significantly smaller (p<0.05) when determined by the maceration method compared to the sectioning method.

2.3.2 Vessel diameters as a function of branch diameter in different plant parts

Mean branch vessel diameter, as measured by the maceration method, significantly increased with increasing branch diameter in both evergreen and deciduous species (p<0.05) (Fig. 2.3, Table 2.5).

Table 2.5  Vessel diameters measured by the maceration method observed in branches of different diameter in evergreen species and deciduous species

<table>
<thead>
<tr>
<th>Branch diameter (mm)</th>
<th>Evergreen mean vessel diameter (µm)</th>
<th>Deciduous mean vessel diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>25.29 ± 1.23</td>
<td>31.14 ± 1.34</td>
</tr>
<tr>
<td>4-6</td>
<td>33.25 ± 1.59</td>
<td>49.25 ± 1.95</td>
</tr>
<tr>
<td>7-10</td>
<td>47.63 ± 1.78</td>
<td>63.63 ± 3.09</td>
</tr>
</tbody>
</table>

Mean vessel diameter in branches, petioles and midribs are shown in (Plate 2.5, 2.6, 2.7). Vessel diameters in branches of evergreen and deciduous species were significantly wider than petioles and midribs (p<0.05) but there was no significant difference in vessel diameter between petioles and midribs (Fig. 2.4). In the conifer, tracheid diameters were significantly wider in branches than in petioles and midribs.
2.3.3 Vessel dimensions in evergreen and deciduous species

The calculated distribution of vessel lengths varied among species, as shown in Fig. 2.6. Evergreen species had maximum vessel lengths of 200 to 280 mm, semi-deciduous species had maximum vessel lengths of 200 mm and deciduous species had maximum vessel length of 120 mm. Using the latex paint infusion method, the vessels of the basal end segment were not all paint-filled (Plate 2.2). The estimated mean branch vessel length for evergreen species (78 mm) was longer than the mean vessel of length of the one deciduous (51 mm) species (*P. careya*). Only one deciduous species was measured because the other deciduous species (*T. ferdinandiana, B. megaphyllus, C. arnhemicus* and *C. fraseri*) contain very viscous and sticky exudate that prevented free flow of the paint solution within the xylem.

The mean vessel diameter of branches was significantly larger in the six deciduous species (50.3 ± 10.3 µm) than in the six evergreen species (31.2 ± 8.6 µm). The mean branch vessel diameter of deciduous species was 1.6 times larger than that of evergreen species (Table 2.6) (P<0.002) when determined using the sectioning method, where the sticky exudate was not a problem.

The coniferous species (*C. intratropica*) only had small tracheids (Plate 2.3 a). The diameter of tracheids was much smaller than vessels of evergreen and deciduous species (Fig. 2.8). Mean tracheid diameter was 10 ± 0.08 µm using the maceration technique and 13 ± 0.34 µm using the sectioning technique (Fig. 2.2). Average tracheid length was 886 ± 36.33 µm.

Vessel elements have a simple perforation on both end walls and some vessel elements have narrow tails on their end walls while others are blunt (Plate 2.3 b,c). Vessel element length decreased as branch diameter increased (Fig. 2.6). Vessel element lengths were, on average, shorter in the six evergreen species (309 µm) than in the six deciduous species (334 µm) (Table 2.6) (P<0.002), as determined by the maceration technique.

Vessel density differed markedly among species. Deciduous species had the lowest vessel density (115 mm⁻²) reflecting the relatively large diameter of these vessels. Evergreen species with small vessel diameters had the highest vessel density (247 mm⁻²) (Table 2.6). A strong negative relationship between maximum vessel density and maximum vessel diameter was observed (Fig. 2.7, r² = 0.86) (P<0.0002).
Fig. 2.2. Comparison of vessel diameter using maceration and sectioning methods. Vessel diameter was smaller when determined by the sectioning than the maceration method. Full species names are given in 2.2.1. Vertical bars represent standard error. (n = 6 evergreen, 6 deciduous and 1 conifer).

Fig. 2.3. Mean vessel diameters (measured using the maceration method) in three difference size classes of branch for evergreen and deciduous species. Vessel diameters were larger in deciduous species than evergreen species. Vertical bars represent standard error. (n = 6 evergreen, 6 deciduous and 1 conifer).
Fig. 2.4. Comparison of mean vessel and tracheid diameters in midribs, petioles and branches of evergreen, deciduous and conifer species. Vertical bars represent standard error.

Fig. 2.5. Mean vessel element length in branches of different diameter of evergreen and deciduous species. Vessel element length is longer in narrower branches than in wider diameter branches. Vertical bars represent standard error.
Fig. 2.6. Frequency of vessels of different lengths, as determined by the paint infusion method, in tree species from different phenological guilds.

☐ evergreen, species. Vertical bars represent standard error.
Fig. 2.6(Contd). Frequency of vessels of different lengths, as determined by the paint infusion method, in tree species from different phenological guilds, □ evergreen, □ semi-deciduous and □ deciduous species. Vertical bars represent standard error.
Plate 2.2. Vessels of *E. tetrodonta* showing green paint stains from paint infusion method. (a) The basal end at the point of paint injection; at (b) 2 cm; (c) 4 cm; (d) 6 cm; (e) 16 cm and (f) 18 cm distance from paint injection point.
Plate 2.3. Tracheids and vessels shown using the maceration method (a) tracheids in *C. intratropica*, (b) vessel elements in *E. tetrodonta* and (c) vessel elements in *B. megaphyllus* (scale bar = 50 µm)
Plate 2.4. Transverse section of lamina showing epidermis, stomata and vascular bundles. (a) *C. intratropica*, (b) *P. falcate* and (c) *T. ferdinandiana* (scale bar = 50µm)
Plate 2.5. Transverse section of midrib showing vascular bundles (a) *E. miniata*, (b) *A. dimidiata* and (c) *P. careya* (scale bar = 50 µm).
Plate 2.6. Transverse section of petiole showing xylem cells (a) tracheid cells in *C. intratropica* (coniferous), (b) vessel cells in *A. auriculiformis*, and (c) *B. megaphyllus* (scale bar = 50 µm).
Plate 2.7. Transverse section of branch showing xylem cells (a) tracheids cells in *C. intratropica*, (b) vessels cells in *M. viridiflora* and (c) *B. megaphyllus* (scale bar = 50 µm).
Table 2.6. Mean vessel diameter and length and vessel density (± 1 SE) for deciduous and evergreen species and the result of ANOVA comparing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Evergreen</th>
<th>Deciduous</th>
<th>N</th>
<th>Mean square</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branch vessel diameter (µm)</td>
<td>31.2 ± 8.6</td>
<td>50.3 ± 10.3</td>
<td>330</td>
<td>8000.9</td>
<td>1</td>
<td>272.4</td>
<td>0.0017</td>
</tr>
<tr>
<td>Petiole vessel diameter (µm)</td>
<td>18.5 ± 5.3</td>
<td>28.8 ± 8.2</td>
<td>330</td>
<td>8729.6</td>
<td>1</td>
<td>195.84</td>
<td>0.0004</td>
</tr>
<tr>
<td>Midrib vessel diameter (µm)</td>
<td>18.3 ± 5.2</td>
<td>28.5 ± 7.4</td>
<td>330</td>
<td>8452.6</td>
<td>1</td>
<td>190.29</td>
<td>0.0014</td>
</tr>
<tr>
<td>Vessel element length (µm)</td>
<td>309.9 ± 83.5</td>
<td>334.8 ± 119.6</td>
<td>660</td>
<td>10180</td>
<td>1</td>
<td>14.5</td>
<td>0.00015</td>
</tr>
<tr>
<td>Vessel density (no./mm²)</td>
<td>247 ± 28.5</td>
<td>115.8 ± 14.1</td>
<td>330</td>
<td>46945</td>
<td>1</td>
<td>86.7</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Fig. 2.7. Mean vessel density and mean vessel diameter for evergreen and deciduous species. There was a negative correlation between vessel diameter and vessel density ($r^2 = 0.86$, $P < 0.002$)

Fig. 2.8. Mean conduit diameter in evergreen and deciduous species (vessel and tracheid diameter added together). Full species names are given in section 2.2.2. Vertical bars represent standard error.
2.3.4 Leaf anatomy

Leaves of evergreen species were sclerophyllous with anatomical characteristics such as abundance of sclerenchyma, thick outer epidermal cell walls and cuticles, thick leaf cross section and sunken stomata (Plate 2.4b). In contrast, leaves of deciduous species were thin in cross section, had thin cuticles and the number of stomata per leaf was less than in evergreen species (Plate 2.4c).

2.3.5 Phloem cross-sectional area in seedlings

Root dry weight, leaf dry weight and phloem cross sectional area increased with seedling age for all species. The absolute rate of increase was slow for months 1 to 3, followed by a larger relative increase in months four and five (Fig. 2.9) for all species.

RGR was, also, not uniform throughout the 5 month study period, generally decreasing with seedling age (Fig. 2.10). Mean RGR varied across species, ranging from 0.062 g g\(^{-1}\) day\(^{-1}\) in \textit{T. ferdinandiana} to 0.01 g g\(^{-1}\) day\(^{-1}\) in \textit{M. viridiflora}. Also, the stem diameter of \textit{C. polysciada} seedling was significantly greater than in the other species at the start of the experiment (p<0.05) (Table 2.7).

There was a strong positive correlation between stem diameter and log transformed leaf and root dry weight, xylem cross-sectional area and phloem cross-sectional area in all species (\(r^2 =0.86\)) (P<0.05) (Fig. 2.11, Fig. 2.12, Fig. 2.13, Fig. 2.14). Increases in stem diameter were correlated with increases in leaf dry weight, root dry weight and xylem cross-sectional area. There was a generally weak but significant relationship between phloem cross-sectional area and root dry weight (root biomass) in \textit{M. viridiflora}, \textit{C. polysciada} and \textit{T. ferdinandiana}. The correlation between phloem cross-sectional area and root dry weight was stronger in \textit{E. tetrodonta} (\(r^2 = 0.95\))(P<0.05)(Fig. 2.15).
Fig. 2.9. Changes in mean (a) leaf dry weight, (b) root dry weight and (c) phloem cross sectional area within monthly seedlings age. Symbols: (●) E. tetrodonta, (○) C. polysciada, (▼) M. viridiflora and (∇) T. ferdinandiana. The bars indicate standard error.
Fig. 2.10. Relationship between whole plant RGR and seedling ages (days) (●). *E. tetrodonta*, (○). *M. viridiflora*, (▼). *C. polysciada*, (▽). *T. ferdinandiana*.

Table 2.7. Initial mean stem diameter at one month age seedling, shoot and root dry weight for 5 specimens in each species (Standard error are also shown).

<table>
<thead>
<tr>
<th>Species</th>
<th>Stem diameter (mm)</th>
<th>Leaf dry weight (g)</th>
<th>Root dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. tetrodonta</td>
<td>0.59 ± 0.02</td>
<td>0.032 ± 0.006</td>
<td>0.011 ± 0.003</td>
</tr>
<tr>
<td><em>M. viridiflora</em></td>
<td>0.60 ± 0.09</td>
<td>0.014 ± 0.003</td>
<td>0.004 ± 0.0007</td>
</tr>
<tr>
<td><em>C. polysciada</em></td>
<td>0.85 ± 0.04</td>
<td>0.095 ± 0.01</td>
<td>0.027 ± 0.001</td>
</tr>
<tr>
<td><em>T. ferdinandiana</em></td>
<td>0.54 ± 0.08</td>
<td>0.067 ± 0.0001</td>
<td>0.176 ± 0.05</td>
</tr>
</tbody>
</table>
Fig. 2.11. Relationship between stem diameter and log root dry weight
(a) *E. tetrodonta* \((r^2=0.83)\), (b) *M. viridiflora* \((r^2=0.83)\), (c) *C. polysciada* \((r^2=0.93)\), and (d) *T. ferdinandiana* 

Fig. 2.12 Relationship between stem diameter and log dry leaf weight

(a) *E. tetrodonta* ($r^2=0.84$)

(b) *M. viridiflora* ($r^2=0.80$)

(c) *C. polysciada* ($r^2=0.87$)

(d) *T. ferdinandiana* ($r^2=0.66$)

Symbols: (●) Month 1, (○) Month 2, (▼) Month 3, (∇) Month 4, (■) Month 5

*E. tetrodonta* ($r^2=0.84$)

*M. viridiflora* ($r^2=0.80$)

*C. polysciada* ($r^2=0.87$)

*T. ferdinandiana* ($r^2=0.66$)
Fig. 2.13. Relationship between stem diameter and xylem cross-sectional area
(a) *E. tetrodonta* ($r^2 = 0.90$), (b) *M. viridiflora* ($r^2 = 0.81$), (c) *C. polysciada* ($r^2 = 0.74$), and (d) *T. ferdinandiana* ($r^2 = 0.72$).

Fig. 2.14. Relationship between stem diameter and phloem cross sectional area
(a) \( E. tetrodonta \) \((r^2=0.93)\), (b) \( M. viridiflora \) \((r^2=0.82)\), (c) \( C. polysciada \) \((r^2=0.86)\), and (d) \( T. ferdinandiana \) \((r^2=0.89)\)
symbols (●). Month 1, (○) Month 2, (▼) Month 3, (∇) Month 4, (■) Month 5
Fig. 2.15  Relationship between stem diameter and log dry root weight
(a) *E. tetrodonta* (*r^2* = 0.95)  
(b) *M. viridiflora* (*r^2* = 0.36)  
(c) *C. polysciada* (*r^2* = 0.38)  
(d) *T. ferdinandiana* (*r^2* = 0.47)  
symbols (●) Month 1, (○) Month 2, (▼) Month 3, (∇) Month 4, (■) Month 5
2.4 Discussion

2.4.1 Sectioning method vs maceration method

Mean vessel diameter was significantly (4%) larger when measured using the maceration method, compared with the sectioning method. There are several possible reasons for this difference. First, during maceration, the samples were crushed on the slide, a drop of glycerine was added and the sample was covered by a cover slip. The glycerine was used to prevent the sample drying out under the microscope. The glycerine and the cover slip may make vessel diameter appear larger. More importantly, however, the vessel diameter may not be truly representative of the average vessel element diameter because it was measured at the mid-point of the vessel element, which is presumably the widest point. In contrast, the sectioning method samples diameters randomly along the length of the vessel, which therefore will include some narrower ends of cells. (Ewers and Fisher 1989) experienced a similar problem when measuring vessel diameter using the same maceration method. However, the advantage of the maceration method is that it can also be used to measure vessel element length.

Sectioning also involves dehydration in ethanol. This could, perhaps, result in a small amount of shrinkage of the dehydrated tissue. The sectioning method randomly samples along the length of the vessel element and this includes the tapered ends. As the sectioning method examines vessels in transverse section, the maximum and minimum diameter of cells that are non-circular can be measured. Thus the sectioning method provides an average diameter, rather than the average maximum diameter, of the vessels. The sectioning method is more useful for determining hydraulic conductance in a stem. The number of vessels, as well as the diameter of each vessel lumen, can be determined. However, a disadvantage of the sectioning method is that the narrowest vessels may be difficult to distinguish from tracheid or fibre cells.

2.4.2 Xylem anatomy

Branch, petiole and mid-rib comparisons

Vessel diameters were widest in branches, intermediate in petioles and narrowest in mid-ribs (Fig. 2.4, Plates 2.5, 2.6, 2.7, Table 2.6). This trend conforms to the

Vessel lengths were measured only in branches in this study. However, vessel lengths in roots are generally longer than the shoots (Zimmermann and Potter 1982, Kolb and Sperry 1999) and vessel length in petioles and twigs can be much shorter than the main branch and trunks (Salleo et al. 1985, Hacke and Sauter 1996). (Leitch 2001) found that vessel elements were wider and longer at the base of the tree and decreased in size with increasing height up the tree. The roots have not only wider but usually also longer vessels than shoots (Zimmermann and Potter 1982, Kolb and Sperry 1999). Therefore, roots are usually more vulnerable to cavitation than stems (Sperry and Saliendra 1994, Hacke and Sauter 1996, Mencuccini and Comstock 1997), but experience less negative water potentials than leaves.

**Phenological comparisons**

The key hypothesis examined in this chapter was that xylem dimensions differ between deciduous and evergreen species. Using a number of measures, this hypothesis was supported by the data presented in this chapter. Mean vessel diameters were largest in deciduous species (50.3 µm), intermediate in evergreen species (31.2 µm) and narrowest in the tracheids of the single coniferous species (10 µm) tested. Our findings for vessel diameters are in agreement with several other studies, including studies of roots of desert species (Hacke et al. 2000), branches of woody American species (Davis et al. 1999), dry rainforest species of Queensland (Choat et al. 2003) and Arctic tundra plants in Alaska (Gorsuch et al. 2001). It is not possible to make conclusions about differences in vessel length between phenological guilds because this study could be measured in only one of the deciduous species.

The vessel diameters in this study for evergreen and deciduous species from savannas were smaller than observed in tropical rainforest evergreen species (*Alphitonia excelsa* 47 µm, *Austromyrtus bidwillii* 32 µm) and deciduous species (*Brachychiton australis* 87 µm, *Cochlospermum gillivraei* 105 µm) in Queensland (Choat et al. 2003). The present study sampled branches diameters of between (7-
10 mm), but (Choat et al. 2003) sampled branches diameter between (10-30 mm). Although the difference in sample branch diameter may explain the difference in measured vessel diameter, the shorter, less severe dry season experienced in the QLD wet tropics may allow larger transpiration rates throughout the year, with consequently larger xylem vessels.

2.4.3 Phloem

There was a strong statistical correlation between stem diameter and dry leaf weight, dry root weight and phloem cross-sectional area. Stem diameters are easy to measure and scaled. Likely to be more variable still when looking at mature plants in the field, for example, varying degrees of termite damage, ratio of live: dead tissue. There was a strong relationship between stem diameter and phloem cross-sectional-area in four species. As the stem diameter increase the phloem cross-sectional should also increases. Strong relationships between stem diameter and leaf area and sapwood area have been identified in a young mountain Ash forest (Vertessy et al. 1995).

Relative growth rate in *T. ferdinandiana* (deciduous species) seedlings was larger than in the two evergreen species (*E. tetrodonda* and *M. viridiflora*). Plant growth strategy theory may be interpreted to suggests that fast-growing species have short-lived leaves and roots of high absorptive capacity, whereas slow-growing species have long-lived leaves and roots of low absorptive capacity (Grime 1977). This is supported by studies showing that fast growing species with short-lived leaves exhibit high leaf area: mass ratio (SLA), high nitrogen concentration, high rates of photosynthesis and low tissue density (Poorter et al. 1990, Wright and Westoby 2000). A decline in RGR with age shown in this present investigation has also been found in many other studies (Castro-Díez et al. 1998). This applies throughout the life cycle, from seedling to mature trees (Wright and Westoby 1999, Koelewijn 2004).
Appendix

Calculations for vessel length distribution

The following text should be read in conjunction with Table 2c. The raw vessel count represents the number of vessels continuous from basal end vessel count \((x_0)\). The first difference (column 3) represents the number of vessel ends that occur between the first and second, or second and third, or third and fourth raw vessel counts and so on. The second difference represents the rate of linear increase for vessels of this length class. The second difference multiplied by the number of increments (steps to zero) gives the number of vessels in that length class. This number can then be expressed as a percent of the paint-filled vessels at the zero point. The sum of the calculated numbers of vessels in each size class should equal the raw vessel count at the zero point (Table 2.4).

As discussed by Zimmermann and Jeje (1981), a negative difference can be attributed to non-random distribution of vessel in the stem segment. These negative numbers were almost always confined to the longer size classes and appear to be result of the small sample size in the longer classes. Negative numbers in the “No. of vessels” column were removed by grouping categories to arrive at positive values under “corrected vessel No. (f)”. To do this, negative numbers were averaged with adjacent positive number (s) in the same column (Ewers and Fisher 1989).

For each species, the mean vessel length was calculated using the mid-point \((x)\) of the length class and frequency of each length class:

\[
x = \frac{\sum xf}{x}
\]

\(x\) = mean vessel length

\(x\) = mid-point of class

\(f\) = frequency

Vessel lengths were compared between deciduous and evergreen species using ANOVA. Prior to analysis, vessel lengths were log-transferred to correct for the distribution of lengths being skewed to the right (Sokal and Rohlf 1981).
Chapter 3: Seasonal changes in hydraulic conductance and xylem vulnerability of deciduous and evergreen species

3.1 Introduction

Water movement from roots to the atmosphere via leaves is controlled by the conductivity of the pathway for conduction water. Trees growing in the seasonally dry tropics of north Australia must either avoid drought (by shedding their leaves) or tolerate it. Evergreen species maintain their leaves, while deciduous species shed their leaves during the dry season (Duff et al. 1997; Williams et al. 1997). There may be differences between deciduous and evergreen species in hydraulic conductance and susceptibility to xylem embolism because of the requirement for evergreen trees to maintain evapotranspiration during the dry season. In the present study, mean vessel diameter and pit membrane pore diameter of six deciduous species were larger than that of six evergreen species (chapter 2 & chapter 4). In this chapter I address the question do these differences influence the hydraulic conductivity of branches?

Seasonal declines in leaf water potential of trees growing in seasonally dry sites are the result of declining soil water availability and declining atmospheric water content (Eamus and Prior 2001). Pre-dawn leaf water potential of woody species in northern Australia is highest during the wet season and decreases with decreasing soil water content as the dry season progresses (Duff et al. 1997, Williams et al. 1997, O’Grady et al. 1999). Evergreen species maintain higher leaf water potentials in the early dry season than deciduous species, but deciduous species are leafless during part of the dry season (Duff et al. 1997). In northern Australia, mid-day evergreen leaf water potential declined to -2.5 MPa during the dry season, and -1.5 MPa during the wet season (Myer et al. 1997, Prior et al. 1997 ab). It is possible that evergreen trees are able to maintain a more favourable plant water status by having a larger, deep root system (Duff et al. 1997, Chen et al. 2002).

Hydraulic conductance is defined as flow rate per unit pressure difference and is proportional to the fourth power of its radius (Poiseuille’s Law). Hydraulic conductivity is defined as the flow rate per unit pressure gradient. Conductance and
conductivity measurements may be divided by leaf area or by branch cross-
sectional area or sapwood area to allow comparisons of different sizes of trees or
branches.

Different components of a plant (eg. roots, branch, stem and leaves) have different
hydraulic conductance with roots tend to have the lowest conductance to flow.
Thus, (Becker et al. 1999) showed that the roots accounted for about 40% of
resistance in several tropical woody plants and that, in comparison, leaves
contributed about 25%. In contrast, about 60% of the total plant resistance to water
flow was located in whole small shoots of Eucalyptus tetrodonta (Prior et al. 1997),
indicating that 40% resided in roots.

Hydraulic conductance may change seasonally, for several reasons, including xylem
embolism (Sperry and Tyree 1990, Lo Gullo and Salleo 1991, Hargrave et al. 1994,
Sperry et al. 1996), and changes in mean path length (Prior and Eamus 2000).
Similarly, the amount of embolism may change seasonally in woody plants. For
examples, in a study of north Australian savannas tree, mean loss of conductance
due to xylem embolism in terminal branches of E. tetrodonta and E. miniata saplings
was less than 15% during seasonal drought (Prior and Eamus 2000). Similarly,
(Sobrado 1997) showed that the loss of conductance due to xylem embolism in
terminal branches of Venezuelan evergreen trees ranged between 30 and 35%
during the wet season, but was between 40 and 48% during the dry season.
Furthermore, deciduous species experienced a loss of conductance of 8% and 19%
during the wet season but 38 to 79% during the dry season. In contrast, several
temperate northern hemisphere trees, embolism ranged between 20 to 25% during
summer and winter (Cochard and Tyree 1990, Tognetti and Borghetti 1994) with
seasonal variation.

To characterise susceptibility to embolism, vulnerability curves can be generated
which show the relationship between percentage loss of hydraulic conductivity and
xylem water potential (Sperry and Tyree 1988, Tyree et al. 1998, Sobrado 1996). In
north Australian savannas, hydraulic conductance of hydrated terminal branches
decreased by 50% for E. tetrodonta (evergreen) between a leaf water potential of
-3.2 and -2.0 MPa (Prior and Eamus 2000). In Queensland’s dry rainforest,
deciduous trees were more vulnerable to water-stress induced xylem embolism
(Choat et al. 2003). Thus, the deciduous species Brachychiton australis and
Cochlospermum gillivraei reached a 50% loss of hydraulic conductivity at -3.57 MPa.
and -1.44 MPa, respectively but evergreen species *Alphitonia excelsa* and *Austromyrtus bidwillii* reached this level of reduced conductivity at -5.57 MPa and at -5.12 MPa respectively, (Choat *et al.* 2003). Thus, there is some evidence that tropical evergreen species may be more resistant to xylem embolism than deciduous species.

The work described in this chapter aimed to determine:

I. Are there any differences in seasonal hydraulic characteristics of evergreen and deciduous species during the late wet season through to the late dry season (February-May-July-September)?

II. Whether there is any relationship between the percentage loss of hydraulic conductance and vessel diameter?

III. Whether there is any relationship between percentage loss of hydraulic conductance and minimum leaf water potential experienced during the dry season in evergreen and deciduous species?

### 3.2 Materials and Methods

#### 3.2.1 Study site and species

Plant samples were collected adjacent to Lee Point Road (15 km from Darwin city), Amy Johnson Drive and from Charles Darwin National Park (5.5 km from Darwin city). Vegetation at the study sites is open forest and dominated by *E. tetrodonta* and *E. miniata*, with other woody sub-dominant trees. Broad-leaved deciduous species such as *T. ferdinandiana* and *P. careya* are common under-storey shrubs with some grasses present in the wet season.

The six species were chosen because they were the most common woody species at the site, and included four evergreen (*E. miniata*, *E. tetrodonta*, *A. auriculiformis*, and *M. viridiflora*), one semi-deciduous (*C. polysciada*) and one deciduous species (*P. careya*). Six terminal branches from each species were collected in the late wet season (February), early dry (May), mid dry (July) and late dry season (September) 2001 for determination of hydraulic conductance, vessel anatomy and vulnerability curves. Preliminary studies have chosen that most deciduous species were not
amenable to measurement of hydraulic characteristics because of the presence of a thick, sticky, latex-like exudate that appears when the branches of many deciduous species are cut (Eamus, pers com). Consequently, fewer one deciduous species was used much smaller than evergreen species.

### 3.2.2 Hydraulic conductance

The maximum vessel length in branches of six species chosen was estimated by blowing low-pressure air (20 kPa) into the cut end of branches while cutting off the branch tips and petioles under water. Bubbles were blown through to the branch tips when the branch length was shorter than the longest vessel (Franks et al. 1995). Once the maximum vessel length for each species had been determined, samples for hydraulic conductance measurement were cut significantly longer than this length to avoid introducing artificial embolism in xylem.

The method for measuring hydraulic conductance of a terminal branch was based on that described by Williams et al. (1997a), Kolb et al. (1996), Prior and Eamus (2000). Terminal branches were collected from the plants in the morning before 09:00 am to minimise the presence of emboli at the time of sampling. The branches were cut under water, and recut under water to remove any vessels embolised by the first cut. The branches were placed upright in a bucket with the cut end under water and covered with a black plastic bag, and transported back to the laboratory. The cut end was recut under water and all leaves were cut from petioles using a fresh razor blade.

Hydraulic conductance was measured using a vacuum chamber, which was constructed from thick PVC pipe of 160 mm diameter and 1 m long. The vacuum chamber was attached to a vacuum pump at one end and the other end was a detachable lid. The cut end of the branch was inserted through a seal in the vacuum chamber lid with the cut end protruding outwards and attached to tubing to 10 ml graduated pipettes. The tubing and pipettes were filled with degassed water filtered through a 0.2 µm filter and acidified with HCl to pH 2 to prevent microbial growth in the tubing system (Sperry et al. 1994). The remaining portion of the branch was placed into the vacuum chamber (Fig. 3.1). Previous comparison of acidified water or acidified salt (KCl) solution revealed no difference in conductance and therefore acidified water was used in this study (MacInnis et al. 2004).
The solution was pulled through the branch in the same direction as the normal transpiration stream by applying a vacuum to the chamber with a vacuum pump. Flow rate was measured at four levels of vacuum (20, 40, 60 and 80 kPa). The initial measurement of hydraulic conductance was followed by a 30 minutes perfusion of acidified, filtered, degassed water at a positive pressure of 175 kPa, to remove air embolism (William et al. 1997a). Pressure was released for 30 minutes before readings were re-started, to allow the tubing and xylem vessels to relax. The measurement of hydraulic conductance was then repeated.

Hydraulic conductance was calculated as the slope of the relationship between flow rate and pressure difference using linear regression (Sigma Plot 6.0, SPSS Science, Chicago, USA). The difference between the initial before applying 175 kPa positive pressure and final after applying the 175 kPa positive pressure values for hydraulic conductance was divided by the final value and multiplied by 100 to calculate the percentage loss in hydraulic conductance due to embolism (Williams et al. 1997). Hydraulic conductivity was also calculated by multiplying hydraulic conductance by branch length. Conductivity was calculated on a leaf area basis and on a branch cross sectional area basis. The diameter of the basal end of the branch was measured with callipers, and length measured with a ruler. Leaf area was measured with a Delta-T Scan (Delta-T Devices Ltd, Cambridge, UK) using Delta-T Scan software to calculate the leaf area of all leaves previously attached to the branch.
Fig. 3.1. Schematic representation of the apparatus for measuring hydraulic conductance (A). vacuum pump; (B). gauge; (C). vacuum chamber; (D). branch; (E). pipette; (F). three-way valve; (G) compressed air tank with regulator; (H). Reservoir. Line connecting is a pressure hose.
3.2.3 Xylem vessel measurement

After conductivity measurements were completed, branches were removed from the apparatus and fixed in FAA solution overnight. The dehydration of the ethanol series and embedding in resin are as described in chapter 2. Cross-sections (30-40 µm thick) were made with a sliding microtome and stained with safranin. Images were obtained from a digital camera attached to a light microscope (DP 11, Olympus, Japan). Each image was imported into the NIH Image 1.23 software for analysis. Twenty vessel diameters were measured from each section.

3.2.4 Vulnerability curves

Vulnerability to water stress-induced embolism was determined by measuring the relationship between leaf water potential to percentage loss of hydraulic conductivity. Branches were cut from the plant and immediately placed in a water filled bucket and covered with a black plastic bag and brought back to the laboratory. The cut end was wrapped in parafilm and moist paper towel and the branch was then placed in a large plastic bag. The bag was partially open to allow the branch to dry slowly. The branches were allowed to dehydrate for 1-6 days on the laboratory bench to water potentials between -2.1 MPa and -5.5 MPa. During dehydration, their water potential was measured at regular intervals using a pressure chamber (Soil Moisture Equipment Corporation, California, USA).

Once water potential was measured and found to be in the desired range, the branch was tightly bagged overnight so that the water content stabilised throughout the branch. The following morning, the water potential for one leaf of the branch was measured with a Scholander pressure chamber (Soil Moisture Equipment Corporation, California, USA). The branch was then placed under water and the basal end was cut with a sharp razor blade. Then the cut end was placed in the vacuum chamber and the percentage loss of hydraulic conductivity due to embolism of the branch was measured by comparing the hydraulic conductance before and after high-pressure (175 kPa) perfusion to remove any air embolism (Sperry et al. 1988).
3.2.5 Statistical analyses

ANOVA was performed using Statistica version 6.0 (Statsoft, Tulsa, OK, USA) to determine effects of month and species on hydraulic conductance. Linear regression analysis was used to determine the relationship between vessel diameters and percentage loss of conductance.

The data from the vulnerability curves were fitted to the exponential sigmoidal equation:

\[ \ln \left( \frac{100}{\text{PLC}} - 1 \right) = a \Psi - ab \]  
Equation........(1)

where \( \Psi \) is the water potential, PLC is the percentage loss in hydraulic conductivity and \( a \) and \( b \) are constants. The coefficient “\( a \)” indicates the vulnerability curve, whereas “\( b \)” is the water potential at which 50% loss of conductivity occurs (Pammenter and Vander Willigen 1998).

3.3 Results

3.3.1 Seasonal patterns of hydraulic characteristics

The mean branch dimensions, hydraulic conductance and conductivity and embolism in terminal branches of four evergreen species, one semi-deciduous and one deciduous species are shown in Table 3.1. Mean hydraulic conductance, conductivity per branch transverse area and leaf area declined as the dry season progressed for all species (Table 3.2). The mean percentage loss of hydraulic conductance increased progressively from February to September in all but one species, \( M. \text{ viridiflora} \) did not show a significant change in loss of conductance through the course of the dry season (Table 3.1, Fig. 3.2). By contrast, mean vessel diameter did not change significantly through the wet season and dry seasons (Table 3.2, p<0.01).

Comparisons among phenological groups must be viewed with great caution because only one semi-deciduous and one deciduous could be examined because most deciduous species have a sticky exudate that precluded measurement of hydraulic conductance. However, the average hydraulic conductance, hydraulic conductivity per branch transverse area and leaf area were higher in the one deciduous species examined than the evergreen and semi-deciduous species.
(Table 3.2). Also the average percentage loss of hydraulic conductance in the dry season (May, July and September) was highest in the one deciduous species (15%) followed by the semi-deciduous species (12%) and lowest in evergreen species (7%) (Table 3.2). The percentage loss of hydraulic conductance between deciduous and evergreen species was significantly different (Fig 3.4, P<0.001). Branch diameter and leaf area were also larger in deciduous species than evergreen species (Table 3.1).
Table 3.1 (a). Branch dimension, % loss of conductance due to embolism and conductance and conductivity of terminal branches of evergreen, semi-deciduous and deciduous species in February (2001).

<table>
<thead>
<tr>
<th>February</th>
<th>Evergreen</th>
<th>Semi-Deciduous</th>
<th>Deciduous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. auriculiformis</em></td>
<td><em>E. miniata</em></td>
<td><em>E. tetrodonta</em></td>
</tr>
<tr>
<td>Branch length (mm)</td>
<td>33 ± 4.3</td>
<td>26 ± 1.6</td>
<td>35 ± 3.1</td>
</tr>
<tr>
<td>Branch diameter (mm)</td>
<td>3.25 ± 0.17</td>
<td>3.75 ± 0.11</td>
<td>4.58 ± 0.23</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>652 ± 80.3</td>
<td>275 ± 14.7</td>
<td>699 ± 33.4</td>
</tr>
<tr>
<td>Hydraulic conductance (g s⁻¹ MPa⁻¹)</td>
<td>0.041 ± 0.004</td>
<td>0.065 ± 0.01</td>
<td>0.08 ± 0.013</td>
</tr>
<tr>
<td>Hydraulic conductivity (g m s⁻¹ MPa⁻¹)</td>
<td>0.142 ± 0.005</td>
<td>0.028 ± 0.004</td>
<td>0.029 ± 0.005</td>
</tr>
<tr>
<td>Hydraulic conductivity per branch tranverse area (g m⁻¹ MPa⁻¹ m⁻²)</td>
<td>1381 ± 317</td>
<td>1291 ± 348</td>
<td>1246 ± 192</td>
</tr>
<tr>
<td>Hydraulic conductivity per leaf area (g m⁻¹ MPa⁻¹ m⁻²)</td>
<td>0.216 ± 0.046</td>
<td>0.363 ± 0.140</td>
<td>0.390 ± 0.071</td>
</tr>
<tr>
<td>Percent loss of hydraulic conductance (%)</td>
<td>3.38 ± 0.96</td>
<td>4.03 ± 1.07</td>
<td>6.3 ± 1.8</td>
</tr>
<tr>
<td>Vessel diameter (µm)</td>
<td>28.4 ± 1.04</td>
<td>33.5 ± 1.45</td>
<td>35.4 ± 1.59</td>
</tr>
</tbody>
</table>
Table 3.1 (b). Branch dimension, % loss of conductance due to embolism and conductance and conductivity of terminal branches of evergreen, semi-deciduous and deciduous species in May (2001).

<table>
<thead>
<tr>
<th>May</th>
<th>A. auriculiformis</th>
<th>E. miniata</th>
<th>E. tetrodonta</th>
<th>M. viridiflora</th>
<th>C. polysciada</th>
<th>P. careya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branch length (cm)</td>
<td>26.8 ± 1.1</td>
<td>26 ± 1.6</td>
<td>31.9 ± 2.2</td>
<td>26.2 ± 0.6</td>
<td>21.3 ± 1.0</td>
<td>22.8 ± 1.42</td>
</tr>
<tr>
<td>Branch diameter (mm)</td>
<td>3.5 ± 0.2</td>
<td>3.8 ± 0.1</td>
<td>4.6 ± 0.2</td>
<td>3.9 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Leaf area (cm$^2$)</td>
<td>522 ± 45</td>
<td>252 ± 17.6</td>
<td>699 ± 33.4</td>
<td>347 ± 21.6</td>
<td>203 ± 20.3</td>
<td>719 ± 18.8</td>
</tr>
<tr>
<td>Hydraulic conductance (g s$^{-1}$ MPa$^{-1}$)</td>
<td>0.032 ± 0.004</td>
<td>0.064 ± 0.023</td>
<td>0.079 ± 0.013</td>
<td>0.038 ± 0.004</td>
<td>0.06 ± 0.009</td>
<td>0.127 ± 0.015</td>
</tr>
<tr>
<td>Hydraulic conductivity (g m s$^{-1}$ MPa$^{-1}$)</td>
<td>0.009 ± 0.001</td>
<td>0.017 ± 0.004</td>
<td>0.025 ± 0.005</td>
<td>0.010 ± 0.001</td>
<td>0.013 ± 0.002</td>
<td>0.030 ± 0.005</td>
</tr>
<tr>
<td>Hydraulic conductivity per branch tranverse area (g m s$^{-1}$ MPa$^{-1}$ m$^{-2}$)</td>
<td>742 ± 157</td>
<td>1285 ± 354</td>
<td>1156 ± 175</td>
<td>682 ± 130</td>
<td>767 ± 195</td>
<td>1015 ± 137</td>
</tr>
<tr>
<td>Hydraulic conductivity per leaf area (g m s$^{-1}$ MPa$^{-1}$ m$^{-2}$)</td>
<td>0.167 ± 0.021</td>
<td>0.661 ± 0.127</td>
<td>0.364 ± 0.071</td>
<td>0.299 ± 0.045</td>
<td>0.643 ± 0.076</td>
<td>0.409 ± 0.065</td>
</tr>
<tr>
<td>Yet loss of hydraulic conductance (%)</td>
<td>4 ± 0.58</td>
<td>4.5 ± 1.32</td>
<td>6.3 ± 1.78</td>
<td>2.75 ± 0.48</td>
<td>9 ± 0.55</td>
<td>12.5 ± 1.66</td>
</tr>
<tr>
<td>Vessel diameter (µm)</td>
<td>29.3 ± 1.36</td>
<td>34.3 ± 1.5</td>
<td>35.4 ± 1.6</td>
<td>32.8 ± 2.3</td>
<td>38.9 ± 2.8</td>
<td>51.51 ± 4.7</td>
</tr>
</tbody>
</table>
Table 3.1 (c). Branch dimension, % loss of conductance due to embolism and conductance and conductivity of terminal branches of evergreen, semi-deciduous and deciduous species in July (2001).

<table>
<thead>
<tr>
<th>July</th>
<th>A. auriculiformis</th>
<th>E. miniata</th>
<th>E. tetrodonta</th>
<th>M. viridiflora</th>
<th>C. polysciada</th>
<th>P. careya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branch length (cm)</td>
<td>35.25 ± 1.8</td>
<td>38.5 ± 2.0</td>
<td>33.9 ± 0.8</td>
<td>30.9 ± 1.0</td>
<td>31.67 ± 2.3</td>
<td>30.8 ± 1.9</td>
</tr>
<tr>
<td>Branch diameter (mm)</td>
<td>3 ± 0.001</td>
<td>3.9 ± 0.2</td>
<td>3.9 ± 0.08</td>
<td>3.8 ± 0.1</td>
<td>3.6 ± 0.2</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>347 ± 56</td>
<td>410 ± 51</td>
<td>475 ± 75</td>
<td>303 ± 22</td>
<td>616 ± 23</td>
<td>186 ± 18</td>
</tr>
<tr>
<td>Hydraulic conductance (g s⁻¹ MPa⁻¹)</td>
<td>0.022 ± 0.004</td>
<td>0.041 ± 0.036</td>
<td>0.057 ± 0.096</td>
<td>0.032 ± 0.006</td>
<td>0.038 ± 0.003</td>
<td>0.102 ± 0.01</td>
</tr>
<tr>
<td>Hydraulic conductivity (g m s⁻¹ MPa⁻¹)</td>
<td>0.008 ± 0.002</td>
<td>0.016 ± 0.002</td>
<td>0.020 ± 0.003</td>
<td>0.010 ± 0.002</td>
<td>0.012 ± 0.007</td>
<td>0.029 ± 0.001</td>
</tr>
<tr>
<td>Hydraulic conductivity per branch transverse area (g m s⁻¹ MPa⁻¹ m⁻²)</td>
<td>993 ± 219</td>
<td>1446 ± 300</td>
<td>1622 ± 356</td>
<td>967 ± 167</td>
<td>1060 ± 155</td>
<td>843 ± 226</td>
</tr>
<tr>
<td>Hydraulic conductivity per leaf area (g m s⁻¹ MPa⁻¹ m⁻²)</td>
<td>0.256 ± 0.036</td>
<td>0.532 ± 0.068</td>
<td>0.572 ± 0.16</td>
<td>0.445 ± 0.07</td>
<td>0.223 ± 0.04</td>
<td>0.308 ± 0.1</td>
</tr>
<tr>
<td>Percent loss of hydraulic conductance (%)</td>
<td>6 ± 0.000</td>
<td>9.8 ± 1.36</td>
<td>9.6 ± 2.8</td>
<td>2.8 ± 0.48</td>
<td>12.3 ± 1.45</td>
<td>15.1 ± 2.35</td>
</tr>
<tr>
<td>Vessel diameter (µm)</td>
<td>30.3 ± 5.0</td>
<td>35.4 ± 3.6</td>
<td>37.37 ± 2.1</td>
<td>34.34 ± 1.7</td>
<td>41.9 ± 3.3</td>
<td>51.45 ± 2.2</td>
</tr>
</tbody>
</table>
Table 3.1 (d). Branch dimension, % loss of conductance due to embolism and conductance and conductivity of terminal branches of evergreen, semi-deciduous and deciduous species in September (2001).

<table>
<thead>
<tr>
<th>September</th>
<th>Evergreen</th>
<th>Semi-Deciduous</th>
<th>Deciduous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. auriculiformis</td>
<td>E. miniata</td>
<td>E. tetrodonta</td>
</tr>
<tr>
<td>Branch length (cm)</td>
<td>30.7 ± 1.12</td>
<td>29.2 ± 0.44</td>
<td>34 ± 1.13</td>
</tr>
<tr>
<td>Branch diameter (mm)</td>
<td>3.2 ± 0.11</td>
<td>3.7 ± 0.17</td>
<td>4.17 ± 0.11</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>284 ± 20</td>
<td>185 ± 11</td>
<td>324 ± 38</td>
</tr>
<tr>
<td>Hydraulic conductance (g s⁻¹ MPa⁻¹)</td>
<td>0.026 ± 0.002</td>
<td>0.054 ± 0.005</td>
<td>0.033 ± 0.006</td>
</tr>
<tr>
<td>Hydraulic conductivity (g m⁻¹ s⁻¹ MPa⁻¹)</td>
<td>0.008 ± 0.001</td>
<td>0.016 ± 0.002</td>
<td>0.011 ± 0.002</td>
</tr>
<tr>
<td>Hydraulic conductivity per branch tranverse area (g m⁻¹ MPa⁻¹ m⁻²)</td>
<td>806 ± 81</td>
<td>1262 ± 270</td>
<td>653 ± 144</td>
</tr>
<tr>
<td>Hydraulic conductivity per leaf area (g m⁻¹ MPa⁻¹ m⁻²)</td>
<td>0.291 ± 0.04</td>
<td>0.894 ± 0.15</td>
<td>0.362 ± 0.08</td>
</tr>
<tr>
<td>Percent loss of hydraulic conductance (%)</td>
<td>9 ± 1.73</td>
<td>11 ± 1.68</td>
<td>13.25 ± 1.9</td>
</tr>
<tr>
<td>Vessel diameter (µm)</td>
<td>31.3 ± 3.0</td>
<td>37.4 ± 2.4</td>
<td>38.4 ± 2.9</td>
</tr>
</tbody>
</table>
Table 3.2 Hydraulic conductance and conductivity of 4 evergreen, 1 semi-deciduous and 1 deciduous tree species from the NT of Australia measured during the late wet season and the early, mid and late dry season. Also shown are the hydraulic conductivities exposed per unit branch transverse area, per unit leaf area, the percentage loss of conductance and average vessel diameter.

<table>
<thead>
<tr>
<th>Species</th>
<th>hydraulic conductance (g s⁻¹ MPa⁻¹)</th>
<th>hydraulic conductivity (g m s⁻¹ MPa⁻¹)</th>
<th>hydraulic conductivity per branch transverse area (g m s⁻¹ MPa⁻¹ m⁻²)</th>
<th>hydraulic conductivity per leaf area (g m s⁻¹ MPa⁻¹ m⁻²)</th>
<th>% loss of hydraulic conductance</th>
<th>vessel diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Evergreen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>0.071 ± 0.005</td>
<td>0.022 ± 0.002</td>
<td>1490 ± 175</td>
<td>0.439 ± 0.041</td>
<td>4 ± 0.55</td>
<td>32 ± 0.87</td>
</tr>
<tr>
<td>May</td>
<td>0.068 ± 0.007</td>
<td>0.017 ± 0.002</td>
<td>1181 ± 101</td>
<td>0.432 ± 0.067</td>
<td>5 ± 0.70</td>
<td>32 ± 0.93</td>
</tr>
<tr>
<td>July</td>
<td>0.048 ± 0.009</td>
<td>0.016 ± 0.003</td>
<td>1097 ± 118</td>
<td>0.468 ± 0.057</td>
<td>7 ± 1.10</td>
<td>33 ± 1.25</td>
</tr>
<tr>
<td>September</td>
<td>0.041 ± 0.004</td>
<td>0.013 ± 0.001</td>
<td>993 ± 96</td>
<td>0.401 ± 0.003</td>
<td>8 ± 1.06</td>
<td>34 ± 1.10</td>
</tr>
<tr>
<td><strong>Semi-deciduous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>0.063 ± 0.008</td>
<td>0.013 ± 0.002</td>
<td>613 ± 107</td>
<td>0.512 ± 0.06</td>
<td>9 ± 0.93</td>
<td>40 ± 2.49</td>
</tr>
<tr>
<td>May</td>
<td>0.060 ± 0.009</td>
<td>0.013 ± 0.002</td>
<td>499 ± 58</td>
<td>0.718 ± 0.05</td>
<td>11 ± 1.59</td>
<td>33 ± 2.81</td>
</tr>
<tr>
<td>July</td>
<td>0.044 ± 0.008</td>
<td>0.014 ± 0.002</td>
<td>1000 ± 130</td>
<td>0.20 ± 0.003</td>
<td>14 ± 1.86</td>
<td>39 ± 2.34</td>
</tr>
<tr>
<td>September</td>
<td>0.037 ± 0.003</td>
<td>0.010 ± 0.005</td>
<td>580 ± 66</td>
<td>0.231 ± 0.027</td>
<td>12 ± 2.0</td>
<td>35 ± 2.33</td>
</tr>
<tr>
<td><strong>Deciduous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>0.149 ± 0.029</td>
<td>0.034 ± 0.006</td>
<td>1061 ± 104</td>
<td>0.374 ± 0.064</td>
<td>10 ± 2.23</td>
<td>51 ± 3.63</td>
</tr>
<tr>
<td>May</td>
<td>0.127 ± 0.015</td>
<td>0.030 ± 0.005</td>
<td>1014 ± 136</td>
<td>0.409 ± 0.065</td>
<td>12 ± 1.66</td>
<td>50 ± 2.22</td>
</tr>
<tr>
<td>July</td>
<td>0.041 ± 0.006</td>
<td>0.013 ± 0.002</td>
<td>843 ± 226</td>
<td>0.708 ± 0.105</td>
<td>15 ± 2.35</td>
<td>50 ± 2.51</td>
</tr>
<tr>
<td>September</td>
<td>0.216 ± 0.0001</td>
<td>0.059 ± 0.005</td>
<td>956 ± 66</td>
<td>0.655 ± 0.065</td>
<td>17 ± 2.93</td>
<td>51 ± 4.68</td>
</tr>
</tbody>
</table>
Fig. 3.2. Percentage loss of hydraulic conductance increased between February (late wet season) and September (late dry season) in (a) four evergreen species and (b) one semi-deciduous species and one deciduous species.
3.3.2 Xylem anatomy and percentage loss in conductivity

Mean vessel diameters of evergreen, semi-deciduous and deciduous species are given in Table 3.1. Mean vessel diameter of the one deciduous species was larger than that all the four evergreen and one semi-deciduous species (Table 3.2, P<0.001). The percentage loss of hydraulic conductance in February, May, July and September increased significantly with vessel diameter across all species. The correlation was strongest early in the year (Fig.3.3).

3.3.3 Vulnerability curves

Hydraulic vulnerability curves for dehydrated branches in the laboratory were constructed for four evergreen species, one semi-deciduous and one deciduous species during September when embolism was likely to be maximal and soil atmospheric water content was very low. Only one deciduous species was selected for this study because the other deciduous species (from chapter 2) exuded a very sticky exudate when branches were excised and this precluded any measurement of conductance. Relationships between percentage loss of conductance and xylem water potential in evergreen and deciduous species are shown in Fig. 3.5. The coefficients determined when equation (1) was fitted to those curves are summarised in Table 3.4.

Two of the four evergreen species (*E. tetrodonta* and *A. auriculiformis*) reached 50% loss of conductance at a xylem water potential of -4.8 MPa to -4.5 MPa, but the one semi-deciduous and one deciduous species and the two remaining evergreen species did not dry to these water potentials (Fig. 3.5). There were differences in vulnerability to water stress-induced embolism, with the deciduous species suffering a larger percentage loss of hydraulic conductance for a given decline in xylem water potentials in the range measured (-1.0 to -2.7 MPa) than the four evergreen and one
Fig. 3.3 The relationship between vessel diameter and percent loss of hydraulic conductance in (a) February, (b) May, (c) July and (d) September.
Fig. 3.4. Percentage loss of hydraulic conductance and in February (wet season), May, July and September (dry season). Vertical bars represent standard error.

Table 3.4. Values of coefficients a and b from exponential sigmoidal equation \( \text{In} \ (100/\text{PLC} - 1) = a\psi - ab \) used to describe the vulnerability curves presented in Fig. 3.4. "b" represents the \( \psi \) at which 50% hydraulic conductance was lost (ie. PLC=50)

<table>
<thead>
<tr>
<th>Species</th>
<th>a</th>
<th>b</th>
<th>( \psi ) at 25% loss of conductance</th>
<th>( r^2 )</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. auriculiformis</em></td>
<td>1.17</td>
<td>-4.5</td>
<td>-3.0</td>
<td>0.73</td>
<td>n=7</td>
</tr>
<tr>
<td><em>E. miniata</em></td>
<td>1.68</td>
<td>-3.5</td>
<td>-2.9</td>
<td>0.50</td>
<td>n=7</td>
</tr>
<tr>
<td><em>E. tetrodonta</em></td>
<td>0.61</td>
<td>-4.8</td>
<td>-2.8</td>
<td>0.63</td>
<td>n=7</td>
</tr>
<tr>
<td><em>M. viridiflora</em></td>
<td>0.88</td>
<td>-4.5</td>
<td>-3.3</td>
<td>0.80</td>
<td>n=6</td>
</tr>
<tr>
<td><em>C. polysciada</em></td>
<td>1.40</td>
<td>-4.0</td>
<td>-3.0</td>
<td>0.96</td>
<td>n=5</td>
</tr>
<tr>
<td><em>P. careya</em></td>
<td>1.10</td>
<td>-2.7</td>
<td>-1.0</td>
<td>0.82</td>
<td>n=7</td>
</tr>
</tbody>
</table>
Fig. 3.5. The relationship between percentage loss of hydraulic conductance due to xylem embolism and water potential in terminal branches of laboratory dried segments of evergreen, semi-deciduous and deciduous species. The data was obtained in 2001 back-transformed from equation (1). (inset: linear Regression of water potential and % loss of hydraulic conductance).
semi-deciduous species. The deciduous species suffered 25% loss of conductance at -1.0 MPa, compared with –3.0 MPa for the semi-deciduous species and –2.8 MPa to –3.3 MPa for the evergreen species.

### 3.4 Discussion

Hydraulic conductance, conductivity, conductivity per branch transverse area and per leaf area all declined as the dry season progressed for all species, even after a pressure flush with acidified water for 30 minutes. Therefore, a modification of xylem structure and possibly increased path length for the water to travel have occurred (Prior and Eamus 2000). Reduced hydraulic conductivity can be caused by xylem cavitation (Tyree and Sperry 1989) or a reduction in vessel diameter or size (Tyree and Ewers 1991), as well as reduced pit pore diameter. Since flushing at 175 kPa removes the emboli I conclude that this is not the cause of the reduced conductance as the dry season progressed.

The values of hydraulic conductance and conductivity of terminal branches of *E. tetrondonta* and *E. miniata* during the dry season were similar to those of saplings measured by Prior and Eamus (2000). Generally, the branch diameters of *A. auriculiformis* and *M. viridiflora* which were smaller than those of the other species. This may cause the conductivity per branch transverse area values to be lower than the other evergreen species. The results suggest that when comparisons among species are made, similar branch diameter should be selected. Percentage loss of hydraulic conductance was higher in the dry season (September, July, May), than the wet season (February).

I was able to measure only deciduous species (*Planchonia careya*) for hydraulic conductance. *Planchonia careya* tended to have higher hydraulic conductivity than the four evergreen species and one semi-deciduous species. However, with only one deciduous species, differences between evergreen and deciduous species could not be demonstrated. The other five species that were used for xylem anatomy (chapter 2 and chapter 4) could not be used for measuring hydraulic conductance because of a sticky sap produced when cut the branches. Some previous work has shown that deciduous species have higher conductance, since they are only active when soil water is readily available, and would be advantaged by wide vessels with a high conductivity. In Venezuela, hydraulic conductance was higher in deciduous species than in evergreen species (Sobrado 1997). However,
Goldstein et al. (1987) found that hydraulic conductivity per leaf area basis in stem segments and in whole trees from Venezuelan was higher in evergreen species than in deciduous species. My result tend to support the Venezuelan findings, but this tendency does not appear universal.

Vessel diameter in *P. careya* was higher than in the four evergreen species and one semi-deciduous species. Wide diameter vessels are more efficient for water conduction and tend to be more vulnerable to embolism than small diameter vessels (Tyree and Sperry 1989, Alder et al. 1996). As would be expected from their greater hydraulic conductivity and wide vessel diameter, the deciduous species are more vulnerable to embolism than evergreen species, due to their wide vessels.

There was a relationship between vessel diameter and percentage loss of hydraulic conductance in this study. The air-seeding mechanism of cavitation suggests that the pit membrane pore size is the critical anatomical feature determining vulnerability to cavitation (Zimmermann 1983, Sperry and Tyree 1988). Vessel diameter and pit membrane pore size tend to be correlated within and individual species. There is a relationship between vulnerability to drought –induced embolism and conduit diameter within one tree (Hargrave et al. 1994), but this does not hold true at the interspecies level (Tyree and Zimmerman 2002). However, in my work there appeared to be a weak relationship between these parameter across species.

In this present study 50% of hydraulic conductivity was lost between -3.7MPa and -4.3 MPa in evergreen species and −2.7 MPa in deciduous species. However, in Venezuelan evergreen savannas trees 50% lost of hydraulic conductivity between -1.5 MPa to −2.4 MPa in three evergreen species and −1.65MPa to −3.82 in four deciduous species (Sobrado 1996, 1997). Thus, there is considerable overlap for the two phenologies, and we need to study more species and data to draw conclusions differences in vulnerability between evergreen and deciduous species.

**Summary and conclusion**

It is likely that the number of species studied was too small and the results were too variable to identify differences in hydraulic conductance between evergreen and deciduous species. In particular, few fully deciduous species were suitable for determination of hydraulic conductance, which generally restricted the ability of the study to compare deciduous and evergreen species. However, the study confirmed
that hydraulic conductance decreases through the dry season, and extended the range of species for which this has been observed. Percentage loss of hydraulic conductance due to xylem embolism increased with mean vessel diameter, in accordance with theory.
Chapter 4: Scanning Electron Microscopy studies of xylem pit membrane pores

4.1 Introduction

The structure of xylem vessels is an important factor in determining the occurrence of water stress-induced embolism (Zimmermann 1983). Xylem vessels are bounded by pit membranes, through which water must pass to move from one vessel to the next, since vessels are not continuous over the entire length of the plant and are interconnected through perforation plates (Plate 4.1). During long distance transport water moves primarily from vessel to vessel or from tracheid to tracheid and pit structure plays an important role in water transport in living plants.

Micromorphological studies illustrate that there is a wide structural variety associated with pits in treachery elements, for instance with respect to pit size, shape and presence of vestures (Jansen et al. 2000). Recent studies highlight the importance of pit membranes in pit function, especially with respect to resistance flow and cavitation vulnerability (Tyree and Sperry 1989, Cochard et al. 1992, Zwieniecki et al. 2001, Becker et al. 2003, Choat et al. 2003 Sperry 2003).

Cavitation induced by water stress occurs through air seeding, when air bubbles are aspirated into water-filled conduits under tension (Zimmermann 1983, Sperry and Tyree 1988, Tyree and Sperry 1989, Jarbeau et al. 1995). The xylem tension required for air seeding to occur is intimately linked to the anatomy of xylem conduits, specifically the structure of the pit membranes that permit flow between adjacent vessels or tracheids (Jarbeau et al. 1995). Water stress-induced embolism is the discrepancy between empirical measurement of pit membrane pore sizes and those calculated from measurements of air seeding pressure differences (Choat et al. 2003)

Vulnerability to drought-induced cavitation has been measured for many plant species and variation in vulnerability among species has been well established (Cochard and Tyree 1990, Cochard, et al. 1992, Sperry et al. 1994, Jarbeau et al. 1995, Choat, et al. 2003).
Plate 4.1. Scanning Electron micrographs tracheary elements in branch (a) longitudinal section of *B. megaphyllus* vessel members with spiral, helical and pitted wall thickening, (b) longitudinal section of *C. polysciada* vessel member with vestured pits on vessel walls.
The vessel wall contains pits that may be simple or bordered. Pit membranes can be considered to act as finely porous filters, allowing the flow of water and nutrients, but at the same time limiting the passage of air bubbles and pathogens between adjacent tracheary elements. Bordered pits occur in vessels and tracheids, and are more complex in structure than simple pits (diagram in chapter 1). A bordered pit consists of a torus in the centre surrounded by a margo or with vested in the centre or non-vestured. The torus acts as a stop-valve preventing the movement of air from an embolised tracheid (Zimmermann 1983). Bordered pits with a torus are a characteristic feature of tracheid pits in conifers (Bauch et al. 1972). In a recent study, (Lancashire and Ennos 2002) showed that resistance to flow through bordered pits on the side walls of tracheids makes up a significant proportion of their total resistance, and that this proportion increased with tracheid diameter.

In conifers, the loss of hydraulic conductance in response to excessive xylem tension, and subsequent cavitation, has been attributed more to the anatomy of the tracheid pit membrane than to conduit size, which varies little in conifers (Sperry and Sullivan 1992, Cochard et al. 1992). The decline in hydraulic efficiency of xylem under high tension results from both cavitation and also from plugging of pit apertures by pressure-displaced torus (Sperry and Tyree 1990).

Vestured pits are defined as bordered pits with the pit chamber or outer pit aperture wholly or partly lined with small projections from the secondary cell wall (Plate. 4.2) (Jansen et al. 1998). Vestured pits are associated with the woods of gymnosperms and angiosperms (Parham and Baird 1974). Vestured pits are regarded as a taxonomic feature characterizing large natural groups including the orders Myrtales (van Vilet and Baas 1984) and Proteales (Cronquist 1988) and are widely found in families Myrtaceae (Leptospermum, Metrosideros, Lophomyrtus and Neomyrtus) ((Meylan and Butterfield 1978), in Eucalyptus regnans (Scurfield et al. 1970) and Proteaceae (Persoonia) (Butterfield and Meylan 1974). The presence of vestures has been associated with prevention of embolism (Carlquist 1982, Carlquist 1983, Carlquist 1988). As Choat et al. (2004) suggested that the presence of vestures would prevent the deflection of pit membranes and reduce the probability of air seeding through pit membranes.

Vesturing of both the pits and the vessel surface may function in embolism control by either preventing it due to the increase of the surface area of the wall and, with this, increased water bonding, or by helping to reabsorb emboli. Vestured pits may
prevent excessive deflection and rupture of the pit membrane resulting from large pressure drops due to an embolism (Zweypfenning 1978). Vesturing on pit membranes also effectively reduces the pore diameter, which also reduces the likelihood of emboli spreading between vessels. A literature survey of 11,843 woody species covering 6,428 genera from diverse climates indicates that the highest frequencies of vested pits occur in deserts and tropical seasonal woodlands (Jansen et al. 2004), environments that are both subject to severe drought stress.

Given that small pit membrane pores are associated with resistance to water-stress induced embolisms, it is likely that evergreen trees, which transport water during the dry season when evapotranspiration is high and soil moisture content is declining, will have smaller pit pores than deciduous trees. Furthermore, evergreen trees may posses features such as vesturing on pit membranes to enhance their drought tolerance. The aim of this study was to investigate pit membrane pore anatomy and size in evergreen, deciduous and coniferous species, from north Australian savannas using electron microscopy.

4.2 Materials and Methods

4.2.1 Study sites and species

Samples for this study were collected from open forest sites in Berrimah, Lee point and Charles Darwin National Park. Seven evergreen and six deciduous or semi-deciduous species (Table 2.1) were selected for study. One of the evergreen Callitris intratropica species was a conifer.
Plate 4.2. SEM photographs of the variation in bordered pits. Bordered pits (a) with non-vestured pits on the vessel wall in *T. ferdinandiana* (b) with a torus at the centre of the tracheid cell wall of *C. intratropica* (c) with vestured pits on the vessel wall of *A. dimidiata.*
4.2.2. Plant materials

Samples were collected from three different trees of each species. From each tree, 3 to 7 mm diameter of branches were collected for determination of pit membrane pore diameter. The specimens were cut to 5 to 7 mm lengths and fixed in a FAA solution. The specimens were cut longitudinally with a sharp razor blade and prepared for scanning electron microscopy. Samples were twice washed in distilled water for 1 hour each and then soaked in oxalic acid (20 mM) and calcium chloride (0.1 mM) for 1 hour and then washed twice in distilled water for 1 hour. Subsequently, the samples were dried at room temperature for 48 hours.

After fixing and washing, samples were then mounted on aluminium stubs and coated with carbon (using a SPI Module carbon coater) followed by a gold-palladium mixture using a SPI Module Sputter coater (Sperry and Tyree 1988). Prepared materials were examined with a JEOL (JSM-5610 LV, Japan) SEM operated at 15 keV. Pit membrane pores were photographed with the SEM and their diameters were measured using NIH Image 1.23 image analysis software. Pits were also examined for vesturing.

4.2.3 Statistical analysis

Differences in pit membrane pores sizes among (i) species and (ii) phenology groups were analysed using ANOVA (Statistica, StatSoft Inc., Tulsa USA, 1999). Linear regression analysis was used to determine the relationship between vessel diameter and pit membrane pore diameter.

4.3 Results

Deciduous species had more damaged pit membrane pores than evergreen species when viewed with SEM. For examples, during samples preparation, I found numerous pit membrane pores damaged by the sectioning knife (Plate. 4.3). Discovering undamaged pit membrane pores was time consuming as the magnification required was high and the instance of undamaged pores was rare. The results involved the measurement of 25 pores in total for each species from three samples with visible membranes and with pores or holes of any type (Plate. 4.4). Pores of pit membranes varied in diameter from almost 0.2 µm in evergreen species (*A. auriculiformis*) to 0.5 µm in deciduous species (*C. arnhemicus*) (Fig. **Plate 4.4**).
4.1). Mean diameter of pit membrane pores in deciduous species (0.44 ± 0.2 µm) was significantly larger (P<0.05) than that of evergreen species (0.25 ± 0.07 µm) (Fig. 4.2). A positive relationship between vessel diameter (using data from chapter 2 as the branches diameter were similar) and pit membrane pore diameter was observed (P<0.0001) ($r^2 = 0.7$) (Fig. 4.3). A positive relationship between pit membrane pores diameter and xylem water potential at 25% loss of hydraulic conductance (using data from chapter 3) was also observed ($r^2 = 0.79$) (Fig.4.4).

There was a variation of bordered pits in this study such as bordered pits with a torus in the centre and bordered pit that were either vestured or nonvestured (Plate 4.2). Vestured pits were found on the vessel walls in six species: *E. tetrodonta*, *E. miniata*, *C. polysciada* and *M. viridiflora* (all Myrtaceae), *P. falcata* (Proteaceae) and *A. auriculiformis* and *A. dimidiata* (Mimosaceae) (Plate 4.5). All evergreen species studied showed vessel pits with vestures. However, nonvestured pits were also common in these species. Most vestures are confined to the pit aperture and chamber. Pit apertures are oval, elongated, slit-like or irregular due to the presence of small vestures. The vestures show a marked difference in the size of the particles of their warty layers (Plate 4.5). However, vestured pits on the vessel wall of *E. tetrodonta* and *E. miniata* were similar (Plate 4.6). Vestured pits were more common in narrow vessels than in wide vessels.

Bordered pits with a torus were present in the coniferous species (*C. intratropica*) which only had small tracheids (Plate 4.7b). Bordered pit diameters were between 10 - 20 µm and pit aperture diameter ranged from between 5 - 8 µm (Plate 4.3). Although many pits on adjacent tracheids were of similar size, there was considerable variation in pit sizes both within the same tracheid and among tracheids (Plate 4.7a).
Plate 4.3. SEM Micrographs of (a) damage to the pit membrane caused by sectioning in *E. miniata*, (b) pit membrane completely removed while preparing samples and an unobscured view of the pit aperture (arrow)
Plate 4.4. SEM micrographs of pit membrane pores (a) pit membrane pores on pit membrane of *C. arnhemicus*, (b) *B. megaphyllus*
Plate 4.5 (a) SEM photographs of vestured pits on the lateral wall of vessels from longitudinal sections of branches of Mimosaceae (a) A. auriculiformis, (b) A. dimidiata, and Proteaceae (c) P. falcata
Plate 4.5(b) SEM photographs of vested pits on the lateral wall of vessels from longitudinal sections of branches of Myrtaceae family (d) *E. tetrodonta*, (e) *E. miniata*, (f) *M. viridiflora* and (g) *C. polysciada*
Plate 4.6. SEM photographs of vestured pits on the vessel wall (a) *E. tetrodonta* vessel with simple perforation plate (x1100, scale bar 10 µm), (b) vestured pits of *E. tetrodonta* (x3500, scale bar 5 µm) (c) *E. miniata* vessel with simple perforation plate (x1100, scale bar 10 µm), (d) vestured pits of *E. miniata* (x3500, scale bar 5 µm).
Plate 4.7. Scanning Electron micrographs of border pits in tracheid of *C. intratropica* (a) tracheids showing uniform bordered pits with pit apertures (small circles in the centre of the border regions) (b) torus can be seen in the centre of the membrane. The surrounding margo has thick radial strands and an interconnecting network of thinner strands.
Fig.4.1. Mean pit membrane pores diameter in evergreen and deciduous species. Full species names are given in Table 2.1. Vertical bars represent standard error.

Fig.4.2. Mean pit membrane pore diameter in evergreen species and deciduous species, showing average pit membrane pore diameter in deciduous was wider than that of evergreen species. Vertical bars represent standard error.(P<0.05)
Fig. 4.3. The relationship between the mean vessel diameter and pit membrane pore diameter in evergreen (●) and deciduous (○) species.

Fig. 4.4. The relationship between pit membrane pore diameter and xylem water potential at 25% loss of hydraulic conductance from the vulnerability curves presented in chapter 3.
4.4 Discussion

Water-stress induced cavitation is caused by air-seeding between inter-conduit pit membranes under high tension (Zimmermann 1983). Thus, pit membrane pore diameters are more important than vessel dimensions in determining susceptibility to embolism (Sperry and Tyree 1990, Sperry and Saliendra 1994). The present study clearly indicated that deciduous species had significantly wider pit membrane pores than evergreen species (Fig. 4.2). Therefore the deciduous species are more likely to be vulnerable to embolism than the evergreen species, because of their larger pit membrane pores.

Previous studies stressed the difficulty in discerning the difference between naturally occurring pores and damage that may occur during sectioning and preparation. In critical-point dried material, the membrane had a flat and featureless appearance, and pore were generally difficult to resolve (Sperry and Tyree 1988). Harvey and Van Den (1997) and Choat et al. (2003) could not detect membrane pores using SEM. Their studies used material that had been dehydrated in the laboratory prior to measurement and this may have contributed to cavitation and loss of pore structure. In air-dried sections, the membrane was tight against the inner wall of the pit, presumably, because of surface tensions developed during dehydration. Pit membranes can be damaged or stretched, after cycles of cavitation and refilling (Hacke and Sperry 2001). By contrast, I used fresh materials, and this may explain why I was successful in being able to view pit membrane pores.

The tracheids of conifers have smaller conduit diameters than do angiosperm vessels, indicating that tracheids should be less vulnerable to water-stress induced embolism than vessels of angiosperms (Sperry and Sullivan 1992, Sperry et al. 1994, Davis et al. 1999). In addition, a more dense and less porous margo would be more effective in holding the torus in the sealing position against air-seeding, but would also afford more resistance to water flow than a more open margo (Sperry and Tyree 1990). The conifer examined in the present study, Callitris intratropica, is often found in rocky areas with skeletal soils, and its survival in such harsh arid environments is likely to be increased by its xylem characteristics. Leaf water potential of Callitris species may be substantially lower than those of co-occurring eucalypts (Clayton-Greene 1983), and their small tracheids are suited to withstand the resulting large pressure gradients in these conifers.
The size of the pit membrane pores that allow air-seeding is probably related to the mechanical properties of the membrane (Hacke and Sperry 2001). Thus, the pores in “relaxed” pit membranes may be smaller than the ones in stretched membranes where air-seeding is occurring. In the present study, a positive relationship between large vessel diameter and large pit membrane pore diameter was observed (Fig. 4.3). Hargrave et al. (1994) found that within a genus, large vessels may have larger pit membrane pores than small vessels. However, Tyree et al. (1994 a) proposed that there is no reason for such a relationship if cavitation is primarily due to air seeding of embolism. However, small pore sizes and small vessel diameters are likely to confer resistance to embolism, and it is noteworthy that evergreen species had smaller pit membrane pores than deciduous species.

Vestures have been suggested to play a functional role in preventing pit membrane rupture by providing mechanical support of the pit membrane and could thereby increase resistance to drought-induced embolism (Zweypfenning 1978). Vestures reduce the probability of air seeding through the pit membrane by preventing deflection of the pit membrane (Choat et al. 2004). Similarly, vestures would permit a greater air-seeding pressure for the membrane conductivity, i.e., a considerable reduction in cavitation resistance after a cavitation-refilling cycle (Hacke and Sperry 2001). However, the properties of pit membranes in non-vestured and vestured pits remain largely unknown.

The present study showed vestured pits on small vessel elements in evergreen species, and non-vestured pits appeared to be more common in wide vessels, although this tendency does not appear to be a general rule. Jansen et al. (2004) found that plant with distinctly developed vestured pits are mainly restricted to warm habitats, such as deserts, seasonal lowland (savannas) and subtropical habitats. Plants in the seasonally dry tropics are likely to be subject to high transpiration rates and large negative xylem pressure (Holbrook et al. 1995, Pockman et al. 1995, Eamus et al. 1999, Prior et al. 1997ab). Drought-induced cavitation has been related to the porosity of pit membranes. When an embolised vessel lies adjacent to a functional vessel under pressure, a substantial pressure difference can develop across a pit membrane that connects this vessels. Gas will penetrate the pit membrane at a critical pressure difference, which may lead to cavitation of the previously water-filled vessel (Tyree and Zimmerman 2002). The presence of vestured pits in evergreen species near Darwin is consistent with Jansen’s findings that vestured pits are common in woody plants of the seasonally dry tropics.
Vestured pits together with small pit pore size are likely to confer drought tolerance to these species by reducing the likelihood of runaway embolism.

Further study is required to determine a greater sample size of species with vested and non-vestured pits. Also should look at different methods to find out to be able to measure pit membrane pores diameter on the dehydrated branches.
Chapter 5 General Discussion and Conclusion

The climate of the Top End of the Northern Territory is dominated by alternating wet and dry seasons, imposed on a background of high year-round temperatures. Consequently native trees of the region have developed a range of responses to this strongly seasonal climate. North Australian savannas have approximately equal number of species (but not standing biomass) in each of four phenological guilds (evergreen, semi-deciduous, brevi-deciduous and deciduous) (Williams et al. 1997). Deciduous species lose all of their leaves every year for 1-3 months or longer (Williams et al. 1997). In contrast, evergreen species retain all or almost all of their canopy through the year.

At the leaf-scale, the physiology of woody species of north Australian savannas is highly seasonal: predawn and mid-day leaf water potentials are higher, and stomatal conductance and carbon assimilation rates are higher during the wet season than in the dry season (Duff et al. 1997, Eamus et al. 1997, Myers et al. 1997, Prior et al. 1997, Eamus et al. 1999). Similarly, Prior (1997) found evidence that whole tree hydraulic conductance decreased as the dry season progressed. Many common tree species of this region are fully or partly deciduous (Williams et al. 1997), and avoid the most severe effects of drought by losing their leaves.

Evergreen trees are generally considered more drought-tolerant than deciduous trees. As they may have a deeper root system and consequently have access to deeper reserves of water in the dry season, and are able to maintain an almost completely full canopy in the dry season and avoid drought (Myers et al. 1997, Sobrado 1986). However, in contrast to this, Chen et al. (2004) observed root biomass was concentrated in the upper layers of the soil profile. Thus, between 77% and 90% of total root biomass of evergreen was found in the upper 0.5 m, with about 5% in the next 0.5 m and 5-15% in the 1-2 m depth range in a tropical savanna of northern Australia. The rapid decline of biomass with depth was because of the steep decline in coarse root biomass as fine root biomass was more evenly distributed with depth (Chen et al. 2004). Concentration of root biomass in the upper 50 cm of soil is generally reported for most woody ecosystems (Snowdon et al. 2000).
The general aim of this project was to study stem anatomy and the hydraulic properties of tree species growing in a northern Australian savanna. Specifically, vessel anatomy in six evergreen, six deciduous species and one coniferous species and the relationship between root biomass and phloem cross sectional area in seedlings were investigated to provide a more detailed understanding of the ecophysiology of tropical deciduous and evergreen species (Chapter 2), and seasonal changes in hydraulic conductance and vulnerability curves between evergreen and one deciduous species (Chapter 3). In addition, SEM studies of pit membrane pore structure were conducted (Chapter 4).

Vessel length of evergreen species tended to be longer than in deciduous species. Evergreen species tend to be taller (hence a longer path for water flow from root to leaf) and may have a smaller vulnerability to xylem cavitation than deciduous species (Sobrado 1997, Eamus and Prior 2001). Furthermore, they tend to have a lower stem hydraulic conductivity (Sobrado 1993, Eamus and Prior 2001), indicative of a smaller xylem (measured as pit membrane pore or xylem diameter). My results show that mean vessel diameter and pit membrane pore diameter were larger in deciduous species than evergreen species, but the vessel length in evergreen species were longer than one deciduous species (chapter 1 and chapter 2). This may explain why deciduous species are more vulnerable to embolism than evergreen species, and are forced to drop their leaves and terminate gas exchange during drought. Trees containing wide vessels and large pit membrane pore diameters are more prone to drought-induced embolism than trees with narrow ones (Salleo and Lo Gullo 1986, Cochard and Tyree 1990; Hargrave et al. 1994, Sperry et al. 1994, Davis et al. 1999). These features are generally associated with large hydraulic conductivities, but I was able to measure this in only one deciduous species because of the presence of a thick, sticky, latex-like exclude that appeared when the branches of many deciduous trees are cut (Eamus, pers com). It is, however, very likely that deciduous species do have larger hydraulic conductance, suggesting that the Huber value (cross sectional area of xylem per downstream leaf area) is lower in deciduous species.

A relatively smaller investment in xylem tissue and roots would be required by trees in which transpiration is restricted to the wet season when water availability is greater. Consequently, a larger allocation of carbon to leaves (higher leaf area ratio) would be expected in deciduous species compared to evergreen species (Prior et al. 2004). In Northern Australia, deciduous species have a higher
photosynthesis rate per unit dry weight than evergreen species (Eamus and Prichard 1998). However, because deciduous species have thinner leaves than evergreen species, there is less leaf tissue per unit leaf area (Eamus and Prichard 1998). The higher photosynthetic rate per unit dry weight of deciduous species results from a larger total investment in leaf N content in deciduous species (Medina and Francisco 1994; Eamus and Prichard 1998). Also, the nitrogen allocation to leaves in deciduous species is larger than in evergreen species.

Zimmermann (1983) postulated that there was a trade-off between xylem efficiency and xylem safety in woody plants. Plants from environments where water is freely available for only part of the year might have large vessels and large root mass to maximise water uptake when water is available. However, plants with wide vessel may be more susceptible to drought-induced embolism than plants with narrow vessels. However, comparisons between sensitivity to embolism and conduit diameters can be misleading (Lo Gullo et al. 1995), because vulnerability to drought-induced cavitation of xylem conduits is influenced by many factors, including pit membrane pore size and the flexibility of the pit membrane (Sperry and Tyree 1990). My results showed that mean vessel diameter, vessel length and pit membrane pore diameter were all larger in deciduous species than evergreen species. These results also showed a strong correlation to the vulnerability to embolism.

Xylem conduit anatomy is often used to explain differences in conductivity and susceptibility to cavitation between species or between individuals (Ewers 1985, Sperry and Sullivan 1992). My results showed a significant correlation between percentage loss of embolism and vessel diameter in evergreen and deciduous species. The air-seeding mechanism of cavitation suggests that water stress-induced cavitation depends on pit membrane pore diameter and then properties, rather than vessel diameter (Zimmermann 1983, Sperry and Tyree 1988). Indeed, a number of other studies, have related pit membrane pore size, measured using SEM (Sperry and Tyree 1988, Sperry et al. 1991, Alder et al. 1996). Some studies stressed the difficulty in discerning the differences between naturally occurring pores and damage caused during sectioning and preparation. It is important to note that the size of pit membrane pores that cause air seedling is probably related to the mechanical properties of the pit membrane (Tyree and Zimmerman 2002; Sperry and Hacke 2004)). The pores in a “relaxed” pit membrane may be considerably smaller than the ones in a stretched membrane where air seeding is actually
occurring (Hacke and Sperry 2001). This may explain why result obtained from SEM studies or micro-bead perfusion studies that examine pit membranes in the relaxed state can find pore sizes that are too small to explain air seedling (Choat et al. 2003).

Vulnerability to water stress-induced embolisms has been related to the porosity of pit membrane by a process known as air seedling (Sperry and Tyree 1988). When an embolised vessel lies adjacent to a functional vessel under pressure, a substantial pressure difference can develop across the pit membrane that connects these vessels. Gas will penetrate the pit membrane at a critical pressure difference (air-seeding pressure), which may lead to cavitation of the previously water-filled vessel (Tyree and Zimmerman 2002). In the present study, I found vestured pits in all evergreen species and bordered pits in the conifer species. Vestures may play a functional role in preventing pit membrane rupture by providing mechanical support of the pit membrane and could thereby increase resistance to drought-induced embolism (Zweypfenning 1978, Choat et al. 2003). Therefore, evergreen species in the present study may have enhanced resistance to embolism compared to deciduous species due to have vestured pits on their xylem wall.

The five evergreen species, one semi-deciduous and one deciduous species examined in this study were predicted to differ in their vulnerability to embolism and in xylem anatomy. My results showed that embolism was low in February (late wet season) May and July, but increased in September (the end of the dry season), as soil moisture decreased. In the present study, the only one deciduous species (P. careya) examined tended to have a higher hydraulic conductivity, and also a larger percentage loss of embolism than the four evergreen species, in agreement with a study of four deciduous and two evergreen trees from a Venezuelan dry forest (Sobrado 1993). Thus, deciduous species were more vulnerable to embolism than evergreen species and developed more embolism than evergreen species. This would be the trigger for leaf fall in the dry season.

Variability in the relationship between water potential and percentage loss of hydraulic conductivity in evergreen and deciduous species was found in the present study. In the evergreen species E. tetrodonta and A. auriculiformis, 50% of the hydraulic conductivity was lost between –4.0 MPa and –4.5 MPa, thus, these species were relatively resistant to xylem embolism. This finding is consistent with Prior and Eamus (2000), who showed that hydraulic conductance of terminal
branches decreased by 50% in E. tetrodonta during the 5 months of the long dry season.

A difference between evergreen and deciduous species vulnerability to embolism could not be shown as I could measure the hydraulic conductance of only one semi-deciduous species and one deciduous species, as well as five evergreen species. The method used to investigate loss of hydraulic conductance in evergreen branches could not be used for deciduous species because of a sticky exudate produced by the cut branches. The relationship between percentage loss of conductance due to xylem embolism and water potential was also highly variable within each of the five evergreen species measured, so that it was not possible to demonstrate significant differences in xylem vulnerability amongst these species. An alternative method such as the acoustic method (Milburn 1973) or the centrifuge method (Pockman et al. 1995), may be better able to establish differences amongst evergreen species, but it is difficult to envisage how the problem of the sticky exudate in deciduous species could be overcome. Other investigators have unsuccessfully attempted to soak and flush the exudate out of T. ferdinandiana branches (Dane Thomas, pers. comm.). The function of this exudate invites speculation; it is possible that it may function as a water absorbing gel and protect vessels from embolism. It may also function to store nutrients, since it contains a high concentration of protein and nitrogen (Dane Thomas, pers. comm.).
Table 5.1. Summary of properties of evergreen and deciduous trees in seasonally dry tropics

<table>
<thead>
<tr>
<th></th>
<th>Evergreen</th>
<th>Deciduous</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydraulic properties</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conductivity</td>
<td>Low</td>
<td>High</td>
<td>Chapter 3</td>
</tr>
<tr>
<td>Vessel length</td>
<td>Long</td>
<td>Short</td>
<td>Chapter 2</td>
</tr>
<tr>
<td>Vessel element length</td>
<td>Short</td>
<td>long</td>
<td>Chapter 2</td>
</tr>
<tr>
<td>Vessel diameter</td>
<td>Small</td>
<td>large</td>
<td>Chapter 2</td>
</tr>
<tr>
<td>Pit membrane pore diameter</td>
<td>Small</td>
<td>Large</td>
<td>Chapter 4</td>
</tr>
<tr>
<td></td>
<td>(vestured)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root system</td>
<td>Large</td>
<td>Small</td>
<td>Chapter 2</td>
</tr>
<tr>
<td><strong>Leaves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leafspan</td>
<td>Long</td>
<td>Short</td>
<td>Prior et al. (2003)</td>
</tr>
<tr>
<td>Max $A_{mass}$</td>
<td>Low</td>
<td>High</td>
<td>II</td>
</tr>
<tr>
<td>Max $N_{mass}$</td>
<td>Low</td>
<td>High</td>
<td>II</td>
</tr>
<tr>
<td>Max $P_{mass}$</td>
<td>Low</td>
<td>High</td>
<td>II</td>
</tr>
<tr>
<td>Thickness</td>
<td>Thick</td>
<td>Thin</td>
<td>II</td>
</tr>
<tr>
<td>Mass per area</td>
<td>Large</td>
<td>Small</td>
<td>II</td>
</tr>
</tbody>
</table>
Stem diameter was a good predictor of dry leaf weight and root weight and phloem cross sectional area. In seedlings, there was a good relationship between stem diameter and phloem cross section area in *E. teterodonta*, but not for other species. Knowledge of root biomass is critical for understanding trade-offs between root and shoot carbon allocation. It would, therefore, be desirable to predict root biomass from easily observable parameters such as stem diameter. Stem diameter is easy to measure and it is possible to make reliable assessments of stem diameter in mature trees in the field, but accurate field estimates of total root biomass are very difficult (Eamus *et al.* 2002).

There are a number of attributes of evergreen and deciduous species that contribute to long term survival. For example, deciduous trees generally maintain larger nitrogen content (per unit dry weight) than evergreen species (Medina and Francisco 1994, Reich *et al.* 1992). The specific leaf area (leaf area divided by leaf dry weight) of evergreen species is generally smaller than that deciduous species (Reich *et al.* 1992, Eamus and Prichard 1998), reflecting the more sclerophyllous nature of evergreen species. In simple terms, deciduous trees take advantage of favourable wet season conditions by producing thin leaves with high nutrient concentrations, that have high rates of photosynthesis per unit mass. Because transpiration occurs only during the early wet to early dry seasons, they do not need to invest as many resources in root mass or in their water transport system, and photosynthetic return is maximised during this time. By contrast, evergreens have a lower return during the wet season on carbon invested in leaves, stems and roots, but the return is continuous throughout the year.

**Conclusion**

There were clear differences in xylem anatomy between evergreen and deciduous species. Vessel length, diameter and diameter of pit membrane pores were all consistently larger in deciduous species, while vessel density was small. These differences are consistent with those found for evergreen and deciduous species in cool temperate areas of the Northern Hemisphere, supporting the idea that these features are characteristic of the deciduous strategy of rapid photosynthesis during the favourable season to compensate for the shorter life-span of their leaves.
References


124


