EFFECTS OF TREE AGE AND SIZE ON GROWTH, PHYSIOLOGY AND WATER USE OF *ACER PSEUDOPLATANUS* L. AND *FRAXINUS EXCELSIOR* L.

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Specially dedicated to my family:

My beloved wife and sons

My parent and parents-in-law, sisters and brothers-in-law

And close friends….

Thank you

ABSTRACT

Forest growth is important both economically and ecologically and it follows a predictable general trend with age. Generally, the growth of all forests accelerates as canopies develop in young forests and declines substantially soon after maximum leaf area is attained. The causes of this decline trend are multiple. Initially, age- and size-related decline was attributed to photosynthesis-respiration imbalance. Subsequently, several competing hypotheses have been proposed over the years although nutrient and hydraulic limitation hypotheses appear to be the most likely.

However, age and size are normally coupled during growth. Therefore, an experimental manipulation has been adopted to separate the effects of size from those of age by using traditional grafting techniques. Genetically identical grafted seedlings were produced from scions taken from trees of four different age classes of two species, ranging from 4 to 162 years of age. The aim of this study is to investigate the effects of tree age and tree size on growth, physiology and water use of two broadleaf species by conducting three major experiments. Growth characteristics, such as relative growth rate and growth efficiency were measured, together with leaf-level gas exchanges and sap flow studies. Comparisons were established among results observed in the field with the ones obtained in the grafted seedlings.

The results showed that relative growth rate and growth efficiency decreased substantially with increasing age of donor trees in the field. In contrast, these parameters seemed almost constant on grafted seedlings, i.e., scions taken from donors with different meristematic ages did not show the age-related trend after they were grafted onto the rootstocks. Similar patterns were also observed in net photosynthesis from leaf-level gas exchange and sap-flow-based parameters for both species. In general, these results suggested that size limitation to water and nutrient transport to the top of the canopy is a primary cause that triggered the decline in production of photosynthate and reduced growth of the trees, and/or increase in maintenance respiration with increasing in tree size rather than controlled by meristematic age.

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TABLE OF CONTENT

Page

CHAPTER 1: INTRODUCTION

1.1 GENERAL BACKGROUND 1
1.2 JUSTIFICATION 3
1.3 AIM OF STUDY 6
1.4 GENERAL OBJECTIVES 7
1.5 GENERAL HYPOTHESES 7
1.6 LITERATURE REVIEW 8
  1.6.1 Forest trees development and ageing 8
  1.6.2 Age- and size-related regulation of net primary production 8
    1.6.2.1 Respiration hypothesis 9
    1.6.2.2 Nutrient limitation hypothesis 10
    1.6.2.3 Hydraulic limitation hvpothesis 12
    1.6.2.4 Maturation hypothesis 14
  1.6.3 Macropropagation approach 16
    1.6.3.1 The techniques 16
    1.6.3.2 Grafting studies 17
  1.6.4 The study site 20
  1.6.5 The study species 22
    1.6.5.1 Acer pseudoplatanus L. 22
    1.6.5.2 Fraxinus excelsior L. 24
1.7 THESIS STRUCTURE 25
REFERENCES 28

CHAPTER 2: AGE- AND SIZE-RELATED CHANGES IN MORPHOLOGY AND GROWTH OF *ACER PSEUDOPLATANUS* AND *FRAXINUS EXCELSIOR* SPECIES

2.1 INTRODUCTION 38
2.2 MATERIALS AND METHODS 40
  2.2.1 Field Study 40
    2.2.1.1 Sampling 40
    2.2.1.2 Diameter, height growth and specific leaf area measurement (SLA) 40
    2.2.1.3 Total leaf area, tree age and sapwood area 41
    2.2.1.4 Tree aboveground biomass, absolute mass growth rate (AGR_{mass}), relative mass growth rate (RGR_{mass}) and growth efficiency (E_G) 42
  2.2.2 Grafting Study 45
    2.2.2.1 Grafted seedling preparation 45
    2.2.2.2 Diameter and height growth, leaf area (A_L), specific leaf area (SLA) and leaf number (L_N) 46
    2.2.2.3 Absolute mass growth rate (AGR_{mass}), relative mass growth rate (RGR_{mass}), growth efficiency (E_G), total biomass (M_T) and root to shoot ratio (Rt:St) 47
  2.2.3 Data analyses 49
2.3 RESULTS 49

2.3.1 Growth characteristics of the donor trees 49
2.3.2 Growth characteristics of the grafted seedlings 54
2.3.3 Relationships between growth characteristic and age in donor trees and grafted seedlings 57
2.4 DISCUSSION 61
REFERENCES 64

CHAPTER 3: AGE- AND SIZE-RELATED CHANGES IN PHYSIOLOGICAL CHARACTERISTICS OF *ACER PSEUDOPLATANUS* AND *FRAXINUS EXCELSIOR* SPECIES

3.1 INTRODUCTION 67
3.2 MATERIALS AND METHODS 68
3.2.1 Field gas exchange, water potential, nitrogen content and $\delta^{13}C$ 68
3.2.2 Grafted seedling gas exchange, nitrogen content and $\delta^{13}C$ 70
3.2.3 Photosynthetic efficiency and capacity of grafted seedling 71
3.2.4 Data analyses 74
3.3 RESULTS 74
3.3.1 Leaf level gas exchange, leaf water potential, nitrogen content and $\delta^{13}C$ of the donor trees 74
3.3.2 Leaf level gas exchange, leaf water potential, nitrogen content and $\delta^{13}C$ of the grafted seedlings 79
3.3.3 Photosynthetic capacity and efficiency 82
3.3.3 Relationship between some physiological parameters and leaf properties in donor trees and grafted seedlings 85
3.4 DISCUSSION 87
REFERENCES 92

CHAPTER 4: THE EFFECT OF AGE AND SIZE IN WHOLE PLANT TRANSPIRATION, HYDRAULIC CONDUCTANCE AND STOMATAL CONDUCTANCE OF *ACER PSEUDOPLATANUS* AND *FRAXINUS EXCELSIOR* SPECIES

4.1 INTRODUCTION 97
4.2 MATERIALS AND METHODS 102
4.2.1 Field study 102
4.2.1.1 Sap flow measurement on donor trees 102
4.2.1.2 Whole plant transpiration (Q_L), canopy stomatal conductance (G_s) and whole tree leaf specific hydraulic conductance (K_L) of donor trees 105
4.2.1.3 Meteorological data 106
4.2.2 Grafting Study 106
4.2.2.1 Stomatal conductance (G_s) versus vapour pressure deficit (D) 106
4.2.2.2 Whole plant transpiration rate (E_L) and whole plant leaf-specific hydraulic conductance (K_L) 107
4.2.3 Data Analysis 108
4.3 RESULTS 109

4.3.1 Variation of sap flow density per unit leaf area (Q_L) and canopy stomatal conductance (G_s) among age classes in the field 109
4.3.2 Age-related trends in sap flow density (Q_L), canopy stomatal conductance (G_s) and whole plant leaf-specific hydraulic conductance (K_L) among tree ages in the field 113
4.3.3 Stomatal conductance (G_s), whole plant transpiration (E_L) and whole plant leaf-specific hydraulic conductance (K_L) among age classes in grafted seedlings 115
4.4 DISCUSSION 118
REFERENCES 122

CHAPTER 5: TESTING THE SUITABILITY OF GRAFTING IN STUDIES AIMED AT SEPARATING THE EFFECTS OF AGE AND SIZE IN TREE GROWTH

5.1 INTRODUCTION 127
5.2 MATERIALS AND METHODS 129
5.2.1 Measurement of hydraulic conductance of grafted seedlings using High Pressure Flow Meter (HPFM) apparatus 129
5.2.2 Growth and physiological characteristics of air-layered plants 131
5.2.2.1 Sample collection and preparation 131
5.2.2.2 Diameter, height and relative growth rate 134
5.2.2.3 Leaf-level gas exchange, transpiration rate (E_L) and leaf-specific hydraulic conductance (K_L) 134
5.2.3 Data analyses 135
5.3 RESULTS 137
5.3.1 Hydraulic resistance and conductivity of the grafted seedlings 137
5.3.2 Growth and physiological characteristics of air-layered plants of *A. pseudoplatanus* 139
5.4 DISCUSSION 147
5.4.1 Comparisons between rootstock (RS), self-grafted (SG) and grafted seedling (GS) 147
5.4.2 Hydraulic conductance of grafted seedling stems 150
5.4.3 Growth and physiology of air-layered plants from two age classes ... 152
REFERENCES 155

CHAPTER 6: GENERAL CONCLUSION 157
APPENDIX 161
VITA 174

LIST OF TABLES

Table		Page
Table 1.1:	Studies reporting a comparison of net photosynthesis in differently aged trees and shrubs.	3
Table 1.2:	Summary of results showing contrasting trends involving grafted scions.	5
Table 2.1:	Characteristics of *A. pseudoplatanus* and *F. excelsior* donor trees used in this study. Mean attributes for each age class in both species used in growth measurements.	42
Table 2.2:	Number of grafted seedlings survived in both species.	47
Table 2.3:	Summary of analysis of variance on growth parameters of *A. pseudoplatanus* and *F. excelsior* donor trees.	50
Table 2.4:	Summary of analysis of variance on growth parameters of *A. pseudoplatanus* and *F. excelsior* grafted seedlings after two growing seasons.	55
Table 2.5:	Summary of growth characteristics of grafted seedling across age classes in both species after two growing seasons.	56
Table 3.1:	Characteristics of *Acer pseudoplatanus* and *Fraxinus excelsior* grafted seedlings used in this study. Mean attributes are based on the sample size of n = 10 for each age class in both species (except in age class 3 of *A. pseudoplatanus* where n = 7) over two growing seasons.	71
Table 3.2:	Summary of ANOVA of leaf-level gas exchange, *SLA* and leaf chemical composition in *A. pseudoplatanus* and *F. excelsior* donors.	75
Table 3.3:	Summary of ANOVA of leaf-level gas exchanges and chemical compositions in *A. pseudoplatanus* and *F. excelsior* grafted seedlings.	80
Table 3.4:	Summary of leaf level gas exchange parameters, leaf water potential and chemical composition of grafted seedlings and controls in both species.	81
Table 4.1:	Summary of main characteristics of donor trees used in this experiment for both species	103
Table 4.2:	Summary of main characteristics of grafted seedlings of both	108

species used in this experiment.

Table 5.1: Summary of main characteristics of *A. pseudoplatanus* (N=16) and *F. excelsior* (N=20) grafted seedlings used in this study. 130

Table 5.2: Some characteristics of *A. pseudoplatanus* air-layered plants used in this study (both N=9). 135

Table 5.3: Summary of ANOVA for stem hydraulic conductance of *A. pseudoplatanus* and *F. excelsior*. 137

Table 5.4: Results of leaf level gas exchange, transpiration rate and leaf-specific hydraulic conductance on the two age classes of *A. pseudoplatanus* air-layered plants. The values presented in the table are mean and standard error with significant level at $p<0.05$. 144

LIST OF FIGURES

Figure **Page**

Figure 2.1: Mean values of A_L:A_S of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied. 50

Figure 2.2: Leaf area to sapwood area ratio (A_L:A_S) plotted against tree height for both species. The *** indicates $p<0.001$. 51

Figure 2.3: Mean values of *SLA* of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied. 51

Figure 2.4: Mean values of AGR_{mass} of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied. 52

Figure 2.5: Mean values of RGR_{mass} of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied. 53

Figure 2.6: Mean values of E_G of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied. 53

Figure 2.7: Regression analyses between specific leaf area (*SLA*) and age of each individual donor tree and grafted seedling. The bar indicates standard error, ns = not significant ($p>0.05$), *=$p<0.05$, **=$p<0.01$ and ***=$p<0.001$. 58

Figure 2.8: Relationship between relative mass growth rate (RGR_{mass}) and age of each individual donor tree and grafted seedling. The bar indicates standard error, ns = not significant and ***=$p<0.001$. 59

Figure 2.9: Relationship between growth efficiency (E_G) and age of each individual donor tree and grafted seedling. The bar indicates standard error, ns = not significant and ***=$p<0.001$. 60

Figure 3.1: The mean values of net photosynthesis (A), internal CO_2 (B), stomatal conductance (C) and leaf water potential (D) of both species. The different letters represent statistically significant 77

differences across age classes.

Figure 3.2: The mean values of specific leaf area (A), nitrogen content (B) and carbon isotope composition (C) of both species. The different letters represent statistically significant differences across age classes. 78

Figure 3.3: The mean values of photosynthetic efficiency and capacity of both species over two growing seasons. The different letters represent statistically significant differences across age classes. 84

Figure 3.4: The relationship between individual net photosynthesis and their respective specific leaf area across age classes in *A. pseudoplatanus* and *F. excelsior* donor trees and grafted seedlings. 85

Figure 3.5: The relationship between individual nitrogen content and specific leaf area of *A. pseudoplatanus* and *F. excelsior* across age classes in donor trees and grafted seedlings. 86

Figure 3.6: The relationship between individual plant leaf nitrogen and net photosynthesis of *A. pseudoplatanus* and *F. excelsior* across age classes in donor trees and grafted seedlings. 86

Figure 3.7: The relationship between maximum photosynthesis and maximum carboxylation rate with their respective specific leaf area across age classes in *A. pseudoplatanus* and *F. excelsior* grafted seedlings. 87

Figure 4.1: Typical diurnal sap flow density in three age classes of *A. pseudoplatanus* and *F. excelsior* species. 110

Figure 4.2: Typical diurnal patterns of four meteorological variables obtained from weather station. 111

Figure 4.3: The relationship between vapour pressure deficit (D) and mean sap flow per unit leaf area (Q_L). Logarithmic regressions were carried out for each age class in both species. 112

Figure 4.4: The relationship between individual tree age and sap flow (A) and hydraulic conductance (B). A linear function was used to fit the data for each parameter. The '*' represents significant difference at $p<0.05$ and 's' represents significance at $p<0.10$. 114

Figure 4.5: The relationship between individual grafted seedling stomatal conductance (G_S) with vapour pressure deficit (D) in each age class of *A. pseudoplatanus* (A) and *F. excelsior* (B). 115

Figure 4.6: The mean values of G_s (A), E_L (B) and K_L (C) among age 117

classes of grafted seedling observed in *A. pseudoplatanus* (•) and *F. excelsior* (•).

Figure 5.1: Means of hydraulic conductivity per unit length between stem and scion sections in each age class of *A. pseudoplatanus*. 138

Figure 5.2: Means of hydraulic conductivity per unit length between stem and scion sections in each age class of *F. excelsior*. 139

Figure 5.3: Mean diameter of *A. pseudoplatanus* air-layered plants of two age classes taken over four sampling times since the initial measurement. Bars indicate standard error. 140

Figure 5.4: Relative diameter growth rate of *A. pseudoplatanus* air-layered plants on two age classes. Same capital and small letters represent no significant difference in first and second measurement respectively. 140

Figure 5.5: Mean height of *A. pseudoplatanus* air-layered plants of two age classes taken over four sampling times since the initial measurements. Bars indicate standard error. 141

Figure 5.6: Relative height growth rate of *A. pseudoplatanus* air-layered plants on two age classes. Same capital and small letters represent no significant difference in first and second measurement respectively. 141

Figure 5.7: Relative diameter growth rate of *A. pseudoplatanus* air-layered plants observed in podded and non-podded plants. Same capital and small letters represent no significant difference in first and second measurement respectively. 142

Figure 5.8: Relative height growth rate of *A. pseudoplatanus* air-layered plants observed in podded and non-podded plants. Same capital and small letters represent no significant difference in first and second measurement respectively. 142

Figure 5.9: Relationship of stomatal conductance with mean diameter (A) and mean height (B), and net photosynthesis with mean diameter (C) and mean height (D) across two age classes. The '**' indicates significant correlation at $p<0.01$, and '*' indicates significant correlation at $p<0.05$. 145

Figure 5.10: Relationship of transpiration rate with total diameter (A) and total height (B), and leaf-specific hydraulic conductance with total diameter (C) and total height (D) across two age classes. The '***' indicates significant correlation at $p<0.001$, and '**' indicates significant correlation at $p<0.01$. 146

Figure 5.11: RGR_{mass} in rootstock (RS), self-grafted (SG) and combination of grafted seedlings from four age classes (GS) in both species. 148

Figure 5.12: E_G in rootstock (RS), self-grafted (SG) and combination of grafted seedlings from four age classes (GS) in both species. 149

Figure 5.13: Net photosynthesis rates in rootstock (RS), self-grafted (SG) and combination of grafted seedlings from four age classes (GS) in *A. pseudoplatanus* over two growing seasons (2003 and 2004). 149

Figure 5.14: Net photosynthesis rates in rootstock (RS), self-grafted (SG) and combination of grafted seedlings from four age classes (GS) in *F. excelsior* over two growing seasons (2003 and 2004). 150

Figure 5.15: Relationship between hydraulic resistance to tip and distance from tip of branches taken from old and young trees in the field, and old and young grafted seedlings of *A. pseudoplatanus* (source: *Petit et al.* in prep) 154

LIST OF PLATES

Plate		Page
Plate 4.1:	Granier-type probes showing heated sensor (left) and unheated sensor (right).	104
Plate 4.2:	Insulation sheets were applied around the stem where sensors were installed to protect them from rainfall and convective heat losses.	105
Plate 5.1:	Graft union of *A. pseudoplatanus* (A) and *F. excelsior* (B), 30 months after grafting.	131
Plate 5.2:	Air-layered branches of age class 2 (left two) and age class 4 (right two) after being cut from the donor trees.	133
Plate 5.3:	Air-layered plants produced flowers and pods in spring.	133
Plate 5.4:	Air-layered plants were assigned in a complete randomised design and rotated every three weeks.	134
Plate 5.5:	The pots of air-layered plants were covered by black plastic bags in order to measure water use by gravimetric method.	136
Plate 5.6:	The pot of air-layered plants was weighed using a dual-face Sartorius bench-top scale in early morning and at midday. Leaf water potentials were also measured at both times.	136
Plate 5.7:	Air-layered plants of age class 2 (left two) and age class 4 (right two) after eight months transplanted into pots.	143

LIST OF SYMBOLS AND ABBREVIATIONS

AC1	Age class 1
AC2	Age class 2
AC3	Age class 3
AC4	Age class 4
AGR_{mass}	Absolute mass growth rate in kg yr^{-1}
A_{L}	Leaf area in m^{2}
A_{max}	Saturated maximum photosynthesis in μmol m^{-2} s^{-1} or μmol g^{-1} s^{-1}
A_{net}	Net photosynthesis in μmol m^{-2} s^{-1} or μmol g^{-1} s^{-1}
A_{S}	Sapwood area in m^{2}
C_a	Ambient CO2 concentration in μmol mol^{-1}
C_i	Internal CO_2 in μmol mol^{-1}
D	Vapour pressure deficit in kPa
DBH	Diameter at breast height in cm
$\delta^{13}C$	Carbon isotope discrimination
E_{G}	Growth efficiency in kg m^{-2} yr^{-1}
E_{L}	Whole plant transpiration
G_{S}	Stomatal conductance and canopy stomatal conductance in mmol m^{-2} s^{-1}
J_{max}	Maximum electron transport in μmol m^{-2} s^{-1}
K_{L}	Whole plant leaf-specific hydraulic conductance in mmol m^{-2} s^{-1} MPa^{-1}
N_{m}	Nitrogen content
Q_{L}	Sap flow density/whole plant transpiration in kg m^{-2} day^{-1}
Q	Light level
R_{graft}	Hydraulic resistance of graft union segment in %
R_{scion}	Hydraulic resistance of scion (stem without graft union) in %
R_{stem}	Hydraulic resistance of entire stem (stem with graft union) in %
$RDGR$	Relative diameter growth rate in mm mm^{-1} week^{-1}
RGR	Relative growth rate
RGR_{mass}	Relative mass growth rate in kg kg^{-1} yr^{-1}
$RHGR$	Relative height growth rate in cm cm^{-1} week^{-1}
$V\mathrm{c}_{\mathrm{max}}$	Maximum carboxylation rate in μmol m^{-2} s^{-1}
Ψ	Water potential in -MPa
Ψ_{leaf}	Leaf water potential in -MPa
Ψ_{midday}	Midday water potential in -MPa
Ψ_{predawn}	Predawn water potential in -MPa
Φ	Apparent quantum efficiency in μmol μmol^{-1}

CHAPTER 1

INTRODUCTION

1.1 GENERAL BACKGROUND

The importance of forests for moderating levels of atmospheric carbon dioxide and in the control of international political climate change processes have created a timely need for reliable and transparent estimates of carbon budgets of forests. Fixed carbon is the source of almost all energy for life and the foundation of food webs, thus influence most ecological processes. Net ecosystem productivity, the net exchange of carbon between the ecosystem and the atmosphere, results from the balance between carbon uptake through photosynthesis and carbon release through respiration and decomposition. The rate at which carbon is sequestered or mobilized from forest ecosystems is of particular interest to forest researchers and managers in light of society's concern over increasing levels of greenhouse gases (particularly CO_2) in the atmosphere.

Forest management practices and activities were one of the main issues discussed during the recent convention on climate change in Kyoto, Japan (Rotter and Danish 2000). The Kyoto protocol, which was designed to mollify the current rise in atmospheric CO_2, suggested managing forests to maximise carbon uptake and sequestration and that nations be responsible for quantifying their carbon sequestration. The potential for carbon sequestration in intensively managed forests is promising under a situation where higher ambient CO_2 concentrations provide a carbon fertilization effect for growing trees through increased water use efficiency, decreased photorespiration during gas exchange and carbon fixation, and higher biomass accumulation over shorter rotation lengths (Groninger *et al.* 1999, Nilsen and Orcutt 1996). In intensively managed forest ecosystems, forest researchers and managers are currently studying the effects of varying silvicultural and harvesting practices on the carbon dynamics of sites differing with respect to vegetation type, age and productivity. Increases in productivity can further enhance the role of forests as a potential carbon sink if a portion of that increased productivity can be incorporated into the soil organic carbon pool (Kirschbaum 2000). However, forests also have the potential to serve as a net source of carbon if they are deforested and lose soil organic carbon (Harmon *et al.* 1990, Rustad *et al.* 2000).

Carbon assimilation through photosynthesis and subsequent carbon dynamics provides the mechanism by which the energy available from solar radiation is converted into substrates useable by all other forms life. Within forest ecosystems, changes in carbon assimilation can have drastic effects on ecosystem metabolism (Waring and Running 1998). Therefore, it is important to understand the constraints on carbon assimilation. Essentially, refining knowledge of the interactions between tree physiology and ecological mechanisms is important in defining management strategies of forest ecosystems.

Numerous studies have highlighted factors that directly or indirectly control photosynthetic carbon gain (net primary production) associated with sapling growth or tree age. For example, a leaf-level impediment to photosynthetic carbon gain would be diminishing stomatal conductance with decreasing water potential (Conroy *et al.* 1986). Stand and leaf level mechanisms may interact to cause decreases in assimilation; thus, net primary production is the result of the interaction of extrinsic (environmental) and intrinsic (physiological) factors (Day *et al.* 2002). Environmental factors consist of all normal biotic, climatic and edaphic factors that influence tree growth. Each of these can interact with each other and concurrently they all can interact with genetic effects. Age- and size-related changes also cannot be examined separately from these effects. According to Day *et al.* (2002), age-related changes in morphology and physiology are likely the result of complex interactions among 'extrinsic', 'intrinsic' and 'intrinsic-extrinsic' pathways, and these interactions may be further complicated by influence of 'stimulus-response' pathways controlled by environmental variables. In order to minimise the influence of both external and internal factors, a common-rootstock approach using scions from juvenile and reproductively mature donors has been proposed as suitable experimental method. Further explanations and the outcomes from such study will be discussed afterward.

1.2 JUSTIFICATION

The production of biomass by both individual trees and forest stands decreases substantially with age (Assmann 1970). Age-related reductions in biomass accumulation is an important consideration in mechanistic models that predict forest growth and determine the capacity of forests to act as carbon sinks. With the current impetus towards silvicultural approaches that employ uneven-aged systems and extended rotations, understanding the physiological basis for age-related decline in productivity of dominant species has became increasingly important. Understanding this component of productivity is crucial to quantifying and manipulating carbon fluxes in forest ecosystems and their influence on global CO_2 cycles.

Despite many studies attempting to explain age-related decline of forest productivity through a number of theories, age-related regulation still remains unclear. There is little direct evidence that old forests assimilate less carbon than young forests. This makes model-based predictions of carbon sequestration suspect, as most physiological process models predict carbon assimilation based on measurements of photosynthesis on young trees (Ryan *et al.* 1997a). For example, Bond (2000) provided a list of studies exhibiting two contrasting results in the changes in net photosynthesis with age of trees and shrubs (Table 1.1).

Table 1.1: Studies reporting a comparison of net photosynthesis in differently aged trees and shrubs.

Decreased net photosynthesis (A_{net}) with increased ages of trees and shrubs		
Species	**Comparison**	**Reference**
Chrysothamnus nauseosus	Maximum A_{net} in summer. Juvenile *versus* mature.	Donovan and Ehleringer (1992)*
Juniperus occidentalis	Juvenile *versus* young mature.	Miller *et al.* (1995)*
Larrea tridentata	Daily maximum A_{net}, three summer months. Juvenile *versus* mature.	Franco *et al.* (1994)*
Picea abies	Light-saturated A_{net}, open-grown trees. Juvenile *versus* mature.	Kull and Koppel (1987)*
Picea rubens	Seasonal A_{net}. Mature *versus* relatively old-growth.	Day *et al.* (2001)

Table 1.1 (continued)

Pinus aristata	Light-saturated A_{net}, optimal conditions. Mature *versus* old-growth.	Schoettle (1994)*
Pinus contorta	Light-saturated A_{net}. Mature *versus* old-growth.	Yoder *et al.* (1994)*
Pinus ponderosa	Light-saturated A_{net}. Mature *versus* old-growth.	Yoder *et al.* (1994)*
Pinus ponderosa	Mean morning A_{net}. Juvenile *versus* mature and old-growth.	Kolb and Stone (2000)*
Prosopis glandulosa	Daily maximum A_{net}, after rainfall. Juvenile *versus* mature.	DeSoyza *et al.* (1996)*
Prunus serotina	Growing season average A_{net}. Seedling *versus* sapling *versus* mature.	Fredericksen *et al.* (1996)*
Sequoiadendron giganteum	Maximum A_{net}. Seedlings *versus* juvenile, mature and old-growth.	Grulke and Miller (1994)*

No difference or increased net photosynthesis with increased ages of trees and shrubs

Species	Comparison	Reference
Acer negundo	Maximum A_{net}, during peak of drought period. Juvenile *versus* mature.	Donovan and Ehleringer (1992)*
Artemesia tridentata	Maximum A_{net}, in mid-summer at a dry site. Juvenile *versus* mature.	Donovan and Ehleringer (1992)*
Chrysothamnus nauseosus	Maximum A_{net}, in mid-summer at a dry site. Juvenile *versus* adult.	Donovan and Ehleringer (1992)*
Prosopis glandulosa	Daily maximum A_{net}. Summer drought. Small (0.5 m) *versus* large (1.1 m).	DeSoyza *et al.* (1996)*
Pseudotsuga menziesii	No clear trend of light-saturated A_{net}. Mature *versus* old growth.	McDowell *et al.* (2002)
Quercus rubra	Light-saturated A_{net}, during growing season. Seedling *versus* mature.	Hanson *et al.* (1994)*

Note: * = cited from Bond (2000).

The causes of this age-related decline are likely multiple, as discussed previously. But the most important potential causes that can explain a decline in forest productivity with stand development are nutrient limitation, hydraulic limitation and maturation (genetic programming) hypotheses. In this thesis, we focus on the last one. According to some authors, the so called "age-related" decline in forest productivity is primarily a "size-related" decline (Weiner and Thomas 2001).

Ideally, a test should be conducted on trees of similar size but different ages or *vice versa*, thereby separating the confounded factors of size and age. An alternative to obtain trees with similar size but different ages is to graft scions of different ages onto young rootstocks.

Table 1.2: Summary of results showing contrasting trends involving grafted scions.

Species	**Result**	**Reference**
Hedera helix	Increased light-saturated A_{net} with increased ages of scions. Juvenile *versus* mature scions grafted to juvenile rootstock.	Bauer and Bauer (1980)
Larix laricina	Height and diameter growth decreased with increased ages of scions. Juvenile *versus* mature scions grafted to juvenile rootstock.	Greenwood *et al.* (1989)
Larix laricina	Total chlorophyll increased with increased ages of scions. Juvenile *versus* mature scions grafted to juvenile rootstock.	Greenwood *et al.* (1989)
Larix laricina (indoor-grown trees)	Increased light-saturated A_{net} with increased ages of scions. Juvenile *versus* mature scions grafted to juvenile rootstock.	Hutchison *et al.* (1990)
Larix laricina (outdoor-grown trees)	No trend observed in light-saturated A_{net} with increased ages of scions. Juvenile *versus* mature scions grafted to juvenile rootstock.	Hutchison *et al.* (1990)
Larix laricina	Increased xylem diameters with increased ages of scions. Juvenile *versus* mature scions grafted to juvenile rootstock.	Takemoto and Greenwood (1993)
Picea rubens	Decreased A_{net} with increased ages of scions. Juvenile *versus* mature scions grafted to juvenile rootstock.	Rebbeck *et al.* (1993)
Picea rubens	Decreased light-saturated A_{net} with increased ages of scions. Mature *versus* old scions.	Day *et al.* (2001)
Picea taeda	Diameter and height growth, leaf area, branch numbers and scion biomass decreased with increased scion ages. Juvenile *versus* young scions grafted to juvenile rootstock.	Greenwood (1984)
Pinus radiata	Diameter and height growth and branch numbers decreased with increased scion ages. Juvenile *versus* mature scions.	Sweet (1973)
Pseudotsuga menziesii	Diameter and height growth, branch numbers and branch length decreased with increased scion ages. Juvenile *versus* young scions grafted to juvenile rootstock.	Ritchie and Keeley (1994)

Studies using grafting have been conducted by a few researchers (as summarised in Table 1.2) with regard to the effect of age on growth, morphology and physiology of grafted scions. However, these studies still showed contrasting outcomes with scion age. Despite the presence of these grafting studies, there are some weaknesses that have never been tackled by previous researchers. For instance, no study so far has compared simultaneously individuals in the field and genetically identical grafted seedlings. Recently, Day *et al.* (2001) have conducted a similar study, but the individuals selected in the field were not genetically identical with the grafted seedlings (although they came from the same population). Moreover, most of the published evidence on grafting has been obtained with very young trees. With regard to the studies presented in Table 1.2, for instance, Hutchison *et al.* (1990) used individuals taken from four age classes ranging from 1 to 45 years whereas Rebbeck *et al.* (1993) only used juvenile and mature (>50 years old) scions. In addition, Greenwood (1984) studied shoot development as a function of age on *Picea taeda* but the comparisons were only made among scions up to 12 years of age. Furthermore, Greenwood *et al.* (1989) also used grafting approach to study the effect of age on morphological characteristics and DNA methylation of *Larix laricina*, but the scions involved were taken from juvenile and mature trees ranging from 1 to 74 years in age. Because our primary objective is to assess the relative roles of size and age in affecting growth reductions in older forests, we decided to expand the existing literature by enlarging the range of ages employed.

1.3 AIM OF STUDY

The overall aim of this study is to add to our understanding of the age- and size related trends on tree growth at the individual level. In other words, how the age and size of trees affect the pattern of growth and how they change the morphological and physiological characteristics of tree species and what factors (i.e. genetic or/and environment control) exactly take place as trees grow taller and older.

1.4 GENERAL OBJECTIVES

1. To compare age- and size-related effects on growth, physiology and water use of *Acer pseudoplatanus* L. (sycamore) and *Fraxinus excelsior* L. (ash) trees.
2. To establish any correlation between age or size and selected morphological and physiological attributes.

1.5 GENERAL HYPOTHESES

Age- and size-related declines in forest productivity have received significant attention from many researchers recently. From these, many hypotheses have been developed and some of them were used in our study. By doing grafting and air layering, we tried to go one step further and test whether maturation (related to age) and hydraulic limitation (related to size) hypotheses are applicable in our study. We hypothesised that age factors (maturation) will not have significant impacts on physiological attributes, but will mostly affect significantly morphological attributes of trees studied. On the other hand, physiological attributes will be affected by differences in size (hydraulic limitation hypothesis). To prove that, we compared the morphological and physiological characteristics of donor trees from different age classes in the field and their grafted scions grown in the glasshouse. We also tested specific hypotheses related to grafting technique whereby, a) There is no effect of graft union on plant hydraulic conductance despite initial disturbance on the xylem structure, and b) There is no complication introduced by the presence of two different genotypes (the rootstock and the scion).

1.6 LITERATURE REVIEW

1.6.1 Forest tree development and ageing

Development and ageing including variation at the leaf level results from the interaction of genetic and environmental controls. It involves differential gene expression and activation together with the effects of biotic and abiotic factors. At the tree level, maturation and ageing are generally thought to be intimately braided. However, the process of maturation which strongly affects plant structure and function and initiates the diversion of resources from vegetative to reproductive growth is thought to be triggered by a shift in hormonal patterns possibly of endogenous genetic origin. In contrast, the age-related decline in net primary productivity (NPP) is generally assumed to be controlled by exogenous factors. A number of theories have been proposed suggesting nutrient or hydraulic limitations, increased respiration or shifts in biomass allocation as possible mechanisms (Magnani and Jarvis 2000). Decline in nutrient availability with stand development may result in increased allocation belowground or decreasing photosynthesis (Gower *et al.* 1996, Ryan *et al.* 1997a). In addition, the influence of genetically based maturational changes in tree meristems on age-related declines in productivity should not be neglected. Such changes could be purely maturational or mediated through physiological pathways related to tree size or external environmental cues (Day *et al.* 2001).

1.6.2 Age- and size-related regulation of net primary production

Observations have showed that aboveground net primary productivity negatively correlates with tree age for both individual trees and single cohort stands (Assmann 1970, Whittaker 1975, Bormann and Likens 1979, Harcombe *et al.* 1990). Long-term studies on even-aged forests showed that maximum bole increment rate occurs shortly after crown closure and declines as trees reach maturity (Assmann 1970). Yoder *et al.* (1994) suggested that age-related declines in photosynthetic rates for lodgepole and ponderosa pine may be a significant cause of net production decreases in old trees. This is supported by studies of age-related differences in photosynthetic rates in Scots pine (Kull and Koppel 1987), bristlecone pines (Schoettle 1994), and hybrid Englemann x white x Sitka spruce (Richardson *et al.* 2000). However, photosynthetic rates in eastern larch have been reported to be higher in grafts from older trees (Hutchison *et al.* 1990).

Explanations for these age-related differences in photosynthetic rates are generally centred on multiple constraints on carbon assimilation. Among the potential constraints on carbon assimilation are genetic-down regulation of photosynthesis, reductions in whole-tree leaf area per unit biomass, nutrient based limitations on photosynthetic capacity and reductions in hydraulic conductance, together with changes in micrometeorological factors such as temperature, light and water availability. A few hypotheses have been developed based on aforementioned constraints such as the respiration hypothesis, the nutrient limitation hypothesis, the maturation hypothesis and the hydraulic limitation hypothesis. In the respiration hypothesis, for instance, the allometry of trees must be such to support the vertical mass and to resist the bending forces from wind, which increase rapidly with tree height. But these support systems require considerable investment in non-photosynthetic tissues, which increase maintenance respiration. Meanwhile, in the nutrient limitation hypothesis, growth in older trees is limited by nutrient supply with nutrients being tied up in living plants and soil litter. Moreover, the hydraulic limitation hypothesis is based on the increase in tree height which may increase hydraulic constraints that limit gas exchange in older or taller trees. Another explanation may be due to ontogenetic changes (maturation hypothesis) that contribute to reduced growth in older trees.

1.6.2.1 Respiration hypothesis

Before the links between respiration and biosynthesis started to become clear in the 1950s, respiration was considered to be an imperfection in the mechanisms which convert substrates into structural dry matters (Lambers 1985). Since then, a rapid expansion of knowledge has taken place. Gradually it became of interest to establish the quantitative relationship between substrate use and processes such as growth and maintenance of plants and plant parts under different environmental conditions (Lambers 1985). The earliest attempt to measure the total respiration of a forest stand was reported by Jensen (1932). Yoda *et al.* (1965) estimated the total aboveground wood respiration of a tree by sorting all shoot parts into diameter classes and multiplying the biomass in each class with the average respiration rate for that class.

For years, many scientists assumed that the most important constraint to large size is the maintenance cost required by living biomass in very large stems and roots systems (Bond 1998). This respiration consumes the photosynthate and thus leaves less for new growth in large trees. Hence, this hypothesis is based on the relative importance of various photosynthate sinks and the observed declines in the ratio of photosynthetic to non-photosynthetic tissues as trees and stands age. Over the years, researchers have expressed respiration rates on the basis of different measures of plant size. Rates expressed on a weight (Yoda *et al.* 1965, Walters *et al.* 1993) or volume (Tranquillini and Schütz 1970, Lavigne *et al.* 1996, Ryan *et al.* 1996) bases decline, while surface area based rates (Tranquillini and Schütz 1970, Lavigne *et al.* 1996) increase with increasing diameter.

Carbon may be allocated away from aboveground growth and toward respiration (Yoda *et al.* 1965), to defend against pathogens or insects, reproduction (Ryan *et al.* 1997b, Becker *et al.* 2000) or belowground growth (Grier *et al.* 1981, Gower *et al.* 1996, Magnani *et al.* 2000). However, tests of the respiration hypothesis in lodgepole pine (Ryan and Waring 1992) and eucalyptus (Ryan *et al.* 2003) have failed to support it. This hypothesis has also been weakly supported by modelling studies (Magnani *et al.* 2000, Mäkelä and Valentine 2001).

1.6.2.2 Nutrient limitation hypothesis

The nutrient limitation hypothesis has been supported by some previous studies and refuted by others (Ryan *et al.* 1997a). As forests age, nutrients may become scarce due to sequestration in biomass and necromass (Gower *et al.* 1996, Ryan *et al.* 1997b). Such scarcity may lead to reductions in nitrogen allocation to thylakoid membranes and enzymes, thereby reducing photosynthetic capacity. This is because photosynthetic capacity is strongly correlated with leaf nitrogen content across a wide range of plant species (Field and Mooney 1986, Pearcy *et al.* 1987, Lambers *et al.* 1990). However, this relationship may be complicated by partitioning between various photosynthetic system and non-photosynthetic components (Evans 1989), and the occurrence of nitrogenous osmoregulatory and storage substances (Sarjala *et al.* 1987; Margolis and Vezina 1988; Lavoie *et al.* 1992; Billow *et al.* 1994). Reich and Schoettle (1988) suggested that photosynthetic response may be more strongly linked to the interaction of nitrogen and phosphorus content than to the former element alone. However, Ryan and Waring (1992), Yoder *et al.* (1994) and Mencuccini and Grace (1996b) found no significant age-related differences in total foliar nitrogen content for chronosequences of *Pinus contorta*, *P. ponderosa* and *P. sylvestris*. However, their analyses were limited to first year foliage. Numerous investigations have shown that foliar nitrogen content is inversely related to leaf age (Lehto and Grace 1994, Field 1983, Field and Mooney 1983, Matyssek 1986, Lang *et al.* 1987). In addition, lower nutrient availability may lead to increased allocation of photosynthetic products to root production especially in older stands and therefore to decreased allocation to aboveground structures (Ryan *et al.* 1997a). Grier *et al.* (1981) found that the allocation to fine roots was dramatically greater in an old rather than in an adjacent young *Abies amabilis* forest. Similar results have also been reported for a *Pinus elliottii* stand where fine-root biomass was greater in a mature stand than in a younger stand (Gholz *et al.* 1982).

Aboveground net primary productivity (ANPP) may decline during stand development due to decreasing availability of nutrients, particularly nitrogen. Generally, nitrogen mineralization and nitrification rates decrease during secondary succession of forests (Vitousek *et al.* 1989). The decline is strongly correlated with litter decomposition which, in turn, is controlled by environmental conditions together with chemical and physical characteristics of litter (Gower *et al.* 1996). Stand-age effects may contribute in two ways. First, the ratio of leaf to woody detritus input (with low and high C:N ratios, respectively) gradually decreases during stand development. Second, the accumulation of woody litter as stands age will slow decomposition due to its low surface area:volume ratio compared with fine litter (Landsberg and Gower 1997). The reductions in litter quality during stand development increase nitrogen immobilization during litter decomposition, which in turn decreases net nitrogen mineralization as stand age (Davidson *et al.* 1992, Hart *et al.* 1994).

1.6.2.3 Hydraulic limitation hypothesis

The hydraulic limitation hypothesis proposes that leaf-specific hydraulic conductance (K_L) declines as trees grow taller, resulting in decreased carbon assimilation ((Ryan and Yoder 1997, Bond and Ryan 2000). Yoder *et al.* (1994) and Ryan and Yoder (1997) proposed this hypothesis to explain the results of their study on ponderosa pine, which indicated that photosynthesis was limited in older trees relative to younger individuals by reductions in midday stomatal conductance (G_s). This hypothesis is based on older trees having lower hydraulic conductivity in the water path between roots and shoots due to longer or more complex hydraulic pathways. Due to this lower conductivity, stomata of older trees show greater sensitivity to evaporative demand and more tightly regulate transpiration to minimize the potential for xylem embolism (Ryan and Yoder 1997). The rate of xylem water flow is determined by Darcy's law (Tyree and Ewers 1991, Margolis *et al.* 1995), in which flow rate is directly proportional to cross-sectional area of the transmitting structure (sapwood xylem), its permeability, and the water potential gradient. As conductivity is equivalent to the combination of area and permeability terms, a decrease in this combined parameter will require an increase in water potential gradient to maintain constant flow. If xylem conductance is lower in older (larger) trees, a critical water potential for stomatal closure will be reached more rapidly than in younger (smaller) trees, as evaporative demand increases.

Studies of tree hydraulic architecture provide evidence that potential xylem flux decreases with tree size. Darcy's law further states that flow is inversely related to pathway length. Thus, maintaining an equal flow to leaves at greater distance from a root absorption point, as in larger trees, requires either an increase in conductivity or water potential gradient. Leaf specific conductivity (*LSC*) is commonly used as a measure of the ability of a particular section of stem or branch to supply water to more distal leaves, and values for this parameter have been found to be relatively constant along the length of tree stems (Tyree and Ewers 1991). However, Ewers and Zimmerman (1984a, b) have found leaf specific conductivity values to be much lower in branches, strongly influenced by branch diameter and order, and subject to significant restriction at branch-stem junctions. Although stem leaf specific conductivity values, if strictly height-determined, would not be expected to differ greatly between younger and older trees of the same height, the longer branches typical of older crowns may provide significantly reduced leaf specific conductivity values.

McDowell *et al.* (2002) found that hydraulic conductance decreased by 44% as tree height increased from 15 to > 32 m, and showed a further decline of 6% with increasing height. Sensitivity analyses based on Darcy's Law to quantify the extent to which compensating mechanisms buffer hydraulic limitations to gas exchange indicated that without the observed increases in the soil-to-leaf water potential differential ($\Delta\Psi$) and decreases in the leaf area/sapwood area ratio, K_L would have been reduced by more than 70% in the 60-m trees compared with the 15-m trees, instead of the observed decrease of 44%. However, compensation may have a cost, for example, the greater $\Delta\Psi$ of the largest trees was associated with smaller tracheid diameters and increased sapwood cavitation, which may have a negative feedback on K_L and G_s as well.

Several studies indicate that larger trees are capable of altering their hydraulic architecture to compensate for the longer, more complex pathways between roots and foliage (Becker *et al.* 2000b, Bond and Ryan 2000, Mencuccini and Magnani 2000). These are summarized in a critique of the hydraulic limitation hypothesis by Becker *et al.* (2000b). Critics of the hydraulic limitation hypothesis have pointed out the various mechanisms by which plants compensate for hydraulic limitation, as evidence that the increased height and structural complexity of old trees are unlikely to constrain hydraulic conductance and hence assimilation. Although tapered xylem conduits may buffer hydraulic resistance from path length effects (West *et al.* 1999, Becker *et al.* 2000a), such buffering appears to be overdriven by hydraulic constraints in tall trees. Further, other buffering factors such as greater water storage in sapwood (Becker *et al.* 2000b) and increased xylem permeability to water flow during tree ageing (Mencuccini and Magnani 2000) may compensate to some extent for long path lengths for water movements in tall trees.

In addition, several attempts to provide direct evidence in support of the hydraulic limitation hypothesis by experimental manipulation have been unsuccessful. Hubbard *et al.* (1999) girdled young lodgepole pine trees to reduce leaf specific conductivity and removed foliage from older trees to increase it, but found that neither manipulation significantly changed stomatal conductance or photosynthetic rates. A similar study, in which foliage of old Douglas-fir was enclosed in plastic bags to reduce transpiration, and thus leaf specific conductivity, was also unsuccessful at increasing gas exchange rates of uncovered foliage on the same branches (Brooks *et al.* 2000).

1.6.2.4 Maturation hypothesis

Genetic regulation of photosynthesis has not been specifically proposed in the literature, nor are there data testing this hypothesis. However, dramatic changes in morphological and physiological attributes of foliage, including photosynthetic capacity, have been described for numerous species during their early development and have been attributed to different challenges to growth and survival at various life-stages (Greenwood 1984, Hackett 1985, Rebbeck *et al.* 1992, Greenwood and Hutchison 1993). Although little is known about age-related trends in foliar attributes beyond reproductive maturity or mid-age, there is some evidence that supports a concept of continuing change. Richardson *et al.* (2000) reported that changes in foliar morphology and gas exchange attributes continue past mid-age in hybrid Englemann x white x Sitka spruce. Similar trends in foliar attributes have been described for Norway spruce (Kull and Koppel 1987), and in needle morphology of Sitka spruce (Steele *et al.* 1989). However, none of these studies have directly addressed the potential for ontogenetic changes in meristematic tissue as a contributing mechanism to age-related declines in productivity, nor did they separate age from size experimentally.

The contribution of maturation-related changes in meristem behaviour to age-related decline in forest productivity is still poorly understood. While changes in morphological and physiological attributes associated with transition from juvenile to reproductively-mature phases have been described for many woody species, few studies have examined maturational changes that occur after the onset of reproductive development (Greenwood 1989, Day *et al.* 2001 and Day *et al.* 2002). In any case, common rootstock studies, in which scions are donated by juvenile and mature trees, hint at the possibility that differences in morphological and physiological traits associated with those life-stages are inherent in meristems. Such studies have been carried out on loblolly pine (Greenwood 1984), eastern larch (Greenwood and Hutchison 1989), red spruce (Rebbeck *et al.* 1992, Day *et al.* 2001).

A field study conducted by Day *et al.* (2001) on *Picea rubens* Sarg. demonstrated significant age-related trends in foliar morphology, including decreasing specific leaf area (*SLA*) and increasing needle width, projected needle area, and needle width-to-length ratio. Similar trends were also apparent in foliage from the grafted trees with different scion ages. Both *in situ* foliage and shoots resulting from grafted scions from the oldest cohort showed significantly lower photosynthetic rates than their counterparts from younger trees; however, differences in stomatal conductance and internal CO_2 concentrations were not significant. They concluded that lower rates of photosynthesis contribute to age-related decline in the productivity of older red spruce and that decline in photosynthetic rates result from non-stomatal limitations. In addition, a study where scions from juvenile and mature red spruce were grafted onto common rootstock (Rebbeck *et al.* 1992) clearly showed that maturation-related changes in meristems of red spruce persist for at least two years after grafting. In their results, scions from mature trees exhibited lower stomatal conductance and net photosynthetic rates than those from juvenile donors.

In addition, Emebiri *et al.* (1998) and Hutchison *et al.* (1990) have implicated a genetic basis for ontogenetic changes by identifying differential patterns of gene expression related to life-stages. However, pathways by which genetic changes in meristems and/or the foliage they produce affect photosynthetic rates have not been described. A possible pathway by which ontogenetic changes in meristems may affect photosynthetic rates is by producing tissue in older trees that has an inherently lower growth rate. Shoots resulting from grafts of meristems from older trees have lower growth rates than scions from young trees. This has been shown for radiata pine (Sweet 1972), loblolly pine (Greenwood 1984), eastern larch (Greenwood *et al.* 1989, Takemoto and Greenwood 1992) and red spruce (Rebbeck *et al.* 1992). Takemoto and Greenwood (1992) speculated that the older meristems may be weak sinks for resources compared to shoots arising from scions taken from younger trees. Weak sinks for carbohydrate, due to reduced growth, can result in feedback limitations to photosynthesis (Stitt 1990). Leverenz (1981) suggested that sink strength limitations may explain differential photosythetic capacity among branches in the crowns of mature Douglas-fir.

1.6.3 Macropropagation approach

1.6.3.1 The techniques

Macropropagation is one of the vegetative propagation methods that has long been recognised and applied mostly in fruit trees and ornamental trees or shrubs. In forestry, this technique is used mainly in clonal propagation to solve problems related to genotype preservation. The common techniques used in macropropagation are grafting, rooting of cutting and layering.

Grafting, in which rootstock and scion are joined to create a composite tree, provide a method of vegetative reproduction in which desired components of the scion are preserved. As the components of the scion are preserved, the memory of their donor trees is directly preserved as well, which may confer benefits that a seedling lacks, such as increased reproductive precocity, increased disease resistance and may reduce tree height (dwarfing) (Hartmann *et al.*, 1997). This technique however has some disadvantages such as the involvement of different genotypes in order to produce a single plant or tree, scion and rootstock incompatibility that reduce the likelihood of grafting success or may alter the physiological condition of scion, and the fact that a tree or plant may be reinvigorated so that the reproductive precocity may be slowed down.

The simplest method of propagating a tree asexually in alternative to grafting is rooting of cuttings. A cutting (a piece of the parent plant) is cut and stuck into the soil or medium. Artificial rooting hormones are sometimes used to ensure success. If the cutting does not die of desiccation first, roots grow from the buried portion of the cutting which then becomes a complete plant. This technique can be used in mass production of desired trees or plants. However, this technique only works well for some plants and most trees are unsuitable to this method.

A refinement on rooting is by layering. This is rooting a piece of a branch that is still attached to its parent and continues to receive nourishment from it. The branch is severed only after it has successfully grown roots. Like grafting, this technique may also increase the reproductive precocity by taking branches from the old donors. This technique carries a few advantages over grafting such as stem characteristics (xylem anatomy) can be maintained by propagating big and lengthy branches. In addition, the size of propagated plants can be determined in advance compared to grafting and rooting of cutting where instead the new stem (new xylem) usually develops from a tiny bud. This technique however does not work as well as rooting of cuttings on most trees.

We used grafting in our study since it has the ability to successfully produce a reasonable amount of grafted seedlings for our selected species. In this study, we employed a side grafting method using different scion ages of two broadleaves species, i.e. *Acer pseudoplatanus* and *Fraxinus excelsior*. Since this technique involved two genotypes (rootstock and scion), we were aware of potential side effects. Hence, we also employed an air-layering technique to make sure we had enough information before making any conclusion on the relative roles of age and size for our trees.

1.6.3.2 Grafting studies

Grafting was known to Chinese and Greeks (Aristotle and Theophrastus) thousand years ago (Roberts 1949, Shen 1980). Renewed interest in grafting during Renaissance increased knowledge of grafting practices. By the 16th century, grafting techniques in Europe were performed with increased attention to cleanliness and proper mechanics of grafting, including matching layers of cambium to each other, resulting in greater success in the production of grafted plants (Hartmann *et al.* 1997). Fundamentally, many contemporary grafting techniques are the same as those reported by Bailey (1891) in the latter 19th century. This technique involves two genotypes of a same species one each from rootstock and scion. The rootstocks provide ready roots that can supply water and nutrients to the scions before and after the graft union heal. However, successful grafting is dependent on the compatibility of rootstock genotypes and scion genotypes. Incompatibility between rootstocks and scions is sometime the cause of the failure or deterioration of the graft union that possibly leads to tree death (Moore and Walker 1981). Incompatibility may not be expressed at the time the graft union is undergoing the initial healing, but deterioration of union connections can take years to occur (Hartmann *et al.* 1997).

The relative importance of rootstock genotypes has been evaluated in various tree species via grafting studies (e.g. Rom and Carlson 1987, Jayawickrama *et al.* 1991). These classic studies, wherein different genotypes or species are grafted interchangeably, have sometimes revealed substantial influence of rootstock on growth performance of scions (*c.f.* Rom and Carlson 1987). In a comprehensive review of rootstock effects in grafting of conifers, Jayawickrama *et al.* (1991) found that in most studies, there were no large effects of rootstock on scion growth and these tend to diminish over time (e.g., Sylvertsen *et al.* 1997). This generally holds for *Pinus taeda* L. (Jayawickrama *et al.* 1997), although in a few studies, substantial effects of rootstock upon growth were found (Schmidtling 1991). Variation in height among different rootstocks of *P. taeda* was positively correlated to foliar potassium concentrations, but not to other foliar nutrients (Schmidtling 1991).

In addition, Mohr and Schopfer (1995) stressed that grafting experiments are a classical approach for studying genetic effects of shoots and roots on plant growth. Although the objectives of most of the studies have been tied to a specific agronomic or horticultural interest, a few studies have been designed to test the fundamental aspect of root-shoot interactions. Many explanations are possible for the lack of root-shoot dependency, but one aspect may relate to the age and source of the grafted plant material. In this study, physiologically mature scions were grafted onto genetically similar and juvenile seedlings as rootstock. Thus, the variation among rootstock plants was less than that within each scion, and so the small effects of rootstock are not surprising. Hence, the traits are more closely related to the genotype of the scion (parent tree). As an example, in Knight's Observations (1795), the grafted trees of apple and pear were found to have inherited all the diseases of the parent trees although they had been re-grafted a few times.

A specialized variation of the typical grafting method is that of reciprocal grafting of very young plants. Allen (1967) employed this technique to assess effects of species as rootstock in the three major southern pine species: loblolly, slash, and shortleaf. Although he included intraspecific grafts, the intraspecific genetic variation was unknown. No treatments were imposed, rather, height growth was assessed annually for five years. The study did reveal evidence that rootstock can affect scion growth, at least for grafts across species.

In studies conducted on *P. radiata* L., *P. taeda* L., *Larix laricina* (Du Roi) K. Koch and *Pseudotsuga menziesii* (Mirb) Franco, growth of grafted scions decreased with increasing age of the donor ortet (Sweet 1973, Greenwood 1984, Greenwood *et al.* 1989, Hutchison *et al.* 1990, Ritchie and Keeley 1994). This reduction in growth was said to be associated with changes in foliar morphology and physiology. Hutchison *et al.* (1990) found that specific leaf area decreased in both species whereas chlorophyll content and net photosynthesis increased in *L. laricina* with increasing maturation. Moreover, in a study related to age and size effects on foliar morphology and physiology, Day *et al.* (2001) found that the age-related trends were retained after three growing seasons in grafted seedlings of *Picea rubens* Sarg. In contrast, Vanderklein *et al.* (*in press*) and Mencuccini *et al.* (2005) found no age-related morphological and physiological trends in *Pinus sylvestris* L., *Acer pseudoplatanus* L., and *Fraxinus excelsior* L. grafted seedlings and a poplar hybrid (*Populus balsamifera* L. ssp. *trichocarpa* and *P. deltoids* Bartr. Ex Marsh) rooting of cuttings.

The conflicting results found in the studies above are puzzling and may suggest either: a) that the relative importance of age and size is species-specific; b) that there are confounding factors which are unaccounted for. For instance whether there is any hydraulic restriction in graft union that influence those results. There was evidence to suggest that the vigour of the rootstock onto which a scion was grafted influenced the scion hydraulic capacity. This occurred independently of conductance changing simply in relation to rootstock-induced changes in stem diameter or supported leaf area. Measurements of stem hydraulics across a combined series of resistances, from the rootstock shank, through the graft union, to the scion, showed that conductance was related to rootstock vigour (Atkinson *et al.* 2001). In addition, Nakano *et al.* (2004) found that compatible graft unions showed higher hydraulic conductance than incompatible grafted unions between peach cultivars and *Prunus tomentosa* Thunb. (Nanking cherry). However, this result was based on different species connection in grafting. Published reports showed considerable tissue disorganisation, particularly within the xylem. These results suggest that the graft union is a region of low hydraulic conductance, relative to the scion in particular, and this may influence the movement of substances in the xylem such as ions, water and plant growth regulating hormones. The experiments reported here describe measurements made with a high-pressure flow system designed to determine *in situ* hydraulic conductance of relatively large stem sections incorporating the graft union (diametric) of two-year-old trees (Atkinson *et al.*, 2003).

1.6.4 The study site

The study site was located in Cramond, in the western part of Edinburgh (55°58’42”N, 3°16’09”W). The study was conducted in a community woodland comprising two dominant species, i.e., *Acer pseudoplatanus* L. (sycamore) and *Fraxinus excelsior* L. (ash) that belongs to Edinburgh City Council under the area of Cramond Village.

Historically, the derivation of Cramond’s name is *Caer Amon*, meaning a fortified place on the River Almond, reflecting the Roman occupation of the area. Cramond contains evidence of the first sites of human settlement in Lothian with recent excavations producing finds such as flint tools, indicating a Middle Stone Age encampment from *c.5000BC.* It is also likely that there was settlement from the Bronze Age as revealed by the discovery of stone burial cists and plough marks. It is thought that the Romans took possession of Cramond in the early 140's AD and established an outpost fort associated with the defensive Antonine Wall located across Scotland. The Roman occupation of Cramond lasted only until 165 AD but it was reoccupied in 208AD as one of three forts in Scotland acting as a rearward depot and supply base for punitive campaigns further north. The Romans finally withdrew from Scotland by 215 AD (*c.f.*, Edinburgh City Council 2005).

Cramond is located in the River Almond Valley which, with its tributaries, is a major landscape feature of the west of Edinburgh. The Almond valley is generally a steep incised valley of sheer and exposed rock faces. To the west, the land rolls up to a local ridge that is reinforced by the woodland plantations on the Rosebery Estate, with its predominant land cover of arable ground and areas of improved pasture. The mixed woodlands, shelterbelts and mature parkland trees follow the localised ridgelines and break down the otherwise open and exposed coastal landscape. The woodlands extend down to and into the Almond Valley itself. This boundary is very rural and open, forming part of the wider Green Belt context that surrounds Edinburgh to the north. The ridgeline provides enclosure to the north-west end of the conservation area as it curves to meet the River Almond. East of the Valley, the land rises more gently to a ridge and a localised high point just to the north of Cramond Bridge.

To the north, the Forth provides an open and natural boundary to the conservation area. Its nature changes between high and low tides ranging from high water to mud flats with geological exposures concentrated to the east and west of Cramond. It is possible to make out the rolling landscape and the mature trees associated with the Estate landscapes right along the coastline. The Cramond Conservation Area consists of clearly defined areas. Although these are physically and historically related, they have distinctive structural parts with varied and contrasting characteristic and spatial patterns.

Cramond Village includes the main village consisting of tightly knit vernacular cottages clustered around the harbour and also the Kirk Cramond area that contains the Roman remains, Cramond Tower and the planned development of Cramond House, Cramond Manse and the Old Schoolhouse. All of which are individual sites of historic, architectural and archaeological interest set in open landscape. A significant proportion of the conservation area is natural environment with the secluded and heavily wooded River Almond valley that contains the surviving relics of the mills.

In the Cramond village, the land rises steeply from the river resulting in a terraced form of development. To the east of the village, this steep slope is covered in trees. There is evidence that this area supports remnants of original woodland planting, as there are a number of mature oak and pine trees enclosing Cramond Tower. The remainder of the area has been disturbed with car parks and archaeological excavation. As a result the area is subject to regeneration of trees such as *A. pseudoplatanus* and *F. excelsior* with a strong under storey of shrubs and ground flora (*c.f.,* Edinburgh City Council 2005). Most of *A. pseudoplatanus* trees in Cramond woodland were found in an area close to the coast at the bottom of the steep slope. Meanwhile, *F. excelsior* trees, especially the older ones, were found at the top of the steep slope.

1.6.5 The study species

1.6.5.1 Acer pseudoplatanus L.

Acer pseudoplatanus, locally known as Sycamore belong to the Aceraceae family. In Britain *A. pseudoplatanus* is considered the invader, i.e., an aggressive naturally-regenerating non native species. Both ecological and historical evidence support the view that the species was introduced around the 15th century. The native range of *A. pseudoplatanus* does not extend as far as the Channel, the North Sea or Atlantic coast, but its introduction and spread has been recorded throughout the coastal areas from Brittany to Norway (Harris 1987). This species remained rare around houses and in hedges throughout the 17th and 18th Centuries when nursery records show stocking and sale of young sycamores (Bleay 1987). However, it was not extensively planted until the late 18th Century (Jones 1944). At that time sycamore was especially popular in amenity planting of some ancient parks and was planted with many other exotics for a classical effect (Mabey 1980) and it is said that this practice encouraged its spread (Pennington 1969). The first British records of this species originate from Scotland. In Scotland the first Gaelic name for sycamore ('the Plinntriinn') was first referred to in 1772 suggesting that the tree was not common enough prior to that date to warrant a name (Fergusson 1878). In 1842, a tree near Dunblane was described as 440 years old (Binggeli 1994). In the Lothian Region of Scotland, this species constitutes 18.4% of the total number of trees in residential areas, 15.3% in lowland rural and 5.5% in upland rural areas. It is the commonest species except in upland areas where soils are poorly drained (Good *et al.* 1978).

This deciduous species can be grown up to 35 m in height with the life span between 400 to 600 years. The growth of the species at seedling stage can be enhanced by the ideal associations with mycorrhizae (Weber and Claus 2000). This species can thrive well in light (sandy), medium (loamy) and heavy (clay) soils, requires well-drained soil but can grow in heavy clay and nutritionally poor soils. The plant is found in acid, neutral and basic (alkaline) soils. Further, it has a capability to grow in semi-shade or under limited light condition. Because of this, it has been classified as a shade-tolerant species (Clapham *et al.* 1962).

The leaves are divided into five lobes which are unevenly toothed (Brimble 1946). The dark green five-lobed leaves turn a deep golden-yellow colour in autumn. Both the shape and size of the leaves vary with the age of the tree. This species is in flower from April to June, and the seeds ripen from September to October. The flowers are monoecious which refers to individual flowers that are either male or female, but both sexes can be found on the same tree. The flowers are pollinated by bees. The paired, winged fruits are known to children as 'helicopters' in England, because of their propeller-like path of descent.

The bark is grey and fissured forming rectangular plates. Orange patches may be revealed as these plates are peeled away. The sapwood is white with a reddish tinge and narrow whereas the heartwood is a uniform light reddish brown. The density of its wood is 520-680 kg m^{-3}. Xylem vessels have essentially equal diameters and are uniformly distributed through a growth ring. This type of wood is called diffuse porous (Esau 1965).

1.6.5.2 Fraxinus excelsior L.

Fraxinus excelsior is a well-known tree species and sometime referred to as common ash. It is belong to the family of Oleaceae which has drawn as much interest as *A. pseudoplatanus*, and a large amount of information is available for the purpose of comparison. It has often been suggested that ash and sycamore are ecologically similar (Oakali 1966). As pointed out by Watt (1925), it is true that many general features such as the frequency of seed production, the age at which this occurs and the adaptations for dispersal are much the same. *F. excelsior* is distributed in Europe from northern Spain to Norway and eastwards to central Russia. It ranges from northern Turkey to the Caucuses and may also be found in north Africa. It is widely distributed throughout Ireland and Britain (Nelson *et al.*, 1993 and Savill, 1991). McCracken (1971) reports that ash became established in Ireland after the major climatic fluctuations of the post-glacial age had given way to the type of climate which, with minor changes, has prevailed since about 500 BC.

The ecology of *F. excelsior* has been reviewed by Wardle (1961) and Grime *et al.* (1988), and some aspects are very well documented including its light requirements (Gardner 1975), seedling and sapling ecology (van Miegroet and Lust 1972, van Miegroet *et al.* 1981), and its silviculture (Thill 1970). The respective ranges of *F. excelsior* and *A. pseudoplatanus* are quite different. The centre of their distributions is in central Europe but *F. excelsior* occurs at higher latitudes and further to the north west and north east whereas *A. pseudoplatanus* occurs at higher altitudes in the Alps. In the British Isles according to Perring and Walters (1962) both species now have a similar distribution.

This species is a broad-spreading deciduous tree which is capable of reaching 30 m or more in height but most often seen growing at a moderate pace at 20 to 25

m. It can thrive in light (sandy), medium (loamy) and heavy (clay) soils whether acidic, neutral or alkaline soils (Bean 1981). But most members of this genus are gross feeders and require a rich soil (Bean 1981, Huxley 1992). Weber and Claus (2000) found that mycorrhizal associations have a strong impact on growth rates of *F. excelsior* saplings as opposed to *A. pseudoplatanus*. This species was said to be successful in very good soil conditions on a sheltered site, with the result that it is usually found in small patches. Plants can succeed in very exposed positions, including maritime exposure, though they can become wind-shaped. Unlike *A. pseudoplatanus*, this species is very intolerant of shade where young plants fail to develop properly in such a position and often die (Beckett and Beckett 1979).

The leaves are dark green and are made up of a group of eleven small spear shaped leaflets or so called pinnately compound, each leaf being about 25 to 30 cm long. It is in leaf from May to October and starts to flower from April to May, and the seeds ripen from September to January. The flowers are dioecious but only one sex is to be found on any one tree. So both male and female plants must be grown if seed is required and are pollinated by wind.

The wood type of *F. excelsior* is ring porous, which means that pore size varies with position in the growth ring, with unequal diameters and early wood pore ring occasionally loosely packed (Esau 1965). Latewood pores are solitary or in short radial multiples (mostly one to two pores), rarely in small groups. The density of its wood is about 690 kg m^{-3}.

1.7 THESIS STRUCTURE

Chapter 1 provides a general introduction on the age- and size-related decline of net primary productivity of the forest. It also provides justification and objectives of the study. It discusses the importance of study and the causes that contribute in reducing net primary productivity in details. The latter part of this Chapter consists of a selected review of the hypotheses followed by information regarding the techniques useful in separating age from size, the study site and a brief description of selected species.

Chapter 2 starts with the investigation of the changes in growth characteristics of both species from the youngest (~ 4 years) to the oldest (~ 162 years) trees. Growth parameters such as relative growth rate and growth efficiency for both donor trees and grafted seedlings are measured. This chapter also provides allometric equations based on diameter and height functions established by previous British investigators to calculate aboveground biomass and leaf area in the field. Equations to calculate those parameters for grafted seedlings are also established through destructive measurements.

Chapter 3 investigates the effects of age and size on gas exchange parameters and leaf chemical compositions on both the donor trees and the grafted seedlings. Parameters such as net photosynthesis, internal CO_2 and stomatal conductance are investigated. Additional parameters investigated in the grafted seedlings included saturated maximum photosynthesis, apparent quantum efficiency, maximum carboxylation rate and maximum electron transport from *A/Q* and *A/Ci* curves response. Correlation analyses are also carried out on some parameters with specific leaf area reported in the previous chapter.

Chapter 4 illustrates experiments regarding sap flow measurement on the donor trees in the field. Whole plant transpiration, canopy stomatal conductance and hydraulic conductance are derived from this measurement. Daily whole plant transpiration and stomatal conductance are also regressed against vapour pressure deficit obtained from nearby meteorological station. Age-related trends are investigated by the establishment of correlation analyses between these parameters and individual tree age. This chapter also investigates whole plant transpiration and hydraulic conductance on grafted seedlings through gravimetric methods. The appropriate values of stomatal conductance are calculated in grafted seedlings through the measurements of stomatal response with different level of vapour pressure deficit. The mean values of those parameters observed in grafted seedlings are statistically grouped.

In Chapter 5, cumulative results from previous chapters are compared between controls and grafted seedlings. The hydraulic resistance of entire stem sections and of scion sections are investigated using the High Pressure Flow Meter apparatus in grafted seedlings of both species. Comparisons are established between them in order to test hypothesis that there are no effect of grafted union on water flow in grafted seedlings. Additional parameters are also derived from these measurements such as hydraulic conductivity of stem and scion sections. Furthermore, air-layered plants are established as an alternative to grafting. With a single genotype involved in air-layered plants, genotypic complication that may exist in grafted seedlings are eradicated. Similar testing such as growth and gas exchange measurements are also carried out on air-layered plants. Due to the variation in terms of size in air-layered plants, correlation analyses are established. Last but not least, general conclusions on previous chapters are presented in Chapter 6.

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CHAPTER 2

AGE- AND SIZE-RELATED CHANGES IN MORPHOLOGY AND GROWTH OF *ACER PSEUDOPLATANUS* AND *FRAXINUS EXCELSIOR* SPECIES

2.1 INTRODUCTION

There is growing interest in understanding the costs and benefits of increased size and lifespan for plants. Some species of trees can grow more than 100 m in height and can live for several millennia, however whether these achievements are obtained at the cost of some other physiological functions is currently unclear. As increases in size are usually associated with ageing, it is also unclear whether observed reductions in growth rates and increased mortality rates are a function of size or of age *per se*. One conjecture proposes that reduced growth after the beginning of the reproductive stage is caused by cellular senescence. A second set of theories has focused instead on plant size and the increased respiratory burdens or excessive height (*c.f.*, Mencuccini *et al.* 2005).

Genetically programmed slowing of tree growth has the potential to explain the decrease in height growth with age (Greenwood 1989, Greenwood and Hutchison 1993, Day *et al.* 2001). In contrast, as the size of trees usually increases with tree age, the decreases in tree growth could be associated with the limitation in hydraulic transport. Bond (2000) stated that the total resistance of the hydraulic pathway increases as trees approach their maximum height owing to a combination of factors including gravity, a longer hydraulic path length through stems and branches, greater tortuosity of the hydraulic path and reduced allocation to roots (see also Chapter 1). Most of the findings discussed before did not separate the possible effect of age from height or size (Niinemets 2002). Hence, which factor, i.e., age or size, play the most important role in tree growth? To answer this question, one should be able to separate the effect of size from age. To do this, macropropagation techniques such as grafting have been identified in order to separate size of trees from their age.

Studies of tree ageing that have compared scions from branches of mature and juvenile trees after grafting onto common rootstocks have shown that stem growth rate varied with tissue age independent of differences in tree height (Greenwood 1995). These studies suggested that some changes in morphological traits during tree maturation result from changes in phytohormones and gene expression. Furthermore, Day *et al.* (2001) also found significant age-related trends of red spruce (*Picea rubens* Sarg.) in foliar morphology and physiology of trees in the field and in grafted scions. They concluded that the expression of genes in meristems is altered as trees grow older or larger beyond reproductive maturity or mid-age and these gene expressions also persisted in meristems of grafted scions. However, some studies showed contrasting results. For instance, net photosynthesis of *Hedera helix* L. and *Larix laricina* K. Koch increased with increasing age of scions (Bauer and Bauer 1980, Hutchison *et al.* 1990). Details related to grafted scion studies have previously been presented in Chapter 1 and will not be discussed further in here.

In this chapter, we studied the growth characteristics of selected *A. pseudoplatanus* and *F. excelsior* trees in the field, comprising different age or size classes. We also present results of experimental manipulations using scions taken from the same trees. The most useful growth characteristics to compare between them used in this study are the absolute growth rate (*AGR*), the relative growth rate (*RGR*) and the growth efficiency (*EG*). The total specific leaf area (*SLA*) and the ratio between total leaf area (A_L) and total sapwood area (A_S) were also determined from allometric equations and increment cores. A comparison was carried out between the donor trees in the field and the grafted seedlings obtained from the same donors, but all now of the same size. We tested a set of hypotheses by decoupling 'intrinsic' from 'extrinsic' factors for each of two tree species and by asking whether growth rates were a function of 'intrinsic' or 'extrinsic' factors *per se* (e.g. Mencuccini *et al.* 2005).

2.2 MATERIALS AND METHODS

2.2.1 Field Study

2.2.1.1 Sampling

A. pseudoplatanus and *F. excelsior* were selected in this study since they are the dominant species in Cramond woodland and are represented by various size and age groups growing at the same site. These species also represented two principal xylem anatomies, i.e. diffuse porous and ring porous, since water transport has been suggested as the main constraint to the growth and physiology of old/tall trees (Yoder *et al.* 1994, Mencuccini and Grace 1996b, Ryan *et al.* 1997). The trees selected varied from 3 to 162 and 3 to 132 years of age in both species respectively. At the beginning, four groups of diameter/age classes were formed. Ten trees were selected in the youngest class for both species, followed by five trees in the second, third and fourth diameter classes.

2.2.1.2 Diameter, height growth and specific leaf area measurement (SLA)

Diameters at breast height (*DBH*) were measured on all selected trees using diameter tape except the youngest ones where the diameters were taken at 10 cm above ground in both species. Current height of all the trees were measured using a Suunto clinometer (Suunto Oy, Vantaa, Finland) and a height stick depending on the height of the trees. The average height growth in the past five years was measured in each tree from the branches taken at the top of the tree. About five to six leaves were excised from the cut branches and were brought back to the laboratory. Leaf area measurements were then made using LI-3100 leaf area meter (LI-COR Inc, Lincoln, Nebraska, USA). The leaves were then dried in the oven at 60°C for 48 hours. Leaf weights were measured afterward using a balance and the specific leaf area (*SLA*) was calculated by dividing leaf area with leaf weight.

2.2.1.3 *Total leaf area, tree age and sapwood area*

Whole tree leaf area of *A. psedoplatanus* and *F. excelsior* in each individual tree was estimated from total leaf mass. Total leaf mass was calculated from equations based on estimated crown biomass and *DBH* as given in Broadmeadow and Matthews (2004). Total crown biomass was first calculated using a specific equation using *DBH* as independent variable, before calculating total leaf mass. Total leaf area in each individual tree was then obtained by multiplying total leaf mass with *SLA* (Table 2.1). The equations used are as given below:

Crown biomass (DBH<7cm):

$$W_{crown} = 0.00005122 * DBH^{2.06704428} * HT_{total}^{0.7321854} \tag{2.1}$$

Crown biomass (DBH>7cm):

$$W_{crown} = 0.00729453 + 0.00003081 * DBH^{3.67047187} * HT_{total}^{-1.44028024} \tag{2.2}$$

Total leaf mass:

$$W_{leaf} = 0.06391085 - 0.06391085 * (0.17108421^{W_{crown}}) \tag{2.3}$$

$$\text{Total leaf area} = W_{leaf} * \text{SLA} \tag{2.4}$$

An increment core was taken at breast height (1.3 m) from trees in each diameter class 2, 3 and 4 in the summer 2003. The growth rings were counted under magnifying glass with a fluorescent lamp. Annual rings may be difficult to distinguish in a sample core due to species-specific characteristics such as in diffusely porous species (*A. pseudoplatanus*). In this case, sample cores were stained with a solution of 1% phloroglucinol in 95% ethyl alcohol and a solution of 50% aqueous hydrochloric acid. For the youngest trees (age class 1), the age was estimated from bud scars along the stem. All the trees were then classed into four age groups (Table 2.1).

Meanwhile, another core was taken from the three older classes in each species to determine the sapwood area. For the youngest class, four trees in each species were cut in order to determine the sapwood area. The width of active sapwood was measured from the cores and stems visually. The sapwood of *A. pseudoplatanus* is white to light yellow, while the heartwood is light to dark brown. However, some of the cores showed little difference in colour between sapwood and heartwood, especially the cores from *F. excelsior*. In this case, the cores were stained with o-toluidine as described by Shain (1967). The sapwood area was estimated afterward.

Table 2.1: Characteristics of *A. pseudoplatanus* and *F. excelsior* donor trees used in this study. Mean attributes for each age class in both species used in growth measurements.

Class	**No. of tree**	**Age at 1.3m (yr)**	**DBH (cm)**	**Height (m)**	**Estimated Leaf Area (m^2)**
A. pseudoplatanus					
1	10	5.2 ± 0.47*	1.95 ± 0.35	2.73 ± 0.41	0.50 ± 0.09
2	5	27.0 ± 0.89	15.20 ± 0.98	7.94 ± 0.32	68.30 ± 14.25
3	5	65.4 ± 3.99	51.70 ± 3.28	16.26 ± 1.19	735.70 ± 89.93
4	5	143.8 ± 6.23	88.20 ± 6.89	24.98 ± 0.58	806.93 ± 75.69
F. excelsior					
1	10	4.6 ± 0.41*	1.83 ± 0.19	2.66 ± 0.31	1.34 ± 0.43
2	5	27.2 ± 1.49	19.40 ± 1.58	12.48 ± 0.91	97.11 ± 27.25
3	5	43.2 ± 3.15	33.70 ± 1.30	15.70 ± 1.36	301.15 ± 51.79
4	5	114.2 ± 8.27	69.30 ± 3.34	22.88 ± 1.18	613.73 ± 45.56

Notes: The '±' represents mean standard error. The '*' indicated that ages were estimated from bud scars on stem surface.

2.2.1.4 Tree aboveground biomass, absolute mass growth rate (AGR_{mass}), relative mass growth rate (RGR_{mass}) and growth efficiency (E_G)

Tree aboveground biomass was estimated by summing the data obtained from the calculation of leaf mass using the equations derived by Broadmeadow and Matthews (2004) with branch and trunk mass equations valid for the United Kingdom obtained from Bunce (1968) for both species, as presented below:

Branch and trunk biomass:

$$\mathrm{Ln}W_{branches+trunk} = -5.570499 + 2.529411 * \mathrm{Ln}DBH \text{ for } \textit{Acer pseudoplatanus} \tag{2.5}$$

$$\mathrm{Ln}W_{branches+trunk} = -5.234459 + 2.480921 * \mathrm{Ln}DBH \text{ for } \textit{Fraxinus excelsior} \tag{2.6}$$

Total aboveground biomass:

$$W_{total} = W_{leaf} + W_{branches+trunk} \tag{2.7}$$

The growth rate of a plant is generally defined as the increase over time in the total dry weight (biomass) of the plant. There are two ways to calculate growth rate whether by the absolute growth rate (*AGR*) or/and the relative growth rate (*RGR*). The *AGR*, representing the average actual rates at which substance is added during each period, is found by subtracting from each value that previously recorded and dividing with the length of the period as shown in the equation below:

$$AGR = \frac{W_{total2} - W_{total1}}{T_2 - T_1} \tag{2.8}$$

where *W* is the plant mass and *T* is the time of measurement. This definition of growth rate is important because *AGR* describes the pattern of biomass accumulation through time in a forest and it is useful in describing the increase in plant size. This determines both the resource requirement and the impact on other plants especially in competition studies. Meanwhile, the *RGR* measure the rate of increase not only per unit of time but per unit of weight (mass) already attained. This definition describes the rate at which a given unit of biomass contributes to growth in an individual tree. The *RGR* is useful in describing the physiological basis for the rate of biomass increase because it can be broken down into several additional components of growth as shown below:

$$RGR = LAR * NAR \tag{2.9}$$

where *LAR* is the leaf area ratio and *NAR* is the net assimilation rate. *LAR* is the amount of leaf area per total plant biomass (A_L/W_{total}). It is the basis for root to shoot ratio acclimation to changing aboveground and belowground resources. The value of

LAR normally declines with increasing root to shoot ratio especially when a plant becomes bigger and it differs among growth forms. Moreover, *LAR* can be further partitioned into two components as shown below:

$$LAR = LWR * SLA \tag{2.10}$$

where *LWR* is the leaf weight (mass) ratio and *SLA* is the specific leaf area. *LWR* is the amount of leaf biomass per total plant biomass whereas *SLA* is the amount of leaf area per leaf mass. By substituting *LAR* from equation 2.10 into equation 2.9, a more detailed definition of RGR is as follow:

$$RGR = LWR * SLA * NAR \tag{2.11}$$

From these expressions, it is clear that there is more than one way for a plant to grow rapidly but the most important determinant of *RGR* is *SLA*. By spreading leaf biomass over a large area, plants can absorb more light and increase photosynthesis. However, in competitive situations where a plant can get overtopped by a neighbour, having a high value of *SLA* is not too useful in the shaded condition. Low values of *SLA* might decrease the amount of light absorbed and carbon gain, and consequently reduce *RGR*.

Both *AGR* and *RGR* are important traits in growth analysis. However, there are some limitations to growth analysis, mainly because these traits are variable over time, since plants produce more unproductive support tissue as they get larger, leaf-level photosynthetic rates decline as leaves age and growth rate becomes resource-limited possibly constrained by hydraulic architecture and cavitation as plants get bigger. In this case, since big trees grow more in an absolute sense than small trees, as to normalize growth for different tree sizes, one often computes the *RGR* rather than *AGR*. As defined above, *RGR* measures the rate of biomass increase per unit of time and weight already attained. Mathematically, the average value of the *RGR* for any period can be obtained from the natural logarithms of the successive weights, just as the actual rates of increase are from the weights themselves as shown below:

$$RGR_{\text{mass}} = \frac{\text{Ln}W_{total2} - \text{Ln}W_{total1}}{\Delta T} \tag{2.12}$$

(Evans 1972, Hunt 1978)

Apart from *AGR* and *RGR*, the growth efficiency (E_G) which is defined as the increase in biomass through time (*AGR*) divided by leaf area, was also computed. The E_G is sometime referred as the *NAR*. The concept of tree E_G, defined originally by Waring *et al.* (1980) as the volume (or biomass) of stemwood increment per unit of foliage, has seen increasing application in studies that attempt to understand and quantify the relationship between tree and stand growth, stand structure (e.g., Smith and Long 1989, Long and Smith 1990, Roberts and Long 1992, O'Hara 1996), and site resource availability (e.g., Binkley and Reid 1984, Kaufmann and Ryan 1986, Vose and Allen 1988, Velazquez-Martinez *et al.* 1992, McCrady and Jokela 1998). For conifers, the widespread development of allometric equations that predict tree leaf area (A_L) from sapwood basal area (e.g., Long and Smith 1989, Gilmore *et al.* 1996, O'Hara and Valappil 1995) or a modified live crown ratio (Valentine *et al.* 1994) has facilitated the estimation of tree leaf areas, and thus E_G, from simple non-destructive procedures.

In our study, AGR_{mass}, RGR_{mass} and E_G were estimated from aboveground biomass growth using measured annual diameter increments over the last five years ($\Delta T = 5$) and allometric equations for branches and trunks as described above.

2.2.2 Grafting Study

2.2.2.1 Grafted seedling preparation

Age-related trends in growth parameters were evaluated using a common-rootstock approach in order to separate age from size. Scions originating as terminal branch shoots with relatively uniform sizes (6 – 8cm) from trees representing four diameter classes (Table 2.1) were collected from selected donor trees during the last two weeks of February 2003. Scions were collected with a hand pruner, pole pruner, and also by climbing the trees depending on tree height. After collection, scions were bagged and tagged, and were then brought to R & B nursery in Roslin, Edinburgh, U.K., where all the grafting works were done. These scions, consisting of a terminal bud with a short twig were side-grafted onto leader stems of similar diameter.

Two hundred seedlings (rootstocks) for each species were used for grafting and 50 seedlings (25 self-grafted and 25 ungrafted) were used as controls. All the grafted seedlings and rootstocks were placed in five trays comprising 100 seedlings per tray due to space limitations. All the grafted seedlings were maintained in a well-ventilated plastic roof greenhouse until danger of frost was past. Trees were transferred in 3-litre polyethylene bags, potted with sphagnum peat, sand and vermiculite mixed 2: 1: 1, and supplied with slow-release fertilizer. Potted trees were then placed in glass frames in School of GeoScience nursery, University of Edinburgh. A Randomised Complete Block Design (RCBD) was used. The arrangement of trees was based on the glass frame space (150cm X 624cm X 2 frames) made after the age of donor trees was determined. Potentially competing leaders from rootstocks were pruned following bud break. All surviving grafted unions were counted in May 2003 after their leaves were fully expanded (Table 2.2). The grafted seedlings were then divided into two groups depending on the purposes of the study. In early 2004, all the grafted seedlings were transferred into 10-litre polyethylene bags.

2.2.2.2 Diameter and height growth, leaf area (A_L), specific leaf area (SLA) and leaf number (L_N)

Ten healthy grafted seedlings of each age class in both species were selected for the study. This included all seven surviving grafted seedlings in age class three of *A. pseudoplatanus* species. Measurements of the diameter were taken at about 10 cm above the graft union using a digital vernier calliper (Mitutoyo Ltd., UK) whereas height measurements were made using a meter ruler on all selected grafted seedlings. First measurement was made in June 2003 after the foliage was fully expanded. These measurements were then carried out on August 2003, June 2004 and August 2004 to determine their growth rate.

All the leaves were excised and counted at the end of each growing season. Total leaf area was determined using LI-3100 leaf area meter (LI-COR Inc, Lincoln, Nebraska, USA). These leaves were then left in a drying oven for four days at 58°C and then weighed. Dry weights of the leaves were used to calculate the specific leaf area (*SLA*) of each selected grafted seedlings.

Table 2.2: Number of grafted seedlings survived in both species.

Age Class	No. of tree grafted	No. of tree survived	% Survival
A. pseudoplatanus			
1	50	25	50
2	50	22	44
3	50	7	14
4	50	12	24
Control (self-grafted)	25	21	42
Total	225	87	38.7%
F. excelsior			
1	50	40	80
2	60	48	80
3	40	30	75
4	50	36	70
Control (self-grafted)	25	25	100
Total	225	179	79.6%

2.2.2.3 Absolute mass growth rate (AGR_{mass}), relative mass growth rate (RGR_{mass}), growth efficiency (E_G), total biomass (M_T) and root to shoot ratio

Three to five grafted seedlings (without leaves) in each age class were destructively harvested in October 2004. The stems and branches (if available) were dried in the oven at 70°C for about four days and weighted. Regressions were then established between stem and branch biomass of each tree and stem diameter using linear regression analysis and were then applied to the rest of the plants. A function based on stem diameter was used since the similar one had been applied to the donor trees. The equations derived from this analysis were applied to the rest of the grafted seedlings. Total aboveground biomass was calculated by summing the stem and branch biomass with the leaf biomass obtained. The equations used to estimate the stem and branch biomass for the rest of undestructed grafted seedlings are as follow:

Stem and branches biomass:

A. pseudoplatanus

$\mathrm{Ln}W_{\mathrm{stem+branches}} = 4.13956 + 1.00067*\mathrm{Ln}Diameter$, n=5, $R^2 = 0.998$ for Age Class 1

$\mathrm{Ln}W_{\mathrm{stem+branches}} = 3.34276 + 2.45623*\mathrm{Ln}Diameter$, n=5, $R^2 = 0.991$ for Age Class 2

$\mathrm{Ln}W_{\mathrm{stem+branches}} = 3.93661 + 0.07494*\mathrm{Ln}Diameter$, n=3, $R^2 = 0.997$ for Age Class 3

$\mathrm{Ln}W_{\mathrm{stem+branches}} = 2.87451 + 5.62113*\mathrm{Ln}Diameter$, n=4, $R^2 = 0.998$ for Age Class 4

F. excelsior

$\mathrm{Ln}W_{\mathrm{stem+branches}} = 3.71985 + 0.98988*\mathrm{Ln}Diameter$, n=5, $R^2 = 0.999$ for Age Class 1

$\mathrm{Ln}W_{\mathrm{stem+branches}} = 3.72016 + 0.98867*\mathrm{Ln}Diameter$, n=5, $R^2 = 0.998$ for Age Class 2

$\mathrm{Ln}W_{\mathrm{stem+branches}} = 3.64250 + 1.14150*\mathrm{Ln}Diameter$, n=5, $R^2 = 0.905$ for Age Class 3

$\mathrm{Ln}W_{\mathrm{stem+branches}} = 3.69586 + 1.26225*\mathrm{Ln}Diameter$, n=5, $R^2 = 0.913$ for Age Class 4

Total aboveground biomass for both species:

$$W_{total} = W_{leaf} + W_{stem+branches} \tag{2.13}$$

E_G and RGR_{mass} of grafted seedling were calculated based on aboveground biomass obtained in 2003 and 2004 using the equations as presented before. Meanwhile, the total mass (M_T) of graft seedlings was calculated by summing the total aboveground biomass with root mass of respective trees used in establishing those equations above. The root masses were measured directly after they were cleaned and oven dried at 70°C for four days, and root dry weight to shoot dry weight ratios were then calculated.

2.2.3 Data analyses

The data obtained were subjected to one-way analysis of variance (ANOVA) for the balanced data and general linear model (GLM) for the unbalanced data among age classes in both species. The statistical analysis system (SAS Institute Inc., 2002) was used for these analyses. Non-linear and linear regression analyses were carried out on some of the data and fitted using Sigma Plot 9.0 (Systat Software Inc., 2004).

2.3 RESULTS

2.3.1 Growth characteristics of the donor trees

Analysis of variance (Table 2.3) indicated that all growth parameters measured from the donor trees were significantly different across age classes in both species. A highly significant difference ($p<0.001$) was found in specific leaf area (*SLA*) and relative mass growth rate (RGR_{mass}) in both species, whereas leaf area to sapwood area ratio ($A_L:A_S$) was found significant at $p<0.05$ and $p<0.01$ in *A. pseudoplatanus* and *F. excelsior*, respectively. Highly significant differences at $p<0.001$ and $p<0.01$ were also found in AGR_{mass} and growth efficiency (E_G) for *A. pseudoplatanus* and *F. excelsior*.

Comparing mean values against age classes, whole tree $A_L:A_S$ was found to vary among age classes, whereby mean values in age class one (AC1) were comparatively higher than the other older age classes for both species (Figure 2.1). The mean value of $A_L:A_S$ in AC1 for *F. excelsior* was found to be more than twice the value recorded in older classes. Age-related trends were clearly observed in this parameter for both species. When $A_L:A_S$ values were regressed against height, negative linear correlations ($p<0.001$) were found in both species suggesting that $A_L:A_S$ decreased with increasing height (Figure 2.2).

Similar patterns were also recorded in *SLA* for both species. The mean values in AC1 for both species were almost double the values of nearest age class (Figure 2.3). The mean values of *SLA* in age class two (AC2), three (AC3) and four (AC4) for *A. pseudoplatanus* were not much different compared with the mean values of the same classes in *F. excelsior* and they were also not statistically different from each other for both species.

Table 2.3: Summary of one-way analysis of variance on growth parameters of *A. pseudoplatanus* and *F. excelsior* donor trees.

Growth Parameters	***A. pseudoplatanus***	***F. excelsior***
	F value	**F value**
A_L:A_S (m^2 cm^{-2})	4.55*	8.64**
SLA (m^2 g^{-1})	8.96***	8.71***
AGR_{mass} (kg yr^{-1})	20.13***	8.18**
RGR_{mass} (kg kg^{-1} yr^{-1})	52.19***	104.27***
E_G (kg m^{-2} yr^{-1})	46.16***	7.84**

Notes: *** Significantly different at $p<0.001$
** Significantly different at $p<0.01$
* Significantly different at $p<0.05$

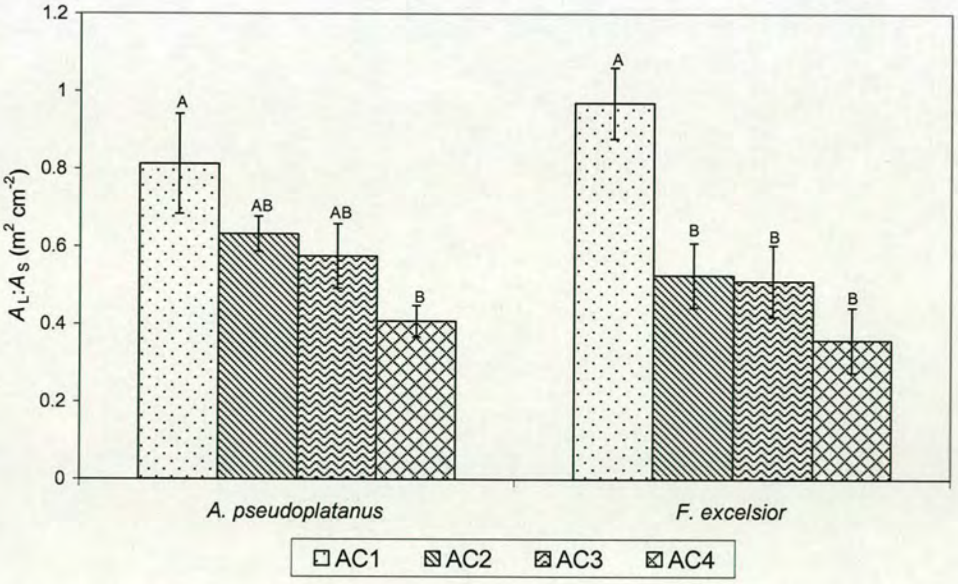

Figure 2.1: Mean values of A_L:A_S of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied.

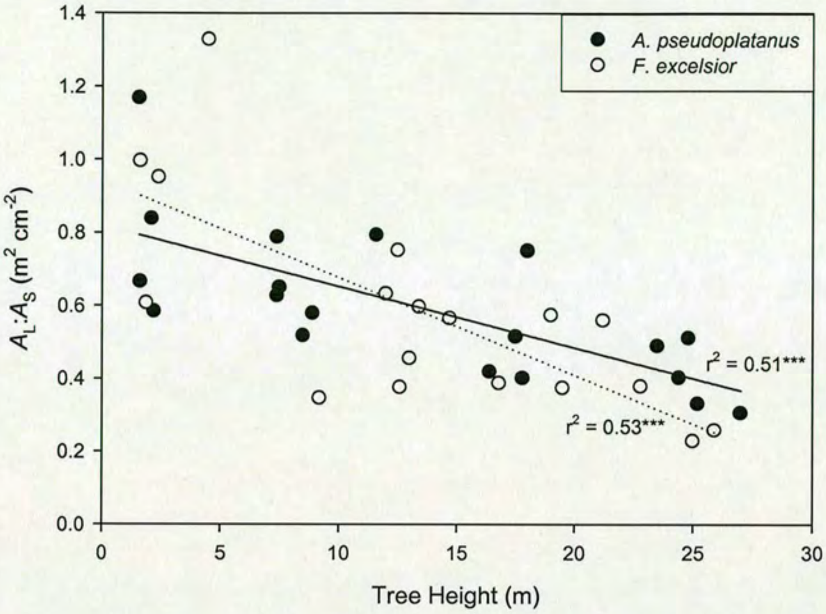

Figure 2.2: Leaf area to sapwood area ratio (A_L:A_S) plotted against tree height for both species. The *** indicates p<0.001.

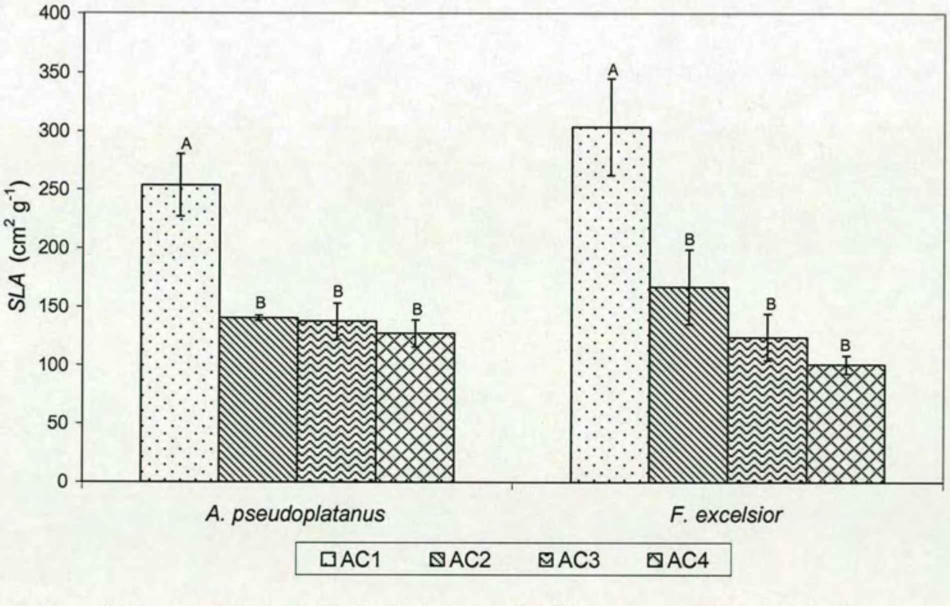

Figure 2.3: Mean values of *SLA* of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences among age classes within species studied.

Total aboveground biomass growth was estimated as annual carbon accumulation from allometric equations as stated before and these values were used in calculating AGR_{mass}, RGR_{mass} and E_G. There was a substantial difference between mean values of AGR_{mass} of AC1 with those observed in AC2, AC3 and AC4 for both species (Figure 2.4). Since AGR_{mass} is a size-dependent parameter, increased trends were observed with increasing size/age of the trees in both species. Hence, the RGR_{mass} was calculated to determine the growth rate without the effect of tree sizes as shown in Figure 2.5. RGR_{mass} showed the most pronounced decreasing trends with increasing age in both species, indicating that the relative growth rates in the youngest trees are much higher compared with older trees. Furthermore, we found that the average E_G of the very young class in *A. pseudoplatanus* was more than twice than that of old-growth trees (Figure 2.6). In *F. excelsior*, mean value of E_G in AC1 was about 62%, 73% and 79% higher than that of AC2, AC3 and AC4 respectively. Age-related decline trends were also observed in E_G but with a minor notable inversion between classes 3 and 4 in *A. pseudoplatanus*. However, E_G in *F. excelsior* showed a very clear age-related decline trend.

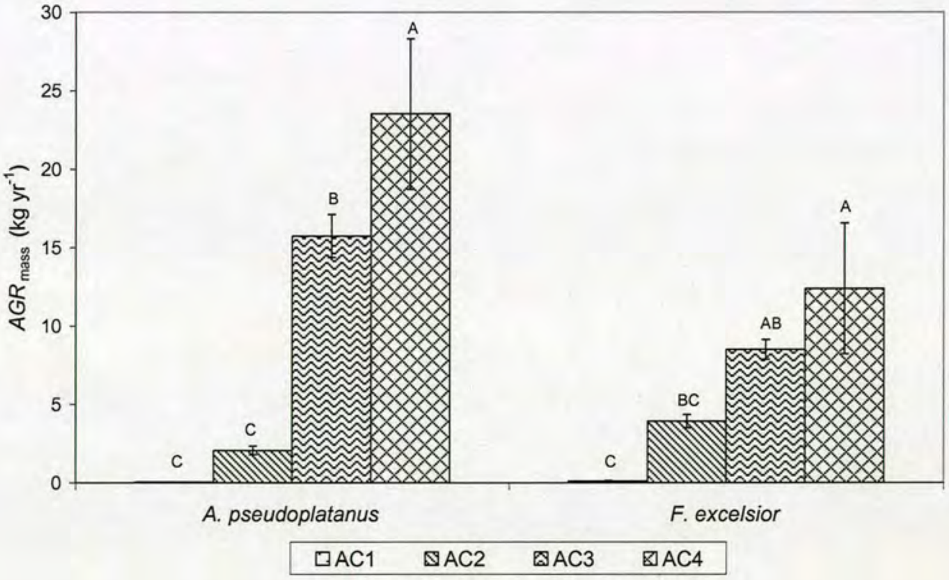

Figure 2.4 Mean values of AGR_{mass} of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied.

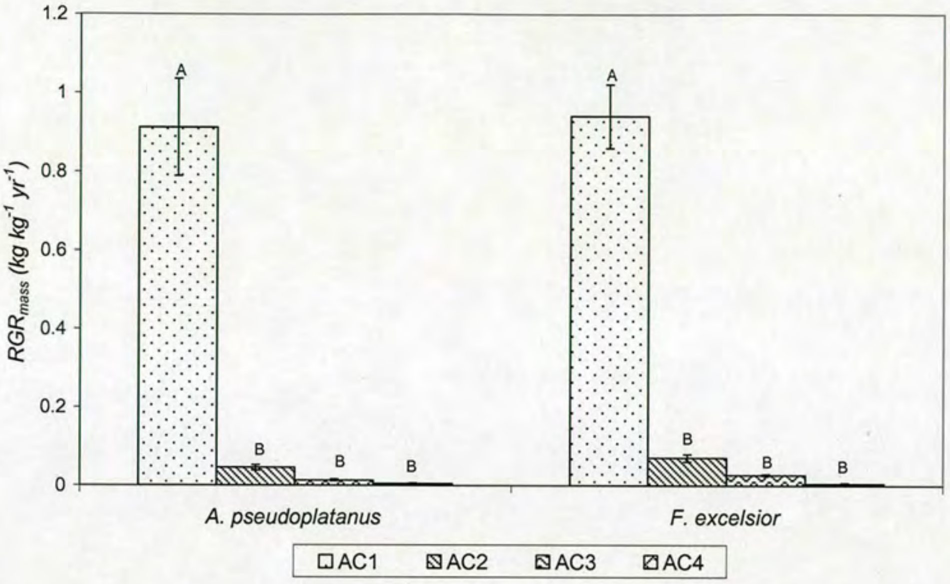

Figure 2.5: Mean values of RGR_{mass} of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied.

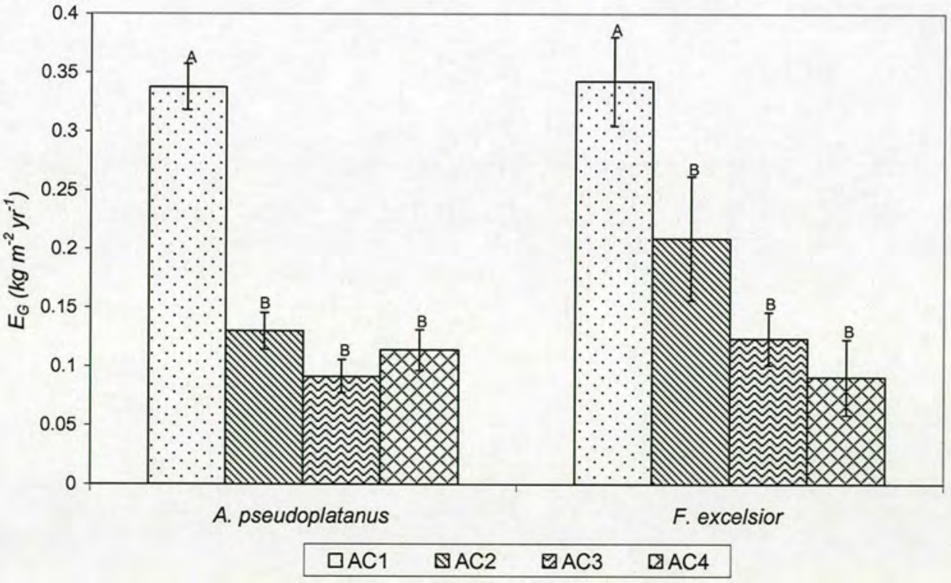

Figure 2.6: Mean values of E_{G} of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied.

2.3.2 Growth characteristics of the grafted seedlings

For our purposes, the grafted seedlings were categorized, at the genotypic level, into four classes of scion age to see whether the growth and morphological characteristics from their donor trees still remained. Rootstock seedlings and self-grafted seedlings obtained from the same rootstock genotype were also used as controls. Table 2.4 showed the analysis of variance (ANOVA) for various growth and morphological traits measured in this study. The ANOVA results combine the effects of scion age as well as the differences with the controls on growth and morphological characteristics. Highly significant differences ($p<0.001$) were found for total diameter in both species, whereas total height was only found significant in *F. excelsior* after two growing seasons. The leaf area (A_L) and specific leaf area (*SLA*) were found highly significant at $p<0.001$ in both species. The effects of scion ages and the controls on L_N and AGR_{mass} were found significant at $p<0.01$ and $p<0.001$ in *A. pseudoplatanus* and *F. excelsior* respectively. In addition, these grafted seedlings were not significantly different among age classes and the controls in RGR_{mass} trait for *A. pseudoplatanus* but a highly significant difference ($p<0.001$) was found in *F. excelsior* with regard to this trait. Similar results were also found for total biomass in both species. The growth efficiencies (E_G) based on the actual records and allometric equations established were significantly affected by scion ages and the controls. However, this trait was found less significant at $p<0.05$ in *A. pseudoplatanus* compared with *F. excelsior*, for which the effect of scion ages and the controls was much higher. However, root to shoot ratio (*Rt:St*) did not differ among scion ages and the controls in both species.

Since the A_L, *SLA,* L_N and AGR_{mass} were highly affected by scion ages and the controls in both species, the mean values of these traits were found to be substantially different between the four age classes and the controls (Table 2.5). Furthermore, our results on *SLA* showed that the trend with scion age still persisted in grafted seedlings. However, the age-related trends in growth traits such as RGR_{mass}, E_G and total biomass (M_{Tot}) tended to diminish or disappear in grafted seedlings, as shown in Table 2.5. Regardless of controls, the mean values of RGR_{mass} and E_G were found higher in AC4 and AC3 for *A. pseudoplatanus* and *F. excelsior* respectively. The lowest mean values of E_G were recorded in AC3 for *A.*

pseudoplatanus and AC1 for *F. excelsior*, while the RGR_{mass} mean values were found lower in AC2 and AC4 for *A. pseudoplatanus* and *F. excelsior* respectively. Furthermore, no age-related trend was found in total biomass (M_{T}) for both species. The mean values of M_{Tot} were found higher in rootstock seedlings and lower in AC3 for both species. The *Rt:St* mean values were also found not significantly affected by scion ages and controls indicating that neither scion nor rootstock genotypes affect the shift in resource allocation to roots or to shoots.

Table 2.4: Summary of analysis of variance on growth parameters of *A. pseudoplatanus* and *F. excelsior* grafted seedlings after two growing seasons.

Growth Parameters	***A. pseudoplatanus***	***F. excelsior***
Total diameter (cm)	6.52***	19.92***
Total height (m)	1.43^{ns}	8.48***
A_{L} (m^2)	7.93***	33.51***
SLA ($m^2\ g^{-1}$)	5.47***	24.21***
L_{N}	3.45**	11.44***
AGR_{mass} ($kg\ yr^{-1}$)	3.94**	8.89***
RGR_{mass} ($kg\ kg^{-1}\ yr^{-1}$)	1.67^{ns}	23.11***
E_{G} ($kg\ m^{-2}\ yr^{-1}$)	2.47*	17.51***
M_{Tot}	1.29^{ns}	7.26***
Rt:St ($g\ g^{-1}$)	0.92^{ns}	1.60^{ns}

Notes:		
	***	Significantly different at $p<0.001$
	**	Significantly different at $p<0.01$
	*	Significantly different at $p<0.05$
	ns	Not significant

Table 2.5: Summary of growth characteristics of grafted seedling across age classes in both species after two growing seasons.

Class	**Scion Age** (yr)	A_L (m^2)	*SLA* (m^2 g^{-1})	L_N	AGR_{mass} (kg yr^{-1})	RGR_{mass} (kg kg^{-1} yr^{-1})	E_G (kg m^{-2} yr^{-1})	M_{Tot} (g)	*Rt:St* (g g^{-1})
A. pseudoplatanus									
1	5.4 ± 0.4	0.39 ± 0.042^{B}	178.85 ± 6.87^{AB}	39.30 ± 4.89^{BC}	0.074 ± 0.006^{B}	0.943 ± 0.111^{AB}	0.205 ± 0.028^{AB}	169.34 ± 14.97^{AB}	0.447 ± 0.030^{A}
2	27.5 ± 0.5	0.38 ± 0.041^{B}	169.39 ± 4.67^{BC}	38.10 ± 3.35^{BC}	0.045 ± 0.005^{B}	0.663 ± 0.088^{B}	0.124 ± 0.018^{B}	146.18 ± 11.78^{AB}	0.467 ± 0.031^{A}
3	64.4 ± 2.9	0.37 ± 0.028^{B}	147.03 ± 10.90^{C}	41.29 ± 3.47^{ABC}	0.043 ± 0.007^{B}	0.922 ± 0.201^{AB}	0.118 ± 0.018^{B}	113.53 ± 1.95^{B}	0.508 ± 0.027^{A}
4	143.4 ± 4.3	0.28 ± 0.023^{B}	139.65 ± 6.42^{C}	31.80 ± 1.65^{C}	0.062 ± 0.007^{B}	1.158 ± 0.145^{A}	0.215 ± 0.027^{AB}	135.18 ± 8.21^{AB}	0.485 ± 0.038^{A}
Self-grafted	3.0	0.49 ± 0.033^{A}	190.2 ± 14.40^{AB}	46.70 ± 3.74^{AB}	0.056 ± 0.003^{B}	0.809 ± 0.049^{AB}	0.116 ± 0.011^{B}	150.08 ± 11.46^{AB}	0.462 ± 0.019^{A}
Rootstock	3.0	0.56 ± 0.043^{A}	202.55 ± 14.40^{A}	50.90 ± 4.10^{A}	0.122 ± 0.032^{A}	0.938 ± 0.164^{AB}	0.235 ± 0.066^{A}	225.04 ± 68.36^{A}	0.402 ± 0.054^{A}
F. excelsior									
1	5.0 ± 0.4	0.48 ± 0.017^{C}	183.66 ± 9.82^{B}	27.4 ± 2.19^{BC}	0.056 ± 0.002^{A}	1.047 ± 0.068^{B}	0.119 ± 0.006^{CD}	135.68 ± 6.53^{B}	0.567 ± 0.055^{AB}
2	25.2 ± 1.1	0.41 ± 0.031^{C}	159.84 ± 6.32^{C}	23.8 ± 2.79^{BCD}	0.054 ± 0.002^{AB}	1.157 ± 0.039^{AB}	0.138 ± 0.009^{BC}	126.24 ± 7.65^{BC}	0.590 ± 0.068^{AB}
3	40.4 ± 0.9	0.24 ± 0.009^{D}	140.15 ± 7.95^{C}	21.5 ± 2.23^{CD}	0.046 ± 0.001^{C}	1.190 ± 0.047^{A}	0.193 ± 0.007^{A}	110.30 ± 4.67^{C}	0.702 ± 0.081^{A}
4	117.8 ± 5.7	0.28 ± 0.041^{D}	145.07 ± 6.25^{C}	17.2 ± 1.50^{D}	0.040 ± 0.003^{D}	0.911 ± 0.023^{C}	0.167 ± 0.022^{AB}	114.96 ± 9.43^{BC}	0.692 ± 0.063^{A}
Self-grafted	3.0	0.56 ± 0.02^{B}	212.92 ± 4.65^{A}	32.9 ± 4.77^{B}	0.052 ± 0.002^{AB}	0.895 ± 0.044^{C}	0.094 ± 0.004^{DE}	133.94 ± 6.01^{B}	0.473 ± 0.044^{B}
Rootstock	3.0	0.66 ± 0.03^{A}	219.35 ± 5.22^{A}	48.5 ± 4.73^{A}	0.049 ± 0.002^{BC}	0.629 ± 0.021^{D}	0.075 ± 0.002^{E}	165.68 ± 8.55^{A}	0.576 ± 0.086^{AB}

The '±' represents mean standard error. Different letters indicate significant differences between age classes within species.

2.3.3 Relationships between growth characteristic and age in donor trees and grafted seedlings

Regression analyses were carried out to compare some growth characteristics between donor trees and grafted seedlings. The regression analysis was found to be a strong tool to compare the data obtained in both donors and grafted scions, with regard to this age- and size-related study. The individual data taken for each tree in the field and grafted seedling was regressed against its individual tree and scion age. Three main parameters, i.e. *SLA*, RGR_{mass} and E_G, were used in this analysis. Non-linear regressions using power functions and linear regressions were used as necessary.

The *SLA* characteristics were assessed in both growing seasons (2003 and 2004) in the grafted seedlings for both species and compared with the ones obtained in the donor trees in 2004 (Figure 2.7). The *SLA* was found to significantly decline at $p<0.001$ with age in the donors. This trait was also declined at $p<0.01$ with increasing scion age in the second growing season for *A. pseudoplatanus*, but no significant decline was observed in the first growing season for this species. However, *F. excelsior* grafted seedlings showed significantly decline with age in *SLA* for both growing seasons.

Figure 2.8 showed the regression analyses between RGR_{mass} and age. The rates of decline in RGR_{mass} with age for the donor trees were very similar in both species. Higher significant decline trends at $p<0.001$ were found in both species. Meanwhile, these trends did not persist in their grafted scions, as found for E_G. The age-related trends in RGR_{mass} disappeared in grafted seedlings indicating that the scion genotypes did not influence the growth characteristics of grafted seedlings.

For each species, E_G strongly declined with age for the donor trees sampled in the field, as shown in Figure 2.9. However, when E_G was compared across scion ages in grafted seedlings, there was no age-related decline observed for either species.

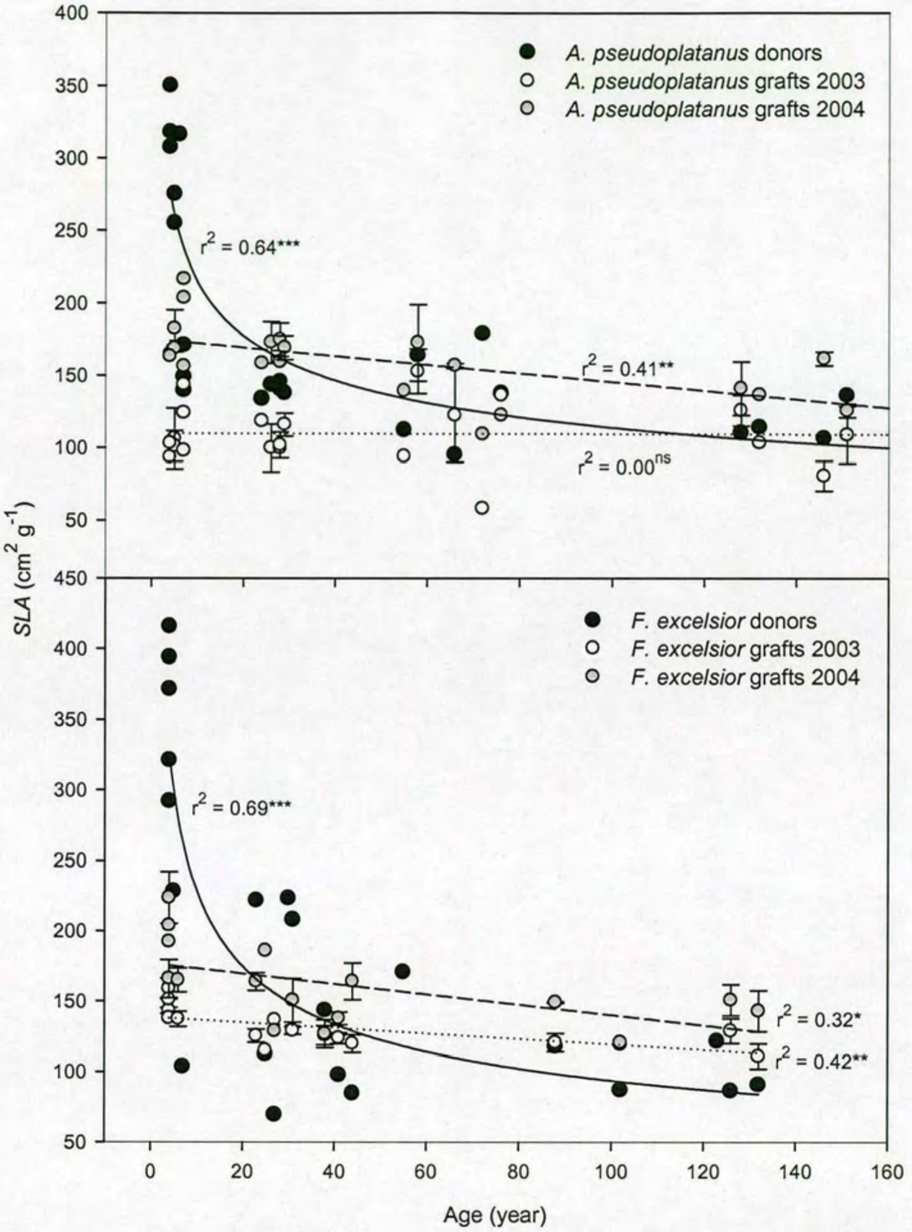

Figure 2.7: Regression analyses between specific leaf area (*SLA*) and age of each individual donor tree and grafted scions. The bar indicates standard error, ns = not significant ($p>0.05$), *=$p<0.05$, **=$p<0.01$ and ***=$p<0.001$.

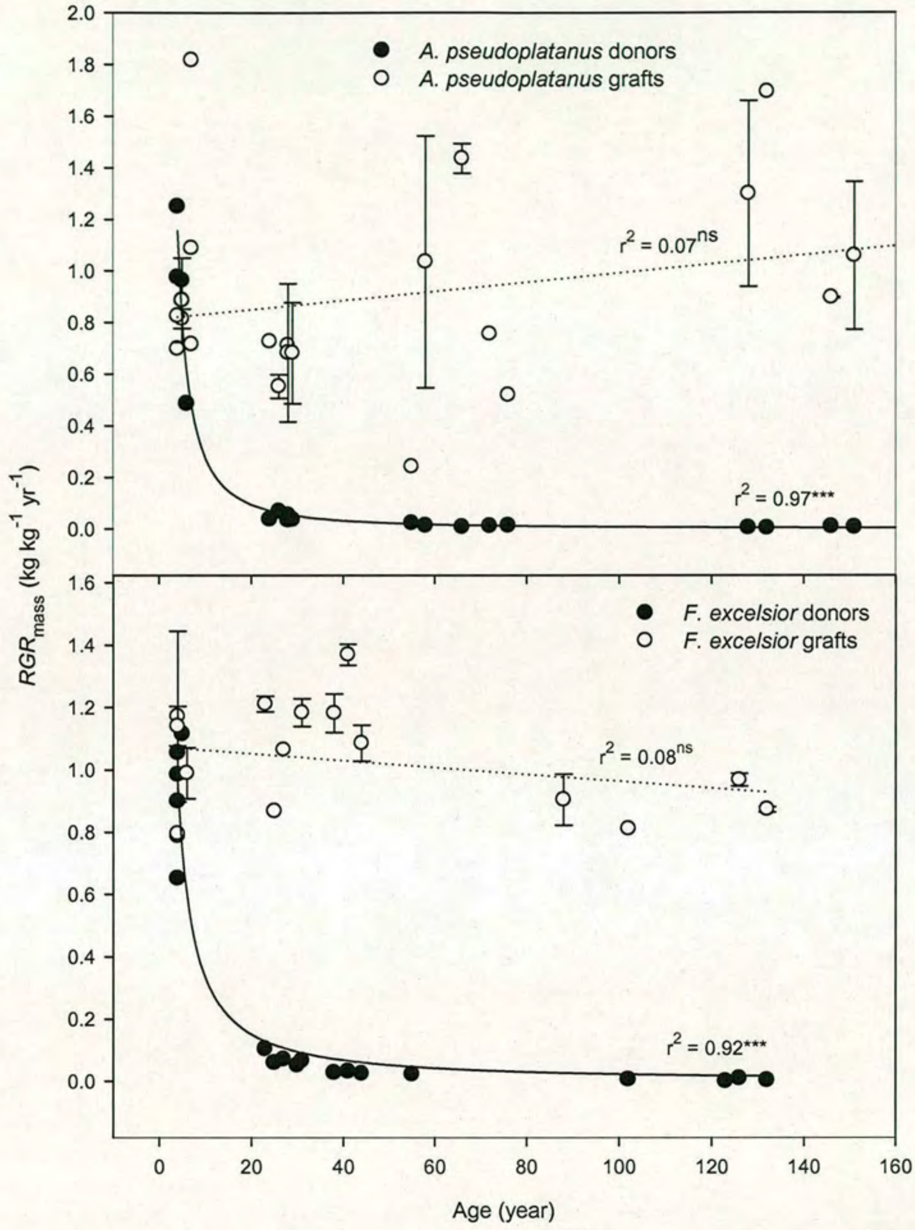

Figure 2.8: Relationship between relative mass growth rate (RGR_{mass}) and age of each individual donor tree and grafted scions. The bar indicates standard error, ns = not significant and ***=$p<0.001$.

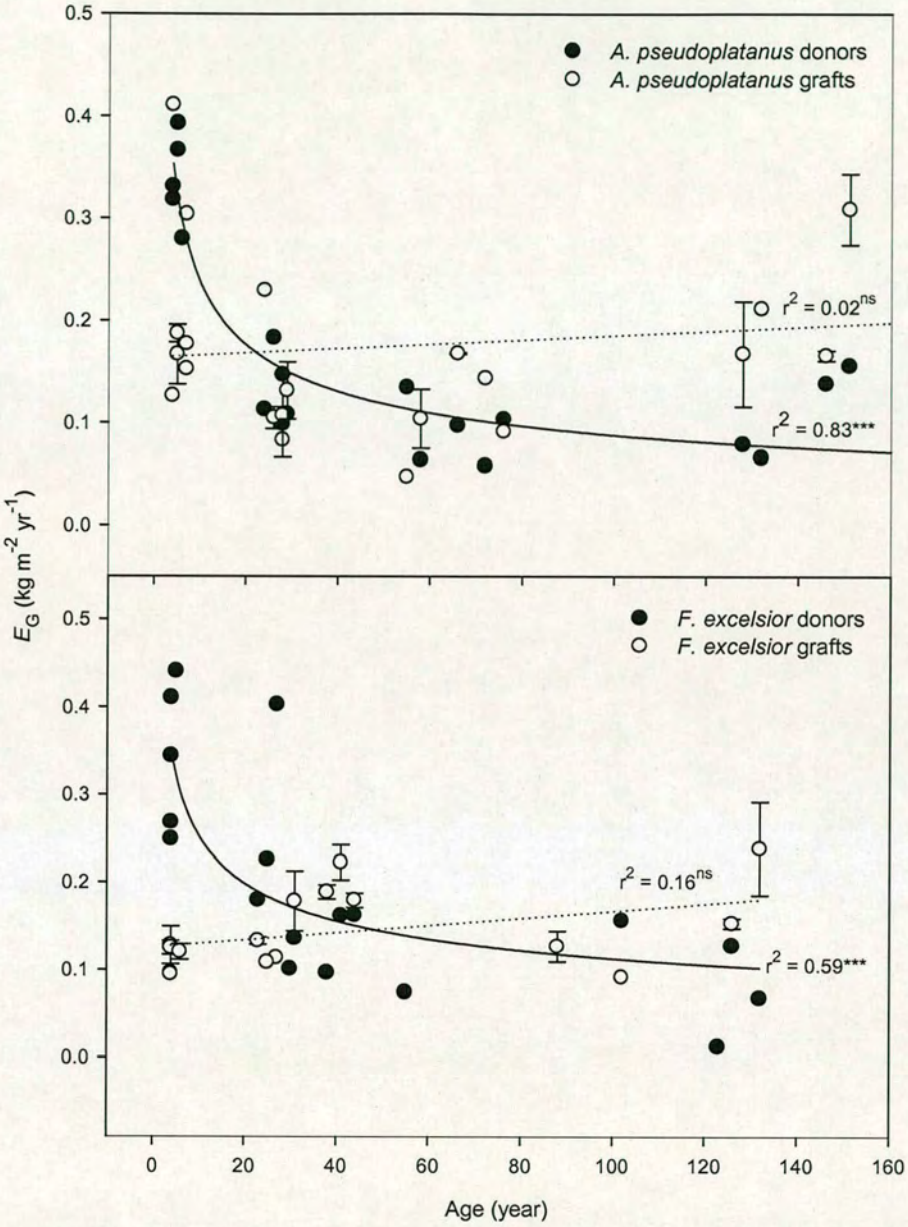

Figure 2.9: Relationship between growth efficiency (E_G) and age of each individual donor tree and grafted scions. The bar indicates standard error, ns = not significant and ***=$p<0.001$.

2.4 DISCUSSION

All growth parameters from donor trees except AGR_{mass} declined with tree age in both species. AGR_{mass} increased with increasing age of donor trees. This is not surprising because absolute mass in new growth is much larger in the big trees compared to the smaller trees, but relative to the size of the individuals, the small trees are growing much faster each year as shown by their relative growth rate.

The trends of age-related decline of tree growth with age as shown by E_G, were more pronounced in *F. excelsior* than in *A. pseudoplatanus* (Figure 2.6) but similar trends were visible when the regression analyses were done for both species (Figure 2.9).These results suggest that, for the two broadleaf species investigated, trees have visible growth declines with regard to growth efficiency after reaching 20 years of age. In both species, the trees were growing very rapidly during younger stages (<7 years) and the growth rates began to decrease afterwards as shown in Figure 2.5. The growth rates were more likely to level-out when the trees reached 40 year-old of age and over (Figure 2.8). Ryan and Waring (1992) observed a decrease in E_G as well as in aboveground net primary production (ANPP) in old lodgepole pine, but their results were obtained on a conifer. Furthermore, Ryan *et al.* (1997) also observed a decline in individual tree growth with advancing age and this decline resulted from reduced efficiency. Furthermore, these results were also consistent with the ones obtained in Scots pine (Mencuccini and Grace 1996a). Parallel work conducted on Scots pine and a poplar clone (*Populus deltoides* x *balsamifera* ssp. *Trichocarpa*) showed also very similar patterns (Mencuccini *et al.* 2005). This confirms what has widely been discussed in various papers regarding the limitation of water and nutrient transport from roots to shoots caused by tree size. This factor has been stated as early as 1960s by Zimmerman (1975), which speculated that the distance between the apical shoots and the roots in large woody trees might be too great to allow efficient transport between them, which in turn causes a decline in growth with increasing size.

Moreover, apart from those growth parameters, *SLA* also showed declining trends with increasing age in the donor trees for both species. Since the leaves from our sampled older trees were found to be larger than those of younger trees (data not shown), these trends may be corresponding to the changes in leaf thickness rather than affected by leaf area. As different age is confounded with different size, it is well known that leaves in shaded areas receive less radiation compared to sun-exposed leaves especially in uneven-aged stands. With this factor, smaller trees tend to have thinner leaves but be more efficient in harvesting light, which contributes to the higher growth rates compared to big trees which canopies are more exposed to the direct sun light. Moreover, the leaves from the tall trees are likely to experience water stress due to the limitation in water transport. Water stress-induced increases leaf dry weight to turgid weight ratio in a drought treatment of *A. pseudoplatanus* seedlings and consequently reduced *SLA* (Khalil and Grace 1992). In eastern larch (*Larix laricina* (Du Roi) K. Koch), the decrease in *SLA* with increasing maturation is associated with increases in the cross-sectional area of the leaf and the size of the vascular cylinder (Takemoto and Greenwood 1993). Furthermore, these results were similar to the ones obtained by Day *et al.* (2001) in red spruce (*Picea rubens* Sarg.). They reported that the age-related trends in *SLA* continued well beyond reproductive maturity, but concluded that foliar morphology was driven by intrinsic factor such as age when they found that the trends occurred in both trees in the field and grafted scions.

Although the age-related trends in *SLA* did exist in our grafted seedlings, the growth characteristics did not follow the same trends. This is not surprising because morphological characteristics such as leaf shape and size may be retained in grafted seedlings, at least initially, that contribute to retaining the *SLA* trends as observed in this study. When scions from mature plants are grafted onto juvenile rootstock, they may retain most of their mature characteristics (Bond 2000). Although there are not many studies related to grafting on forest trees to support the argument that phenological characteristics are retained in grafted scions, alternative evidence can be obtained from agriculture- or horticulture-based tree species studies. For example, Knight (1975) found that scions taken from very young apple trees had not produced a single blossom compared to the ones taken from old trees. Furthermore, there were variations among age classes of grafted seedlings but none of the growth parameters showed clear age-related trends. The growth characteristics of our grafted seedlings likely corresponded to the ones obtained in Scots pine by Vanderklein *et al.* (2006). They found some significant differences among seedlings with different scion ages but they did not find a consistent trend of increasing or decreasing growth with increasing age of parent tree. They suggested that growth variation found in grafted seedlings were due to grafting success since this variation was age independent. In our case, since the survival rates were reasonable and root pruning had also been applied on rootstocks after grafting, the factor that most likely contributed to the significant differences among grafted seedlings was probably seedling size. Since there were no initial size difference, the grafted scions from older trees may become reinvigorated after grafting onto young rootstocks. Takemoto and Greenwood (1993) found that when the mature scion of eastern larch no longer had to compete with juvenile rootstock shoots, its vigour tend to increase.

In this chapter, the results showed that age-related trends of growth only occurred in donor trees but not in grafted seedlings, suggesting that the growth attributes are size-dependent rather than controlled by maturation (genetic) factor. Furthermore, *SLA* was found to decrease with increasing age in donor trees and grafted seedlings for both species about at a reduced rate. Similar results were obtained on two other species in a parallel study (Mencuccini *et al.* 2005). Overall, these results once again supported the fact that growth is reduced in aging trees but the underlying mechanism is still not well understood. Since this chapter mainly focused on growth characteristics in donor trees and grafted seedlings, further studies have to be done in order to provide a clearer picture in supporting this phenomenon. In the following chapters, we provide more evidence related to physiological characteristics as well as sap flow.

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CHAPTER 3

AGE- AND SIZE-RELATED CHANGES IN PHYSIOLOGICAL CHARACTERISTICS OF *ACER PSEUDOPLATANUS* AND *FRAXINUS EXCELSIOR* SPECIES

3.1 INTRODUCTION

Approximately 90% of a plant's dry weight originates from products fixed in photosynthesis (Poorter *et al.* 1990) and may reach 100% in tall trees. It is therefore not surprising, that photosynthesis has been the subject of many studies which sought to understand the basis of variation in tree growth. For many years, scientists have measured photosynthesis with various cuvette-based machines or concluded from leaf chemical composition such as nitrogen. Although photosynthesis is responsible for tree growth, causes of the decreased or increased growth rates are still not well understood. It is true that when photosynthesis is greatly impaired, growth declines. However, there appears to be a wide range of photosynthetic rates which do not appear to be related to growth. In fact there are situations where growth appears to control photosynthesis. For example, when the trees are getting older or taller, photosynthesis normally appears to decrease. But in certain cases the effect is reversed particularly in uneven age stands comparing between shaded and un-shaded trees. The causes for the decrease or increase in photosynthesis are likely multiple. Recently, many hypotheses have been proposed to explain this phenomenon as given in Chapter 1. Among those hypotheses, maturation and hydraulic limitation hypotheses are the most likely to explain this.

Studies of tree ageing from grafting have shown that physiological traits such as net photosynthesis and stomatal conductance are reduced with tree age independent of differences in tree size (Rebbeck *et al.* 1993, Day *et al.* 2001). Furthermore, height and diameter growth together with branch numbers have found to decrease with increased age of scions in Douglas-fir (Ritchie and Keeley 1994), eastern larch (Greenwood *et al.* 1989) and radiata pine (Sweet 1973). Thus, these grafting studies demonstrate that maturation results in changes in the growth habits of the apical meristem that persist even when the mature meristem is re-exposed to physiological conditions associated with a young plant, including input from a juvenile rootstock (Greenwood 1995). In contrast, net photosynthesis of *Hedera helix* (Bauer and Bauer 1980) and *Larix laricina* (Hutchison *et al.* 1990) have been found to increase with increases in scion ages. Therefore, for this case, the maturation hypothesis seems unlikely. With regard to age- and size related changes, some studies have shown that photosynthesis is reduced in tall or old trees because of the limitation of hydraulic transport. The age-related or size-related reductions in leaf-specific hydraulic conductance has been suggested as a main mechanism that constraint stomatal conductance of tall trees and consequently reduces photosynthesis and subsequently primary productivity (Yoda *et al.* 1994, Ryan and Yoder 1997). Leaf-specific hydraulic conductance may decrease with tree size as a result of a larger path length from soil to stomata, causing a reduction in stomatal conductance and photosynthesis that directly affects tree growth.

Considering the obvious difference in growth trends in Chapter 2, one would expect strong differences in carbon assimilation as well. Do these characteristics of carbon gain imply any differences in age or size? So, in the field, physiological characteristics and chemical composition at the leaf level were measured and compared to their grafted scion seedlings. The first aim is to link the reduction in tree growth when ageing with their physiological characteristics.

3.2 MATERIALS AND METHODS

3.2.1 Field gas exchange, water potential, nitrogen content and $\delta^{13}C$

Field gas exchange was carried out in summer 2004. Four sampling dates were chosen (Julian dates 175, 176, 177 and 178) and about eight to ten trees from four age classes in both species were selected randomly in each day. The measurements were taken between 1030 and 1430 GMT with ambient irradiance ranging between 1100 μmol photons m^{-2} s^{-1} and 2800 μmol photons m^{-2} s^{-1} (data from Edinburgh Gogarbank meteorological station) and ambient temperature was around 22°C to 26°C except Julian date 175 when the day was overcast .

The measurements were carried out using LCpro Portable Photosynthesis System (ADC, Inc., Lincoln, UK). This open-type photosynthesis system was equipped with a standard 2.5 X 2.5 cm broadleaf cuvette. Prior to measurement, calibrations for flow meter and CO_2 zero values were made. To avoid the effects of fluctuating environmental conditions, the cuvette irradiance was set at 1200 μmol photons m^{-2} s^{-1} (saturating irradiance) for both species based on trial measurements where photosynthesis saturates at >1000 μmol photons m^{-2} s^{-1} using external light unit with Red/Blue LED array. Cuvette C_a, temperature and relative humidity were set at 360 ppm CO_2, 25°C and 40% respectively. Branches about two to four meters long were taken from the top third of the donor tree crowns using a climber and pole pruner. Four to six leaves were measured directly from the cut branches within three minutes after the branches were cut. Trials were conducted prior to taking actual measurements where excised branches have not showed deleterious effects on photosynthetic rates within five to eight minutes. Day *et al.* (2001) also conducted similar procedure on *Picea rubens* Sarg. where they found that preliminary measurements indicated that excision of branches did not affect photosynthetic rates for at least six to ten minutes.

The measured leaves were then excised from the branch and placed in a black bag with wet tissues to avoid evaporation. These samples were brought back to the laboratory and the leaf water potential was taken using Portable Plants Moisture System (Skye Instruments Ltd, Powys. UK) with N_2. Leaf area was measured on these samples using a LICOR 3100 leaf area meter (LI-COR Inc, Lincoln, Nebraska, USA) and specific leaf area (*SLA*) was calculated after the leaves were oven dried at 60°C for about 48 hours.

The same samples were used to determine the leaf nitrogen content on a mass basis (N_m) and the $\delta^{13}C$ isotopes. These samples were first ground in the automatic freezer miller with liquid nitrogen and the ground samples were then placed in Eppendorf tubes before being sent to the Cornell University stable isotope laboratory, Ithaca, USA for analysis. Measurements of $\delta^{13}C$ isotopes were done on a Finnigan MAT Delta Plus mass spectrometer that was interfaced to a Carlo Erba NC2500 elemental analyzer (EA). The ^{13}C delta ($\delta^{13}C$) values were measured against the PDB scale, which is the international scale for measuring stable isotopes of carbon. Most organic plant samples have a negative value between -25 and -35 at natural abundance levels.

3.2.2 Grafted seedling gas exchange, nitrogen content and $\delta^{13}C$

The gas exchange was compared between four age classes together with two controls, i.e. self-grafted and rootstock in the first and the second growing season. In both growing seasons, ten trees from each age class in two species were randomly selected for gas exchange measurements. This included all seven surviving trees in age class three (AC3) of *A. pseudoplatanus*. The main characteristics of grafted seedlings used in this study were presented in Table 3.1. The selected seedlings were then placed in a constant environment such as a glasshouse prior to gas exchange measurements. Two sampling dates, i.e., June and August were selected in both growing seasons on the basis of seasonal changes of foliar attributes. Because the number of trees is high, the measurements were conducted on different days for both species. The gas exchange measurements were made on fully expanded leaves from the uppermost part of each selected grafted scion and rootstock. The LCpro Portable Photosynthesis System (ADC, Inc., Lincoln, UK) was used and cuvette climate was set as described in the field gas exchange. Three sequential measurements were made within 1 to 4 minutes, and the average values were used for analyses. After sampling, the leaves used in gas exchange measurements were severed and leaf water potentials were measured. In addition, predawn water potential measurements were made on all selected seedlings between 0230 and 0530 hours. The leaf area was also measured on the leaves taken in both water potential measurements for further study.

The leaves from six grafted seedlings in each age class taken in the first and the second growing season during gas exchange and water potential measurements were randomly selected from ten grafted seedlings in order to determine nitrogen content and $\delta^{13}C$. These leaves were oven dried at 60°C for about 48 hours before being weighed and ground in liquid nitrogen using freezer miller. The procedures for nitrogen and $\delta^{13}C$ analyses were similar to the ones applied in donor tree leaves.

Table 3.1: Characteristics of *Acer pseudoplatanus* and *Fraxinus excelsior* grafted seedlings used in this study. Mean attributes are based on the sample size of n = 10 for each age class in both species (except in age class 3 of *A. pseudoplatanus* where n = 7) over two growing seasons.

			2003			**2004**	
Class	**Scion Age**	**Diameter (cm)**	**Height (cm)**	***SLA*** **(cm^2 g^{-1})**	**Diameter (cm)**	**Height (cm)**	***SLA*** **(cm^2 g^{-1})**
Acer pseudoplatanus							
1	5.4 ± 0.4	0.94 ± 0.06	90.2 ± 9.6	108.3 ± 7.3	1.55 ± 0.05	175.9 ± 12.3	178.9 ± 6.9
2	27.5 ± 0.5	0.95 ± 0.06	102.0 ± 8.7	107.0 ± 4.7	1.45 ± 0.05	162.2 ± 6.4	169.4 ± 4.7
3	64.4 ± 2.9	0.81 ± 0.07	66.1 ± 12.4	119.8 ± 15.0	1.38 ± 0.07	140.6 ± 8.8	147.0 ± 10.9
4	143.4 ± 4.3	0.79 ± 0.07	61.5 ± 9.6	110.5 ± 7.3	1.32 ± 0.05	153.7 ± 6.5	139.7 ± 6.4
Fraxinus excelsior							
1	5.0 ± 0.4	1.03 ± 0.07	65.9 ± 7.6	148.5 ± 4.2	1.49 ± 0.07	148.3 ± 8.6	183.7 ± 9.8
2	25.2 ± 1.1	0.84 ± 0.04	29.6 ± 2.3	126.1 ± 3.2	1.31 ± 0.05	106.9 ± 7.8	159.8 ± 6.3
3	40.4 ± 0.9	0.75 ± 0.03	26.6 ± 3.3	123.5 ± 3.8	1.19 ± 0.04	98.4 ± 13.2	140.2 ± 7.9
4	117.8 ± 5.7	0.93 ± 0.04	27.1 ± 3.0	120.8 ± 4.8	1.18 ± 0.04	94.5 ± 10.5	145.1 ± 6.3

The '±' represents mean standard error.

3.2.3 Photosynthetic efficiency and capacity of grafted seedling

An extended experiment was carried out on the grafted seedlings to determine the response of photosynthesis to different light regimes (efficiency) and CO_2 concentrations (capacity). Photosynthetic efficiency and capacity were assessed using different light intensities (*A/Q* curve) and response curves of photosynthesis to intercellular CO_2 (A/C_i curve). In *A/Q* response curve, cuvette C_a was kept at ambient level (360 μmol mol^{-1}) and constant temperature under high and stable humidity conditions. Measurements were started from 530 μmol mol^{-1} and increased in three steps, i.e., 760, 950 and 1190 μmol mol^{-1} until complete light saturation was reached and then decreased in five steps, i.e., 330, 150, 100, 50 and 0 μmol mol^{-1}. Quantum efficiency and saturation photosynthesis were derived from these curves. The response of net photosynthesis (*A*) to light level (*Q*) can be modelled by a non-rectangular hyperbola where the initial slope is Apparent Quantum Efficiency (Φ), the light compensation point and apparent respiration are estimated from axis intercepts and the light saturated maximum photosynthesis ($A_{\max}$) is the upper asymptote. An additional parameter k (convexity) is required to describe the progressive rate of bending between the linear gradient and maximum value. All these parameters can be determined by fitting data to the model function expressed as a quadratic equation by Prioul and Chartier (1977) as below:

$$A = \frac{\Phi \cdot Q + A_{\max} - \sqrt{(\Phi \cdot Q + A_{\max})^2 - 4\Phi \cdot Q \cdot k \cdot A_{\max}}}{2k} - R_{day} \tag{3.1}$$

where R_{day} refers to the release of CO_2 in the light by processes other than photorespiration (Brooks and Farquhar 1985).

Meanwhile, the A/C_{i} response curve was determined by measuring photosynthesis rate (A) and intercellular CO_2 (C_{i}) at a series of ambient CO_2 concentrations (C_a). A protocol in determining this response (e.g Ainsworth *et al.* 2002) was used in this study as follows: (1) Induce photosynthesis at the growth C_a until a steady-state A is obtained (2) Then C_a is decreased first to 300 μmol mol^{-1}, A will drop and as soon as C_a is stable (3) A and C_{i} are recorded, and C_a is again dropped to 250, 200, 150, 100 and finally 50 μmol mol^{-1} (4) C_a is then returned to 370 μmol mol^{-1} (ambient) to check that the original A can be restored. If this achieved then C_a is increased in the following order, 450, 550, 650, 800 and 1000 μmol mol^{-1}. Steady-state photosynthesis needs to be obtained at each step (Long and Bernacchi 2003). The maximum carboxylation rate (Vc_{max}) and maximum electron transport rate (J_{max}) were calculated from A/C_{i} curves using non-linear least squares regression to fit the values of these parameters to the best fit equation of von Caemmerer and Farquhar (1981) photosynthesis model (Harley *et al.* 1992). The A/C_{i} response curve consists three phases. The first phase is the initial response below C_{i} concentrations of approximately 20 Pa where ribulose bisphosphate (RuBP) is saturated and Rubisco activity limits carboxylation. The second phase is the slower rise of the curve beyond its inflection point. The higher C_{i} levels present within this phase result in the limiting factor being the supply of RuBP. This model can be used to provide estimations of the $Vc_{\max}$ and $J_{\max}$. The calculation of these parameters according to the model uses equations as below:

$$A = \left(1 - \frac{0.5O}{\tau \cdot C_i}\right) \cdot \min(W_c, W_j, W_p) - R_{day} \tag{3.2}$$

where O is the concentration of oxygen in the stroma in unit Pascal (Pa) and τ (Tau) represents the specificity factor for Rubisco (Jordan and Ogren 1984). When the rate of carboxylation is solely limited by the activity of Rubisco, carboxylation can be described by the equation:

$$W_c = \frac{Vc_{\max} \cdot C_i}{\left[C_i + K_c(1 + O / K_o\right]} \tag{3.3}$$

where K_c and K_o respectively are the Michaelis-Menten constants of Rubisco for CO_2 and O_2. The conditions of this limitation can be imposed by low C_i levels (<20 Pa) and high irradiance (>1500 μmol m^{-2} s^{-1}). When electron transport limits photosynthesis by the regeneration of RuBP, carboxylation rate can be expressed by the following equation:

$$W_j = \frac{J \cdot C_i}{4(C_i + O / \tau)} \tag{3.4}$$

The factor 4 represents the fact that four electrons will generate sufficient ATP and NADPH to regenerate RuBP (Farquhar and von Caemmerer 1982). J, the potential rate of electron transport, is calculated using the empirical relationship (Harley *et al.* 1992) as below:

$$J = \alpha \cdot I \div \sqrt{1 + \left(\frac{\alpha \cdot I}{J_{\max}}\right)^2} \tag{3.5}$$

where α is the efficiency of the light conversion and $J_{\max}$ is the light saturated rate of electron transport and I is the incident radiation.

Photosyn Assistant Software package (Dundee Scientific, UK) was used for calculations and data interpretation using abovementioned equations for both A/C_i and A/Q curves.

3.2.4 Data analyses

The data obtained from gas exchange of repeating measurements were summed and averaged for each individual tree prior to any data analysis. If necessary, data transformations (normalised) were applied to stabilise error variance. These data were then analysed using one-way analysis of variance (ANOVA) and general linear model (GLM) for balanced and unbalanced data among age classes in both species respectively. The mean values obtained were compared among age classes using Duncan Multiple Range Test (DMRT). All the statistical analyses were performed using Statistical Analysis System version 9.0 (SAS Institute Inc. 2002) and the significance level was set at 0.05. The linear regression analyses were also carried out on some of the data and plotted against tree age and leaf characteristics using Sigma Plot 9.0 (Systat Software Inc. 2004). The slopes of the regression equations were tested by analysis of covariance (ANCOVA) in SPSS version 12.0 (SPSS Inc. 2003).

3.3 RESULTS

3.3.1 Leaf level gas exchange, leaf water potential, nitrogen content and $\delta^{13}C$ of the donor trees

Analysis of variance (ANOVA) showed variable effects of age class on physiological parameters such as net photosynthesis (A_{net}), internal CO_2 (C_i), stomatal conductance (G_s) or even leaf characteristics and chemical compositions such as leaf water potential, specific leaf area (*SLA*), nitrogen content (N_m) and carbon isotope discrimination ($\delta^{13}C$) in both species (Table 3.2). The A_{net} was found significantly different among age classes at $p<0.01$ in *A. pseudoplatanus* and $p<0.05$ in *F. excelsior*. A highly significant difference ($p<0.01$) was found in C_i in *A. pseudoplatanus* but this parameter was not significantly different among age classes in *F. excelsior*. In contrast, G_s was not significantly different among age classes for either species. The differences in result obtained between A_{net} and G_s or even C_i in both species could be due to the differences in leaf characteristic such as leaf water potential (Ψ_{leaf}) and *SLA*. A highly significant difference at $p<0.001$ was found in Ψ_{leaf} and *SLA* among age classes in *A. pseudoplatanus*. In contrast, no significant difference was found in Ψ_{leaf} in *F. excelsior* but only *SLA* showed significant difference ($p<0.01$) in this species. Furthermore, the most promising parameters to show the differences among age classes in the donor trees were contained in the leaf chemical compositions such as nitrogen content (N_m) and carbon isotope discrimination ($\delta^{13}C$). There was no significant differences among age classes detected in N_m in both species but $\delta^{13}C$ was found significant at $p<0.01$ in *A. pseudoplatanus* and at $p<0.001$ among age classes in *F. excelsior* respectively.

Table 3.2 Summary of ANOVA of leaf-level gas exchange, *SLA* and leaf chemical composition in *A. pseudoplatanus* and *F. excelsior* donors.

	Source of Variation AGE CLASS	
	A. pseudoplatanus	***F. excelsior***
Parameters	**F value**	**F value**
Net assimilation rate, A_{net} (μmol g^{-1} s^{-1})	5.20**	2.57*
Internal CO$_2$, C_i (μmol mol^{-1})	5.67**	1.66^{ns}
Stomatal conductance, G_s (mmol m^{-2} s^{-1})	0.85^{ns}	0.34^{ns}
Leaf water potential, Ψ_{leaf} (-MPa)	9.84***	0.02^{ns}
Specific leaf area (*SLA*)	8.96***	8.71**
Nitrogen content, N_m (% mass)	0.99^{ns}	2.91^{ns}
Carbon isotope discrimination, δ^{13}C	7.79**	11.25***

Notes: *** Significantly different at $P < 0.001$
** Significantly different at $P < 0.01$
* Significantly different at $P < 0.05$
ns Not Significant

The measured values of A_{net} on the basis of leaf area ranged from 7.19 to 8.76 and 6.48 to 9.51 μmol m^{-2} s^{-1} in *A. pseudoplatanus* and *F. excelsior*, respectively, but no age-related trend was observed. Furthermore, the age-related trends were only found in both species in A_{net} on the basis of leaf mass as shown in Figure 3.1A. This trend was not observed in other gas exchange parameters such as C_i and G_s in either species (Figures 3.1B and 3.1C). The C_i was also found higher in the youngest class in *A. pseudoplatanus*, followed by AC4, AC2 and AC3 as opposed to A_{net} in leaf mass basis. Similar trends were also observed in *F. excelsior*, as illustrated in Figure 3.1B. An age-related trend in G_s was found for the first three age classes in *A. pseudoplatanus*. However, no age-related trend was found for this parameter in *F.*

excelsior, for which AC3 showed highest value, followed by AC4, AC2 and AC1. Furthermore, higher value of Ψ_{leaf} was found in AC1 followed by AC2, AC4 and AC3 for *A. pseudoplatanus*, whereas this parameter was found higher in AC3 for *F. excelsior* followed by AC4, AC2 and AC1 (Figure 3.1D). In contrast, leaf characteristics such as *SLA* sampled during gas exchange showed an age-related trend in both species (Figure 3.2A). This parameter may contribute to the age-related trend observed in A_{net} compared with other gas exchange parameters. Further observations found that the trends recorded in Ψ_{leaf} were similar with the ones recorded in G_{s} in both species respectively. Hence, the G_{s} values obtained during one-time leaf level gas exchange measurements may have been directly affected by Ψ_{leaf}.

Since gas exchange parameters are directly affected by timing and environmental conditions, the leaf chemical compositions were also determined in the same samples taken during gas exchange measurements. Although the mean values of N_{m} were not found to significantly differ among age classes in either species as indicated in Table 3.2, age related trends were clearly observed as shown in Figure 3.2B. Similar patterns were also found in $\delta^{13}C$ in both species in the field (Figure 3.2C).

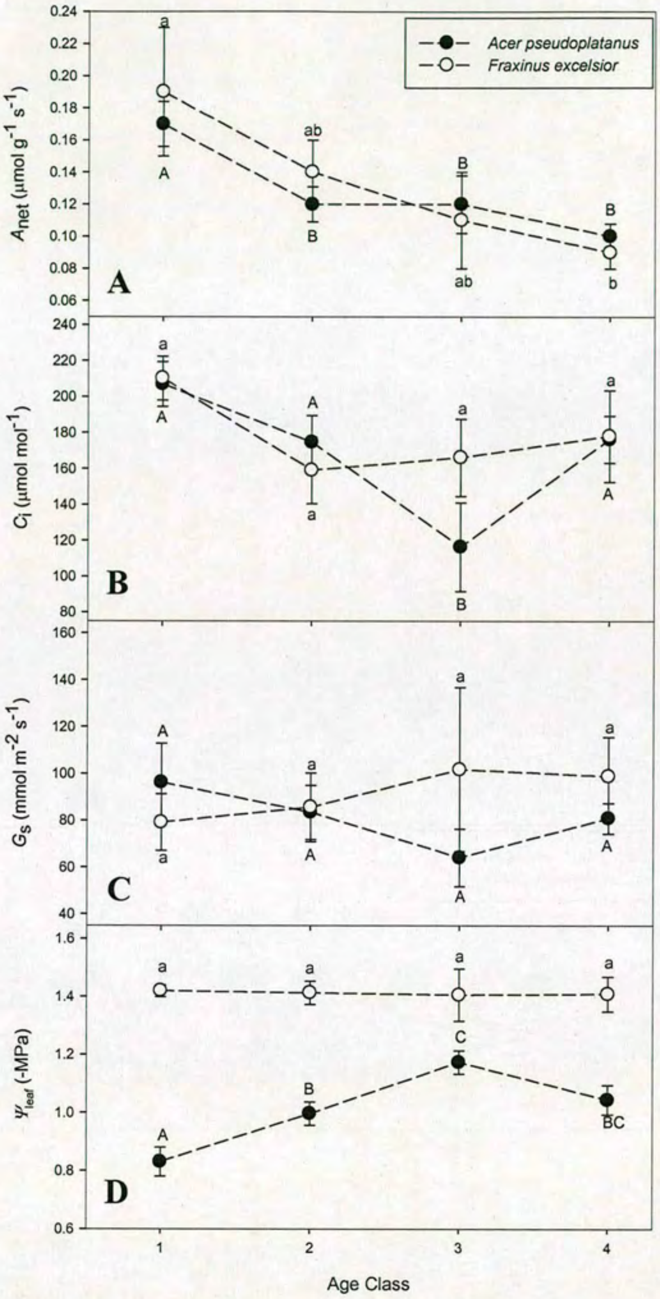

Figure 3.1: The mean values of net photosynthesis (A), internal CO_2 (B), stomatal conductance (C) and leaf water potential (D) of both species. The different letters represent statistically significant differences across age classes.

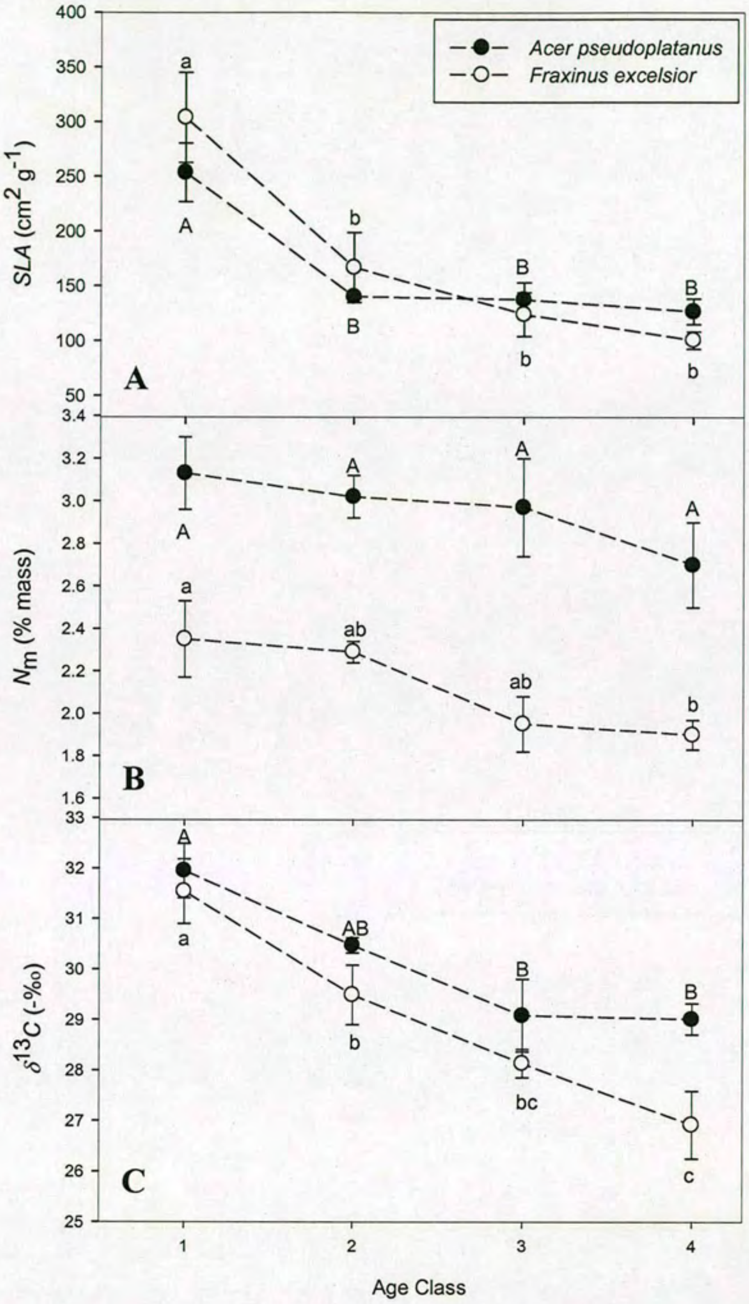

Figure 3.2: The mean values of specific leaf area (A), nitrogen content (B) and carbon isotope composition (C) of both species. The different letters represent statistically significant differences across age classes.

3.3.2 Leaf level gas exchange, leaf water potential, nitrogen content and $\delta^{13}C$ of the grafted seedlings

Analogous measurements were also carried out on grafted seedlings to determine whether the scion ages behaved similarly to their donors. Leaf level gas exchange, leaf water potential (Ψ_{leaf}) and leaf chemical composition from the same leaf samples were determined in grafted seedlings from four classes of scion age and two types of control, i.e., rootstocks and self-grafted in the first and the second growing season. ANOVA and GLM procedures were used as necessary, and DMRT was used for grouping. The data were first normalised prior to these analyses. Table 3.3 showed the summarised outcomes from these analyses on those parameters stated above.

Net assimilation rates (A_{net}) on the basis of leaf mass were found to be significantly different among scion ages and controls of *A. pseudoplatanus* and *F. excelsior* for both growing seasons. A highly significant level at $p<0.001$ was found for the first growing season for *A. pseudoplatanus*, whereas this significance level was detected in both growing seasons for *F. excelsior*. The A_{net} was found to be less significantly difference in the second growing season for *A. pseudoplatanus*. However, internal CO_2 (C_i) was not significantly different among scion ages and controls in *A. pseudoplatanus* in either seasons, but this parameter did differ only in the first growing season for *F. excelsior*. The G_s values from both growing seasons were found to be significantly different among scion ages and controls in both species. The G_s did differ at $p<0.01$ in the first growing season and at $p<0.001$ in the second growing season for *A. pseudoplatanus*, whereas this parameter was found to have a highly significant difference at $p<0.001$ in both growing seasons for *F. excelsior*. Differences in Ψ_{leaf} were found to be not significant in the first growing season for *A. pseudoplatanus*, but a less significant difference was detected in the second growing season. Meanwhile, this parameter was found less significant in the first growing season, whereas higher significant level was observed in the second growing season in *F. excelsior*. The effects of scion age and control in N_m were significantly different in both species in the first growing season but no significant effect was found in the second growing season. However, the $\delta^{13}C$ parameter of both species was consistently affected by scion age and control in both growing seasons.

Table 3.3: Summary of ANOVA of leaf-level gas exchanges and chemical compositions in *A. pseudoplatanus* and *F. excelsior* grafted seedlings.

	Source of Variation AGE CLASS			
	A. pseudoplatanus		***F. excelsior***	
Parameters	**F value**		**F value**	
	2003	2004	2003	2004
Net assimilation rate, A_{net} (μmol g^{-1} s^{-1})	5.18***	2.85*	21.21***	4.64***
Internal CO_2, Ln C_i (μmol mol^{-1})	2.13^{ns}	0.52^{ns}	4.62**	0.83^{ns}
Stomatal conductance, Ln G_s (mmol m^{-2} s^{-1})	3.58**	14.7***	5.49***	9.11***
Leaf water potential, Ψ_{leaf} (-MPa)	0.73^{ns}	2.89*	3.31*	17.18***
Nitrogen content, N_m (% mass)	4.47**	0.95^{ns}	4.37**	2.27^{ns}
Carbon isotope discrimination, $\delta^{13}C$	8.41***	2.71*	9.35***	17.37***

Notes: *** Significantly different at $P < 0.001$
** Significantly different at $P < 0.01$
* Significantly different at $P < 0.05$
ns Not Significant

Table 3.4 shows the mean values of those parameters discussed above. With no regards to controls, the mean values of A_{net} on the basis of leaf mass were found higher in AC2 followed by AC3, AC4 and AC1 in first growing season. Meanwhile this parameter was found greater in AC1 followed by AC4, AC2 and AC3 of *A. pseudoplatanus* in second growing season. In *F. excelsior*, highest values of A_{net} in leaf mass basis were found in AC1 and AC4, and lowest values were observed in AC2 and AC1 in both growing seasons respectively. Obviously, the age-related decline trend in A_{net} did not persist in the grafted seedlings of either species. Moreover, the mean values of A_{net} on the basis of leaf area did not show any age-related trend. In *A. pseudoplatanus*, these values ranged from 8.08 to 10.06 and 8.20 to 13.31 μmol m^{-2} s^{-1} in first and second growing season respectively. In *F. excelsior*, the mean values of A_{net} in leaf area basis were ranged from 14.42 to 19.48 and 8.66 to 13.90 μmol m^{-2} s^{-1} in first and second growing season respectively. Furthermore, the mean values of C_i did not vary among four classes of scion ages and controls in first and second growing season in either species, except in the first growing season in *F. excelsior*. Again, this parameter did not show any declining trend with increasing scion ages. Moreover, the G_s parameter was highly affected by scion ages and controls but apparently there was no trend with regards to their scion ages observed in either species. The mean value of G_s was found higher in AC1 followed by AC2, AC4 and AC3 in first growing season, whereas this mean value was found greater in AC4 followed by AC1, AC3 and AC2 during the second growing season in *A. pseudoplatanus*. In *F. excelsior*, this parameter was found greater in AC1 followed by AC3, AC4 and AC2 in first growing season. However, an age-related trend in G_s was observed in the second growing in *F. excelsior*, although this trend was found in reverse order with greater values recorded in AC4 followed by AC3, AC2 and AC1. Generally, none of the observed parameters showed any age-related decline trends, indicating that the differences with regards to age in physiological characteristics or even leaf chemical compositions tended to disappear in the grafted scions.

Table 3.4: Summary of leaf level gas exchange parameters, leaf water potential and chemical composition of grafted seedlings and controls in both species.

Class	A_{net} **(μmol g^{-1} s^{-1})**	**Ln *Ci* (μmol mol^{-1})**	**Ln G_S (mmol m^{-2} s^{-1})**	Ψ_{leaf} **(MPa)**	N_m **(%)**	$\delta^{13}C$
Acer pseudoplatanus						
					2003	
1	0.087 ± 0.008^{A}	2.460 ± 0.006^{A}	2.440 ± 0.048^{A}	-0.396 ± 0.04^{A}	2.30 ± 0.13^{C}	-29.55 ± 0.48^{B}
2	0.099 ± 0.006^{A}	2.398 ± 0.012^{A}	2.344 ± 0.041^{AB}	-0.369 ± 0.03^{A}	2.64 ± 0.04^{BC}	-28.91 ± 0.30^{B}
3	0.098 ± 0.012^{A}	2.380 ± 0.017^{AB}	2.159 ± 0.058^{B}	-0.408 ± 0.02^{A}	3.09 ± 0.06^{A}	-29.54 ± 0.19^{B}
4	0.091 ± 0.008^{A}	2.408 ± 0.013^{A}	2.232 ± 0.052^{B}	-0.391 ± 0.03^{A}	2.55 ± 0.18^{BC}	-28.97 ± 0.48^{B}
SG	0.095 ± 0.009^{A}	2.399 ± 0.006^{A}	2.200 ± 0.047^{B}	-0.396 ± 0.03^{A}	2.49 ± 0.15^{BC}	-27.73 ± 0.38^{A}
RS	0.052 ± 0.005^{B}	2.214 ± 0.135^{B}	1.769 ± 0.101^{C}	-0.450 ± 0.03^{A}	2.89 ± 0.17^{AB}	-26.92 ± 0.25^{A}
					2004	
1	0.223 ± 0.022^{A}	2.254 ± 0.024^{A}	2.162 ± 0.061^{AB}	-0.598 ± 0.03^{B}	2.32 ± 0.26^{A}	-29.78 ± 0.53^{B}
2	0.150 ± 0.018^{BC}	2.256 ± 0.034^{A}	1.935 ± 0.078^{C}	-0.580 ± 0.02^{B}	2.42 ± 0.04^{A}	-28.98 ± 0.47^{AB}
3	0.123 ± 0.018^{C}	2.301 ± 0.016^{A}	2.010 ± 0.069^{BC}	-0.478 ± 0.02^{A}	2.35 ± 0.17^{A}	-28.42 ± 0.61^{AB}
4	0.185 ± 0.010^{AB}	2.265 ± 0.016^{A}	2.235 ± 0.029^{A}	-0.465 ± 0.03^{A}	2.10 ± 0.18^{A}	-28.83 ± 0.72^{AB}
SG	0.186 ± 0.019^{AB}	2.263 ± 0.015^{A}	2.047 ± 0.064^{BC}	-0.530 ± 0.04^{AB}	2.49 ± 0.13^{A}	-27.83 ± 0.31^{A}
RS	0.191 ± 0.025^{AB}	2.258 ± 0.015^{A}	2.007 ± 0.049^{BC}	-0.501 ± 0.04^{AB}	2.58 ± 0.15^{A}	-27.42 ± 0.33^{A}

Table 3.4 (continued)

Fraxinus excelsior						
					2003	
1	0.289 ± 0.012 ^{B}	2.358 ± 0.009 ^{AB}	2.693 ± 0.051 ^{AB}	-0.346 ± 0.02 ^{AB}	2.87 ± 0.09 ^{C}	-27.64 ± 0.32 ^{B}
2	0.179 ± 0.013 ^{C}	2.350 ± 0.008 ^{B}	2.439 ± 0.069 ^{C}	-0.374 ± 0.02 ^{A}	3.35 ± 0.06 ^{A}	-26.93 ± 0.06 ^{A}
3	0.219 ± 0.008 ^{C}	2.375 ± 0.008 ^{A}	2.683 ± 0.048 ^{AB}	-0.297 ± 0.02 ^{BC}	3.21 ± 0.08 ^{AB}	-28.14 ± 0.17 ^{B}
4	0.204 ± 0.008 ^{C}	2.351 ± 0.005 ^{B}	2.584 ± 0.048 ^{B}	-0.355 ± 0.03 ^{AB}	3.30 ± 0.05 ^{A}	-27.64 ± 0.14 ^{B}
SG	0.374 ± 0.032 ^{A}	2.350 ± 0.006 ^{B}	2.786 ± 0.040 ^{A}	-0.261 ± 0.02 ^{C}	3.28 ± 0.12 ^{A}	-27.82 ± 0.06 ^{B}
RS	0.377 ± 0.026 ^{A}	2.329 ± 0.004 ^{C}	2.621 ± 0.039 ^{B}	-0.306 ± 0.03 ^{ABC}	2.98 ± 0.12 ^{BC}	-26.84 ± 0.08 ^{A}
					2004	
1	0.159 ± 0.014 ^{C}	2.112 ± 0.064 ^{A}	1.886 ± 0.072 ^{CD}	-0.706 ± 0.03 ^{B}	2.27 ± 0.04 ^{A}	-26.36 ± 0.40 ^{A}
2	0.191 ± 0.008 ^{ABC}	2.187 ± 0.042 ^{A}	2.119 ± 0.057 ^{AB}	-0.624 ± 0.02 ^{C}	2.61 ± 0.12 ^{A}	-27.59 ± 0.16 ^{B}
3	0.189 ± 0.003 ^{BC}	2.194 ± 0.037 ^{A}	2.197 ± 0.032 ^{A}	-0.565 ± 0.02 ^{C}	2.61 ± 0.16 ^{A}	-29.02 ± 0.17 ^{C}
4	0.196 ± 0.007 ^{AB}	2.226 ± 0.021 ^{A}	2.205 ± 0.025 ^{A}	-0.581 ± 0.03 ^{C}	2.24 ± 0.05 ^{A}	-28.34 ± 0.32 ^{C}
SG	0.221 ± 0.014 ^{A}	2.129 ± 0.060 ^{A}	1.986 ± 0.063 ^{BC}	-0.705 ± 0.01 ^{B}	2.25 ± 0.14 ^{A}	-26.85 ± 0.11 ^{A}
RS	0.167 ± 0.010 ^{BC}	2.146 ± 0.041 ^{A}	1.825 ± 0.051 ^{D}	-0.868 ± 0.03 ^{A}	2.21 ± 0.17 ^{A}	-26.97 ± 0.13 ^{AB}

The '±' represents mean standard error. Different letters indicate significant differences between age classes within species.

3.3.3 Photosynthetic capacity and efficiency

An extensive study was conducted on the grafted seedlings by looking at their photosynthetic efficiencies and capacities. These experiments were conducted over two growing seasons. All the results obtained over both growing seasons were summarised in Figure 3.3. The mean values of apparent quantum efficiency (Φ) observed in the two growing seasons were not significantly different among scion ages and controls in both species respectively. Regardless of the controls, no age-related trend was observed in either the first or the second growing season in *A. pseudoplatanus* as well as in *F. excelsior*.

The mean values of maximum photosynthetic rates ($A_{\max}$) did not differ among scion ages and controls in either the first or the second growing season in *A. pseudoplatanus*. But these values were found higher in the second growing season compared to the first growing season. In *F. excelsior*, $A_{\max}$ showed very similar values in the second growing season across all groups. However, the mean values of this parameter were found to be quite different among different scion ages and controls during the first growing season. Again, no age-related trend was found in either species, although the experiments were carried out over two growing seasons.

The mean values of maximum carboxylation rate (Vc_{max}) and maximum electron transport rate (J_{max}) in each scion age and control over both growing seasons were also presented in the figure above. In the first growing season, the mean value of Vc_{max} was found higher in AC1 in *A. pseudoplatanus* and AC2 in *F. excelsior*. In the second growing season, self-grafted seedlings showed highest mean value of Vc_{max} compared to rootstock and scion age classes in *A. pseudoplatanus* but this parameter was found higher in AC3 in *F. excelsior*. Meanwhile, AC1 and AC2 showed the highest mean value of J_{max} in the first growing season for both species respectively. In the second growing season, AC4 in both species showed highest mean value of J_{max} compared to the rest of the treatments. With regards to the age-related trend, a similar result as observed in parameters obtained in *A*/*Q* curve analyses was also observed in these two parameters obtained from the mechanistic A/C_{i} response curve analyses.

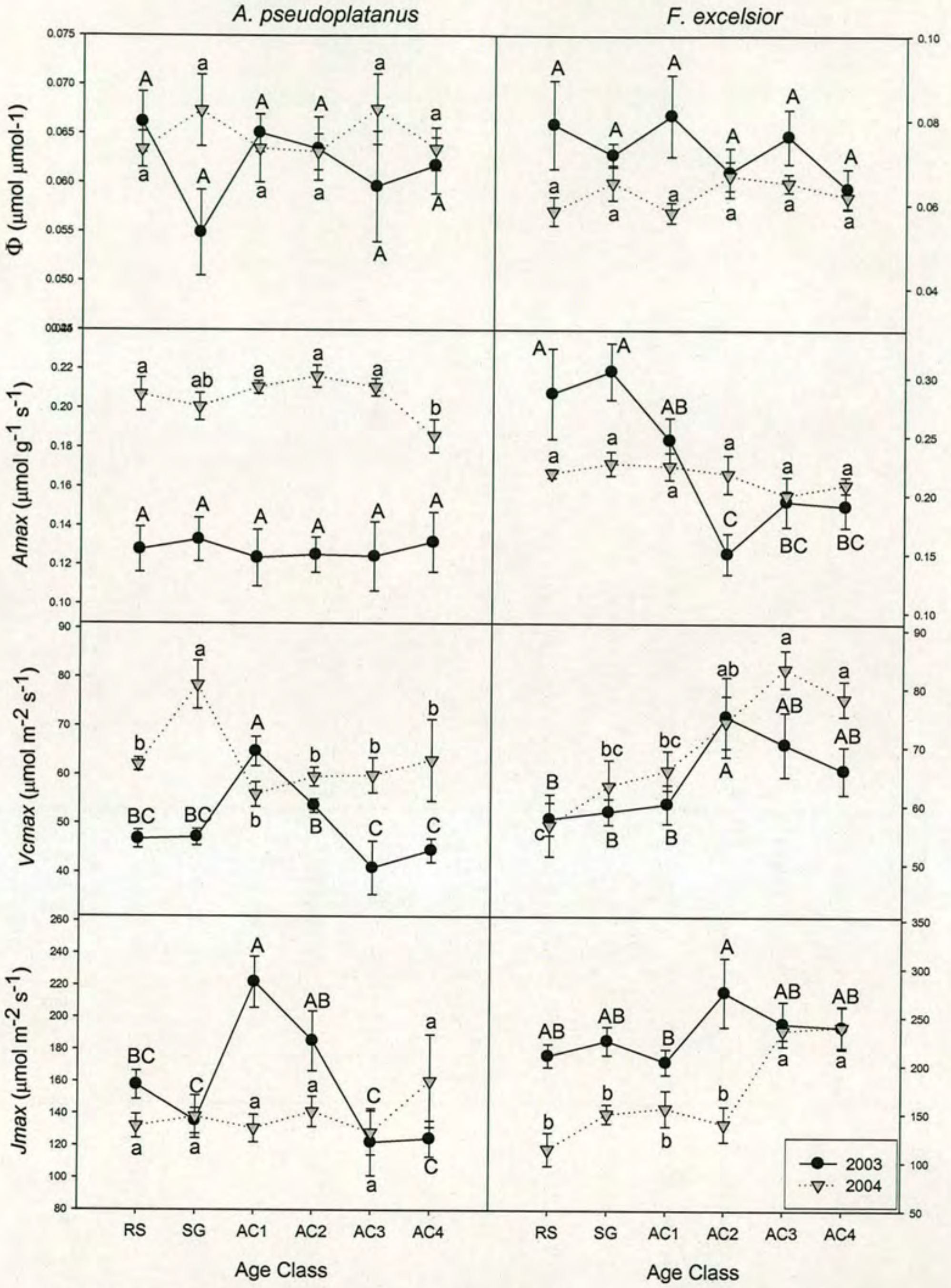

Figure 3.3: The mean values of photosynthetic efficiency and capacity of both species over two growing seasons. The different letters represent statistically significant differences across age classes.

Overall, these results showed that no age-related trend was found in the grafted seedlings despite the extensive study carried out on them with different light regimes and CO_2 concentrations.

3.3.3 Relationship between some physiological parameters and leaf properties in donor trees and grafted seedlings

Gas exchange parameters and leaf chemical compositions are normally affected by their leaf characteristics, especially *SLA*. Since the age-related decline trends were observed in *SLA* for both donor trees and grafted seedlings, this parameter was regressed against A_{net} and N_{m} for donor trees, and A_{net}, A_{max}, Vc_{max} and N_{m} for grafted seedlings, to conclude the results obtained above with no regards to their age factor. The correlation analyses were also carried out between A_{net} and N_{m} in both donor trees and grafted seedlings for the two species.

Figure 3.4 shows the relationships between *SLA* and A_{net} on leaf mass basis for both donor trees and grafted seedlings. Highly significant positive correlations ($p<0.001$) were found between *SLA* and A_{net} in donor trees of *A. pseudoplatanus*, as well as in their grafted seedlings for the two growing seasons. Meanwhile, strong correlations ($p<0.001$) were also found between *SLA* and A_{net} in *F. excelsior* in donor trees and their grafted seedlings in first growing season, but no significant correlation was observed in their grafted seedlings in second growing season. When *SLA* was held constant, the ANCOVA analysis revealed that there were significant differences ($p<0.001$) between values for donor trees and grafted seedlings in both species, with higher values for the seedlings.

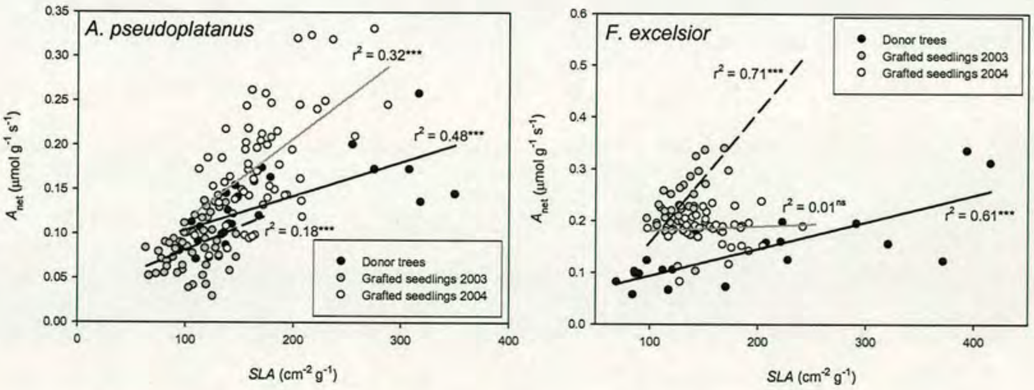

Figure 3.4: The relationship between individual net photosynthesis and their respective specific leaf area across age classes in *A. pseudoplatanus* and *F. excelsior* donor trees and grafted seedlings.

Meanwhile, Figure 3.5 shows the regression analyses between N_m and *SLA* in both species. There were highly significant correlations ($p<0.001$) between these two parameters found in both donor trees and grafted seedlings for *A. pseudoplatanus*, but, significant correlation was only observed in donor trees for *F. excelsior*. Further, ANCOVA once again revealed that there were highly significant differences at $p<0.001$ for *A. pseudoplatanus* and $p<0.01$ for *F. excelsior* in nitrogen concentration between the donor trees and the grafted seedling, when *SLA* was held constant.

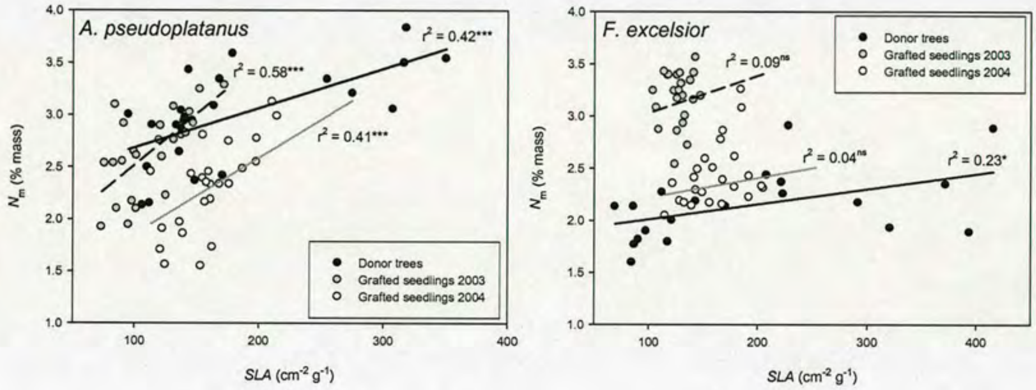

Figure 3.5: The relationship between individual nitrogen content and specific leaf area of *A. pseudoplatanus* and *F. excelsior* across age classes in donor trees and grafted seedlings.

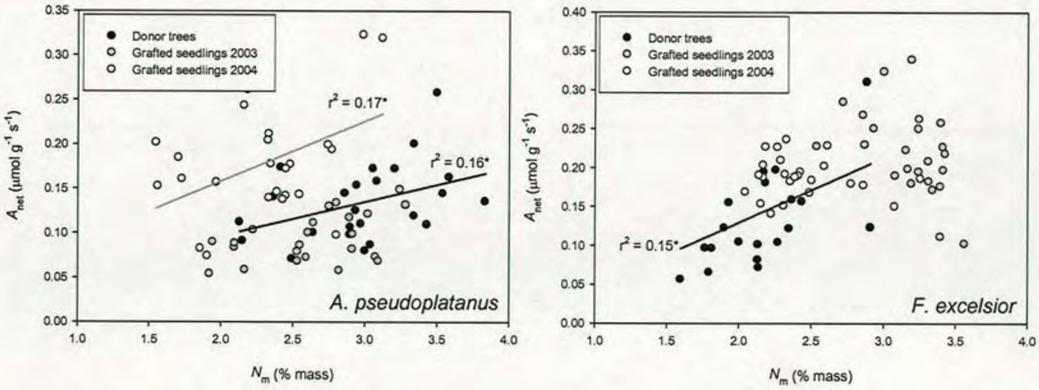

Figure 3.6: The relationship between individual plant leaf nitrogen and net photosynthesis of *A. pseudoplatanus* and *F. excelsior* across age classes in donor trees and grafted seedlings.

In parallel with many studies, nitrogen concentrations were positively correlated with A_{net} in donor trees for both species (Figure 3.6). For the grafted seedlings, however, a significant positive correlation was only found in *A. pseudoplatanus* in the second growing season.

Further, the A_{max} and Vc_{max} values obtained from the responses of assimilation rate with different light intensity (*A/Q* curves) and different CO_2 concentrations (*A/C_i* curves) experiments in second growing season were regressed against their respective *SLA* as shown in Figure 3.7. Interestingly, both species showed similar correlation lines in A_{max} (both $p<0.001$). For Vc_{max}, negative correlations were also found as A_{max}. However, a significant correlation was found only in *F. excelsior* but no significant correlation was found in *A. pseudoplatanus*.

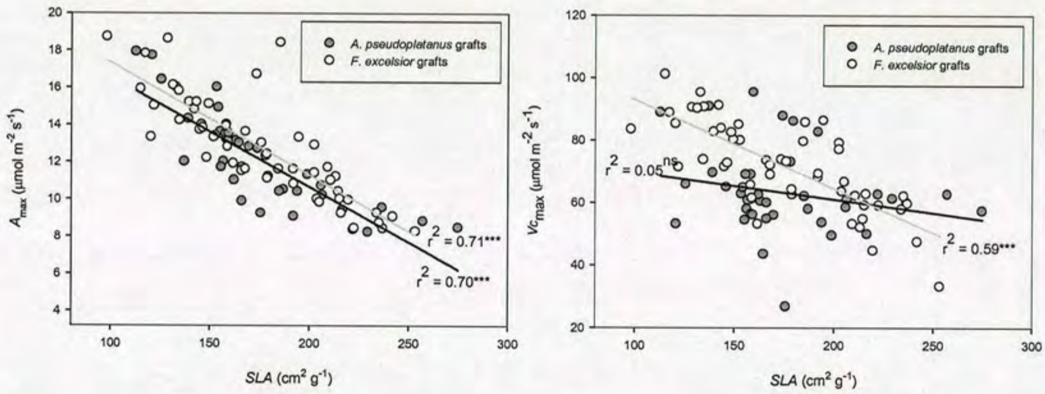

Figure 3.7: The relationship between maximum photosynthesis and maximum carboxylation rate with their respective specific leaf area across age classes in *A. pseudoplatanus* and *F. excelsior* grafted seedlings.

3.4 DISCUSSION

Generally, our gas exchange results of donor trees did not strongly support the decline in growth rates observed for the donor trees in Chapter 2. Thus measurements of photosynthesis on the leaf level do not easily allow for conclusions to be reached on tree growth (Küppers andd Küppers 2003). Age-related decline trends were only observed in A_{net} per unit leaf mass in both species (Figure 3.1A).

Despite the fact that, values of A_{net} per unit leaf area basis did not show age-related trends, these values were found to be within the range recorded by Morecroft and Roberts (1999) for *A. pseudoplatanus* in unmanaged old woodland and by Hölscher (2004) for *F. excelsior* in old-growth forest of broad-leaved deciduous tree species. Day *et al.* (2001) also found similar trends of A_{net} on the leaf mass basis in red spruce, but they also found other parameters such as C_{i} and G_{s} were also reduced in older trees compared to younger trees. Lower G_{s} of large trees compared with small trees under unshaded conditions is consistent with the hydraulic limitation hypothesis that water movement to the top of tall trees is restricted because of the gravitational hydrostatic gradient and cumulative hydraulic resistance (Ryan and Yoder 1997, Ryan *et al.* 1997, Bond and Ryan 2000), thus directly reducing photosynthesis rate and C_{i}. In our case, C_{i} and G_{s} partially showed the age-related decline trends in *A. pseudoplatanus* with a recovery observed for values in trees above 100 year-old of age (AC4). However, no age-related trend was observed in *F. excelsior* (Figure 3.1). The lowest value of G_{s} for *F. excelsior* was found in AC1. Since the study site is a mixed uneven-aged species woodland, the results observed in G_{s} for both species may reflect the attribute of both species. It is well known that *A. pseudoplatanus* is a shade tolerant species, whereas *F. excelsior* is a shade intolerant species (Beckett and Beckett 1979). In particular, partially shaded seedlings and saplings often have lower G_{s} than un-shaded dominant trees, despite limitations to water movement in large trees (Köstner *et al.* 1992, Fredericksen *et al.* 1995, Martin *et al.* 1997, Samuelson and Kelly 1997) as found in *F. excelsior*. Moreover, other potential reasons may lie in 1) the measurement of gas exchange was not carried out at the same time on each tree due to accessibility problems, 2) the Ψ_{leaf} values at the time of measurements had similar trends with G_{s} in both species as indicated in Figure 3.1, and they may have played an important role in determining stomatal conductance values. It is well known that stomatal closure has been associated with lower soil to leaf hydraulic conductance (Kolb and Stone 2000) and the differences in Ψ_{leaf} (Hubbard *et al.* 1999).

Because of these limitations, the chemical composition such as nitrogen content and carbon isotope discrimination observed in the leaves taken during gas exchange measurements may become the best parameters to described age-related decline trends. Leaf nitrogen content has been shown to be a good predictor of A_{net} and G_s (Field and Mooney 1986, Reich *et al.* 1997, Samuelson and Kelly 1997), thus information on variation in leaf nitrogen content with tree ageing might improve the understanding of changes in leaf gas exchange. Nitrogen contents in mass basis (N_m) clearly decreased with increasing age of the trees in both species, although this trend was significant only for *F. excelsior* (Figure 3.2B). These results also indicated that lower photosynthetic capacity was observed in older trees. Many studies have shown that the nitrogen content of leaves is closely correlated with photosynthetic capacity (e.g. Hackett 1985, Kolb *et al.* 1998). In concordance with our results, Kull and Koppel (1987) observed declines in leaf nitrogen and photosynthetic capacity as tree aged. Furthermore, Schoettle (1994) found that leaf nitrogen contents in old *Pinus aristata* were lower than the ones observed in young trees. In contrast, other studies have shown no change in nitrogen concentration or photosynthetic capacity with tree age (e.g. Schoettle 1994, Mencuccini and Grace 1996b, Hubbard *et al.* 1999, McDowell *et al.* 2002a, Barnard 2003). Apart from N_m, $\delta^{13}C$ was also determined. This parameter can act as an independent test of the hypothesis that G_s declines with increasing height (McDowell *et al.* 2002a) and age in our case. The $\delta^{13}C$ showed age-related trends in both species in the field with the values becoming less negative with increasing age (Figure 3.2C). The trends in $\delta^{13}C$ were not similar with the trends obtained in G_s. McDowell *et al.* (2002a) also found different trends between cuvette-based G_s and G_s which were inferred from $\delta^{13}C$ of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). They stated that hydraulic limitations to gas exchange may occur in the spring months but not during summer drought. Furthermore, Bauerle *et al.* (1999) concluded that cuvette-based G_s was a poor measure compared with $\delta^{13}C$ data because of the limitation in temporal integration of gas exchange instrument. With that factor in mind, our $\delta^{13}C$ results have clearly provided evidence that G_s may decrease with increasing age or size of trees. Livingston *et al.* (1998) found that G_s decline linearly with height provided that $\delta^{13}C$ was not a result of photosynthetic capacity or light availability.

Studies of tree ageing that have compared scions from branches of mature and juvenile trees grafting onto seedling rootstocks have shown that physiological characteristics such as A_{net} and G_s can also vary with tissue age independent of differences in tree height (Greenwood 1995). Rebbeck *et al.* (1993) found that G_s was lower for scions from old *Picea rubens* trees compared with scions from seedlings, when both were grafted onto similar sized rootstocks. Similar results were also found in red spruce when scions from older and younger trees were grafted onto young rootstocks (Day *et al.* 2001). They concluded that differences in physiological characteristics between young and old trees can arise from tree maturation processes. These experiments showed that grafting may also imply the existence of genetic controls. However, in our grafted seedlings, there was no age-related trend of leaf gas exchange parameters nor of leaf chemical compositions in either species, despite measurements were taken in both first and second growing season (Table 3.4). Furthermore, results obtained from photosynthetic capacity and efficiency measurements using response curves of A_{net} against different levels of carbon dioxide and light intensity on grafted seedlings also did not show any age related trend (Figure 3.3). In fact, those results did not follow the trends in their growth traits as illustrated in Chapter 2. Poorter *et al.* (1990) has pointed out that the relationship between photosynthesis and growth is a complex one, with growth rate not being well correlated with the rate of photosynthesis on a leaf-area basis. This is because growth also depends on the investment of biomass in growing sinks and investment in leaf area (Chapin *et al.* 1990, Poorter and Remkes 1990). Increases in photosynthesis rates and decrease in growth rates is sometime associated with fruit production (Bond 2000).

Since *SLA* did change in grafted seedlings as observed in Chapter 2, regression analyses were conducted on some physiological parameters such as A_{net}, A_{max} and Vc_{max}, and leaf N_m with *SLA* on grafted seedlings without taking into account of scion ages. The regressions were also carried out on A_{net} and N_m with their respective *SLA* in donor trees regardless of age factor. Interestingly, A_{net} and N_m correlated well with *SLA* in first and second growing season for both species in grafted seedlings (Figure 3.5 and 3.7). An exception only occurred in A_{net} of *F. excelsior* grafted seedlings in second growing season. Furthermore, similar trends were also observed in donor trees (Figure 3.4). These results clearly support the general correlation between *SLA*, A_{net} and N_m for six biomes and different plant life forms by Reich *et al.* (1997) (*c.f.* Gulias 2003). In addition, A_{net} on a leaf-mass basis has been positively correlated with *SLA* and N_m (Poorter *et al.* 1990, Reich *et al.* 1994), and these patterns are common to all species (Reich 1999). This is because significant nitrogen per unit leaf mass accumulation would be required in leaves to achieve a high A_{net}. According to Hunt and Cornelissen (1997), species with high *SLA* and N_m usually show high potential relative growth rates. This is supported by our results where trees in younger classes tend to have higher relative growth rates compared with older trees as given in Chapter 2.

Generally, the results of gas exchange only partially supported the age- or size-related changes in growth parameters. The age- or size-related decline trends were only observed in A_{net} on the basis of leaf mass for both species. Furthermore, the evidence supporting the size-related hypotheses lies in leaf chemical composition, such as N_m and $\delta^{13}C$. Overall, the results showed that the changes in A_{net} and leaf chemical compositions were triggered by size, not age. This is supported by results observed in the grafted seedlings for both species. One time leaf-level gas exchange measurements may insufficient to test the hydraulic limitation hypothesis. Further investigations have to be carried out, especially involving stomatal response and hydraulic conductance. Hence, the following chapter focuses on those parameters.

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CHAPTER 4

THE EFFECT OF AGE AND SIZE IN WHOLE PLANT TRANSPIRATION, HYDRAULIC CONDUCTANCE AND STOMATAL CONDUCTANCE OF *ACER PSEUDOPLATANUS* AND *FRAXINUS EXCELSIOR* SPECIES

4.1 INTRODUCTION

Water, especially the availability of soil water, in the soil-plant-atmosphere system is one of the most important factor controlling the distribution and growth of forest trees. Water affects all phases of tree growth, because it is involved in various vital processes such as photosynthesis, respiration, mineral nutrition, enzymatic activity and nitrogen metabolism (Kozlowski 1982). Water deficits reduce tree growth directly through effects on cell turgor that affects cell enlargement and differentiation, and indirectly through perturbation of various essential physiological processes.

Water movement from soil through the tree to the atmosphere involves different mechanisms of transport. In the soil and xylem, water moves by bulk flow in response to water potential gradient. In the vapour phase, movement is primarily by diffusion, at least until water reaches the outside air, where convection (a form of bulk flow) becomes dominant. When water transport occurs across membranes, the driving force is the water potential gradient across the membrane (Taiz and Zeiger 1998). Such flow occurs when cells absorb water and when roots transport water from the soil to the xylem. Water movement is largely believed to be a passive process in which water flow in response to physical forces and no energy is expended directly by the plants to transport water.

Usually, plant water relations are dominated by transpiration. Transpiration is the evaporation of water from plants and involves the movement of water through the soil-plant-atmosphere continuum. High transpiration may cause plant injury, however, it is ultimately unavoidable because leaf structure that is favourable for the entrance of CO_2 is also favourable for the loss of water vapour (Kozlowski 1991). In general, the rate of transpiration depends on environmental conditions such as light, temperature, humidity and the relative availability of soil moisture, and plants characteristics such as canopy leaf area and xylem structure. However, there is a growing interest in the role of hydraulic resistance to water transport from the soil to the leaf and the changes in stomatal conductance at different heights that directly influence the rate of transpiration and the inhibition of photosynthesis. Hydraulic resistance may increase in response to increased path length in growing trees, thereby reducing leaf stomatal conductance. The relationship between hydraulic conductance and tree height has been well documented in Bond (2000). In addition, stomata may also become very sensitive to vapour pressure deficit (*D*) with increased tree height. *D* normally varies spatially within crown height. Tang *et al.* (1999) showed that the upper crown foliage of loblolly pine had a significantly greater *D*, corresponding to a lower stomatal conductance, albeit higher transpiration rates compared to lower crown foliage. These two assumptions clearly indicated the role of tree height in conjunction with physiological studies, especially stomatal conductance.

The resistance to water movement in plants has been described using Ohm's Law analogy which is often referred to as van den Honert's equation (van den Honert 1948). This equation has gained wide acceptance amongst plant scientists (Cowan 1965, Feddes and Rijtema 1972, Lynn and Carlson 1990, Ewers and Cruiziat 1991, Mencuccini and Grace 1996a). From the Ohm's law electrical analogy, water flow through a path of the soil-plant-atmosphere continuum is analogous to current in an electrical circuit composed of a series of resistances (Ewers and Cruiziat 1991). The total resistance of the tree includes the root, stem, leaf, stomata, and boundary layer resistances. Ewers and Cruiziat (1991) described that the flow rate between two points A and B in the plant equals the difference in water potentials at these points divided by the resistance of the path between A and B.

$$\mathrm{Flux}_{\mathrm{AB}} = \frac{(\psi_{\mathrm{A}} - \psi_{\mathrm{B}})}{R_{\mathrm{AB}}} \tag{4.1}$$

where $\mathrm{Flux}_{\mathrm{AB}}$ is flow rate between points A and B ($\mathrm{m}^3\ \mathrm{s}^{-1}$), Ψ_{A} and Ψ_{B} are water potentials at points A and B (MPa), and R_{AB} is the resistance of the path between A and B (MPa m^{-3} s). Hydraulic conductance is the reciprocal of resistance: $K_{\mathrm{AB}} = 1/R_{\mathrm{AB}}$. The Ohm's Law analogy is useful to calculate water flow and hydraulic conductance under constant conditions, because it assumes that the resistance offered by a system is constant. It requires that the quantity of water transpired by the plant should equal the quantity of water absorbed by plant roots to maintain or reach a steady state. However, the steady-state condition is not easy to meet because transpiration changes during the day as influenced by environmental conditions and water storage varies inside the plant. Therefore, it is necessary to measure the sap flow density during different times in the day.

Since there is a direct link between transpiration and stomatal conductance, any changes in stomatal response to fluctuating environmental conditions will also change the rate of transpiration. One of the most influential environmental variables governing stomatal conductance is the vapour pressure deficit (*D*) between the saturated leaf intercellular air spaces and air surrounding the leaf. Saturation vapour pressure in the leaf is a function of leaf temperature, which fluctuates according to air temperature and radiation (Nobel 1991). Absolute humidity in the ambient air, which is also a function of temperature, is rarely at a maximum and thus some *D* almost always exists. The relationship between conductance and *D* has been demonstrated in numerous reports from various species including loblolly pine (Pataki *et al.* 1999), tropical trees (Meinzer *et al.* 1995), *Pinus sylvestris* (Scots pine, Beadle *et al.* 1985), *Elates suineans* (oil palm, Dufrene and Saugier 1993), *Vigna unguiculata* (cowpea, Bates and Hall 1982) and several others. In all cases, an increase in *D* decreased stomatal conductance. Also, it should be noted that in several of the above cases, low soil water potential coupled with a high *D* contributed to accelerated stomatal closure.

The typical stomatal response to a high *D* is thought to be the result of either increasing water losses directly from the stomata (peristomatal or epidermal hydropassive mechanism) or via complex integrated metabolic responses resulting from water stress. Transpiration will generally increase when the *D* is increased until rapid water loss triggers a stress response. This change in *D* eventually causes stomatal closure, resulting in water conservation. This process improves leaf water status and will often decrease or maintain transpiration rates despite an increase in *D*. However, an increase in *D* may be enough to overcome increasing stomatal resistance, resulting in a potential increase in transpiration with increasing *D*.

Assuming hypothetically that conductance is constant, an increasing *D* will directly increase transpiration since transpiration is equal to stomatal conductance multiplied by the driving force (*D*).

Photosynthesis is indirectly related to *D* since stomatal conductance influences internal carbon (C_i) concentrations and therefore affects the amount of CO_2 available for reduction via photosynthesis. Conifer gas exchange studies, including loblolly pine studies, have consistently shown that photosynthesis and stomatal conductance are closely coupled (Teskey *et al.* 1986, Mitchell and Hinckley 1993, Tang *et. al* 1999). Increases in *D* reduce photosynthesis by decreasing stomatal aperture and reducing C_i. In Scots pine, the relationship between photosynthesis and stomatal conductance was directly correlated with *D* at large deficits (Beadle *et al.* 1985). The relationship was curvilinear at a high *D*, but independent of *D* at lower deficits. *D* therefore plays a vital role in governing the resources available for carbon assimilation, which directly influences productivity.

As transpiration by trees is a key component in the water use of forest stands, the direct measurement of water use by trees permits the partitioning of this component of total stand evapo-transpiration from soil evaporation and the loss of intercepted or condensed water from the canopy. Such estimates are necessary for modelling biological control of transpiration with respect to evaporative demand, soil water levels, and canopy wetness (Hatton and Vertessy, 1990). Unfortunately, direct measures of tree and stand transpiration are difficult and rare. Some canopy transpiration models based on energy balance parameters with refinements such as soil moisture limitation parameters or stomatal responses to humidity fail to provide information on the variation of transpiration among trees within a stand (Werk *et al.* 1988). Rapid and effective methods of directly measuring the mass flow of water through the xylem of a tree, and thus whole-tree transpiration have been developed and tested (Čermák *et al.* 1973, Edwards and Booker 1984, Sakuratani, 1984, Granier 1987, Hatton and Vertessy 1990, Oren *et al.* 1999).

An understanding of the transpiration by individual trees of different heights and forest stands of different strata is a necessary component of assessment of stand water use. However, it is difficult to quantify the transpiration of large trees.

Techniques to measure sap flow remain the most economical and practical approach for the direct estimation of water use by individual trees. The most widely applied techniques are based on the use of heat as a tracer for sap flow. Methods for measuring sap flow include the heat pulse velocity (Edwards and Booker 1984, Hatton and Vertessy 1990), the stem heat balance techniques (Čermák *et al.* 1973, Sakuratani 1984), and the heat dissipation method (Granier 1987, Oren *et al.* 1999). The stem heat balance technique measures sap flow directly but can not be used for large tree measurements. The heat pulse velocity and heat dissipation methods can be used in large tree measurements of sap flow density (sap flow per unit sapwood area), but variation in different positions of xylem need to be considered for estimating whole-tree sap flow. Variations of sap flow density between outermost and inner xylem have been reported in some studies (Dye *et al.* 1991, Phillips *et al.* 1996, Zang *et al.* 1996, Oren *et al.* 1999, Wullschleger and King 1999, Jiménez *et al.* 2000, Lu *et al.* 2000).

In addition, Granier (1987) improved the heat dissipation method of the constant-heat flow to measure sap flow density for large trees. This method uses two cylindrical probes with a diameter of 2 mm and an effective measuring length of 20 mm. The two probes are inserted into the hydroactive xylem of the tree stem with a vertical spacing of 10-15 cm. The upper probe is heated with a constant energy (200 mWatt DC), which is dissipated as heat into the sapwood and vertical sap flow surrounding the probe. The lower probe is left unheated to monitor the ambient temperature of sapwood. Both thermocouples are connected at the constantan end and thus give an output representing the temperature difference between the two probes (1°C = 40 μVolt for copper-constantan at 20°C). The sap flow density u (m s^{-1}) is calculated as follows:

$$u = 119 \times 10^{-6}.K^{1.231} \tag{4.2}$$

where $199 \text{x} 10^{-6}$ and 1.231 are empirical constants from the calibration, and K is sap flow index that is related to the temperature difference between the two probes (ΔT) as calculated below:

$$K = \frac{(\Delta T_m - \Delta T)}{\Delta T} \tag{4.3}$$

where ΔT_{m} is the temperature difference at zero flow ($u = 0$). Total sap flow (flow) of a tree ($\mathrm{m}^3\ \mathrm{s}^{-1}$) is estimated as follow:

$$F = u.S_A \tag{4.4}$$

where S_A is the cross-sectional area of sapwood at the point of insertion of the heated probe.

Many studies have used this method to study sap flow of different forest strata, age or even size of trees (e.g. Meinzer *et al.* 1997, Hubbard *et al.* 1999, Oren *et al.* 1999, Schäfer *et al.* 2000, Phillips *et al.* 2002, Phillips *et al.* 2003, Barnard and Ryan 2003, Delzon *et al.* 2004, Unsworth *et al.* 2004, Meinzer *et al.* 2005). In our study, we used the same method to study age- and size-related decline trends on *A. pseudoplatanus* and *F. excelsior* in the field. This study was conducted to estimate the whole plant transpiration and whole plant leaf-specific hydraulic conductance. Canopy stomatal conductance was also estimated from the data obtained in sap flow measurements and meteorological station. In addition, a gravimetric method was used to determine the whole plant transpiration and whole plant leaf-specific hydraulic conductance in grafted seedlings. Stomatal conductance values of grafted seedlings from different age classes were also estimated from an experiment relating stomatal conductance to D. These studies may provide further information regarding the tested hypotheses.

4.2 MATERIALS AND METHODS

4.2.1 Field study

4.2.1.1 Sap flow measurement on donor trees

In this experiment, five trees from age class 2 and three trees from each of age classes 3 and 4 in both species were selected in the field (Table 4.1). Due to technical limitations in cable lengths and distances from loggers, trees from age class

1 could not be sampled in either species. The sap flow measurements were done using constant heating Granier probes (Granier 1987). Two sensors, approximately 2.2 cm long and 0.2 cm in diameter were constructed using cylindrical probes. These sensors comprise one heated probe with a constantan resistor which is inserted in the sapwood at the top and an unheated probe which is inserted at the bottom (Plate 4.1).

Table 4.1: Summary of main characteristics of donor trees used in this experiment for both species

Age class	**Age (year)**	***DBH* (cm)**	***Height* (m)**	**A_L:A_S (m^2 cm^{-2})**
A. pseudoplatanus				
2	27.00 ± 0.89^{A}	15.20 ± 0.98^{A}	7.94 ± 0.32^{A}	0.63 ± 0.04^{A}
3	71.33 ± 2.91^{B}	56.83 ± 1.59^{B}	17.77 ± 0.15^{B}	0.56 ± 0.10^{A}
4	135.33 ± 5.46^{C}	78.67 ± 0.73^{C}	24.23 ± 0.38^{C}	0.47 ± 0.03^{A}
F. excelsior				
2	27.20 ± 1.50^{A}	19.40 ± 1.58^{A}	12.48 ± 0.91^{A}	0.53 ± 0.07^{A}
3	44.67 ± 5.24^{A}	33.83 ± 2.20^{B}	15.93 ± 2.07^{A}	0.53 ± 0.07^{A}
4	104.33 ± 10.17^{B}	68.33 ± 5.33^{C}	21.17 ± 0.95^{B}	0.44 ± 0.06^{A}

The '±' represents mean standard error. Different letters indicate significant differences between age classes within species.

Two small holes were drilled accordingly on the north facing side of the stem at tree breast height (1.3 m). The sensors were coated with conducting pastes before being inserted into the drilled holes. The conducting pastes filled excess space in the drilled holes to ensure thermal contact between probes and xylem. The sensors were then covered with an aluminium box, and insulation sheeting was applied around the tree stem, protecting them from rainfall and the effects of radiant heating and convective heat losses (Plate 4.2).

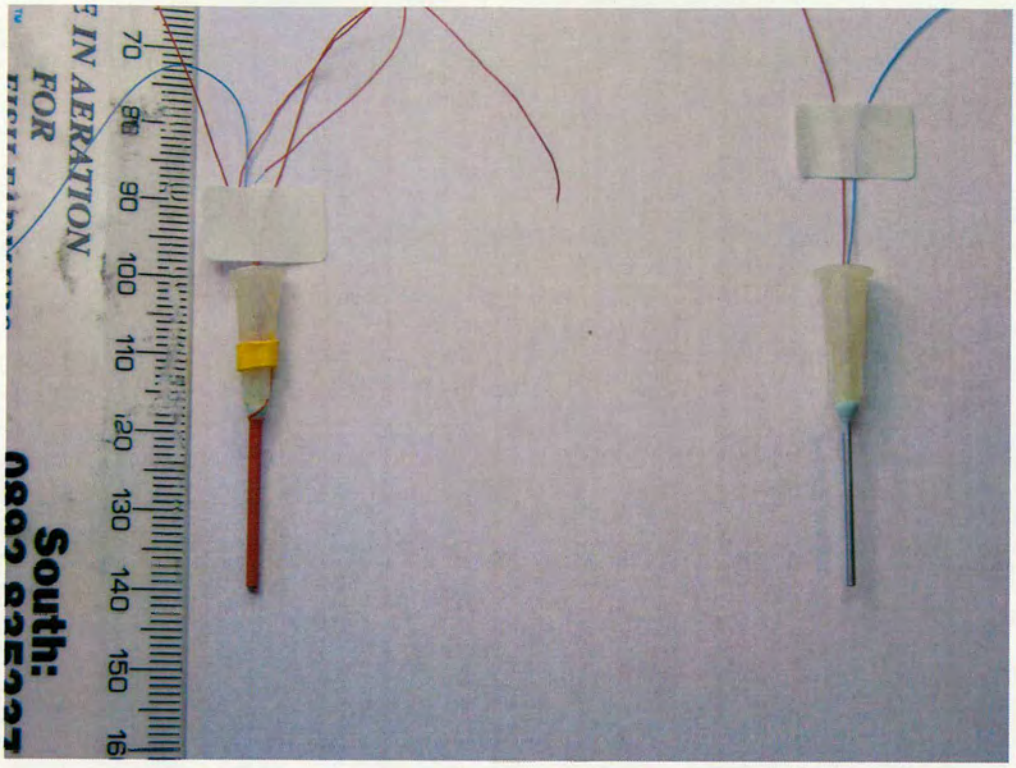

Plate 4.1: Granier-type probes showing heated sensor (left) and unheated sensor (right).

All sensors were connected to dataloggers and heating boxes for heating purposes. These equipments were powered with 12 V batteries. The batteries were replaced between five to seven days when all the data were downloaded into a laptop. The data were recorded every 10 seconds and stored every 15 minutes with two dataloggers (Model 21X and CR10, Campbell Scientific Inc., Utah, U.S) and a multiplexer (Model AM416, Campbell Scientific Inc., Utah, U.S). This experiment was carried out for about three weeks starting from July 4 to July 23, 2004.

Plate 4.2: Insulation sheets were applied around the stem where sensors were installed to protect them from rainfall and convective heat losses.

4.2.1.2 Whole plant leaf-specific transpiration (Q_L) and whole tree leaf- specific hydraulic conductance (K_L) of donor trees

The estimation of total sap flow was done using the equations stated above for Granier-type sensors. However, in the case of *A. pseudoplatanus* and *F. excelsior* where sapwood thickness is normally shorter than the sensor length, we used equation 4.2 to represent sap flow density per sapwood area instead of using equation 4.4. These sap flow values were then multiplied with sapwood area to leaf area ratio values to obtain whole plant transpiration per unit leaf area (Q_L) (Oren *et al.* 1999). Sapwood area was estimated using tree-cores for each tree and visually distinguished between light coloured sapwood and dark coloured heartwood, while leaf area was estimated from allometric equations as given in Chapter 2.

Meanwhile, the whole plant leaf-specific hydraulic conductance (K_L, in mmol m^{-2} s^{-1} MPa^{-1}) was estimated for each tree from both species using the equation given in Wullschleger *et al.* (1998) as follow:

$$K_L = \frac{Q_{\mathrm{L\,max}}}{\psi_{\mathrm{predawn}} - \psi_{\mathrm{midday}}} \qquad (4.5)$$

where Q_{Lmax} is the average sap flow at radiation above 1000 KJ m^{-2} from 11.00 am to 16.00 pm, Ψ_{predawn} is the predawn water potential (MPa) and Ψ_{midday} is the midday water potential (MPa) taken on Julian date 209.

4.2.1.3 Meteorological data

Meteorological data were obtained from Edinburgh Gogarbank meteorological office. The data comprise five important variables, i.e., air temperature, relative humidity, rainfall, wind speed and radiation. Vapour pressure deficit (*D*) was calculated based on temperature and relative humidity data. These variables were measured every hour.

4.2.2 Grafting Study

4.2.2.1 Stomatal conductance (G_s) versus vapour pressure deficit (D)

A study of stomatal conductance against vapour pressure deficit (*D*) was conducted to obtain appropriate values of stomatal conductance in each individual from four age classes and two controls in both species. One fully expanded and healthy leaf was chosen from six out of ten grafted seedlings in each age class that were picked up randomly and placed in the glasshouse to obtain relatively constant conditions. The LCPro portable photosynthesis system was used. At the beginning, about ten different *D* ranging from 0.5 kPa to 2.5 kPa were set using climate control on LCPro portable photosynthesis system. However, since ambient vapour pressure and air temperature in the glasshouse fluctuated throughout the experiment and caused the cuvette vapour pressure and temperature to change, we decided to calculate the actual *D* from the raw data obtained by using equations cited in Jones (1992) as given below:

$$D = e_{s(T)} - e \tag{4.6}$$

where e is the actual ambient vapour pressure in the air and $e_{s(T)}$ is the saturated or maximum vapour pressure at the ambient air temperature calculated as follow:

$$e_{s(T)} = \mathrm{a}.\exp\left(\frac{\mathrm{b}T}{\mathrm{c}+T}\right) \tag{4.7}$$

where T is temperature (°C) and a (= 613.75), b (= 17.502) and c (= 240.97) are the empirical coefficients.

The stomatal conductance values for each individual seedling were then plotted against D using logarithmic regressions and the equations obtained were used to calculate stomatal conductance at a reference ambient D (1.5 kPa).

4.2.2.2 Whole plant leaf-specific transpiration rate (E_L) and whole plant leaf-specific hydraulic conductance (K_L)

Whole plant leaf-specific transpiration rate (E_L) and whole plant leaf-specific hydraulic conductance (K_L) were measured using the gravimetric method. Five grafted seedlings from a total of 60 seedlings were selected for both species (Table 4.2). Prior to taking the measurement, the pots were covered in black bags after they were watered at field capacity to avoid any water loss from the soil. The trees were weighed using a 12 kg dual face balance in early morning around 3.30 to 5.30 BST (T_1) . One leaf was excised in each individual after the weight was measured in order to estimate the leaf water potential (Ψ_{predawn}). Predawn water potential was measured as an estimation of soil water potential that can be used to calculate leaf-specific hydraulic conductance (K_L). This leaf was then placed in a plastic bag with a small lump of moist tissue in it to avoid evaporation. The water potential measurement was carried out in the laboratory using a Portable Plants Moisture System (Skye Instruments Ltd, Powys. UK) with N_2. Those seedlings were then weighed again (T_2) at around 10.30 BST and a leaf was also excised in order to estimate the midday water potential (Ψ_{midday}). The E_L and K_L values were calculated using equations as follow:

$$E_{\mathrm{L}} = \frac{\mathrm{d}W}{\mathrm{d}T} \tag{4.8}$$

where dW is the different in weight (W_1-W_2) at T_1 and T_2 and dT is the different in time (T_2-T_1).

$$K_{\mathrm{L}} = \frac{E_{\mathrm{L}}}{\psi_{\mathrm{predawn}} - \psi_{\mathrm{midday}}} \tag{4.9}$$

Table 4.2: Summary of main characteristics of grafted seedlings of both species used in this experiment.

Age class	***Diameter* (cm)**	***Height* (cm)**	**A_{L} (m^2)**
A. pseudoplatanus			
1	1.52 ± 0.08	176.43 ± 13.54	0.37 ± 0.06
2	1.45 ± 0.06	165.92 ± 8.16	0.38 ± 0.06
3	1.35 ± 0.06	135.68 ± 8.62	0.35 ± 0.02
4	1.38 ± 0.06	157.65 ± 7.77	0.27 ± 0.04
SG	1.45 ± 0.04	177.23 ± 20.51	0.52 ± 0.04
RS	1.66 ± 0.09	161.50 ± 15.90	0.56 ± 0.06
F. excelsior			
1	1.51 ± 0.08	151.80 ± 14.64	0.46 ± 0.02
2	1.25 ± 0.04	110.03 ± 11.01	0.39 ± 0.05
3	1.13 ± 0.04	104.97 ± 20.38	0.24 ± 0.02
4	1.21 ± 0.04	101.00 ± 11.61	0.29 ± 0.06
SG	1.56 ± 0.08	139.42 ± 10.80	0.54 ± 0.03
RS	1.76 ± 0.11	151.30 ± 12.24	0.63 ± 0.04

The '±' represents standard error of the mean.

4.2.3 Data Analysis

The data obtained from sap flow measurements in the field were analysed using equations given above for every parameter studied. The average value of Q_{L} from each age class and from each day was plotted against daytime D and fitted using logarithmic regression. The slopes of the regression equations were tested by analysis of covariance (ANCOVA) in SPSS version 12.0 (SPSS Inc. 2003). Age-related trends were determined using non-linear regression analyses of mean tree values for Q_L at a given D, and K_L with tree age.

In grafted seedlings, the values of G_s were specifically obtained from the response curves between G_s and D using logarithmic regression analyses. The data were analysed using ANOVA and compared using DMRT. Similar analyses were also done on E_L and K_L in grafted seedlings.

4.3 RESULTS

4.3.1 Variation of sap flow density per unit leaf area (Q_L) among age classes in the field

The typical diurnal patterns of sap flow density per unit leaf area (Q_L) among age/sizes classes in both species are shown in Figure 4.1. These diurnal sap flow density patterns (the amplitudes) were found similar with the patterns observed in meteorological parameters especially temperature and vapour pressure deficit (D) as shown in Figure 4.2. Similar patterns were observed with regard to each age class of both species. However, the separation of the amplitudes among age classes in *A. pseudoplatanus* was not as distinct as the differences observed in *F. excelsior* especially towards the end of the experiment. Diurnal patterns of Q_L were generally found to be lower in the oldest class of both species.

In addition, the variation observed in daily mean values of Q_L was regressed against D (Figure 4.3). Apparently, with similar daytime values in D, mean values from trees in age class 2 (AC2) showed highest mean values of Q_L followed by age class 3 (AC3) and age class 4 (AC4) in both species. The amount of separation of the data obtained between age classes seem to be fair in *A. pseudoplatanus*. However, the values obtained in AC2 for *F. excelsior* were only slightly higher than the ones observed in AC3. This could be due to the fact that height of trees in AC2 and AC3 are not significantly different, as shown in Table 4.1. The ANCOVA analysis revealed that, once D was held constant, there were significant differences ($p<0.001$) in the values of sap flow per unit leaf area (Q_L) among age classes in both species.

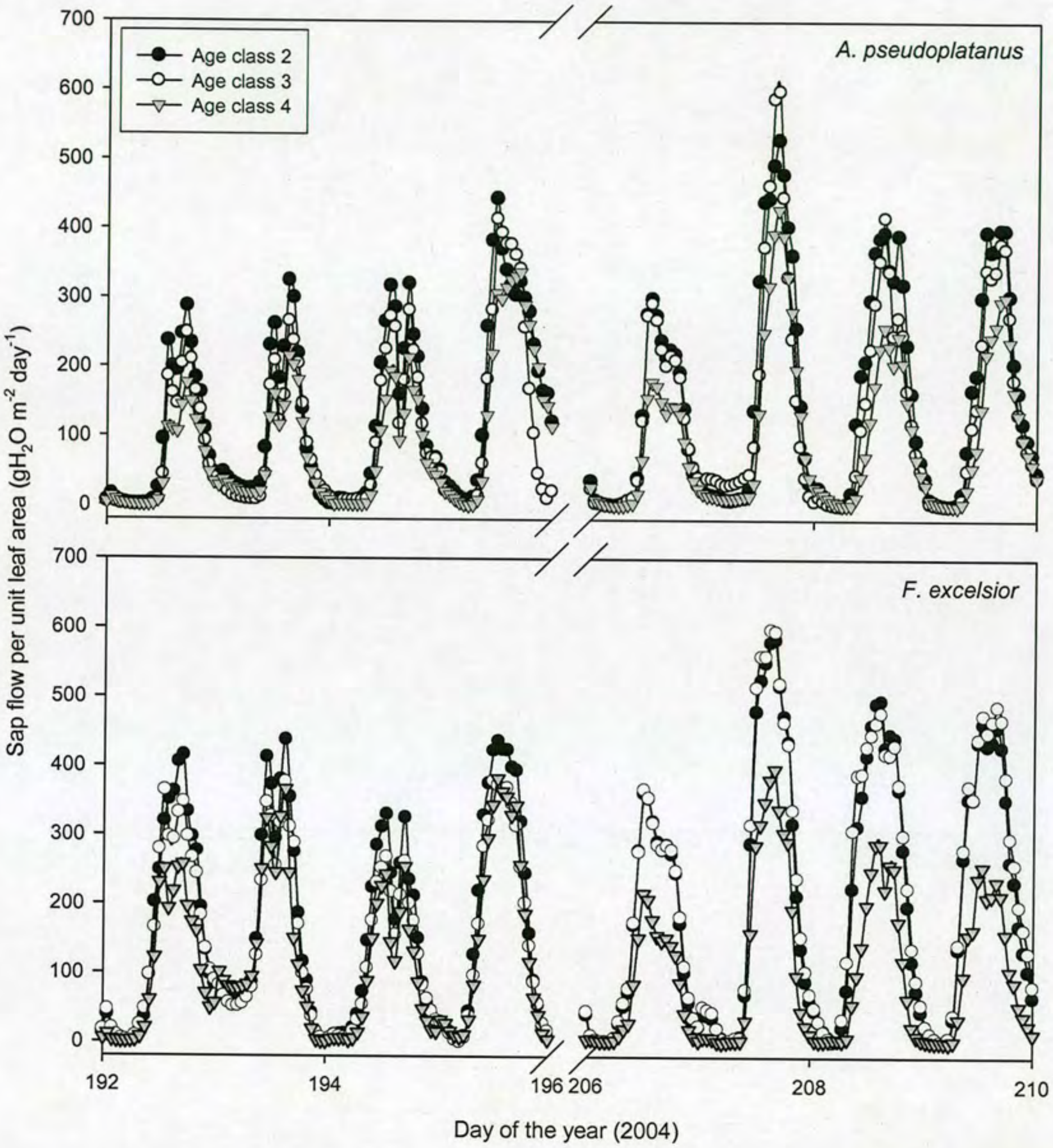

Figure 4.1 : Typical diurnal sap flow density in three age classes of *A. pseudoplatanus* and *F. excelsior* species.

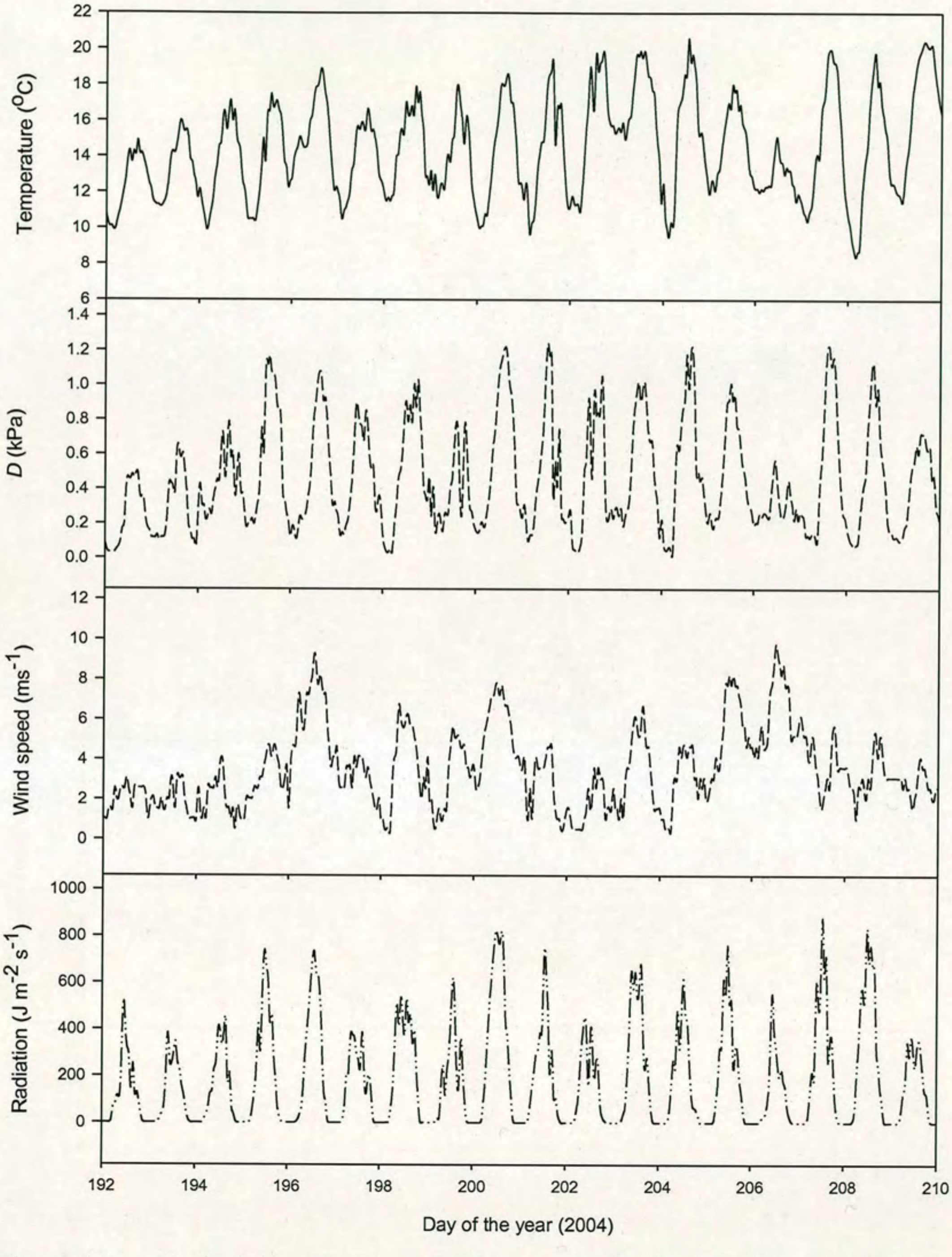

Figure 4.2 : Typical diurnal patterns of four meteorological variables obtained from weather station.

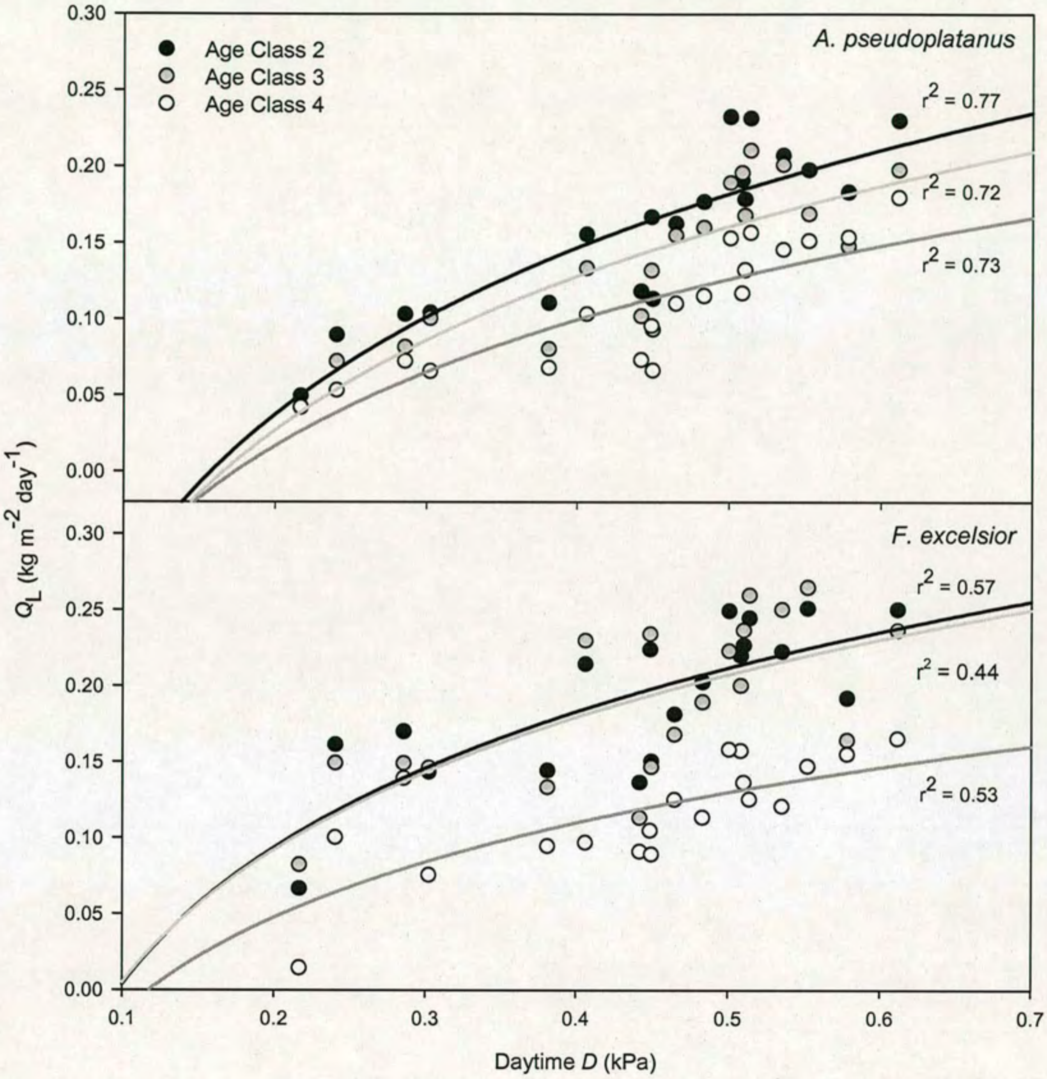

Figure 4.3 : The relationship between vapour pressure deficit and mean sap flow per unit leaf area (Q_L). Logarithmic regressions were carried out for each age class in both species.

4.3.2 Age-related trends in sap flow density (Q_L) and whole plant leaf-specific hydraulic conductance (K_L) among tree ages in the field

The mean values of Q_L and K_L from each tree were then regressed against tree age using non-linear model, as shown in Figure 4.4. In these analyses, the level of statistical significance was set to $\alpha = 0.1$ due to the relatively small sample size and small range of values for sap flow. In Q_L, significant negative correlations ($p<0.1$) were observed in both species. The trees from youngest/smallest class tended to have higher values of Q_L compared to the older/bigger classes. Comparing Q_L values between species, *F. excelsior* trees showed higher values in the youngest/smallest class than the ones observed in *A. pseudoplatanus*. However, *A. pseudoplatanus* trees showed the opposite results compared with *F. excelsior* trees in the oldest/biggest class. Hence a steeper regression line was observed in *F. excelsior* compared with *A. pseudoplatanus*, suggesting that the stomata from older *F. excelsior* trees are more sensitive than the ones from *A. pseudoplatanus*. This could be due to the older trees of *F. excelsior* being located at a higher altitude compared to *A. pseudoplatanus* which was exposed to higher *D*. Furthermore, significant regressions ($p<0.05$ and $p<0.1$) were observed in K_L for *A. pseudoplatanus* and *F. excelsior* respectively.

Overall, the results showed the age-related decline trends to be clearly observed in all parameters obtained from sap flow measurements except for one exceptional circumstance observed in *F. excelsior* between AC2 and AC3.

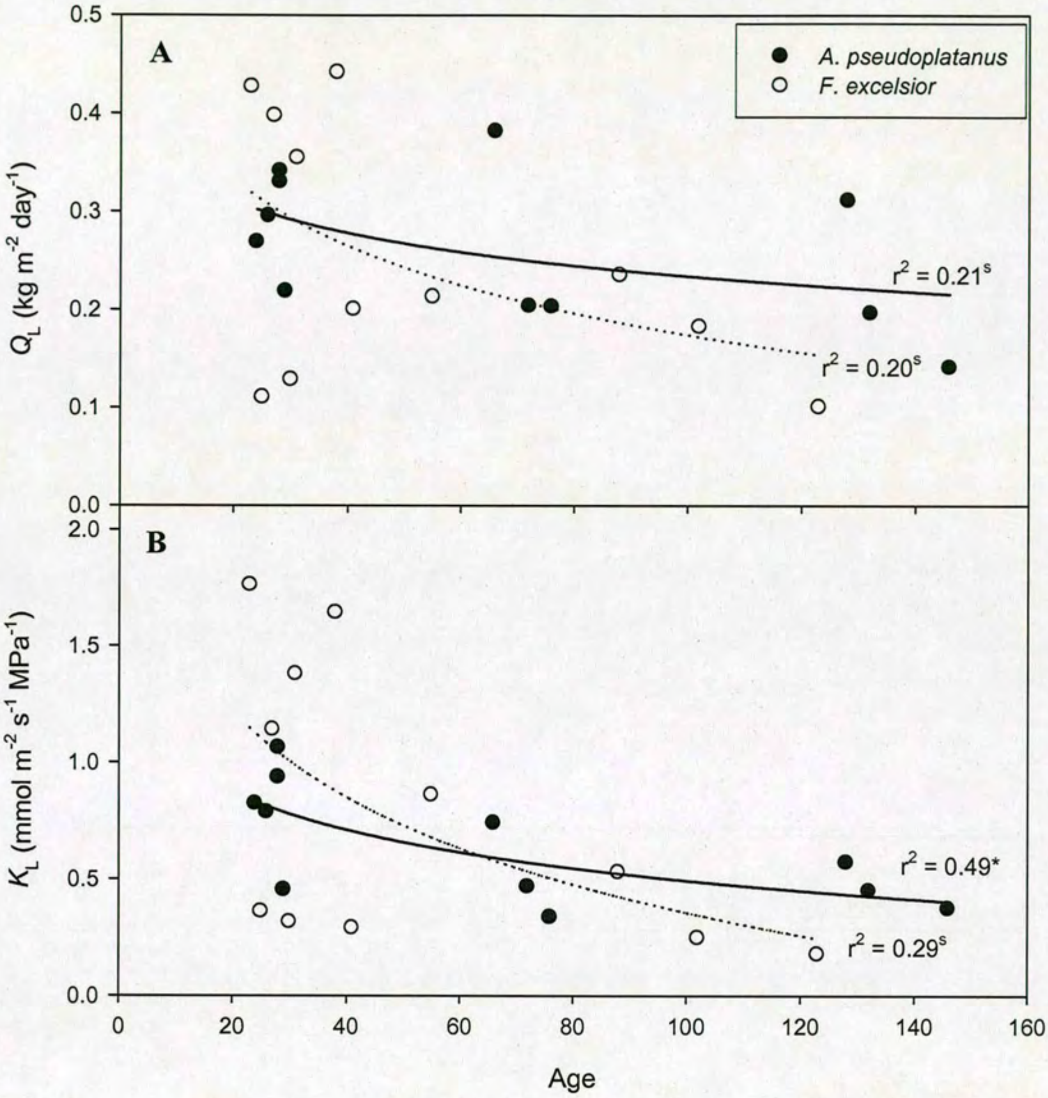

Figure 4.4: The relationship between individual tree age and sap flow (A) and hydraulic conductance (B). A non linear function was used to fit the data for each parameter. The '*' represents significant difference at $p<0.05$ and 's' represents significance at $p<0.10$.

4.3.3 Stomatal conductance (G_s), whole plant transpiration (E_L) and whole plant leaf-specific hydraulic conductance (K_L) among age classes in grafted seedlings

The data obtained on the response of G_s to D for both species are shown in Figure 4.5. These results suggested that the influence of D on G_s was much weaker at lower D, which is contributes to the higher values of G_s, but the values of G_s tended to decrease and level-out with increasing D.

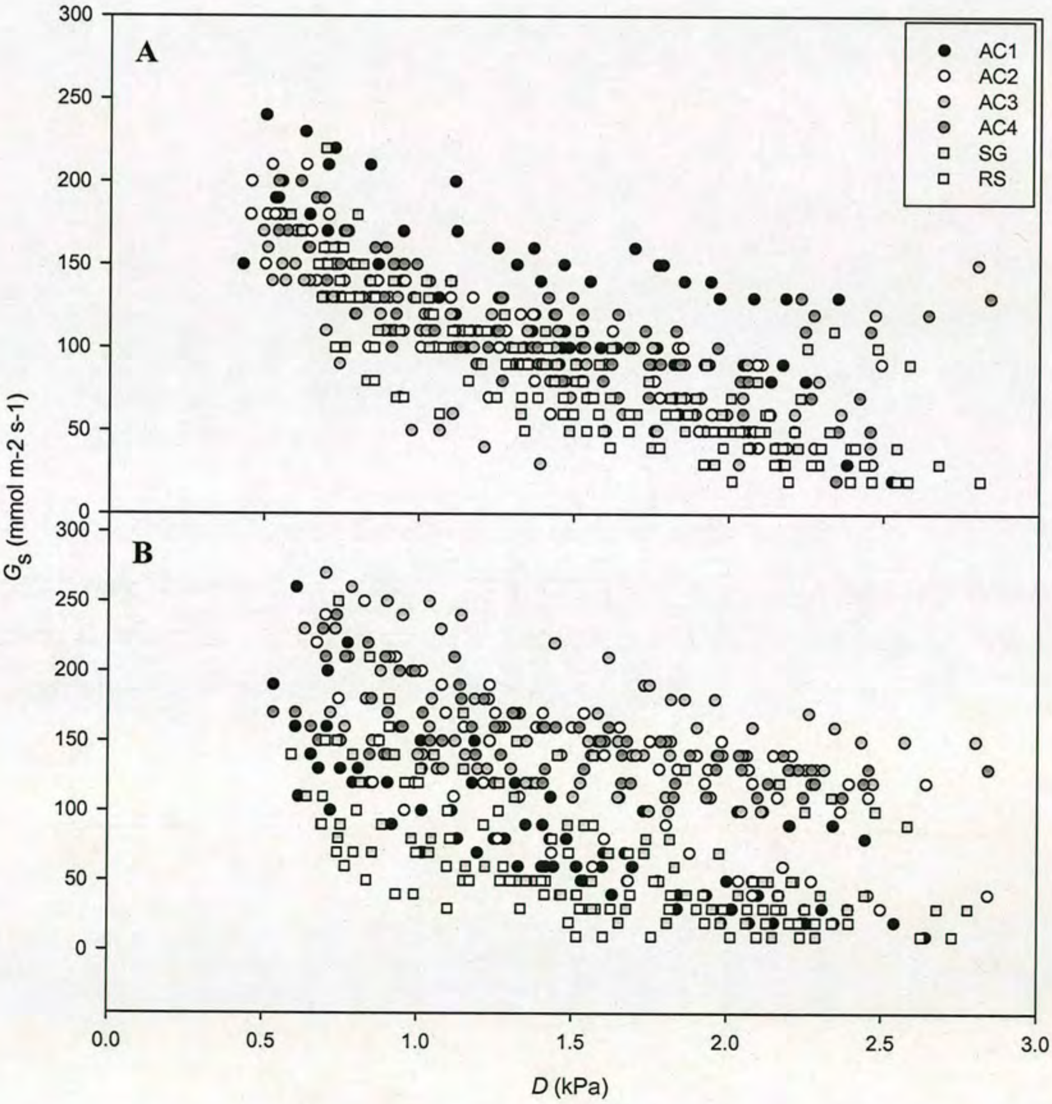

Figure 4.5: The relationship between individual grafted seedling stomatal conductance (G_S) with vapour pressure deficit (D) in each age class of *A. pseudoplatanus* (A) and *F. excelsior* (B).

The comparison of appropriate G_s values among age classes together with controls was done using logarithmic regression equations. These values were then averaged and grouped using DMRT from ANOVA. Significant differences among age classes and controls were observed. However, no age-related trend was observed in either species as shown in Figure 4.6A. Regardless of controls, highest mean values of G_s were found in AC1 and AC3 whereas the lowest mean values were found in AC3 and AC1 for *A. pseudoplatanus* and *F. excelsior*, respectively. Generally, the mean values of G_s were found higher in *F. excelsior* than *A. pseudoplatanus*.

The gravimetric method was used to calculate whole plant transpiration rate per unit leaf area (E_L) of grafted seedlings and the results obtained are presented in Figure 4.6B. The mean value of E_L was found higher in AC4 and lower in AC1 for *A. pseudoplatanus*. Meanwhile, this parameter was found higher in AC3 and the lowest was found in SG for *F. excelsior*. However, the trends were similar in both species.

Whole plant hydraulic conductance (K_L) was calculated by dividing E_L with the differences in leaf water potential between midday and predawn. Regardless of controls, the mean value of K_L was found to increase with increasing age of grafted seedlings in *A. pseudoplatanus* (Figure 4.6C). A similar trend was found in *F. excelsior*, except for the value observed in AC4. These results could be a reflection of the physical sizes of grafted seedlings used in this study as given in Table 4.2 (i.e., seedlings from older donors tended to be smaller).

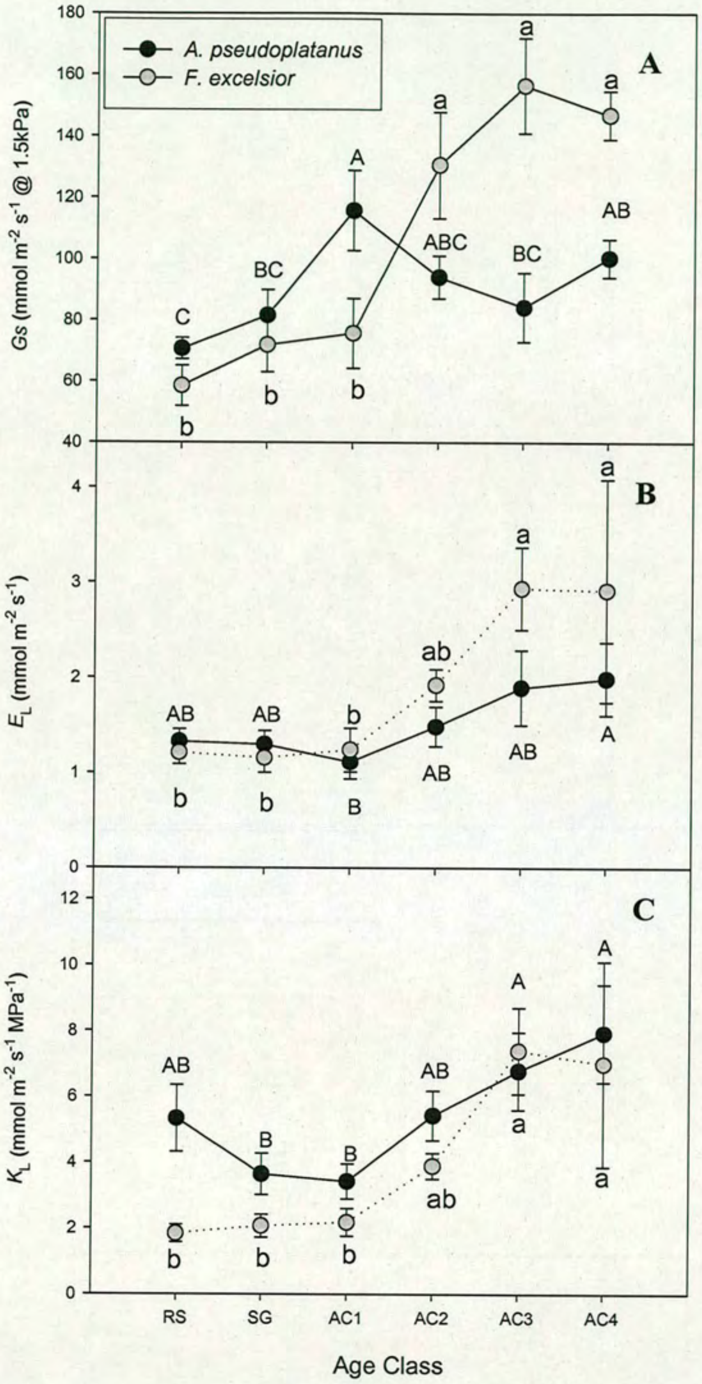

Figure 4.6: The mean values of G_s (A), E_L (B) and K_L (C) among age classes of grafted seedling observed in *A. pseudoplatanus* (•) and *F. excelsior* (•).

4.4 DISCUSSION

The sap flow experiments carried out on donor trees in the field can provide additional information regarding the age and size effects on morphological and physiological characteristics observed in previous chapters. As productivity is always referred to physiological processes such as photosynthesis, reduced photosynthesis may directly reduce productivity. Vapor pressure deficit (*D*) is known to indirectly affect photosynthesis by influencing stomatal conductance (Grantz 1990). A feedback system for the stomata response to *D* is based on the effect of whole leaf transpiration rate on leaf water status or the gradient of water potential between guard cells and other epidermal cells (Yong *et al.* 1997). However, we did not calculate the canopy stomatal conductance due to the lack of support from the available meteorological data. Nevertheless, since stomatal conductance is closely coupled with photosynthesis (Teskey *et al.* 1996, Tang *et al.* 1999) and directly link with transpiration rate, whole plant leaf-specific transpiration rate (Q_L) observed in sap flow experiment may become an important parameter to explain the effect of size and age on stomatal response in our study.

Although this experiment was carried out over a short period, the results obtained clearly indicated that Q_L tended to be increased and leveled out, and may subsequently be reduced when *D* increased regardless of species involved. This is because stomata responses to close as the *D* between a leaf and surrounding air increases is caused by an increase in the rate of transpiration. Almost without exception, studies of the stomatal response to increases in *D* report a decline in stomatal conductance (Whitehead *et al.* 1984, Meinzer *et al.* 1997, Oren *et al.* 1999). Furthermore, as *D* increases, stomata generally respond by partial closure (Lange *et al.* 1971). However, in most cases, stomatal conductance decreases exponentially with increasing *D* (McCaughey and Iacobelli 1994, Monteith 1995). The stomatal closure response to increasing *D* generally results in a non-linear increase in transpiration rate with *D*, as shown in Figure 4.3, leading to a plateau and in some cases a decrease at high *D* (Jarvis 1980, Monteith 1995, Pataki *et al.* 1999). By avoiding high rates of transpiration which would otherwise be caused by increasing *D,* stomatal closure avoids the corresponding decline in plant water potential (Saliendra *et al.* 1995). It is a reasonable premise that the closure response evolved to prevent excessive dehydration and physiological damage.

When Q_L values from trees of different ages/sizes were regressed against their respective individual tree age, we found that Q_L showed the age related decline trends with increasing age in both species (Figure 4.4A). These results do not coincide with the ones obtained from leaf-level gas exchanges especially stomatal conductance values in Chapter 3. In leaf-level gas exchanges, we found that only net photosynthesis on a mass basis (A_{net}) showed the age-related decline trends, but not stomatal conductance and internal CO_2 in both species. On the other hand, these results are less at variance with the age-related decline trends observed for the leaf chemical compositions observed in the field (i.e., N_m and $\delta^{13}C$). As discussed in previous chapter, leaf-level gas exchange could be synchronised over time due to accessibility problems and temporal coverage was more limited. Overall, these results are similar to the ones observed by Bond *et al.* (1999). They found that the rate of sap flow per unit xylem area was slightly lower for large/old trees compared with small/young trees but stomatal conductance and photosynthesis showed the opposite trend. They concluded that the difference occurred because branches of older trees had a much higher sapwood area to leaf area ratio. However, the cause of

It is well known that hydraulic resistance increases as trees grow taller. Hence, the decreases in Q_L were found to be associated with increased hydraulic resistance. Hydraulic conductance, which is the inverse of hydraulic resistance, is an important regulator of stomatal conductance via a supply-and-demand relationship. In our results, hydraulic conductance (K_L) showed declining trends with increasing age of trees in both species (although the difference was only significant at $p<0.10$ for *F. excelsior*) (Figure 4.4B) and the trends are similar to those for Q_L as shown in Figure 4.4A. It is a shame that we could not instrument our AC1 for sap flow measurements, as many other measurements (e.g., N_m and $\delta^{13}C$) showed a very strong decline between AC1 and AC2. Evidence showing reduced hydraulic conductance with increasing tree size has been found in numerous species (e.g. Mencuccini and Grace 1996b, Hubbard *et al.* 1999, Ryan *et al.* 2000). Our results also paralleled to those obtained by Vilalta *et al.* (in press) for Scots pine. They found that Q_L and K_L were reduced in older trees compared with young trees. In contrast, Philips *et al.* (2002) found that K_L did not differ between 32 m tall trees and

60 m tall trees in their sap flow experiment. They suggested that the structural change in the aboveground portions of the trees may moderate the influence of size on K_L. Furthermore, hydraulic limitation hypothesis is also based on the interactions between cavitation avoidance and water transport capacity (McDowell *et al.* 2002). Moreover, this hypothesis also predicts that xylem water potential at the top of tall trees is close to the water potential that causes xylem cavitation. Foliage tends to hold a minimum water potential beyond which cavitation will occur. Ryan and Yoder (1997) stated that stomates close more often in old trees than in young trees to prevent such cavitation. Stomatal closure prevents leaf water potential (Ψ_L) from dropping below this minimum (Ψ_L threshold). In our leaf-level gas exchange study (Chapter 3), similar levels of stomatal conductance in old trees compared to young trees were found to be accompanied by lower Ψ_L (at least in *A. pseudoplatanus*) in old trees, suggesting that the closure is a response to a decrease in water status (Bauerle *et al.* 1999). However, at least for *F. excelsior*, the leaf-level data showed no difference in either stomatal conductance or water potential across age classes.

In grafted seedling, an intended experiment with regard to stomatal conductance (G_s) *versus* different levels of D was carried out. This experiment was done to gain appropriate values of G_s since leaf-level gas exchange in Chapter 3 was more limited in its coverage of a range of environmental conditions (thanks to climate control in the cuvette). Furthermore, one would expect that if the memory of donors trees persisted in grafted scions, G_s of grafted seedlings would not changed dramatically. In this chapter, however, G_s of grafted seedlings did not show any age-related decline trend in either species. Thus, we can conclude that the response of stomatal conductance does not depend on maturation characteristics persisting in grafted seedlings. These results suggest that the factor affecting the regulation of stomata is again size, rather than age. This can be supported by the results of K_L especially the ones observed in *F. excelsior* where the trend is similar to G_s, despite being obtained in two different experiments. In both experiments, neither G_s nor K_L showed an age-related trend, although they are different in terms of scion ages. In contrast, Day *et al.* (2001) found the opposite results, whereby grafted scions of red spruce showed a declining trend of G_s with increasing age, but no age-related trend was observed in donor trees. However, their results were based on small sample size.

Together with the results obtained in previous chapters, the results obtained in this chapter strengthen the hypothesis that age- or size-related reductions in hydraulic conductance in older or tall trees, causing reduced photosynthesis and growth. Several studies have also reported a decline in whole-plant hydraulic conductance with height (Mencuccini and Grace 1996a, Ryan *et al.* 2000, McDowell *et al.* 2002). The association between stomatal and hydraulic conductance with the transpirational flux has now been widely observed, but less is understood about how stomatal conductance is affected by a decline in hydraulic conductance as trees grow taller. It has been proposed that the stomatal control mechanism is affected directly by these-height related or age-related changes in plant hydraulic conductance, and that this could contribute to a declining productivity as trees get taller (Ryan and Yoder 1997).

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CHAPTER 5

TESTING THE SUITABILITY OF GRAFTING IN STUDIES AIMED AT SEPARATING THE EFFECTS OF AGE AND SIZE IN TREE GROWTH

5.1 INTRODUCTION

Previous chapters have shown that the growth and physiological characteristics of *A. pseudoplatanus* and *F. excelsior* generally decreased with increasing age. However, these trends were observed in donor trees in the field but not in their grafted scions. Since the trees in the field are significantly variable in terms of size, we concluded that the size limitation to water or nutrient transport (related to hydraulic limitation) is the major factor that contributes to these findings rather than age itself. Hence, we found no evidence that the morphological and physiological changes are genetically programmed inside the meristems. Nevertheless, we could not finalise our study without taking into consideration the problems that may rise from the grafting technique.

Manipulative experiments such as taking scions from different tree age groups to separate the size effect through grafting have to be carefully investigated. Since grafting involves two relatively different genotypes (rootstock and scion), two potential problems should be considered; 1) the potential constraint on water movement created by the graft union itself, and 2) the physiological and morphological effects of the fact that the grafted seedlings contain two different genotypes.

When scions are grafted onto rootstocks, a graft union is formed at the junction of the two tissues (Atkinson *et al.* 2001). The gross morphology of this union tissue can be quite distinctive and in some cases a considerable swelling exists at the tissue junction, predominantly on the scion side (Warne and Raby, 1938). This type of swelling is often much more pronounced when a scion is grafted onto a rootstock which restricts its vegetative growth, i.e. a dwarfing rootstock (Atkinson *et al.* 2001). The internal anatomy of these graft unions found in apple has revealed that the structure of the union tissue is different from that of either the scion or the rootstock (Simons, 1986; Soumelidou *et al.*, 1994). Atkinson *et al.* (2001) reported that there is considerable tissue disorganisation, particularly within the xylem in the graft union of two-year-old grafted apple clone (Queen Cox). The results from this study suggest that the graft union is a region of low hydraulic conductance, relative to the scion in particular, which may influence the movement of substances in the xylem, such as ions, water and plant growth regulating hormones. There have been many suggestions that the graft union itself plays a role in a causing dwarfing effect by apparently restricting water flow, or by removing substances, particularly minerals and plant growth regulators, from the xylem sap (Knight 1926, Jones 1984). Such a hypothesis is consistent with the observed anatomical changes in the graft union (Simons, 1986; Soumelidou *et al.*, 1994).

In this chapter, we initially compare the cumulative results obtained from growth characteristics and net photosynthesis rates from previous chapters between rootstock, self-grafted and the combination of grafted seedlings from four age classes. These comparisons can provide us early indicators of incompatibility issues. Later, we also report results of measurements of the hydraulic conductance of entire stems (stem with graft union) and scions (stem without graft union) of seedlings of the two studied species. The aim of the experiments described here was to determine if the graft union provides a hydraulic bottleneck to the movement of water between the rootstock and the scion. Additionally, we also recorded the hydraulic conductance of entire stems with and without leaves, and of graft sections across the four age classes in both species.

Another potential issue with regards to grafting technique is the involvement of two genotypes in the process of producing a single tree. In our case, it was not possible to separate the effects of age and size while at the same time obtaining survival rates high enough to ensure a reasonably large sample size merely by selecting grafting as the sole method of propagation. Therefore, we conducted a simple test by also propagating some branches of variable lengths through the air layering technique. This technique holds three main advantages over grafting; 1) only one genotype is involved, 2) it can produce various seedlings sizes by propagating different branch lengths, and 3) there is no alteration in wood structure at the beginning of growth since the sample branches are relatively long compared to grafted scions. The plants that have been propagated using this technique can be used to test our hypothesis that there is no adverse effect of the genotype complication in grafting and also to provide additional evidence on the role of plant size. To our knowledge, there is no published account on this kind of study to date.

5.2 MATERIALS AND METHODS

5.2.1 Measurement of hydraulic conductance of grafted seedlings using High Pressure Flow Meter (HPFM) apparatus

The hydraulic conductance of xylem was measured using HPFM (Dynamax Inc., Texas, USA). Four and five grafted seedlings were measured within each age class of *A. pseudoplatanus* and *F. excelsior* respectively. These plants were brought into the laboratory under low light condition and the entire pot was immersed in water for at least a day to avoid the introduction of air embolisms into the xylem after cutting the stem prior to measurement. The stem was cut about five centimetre below the graft union (refers to the point at which the scion and understock of a graft meet) and was attached to the HPFM by compression couplings. A leaf was excised from the top of the plants and bagged with wet tissues before hydraulic conductance measurements took place. Leaf water potential was measured on those leaves using a Portable Plants Moisture System (Skye Instruments Ltd, Powys. UK). Leaf water potentials for all the studied plants were above 1.5 bar (0.15 MPa) suggesting that the entire plants were effectively saturated with water and having low transpiring leaves. Diameter and height (length) of the entire stem and the scion were also measured (Table 5.1). The hydraulic conductance of the entire stem segment with leaf (refers to entire aboveground stem, where the cut was made 5 cm below graft union) was measured first, then, followed by entire stem without leaf (refers to entire aboveground stem after the leaves were removed) and graft union (refers to grafted union segment where another cut were made 5 cm above graft union with the total length of about 10 cm). The hydraulic conductance of the scion segment (refers to the entire stem after the graft union was removed) was calculated by subtracting the value of the graft union from the value of the entire stem segment. In all measurements, hydraulic conductance was measured using the transient method (Tyree *et al.* 1995). Three sequential measurements were made on each section and the readings were only recorded after water could be seen coming out from the tips. The comparison between sections was established by calculating the hydraulic resistances for each age class. In addition, hydraulic conductivity for each section was also determined by dividing hydraulic conductance by the length of each measured section.

Table 5.1: Summary of main characteristics of *A. pseudoplatanus* (N=16) and *F. excelsior* (N=20) grafted seedlings used in this study.

Age class	**Stem diameter (cm)**	**Stem length (cm)**	**Scion diameter (cm)**	**Scion length (cm)**	**Leaf area (m^2)**
A. pseudoplatanus					
1	2.19 ± 0.19a	219.63 ± 22.78a	1.68 ± 0.13a	208.05 ± 23.54a	0.330 ± 0.05a
2	2.23 ± 0.12a	181.25 ± 6.82ab	1.62 ± 0.15a	170.23 ± 6.66ab	0.342 ± 0.05a
3	2.34 ± 0.13a	169.35 ± 17.92ab	1.61 ± 0.11a	159.30 ± 17.92ab	0.198 ± 0.02b
4	2.17 ± 0.05a	161.85 ± 14.31b	1.61 ± 0.09a	151.80 ± 14.28b	0.245 ± 0.03ab
F. excelsior					
1	1.93 ± 0.11a	181.90 ± 6.56a	1.54 ± 0.13a	170.82 ± 6.31a	0.532 ± 0.04a
2	1.79 ± 0.10a	152.30 ± 9.97a	1.42 ± 0.04a	142.24 ± 9.97a	0.516 ± 0.02a
3	1.95 ± 0.06a	150.84 ± 14.08a	1.29 ± 0.05a	140.80 ± 14.10a	0.465 ± 0.03a
4	1.72 ± 0.09a	161.50 ± 15.63a	1.31 ± 0.07a	150.90 ± 15.94a	0.469 ± 0.03a

Note: Stem represents the whole shoot from 5 cm below graft union to the top and scion represents the shoot from 5 cm above graft union to the top. Same letter within each column indicates that age classes were not significantly different (using Duncan Multiple Range Test).

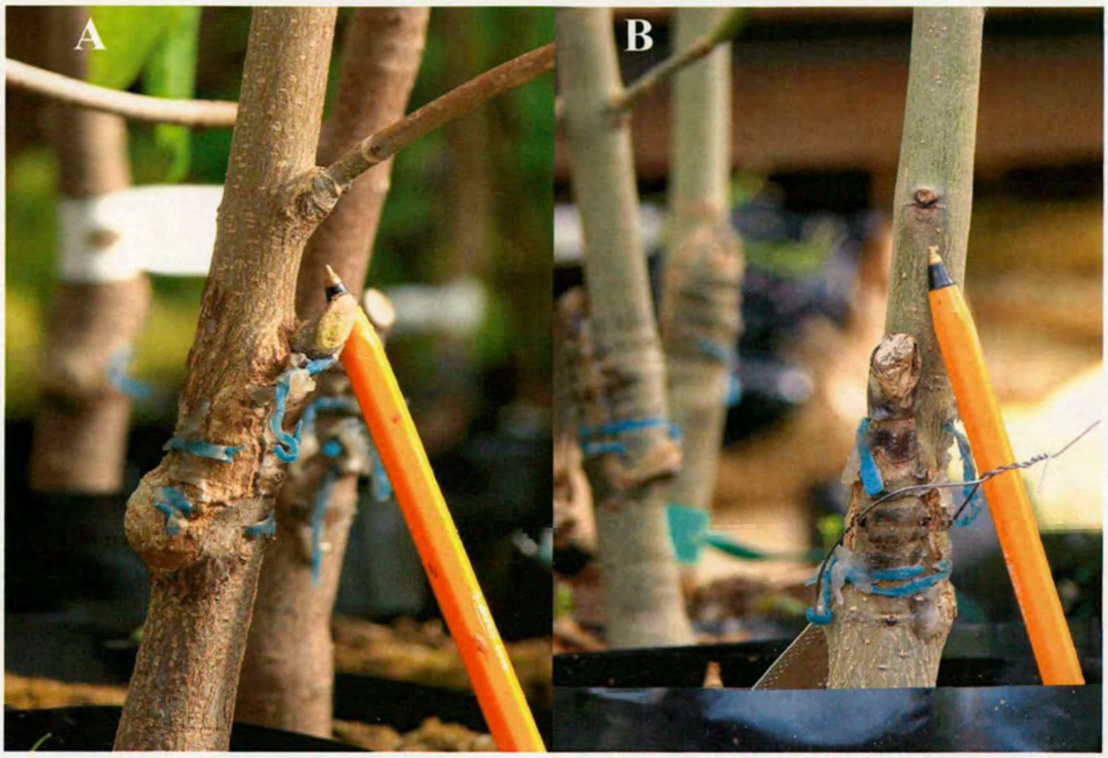

Plate 5.1: Graft union of *A. pseudoplatanus* (A) and *F. excelsior* (B), 30 months after grafting.

5.2.2 Growth and physiological characteristics of air-layered plants

5.2.2.1 Sample collection and preparation

For the two species, six branches of three different lengths were selected in each of three trees of age class 2 (~27 years) and age class 4 (~140 years) for air layering. Due to accessibility problem, lower or middle branches were selected. Branches that grew more than 60° upright and receiving full sunlight were chosen in order to avoid the air-layered plants to grow laterally after being transplanted into pots. The air layering was conducted in early spring 2004 after a trial was made in 2003. From the trial, we only succeeded to propagate one branch of *A. pseudoplatanus* but none for *F. excelsior* probably due to unsuitable timing. Some samples were also damaged by birds and squirrels. In 2004, the works were started in early spring (March). The selected branches were girdled in an area about 2.5 cm to

3.5 cm wide to remove the bark. This girdled area was then scrapped gently to remove the cambium layer that can prevent the formation of roots. A commercial rooting hormone (Homebase Rooting Hormone powder, PSD 07630) was applied on the girdled area before being covered by a lump of medium. The material used as the medium was sphagnum peat, and aluminium foil was used to make a lump which was then covered by a thick plastic sheet in order to prevent them from being damaged by birds and squirrels. Normally, the air-layered branch will root after four to eight weeks. In our case, for unknown reasons they took about five months to root and the branches were ready to cut after six months. We only succeeded at propagating 12 branches out of 18 from age class 2 and 9 out of 18 from age class 4 in *A. pseudoplatanus*. No survival was recorded for *F. excelsior*.

After the branches rooted (Plate 5.2), they were transferred into 10-L pots, potted with sphagnum peat, sand and vermiculite mixed 2: 1: 1, and supplied with slow-release fertilizer. The initial diameter and height (length) were recorded after being transplanted (Table 5.2). These plants were then placed in the glasshouse until the 2005 growing season. In 2005, only nine air-layered plants had survived in each age class. About 50% of them started flowering and podding (Plate 5.3). They were then arranged in a complete randomised design and rotated every three weeks.

Plate 5.2: Air-layered branches of age class 2 (left two) and age class 4 (right two) after being cut from the donor trees.

Plate 5.3: Air-layered plants produced flowers and pods in spring.

Plate 5.4: Air-layered plants were assigned in a complete randomised design and rotated every three weeks.

5.2.2.2 Diameter, height and relative growth rate

Measurements of total diameter (taken about 10 cm above the soil and marked with a permanent marker) and total height were taken in early May 2005 and subsequent measurements were taken every four weeks until July 2005. A digital calliper and a measuring tape were used for diameter and height measurements respectively. Relative growth rate for both parameters were calculated using the equations given in Chapter 2.

5.2.2.3 Leaf-level gas exchange, transpiration rate (E_L) and leaf-specific hydraulic conductance (K_L)

Gas exchange was measured in three sampling dates: May 7th, June 26th and July 31st. In each sampling date, the measurements were conducted on the three uppermost leaves of each individual plant. The LCPro portable photosynthesis system with climate control as described in Chapter 3 was used and average values across three sampling dates were obtained.

Transpiration rate per unit leaf area (E_L) and leaf-specific hydraulic conductance (K_L) were also estimated on July 31st using the gravimetric method. All the air-layered plants were watered to field capacity and covered by black plastic bags about 8 hours before taking the measurement (Plate 5.5). The first measurement was taken around 3.00 am by weighing the pot on a dual face balance (Plate 5.6). A leaf was excised from each plant to measure the leaf water potential. The second measurement was carried out around 12.00 pm (after 9 hours). All the leaves were excised from the air-layered plants and total leaf area was measured using LI-3100 leaf area meter (LI-COR Inc, Lincoln, Nebraska, USA). The difference of plant weight was divided by the time interval and total leaf area in order to calculate E_L. The leaf specific hydraulic conductance (K_L) was estimated by dividing E_L by the difference in leaf water potential between predawn and midday. The equations used to calculate E_L and K_L are as shown in Chapter 4.

Table 5.2: Some characteristics of *A. pseudoplatanus* air-layered plants used in this study (both N=9).

Age class	**Diameter range (mm)**	**Height range (cm)**	**Total leaf area range (m^2)**	**Podding (%)**
2	6.62 – 16.55 (10.79 ± 1.19)	38.7 – 147.1 (80.5 ± 12.6)	0.02 – 0.36 (0.12 ± 0.03)	44.4
4	8.51 – 19.36 (12.71 ± 1.24)	60.0 – 121.1 (91.1 ± 8.4)	0.03 – 0.33 (0.16 ± 0.03)	55.6

Note: Numbers in parentheses are mean values and standard errors.

5.2.3 Data analyses

Means and standard errors calculated for each parameter in the first experiment by analysing the data using SPSS (SPSS Inc. 2003). ANOVA was carried out in second experiment and the mean values were compared using Duncan Multiple Range Test (DMRT) using SPSS. Some of the data were also fitted by regression analysis using Sigmaplot 9.0.

Plate 5.5: The pots of air-layered plants were covered by black plastic bags in order to measure water use by gravimetric method.

Plate 5.6: The pot of air-layered plants was weighed using a dual-face Sartorius bench-top scale in early morning and at midday. Leaf water potentials were also measured at both times.

5.3 RESULTS

5.3.1 Hydraulic resistance and conductivity of the grafted seedlings

Table 5.3 shows the mean values and standard errors of each parameter measured among four age classes of *A. pseudoplatanus* and *F. excelsior*. The mean values of the whole stem resistance (R_{stem}) ranged from 66.21% to 80.83% in *A. pseudoplatanus* and from 44.63% to 53.94% in *F. excelsior*. Meanwhile, the mean values of the hydraulic resistance observed in scion sections (R_{scion}) ranged from 45.67% to 58.12% and from 39.42% to 51.58% in *A. pseudoplatanus* and *F. excelsior* respectively. The mean values of the graft union hydraulic resistance (R_{graft}) ranged from 12.54% to 35.15% in *A. pseudoplatanus* and from 1.44% to 2.85% in *F. excelsior*. An ANOVA analysis conducted on stem and scion values showed that there were no significant differences among age classes for either species, indicating that graft union did not differ depending on donor age effects on hydraulic transport.

Table 5.3: Means and standard errors of hydraulic resistance from various parts of *A. pseudoplatanus* and *F. excelsior* grafted seedlings.

Age class	**n**	$R_{stem+leaf}$ **(x 10^3)**	R_{stem} **(%)**	R_{scion} **(%)**	R_{graft} **(%)**
A. pseudoplatanus					
1	4	8.35 ± 2.04	73.10 ± 5.77	58.12 ± 4.24	14.99 ± 2.44
2	4	8.15 ± 0.71	80.83 ± 6.10	45.67 ± 3.02	35.15 ± 5.79
3	4	9.87 ± 1.86	66.21 ± 4.29	53.67 ± 4.40	12.54 ± 0.87
4	4	9.85 ± 2.07	79.81 ± 5.76	49.21 ± 9.49	30.60 ± 13.97
F. excelsior					
1	5	10.99 ± 1.25	53.94 ± 8.60	51.58 ± 6.41	1.89 ± 0.27
2	5	12.72 ± 1.19	49.23 ± 3.40	43.59 ± 1.68	2.39 ± 0.83
3	5	13.32 ± 1.94	44.63 ± 4.65	39.42 ± 3.39	1.44 ± 0.10
4	5	16.20 ± 4.98	45.03 ± 6.07	39.90 ± 3.38	2.85 ± 0.63

Note: R_{stem} = stem hydraulic resistance for the whole tree excluding root system, R_{scion} = scion hydraulic resistance, and R_{graft} = graft section hydraulic resistance.

The hydraulic conductivity (i.e., conductance per unit length) was also calculated in stem and scion segments of each age class. The mean values and standard errors of these parameters were plotted for each age class of both species, as shown in Figure 5.1 and Figure 5.2 respectively. The mean values of hydraulic conductivity for both stem and scion sections were found higher in AC1 of both species but no trend related to age was observed. These results also showed no significant difference in hydraulic conductivity between stem and scion sections.

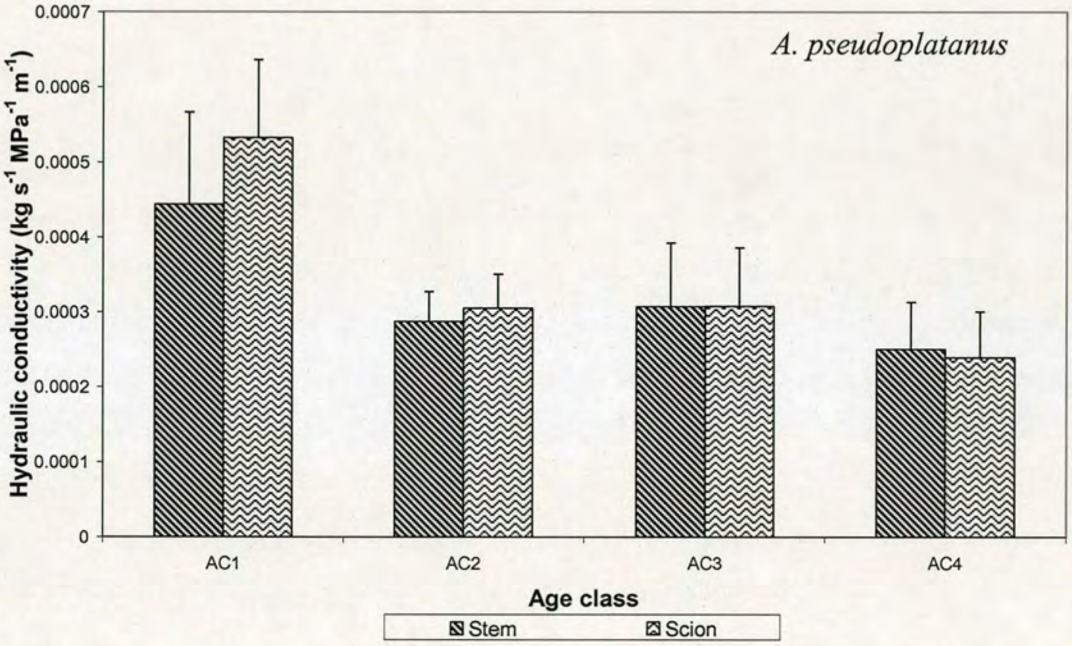

Figure 5.1: Means of hydraulic conductivity per unit length between stem and scion sections in each age class of *A. pseudoplatanus*.

Figure 5.2: Means of hydraulic conductivity per unit length between stem and scion sections in each age class of *F. excelsior*.

5.3.2 Growth and physiological characteristics of air-layered plants of *A. pseudoplatanus*

Total diameter and total height growth were almost constant throughout the sampling period for both age classes (Figures 5.3 and 5.5). The growth changes were very small as shown by diameter growth increment over time (*RDGR*) and height growth increment over time (*RHGR*) (Figures 5.4 and 5.6). These low rates of growth over time in air-layered plants after they were transplanted into pots could be due to allocation of photosynthates or other resources to belowground structures such as roots. Unfortunately, root biomass could not be measured due to the small number of available plants. Plate 5.7 shows evidence of substantial growth of roots eight months after transplanting (compare Plate 5.7 with Plate 5.2). When these parameters were analysed according to whether plants were non-podded and podded, we also found that the mean values of *RDGR* and *RHGR* were higher in non-podded plants (Figures 5.7 and 5.8), suggesting that an influence of this sink factor at the time of fruit growth.

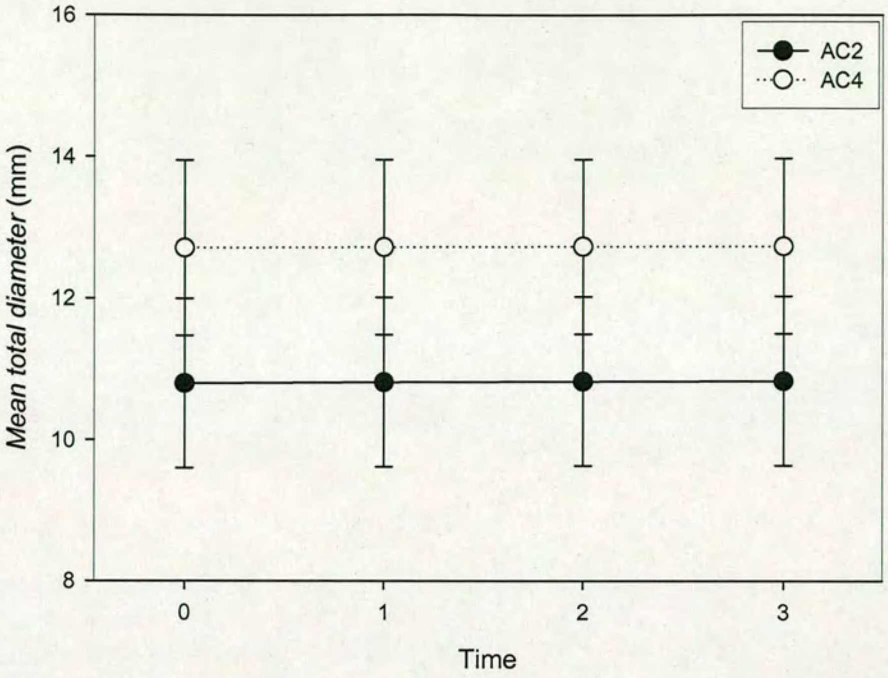

Figure 5.3: Mean diameter of *A. pseudoplatanus* air-layered plants of two age classes taken over four sampling times since the initial measurement. Bars indicate standard error.

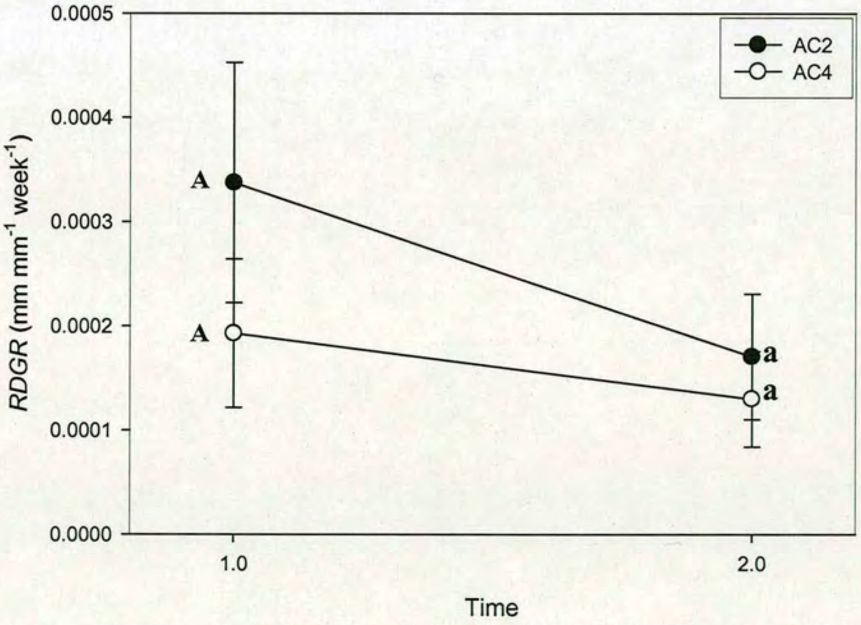

Figure 5.4: Relative diameter growth rate of *A. pseudoplatanus* air-layered plants on two age classes. Same capital and small letters represent no significant difference in first and second measurement respectively.

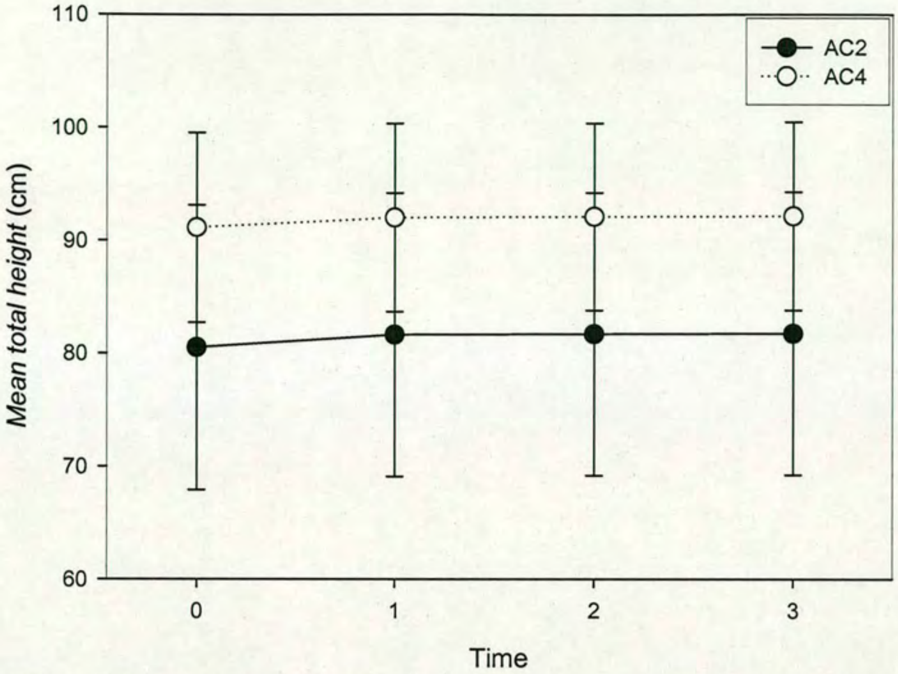

Figure 5.5: Mean height of *A. pseudoplatanus* air-layered plants of two age classes taken over four sampling times since the initial measurements. Bars indicate standard error.

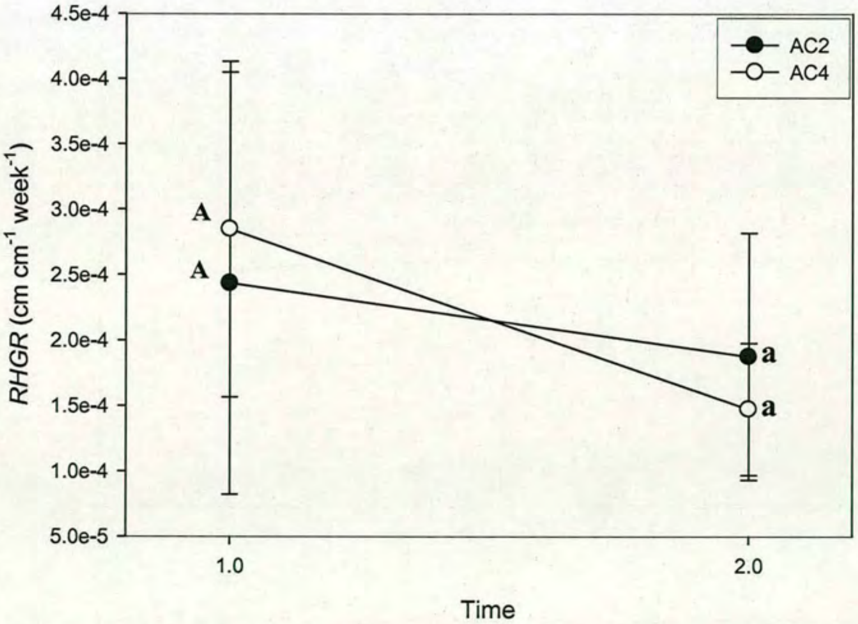

Figure 5.6: Relative height growth rate of *A. pseudoplatanus* air-layered plants on two age classes. Same capital and small letters represent no significant difference in first and second measurement respectively.

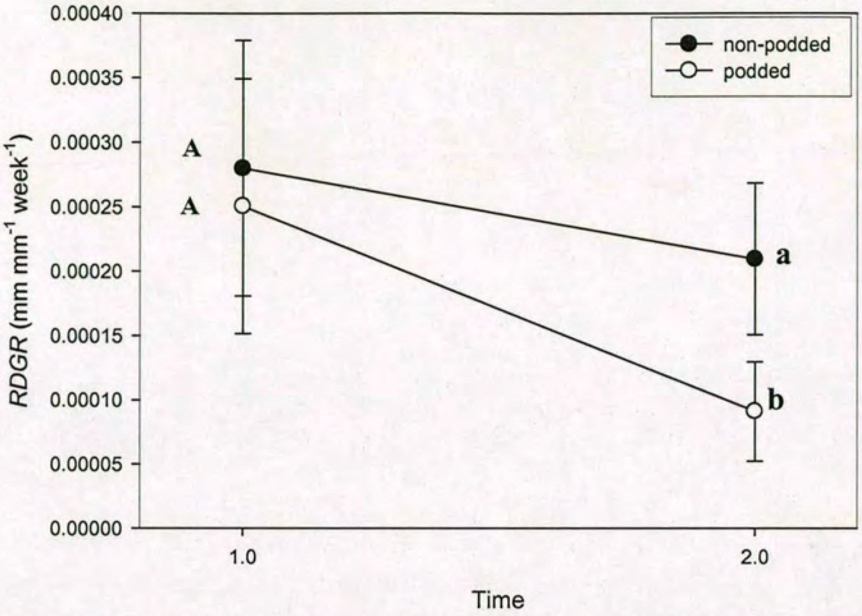

Figure 5.7: Relative diameter growth rate of *A. pseudoplatanus* air-layered plants observed in podded and non-podded plants. Same capital and small letters represent no significant difference in first and second measurement respectively.

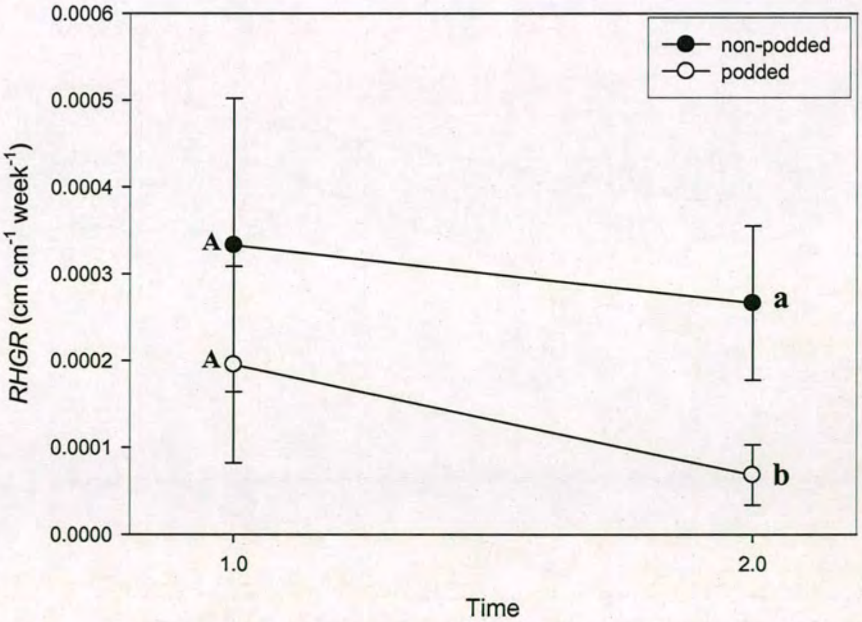

Figure 5.8: Relative height growth rate of *A. pseudoplatanus* air-layered plants observed in podded and non-podded plants. Same capital and small letters represent no significant difference in first and second measurement respectively.

Plate 5.7: Air-layered plants of age class 2 (left two) and age class 4 (right two) after eight months transplanted into pots.

In concordance with growth studies, physiological measurements were also taken in these air-layered *A. pseudoplatanus* plants. ANOVA was carried out to compare these two age classes for their leaf-level gas exchange parameters, such as net photosynthesis (A_{net}), internal CO_2 concentration (C_{i}), transpiration rate (E) and stomatal conductance (G_{S}). The whole-plant transpiration rate (E_{L}) was also estimated using gravimetric methods. Measurements of predawn water potential (Ψ_{predawn}) and midday water potential (Ψ_{midday}) were also taken to calculate whole-plant hydraulic conductance (K_{L}). Table 5.4 shows the ANOVA summary of parameters mentioned above. Although the mean values of those parameters were generally higher for the younger class, no significant difference was found between these two age classes.

Table 5.4: Results of leaf level gas exchange, transpiration rate and leaf-specific hydraulic conductance on the two age classes of *A. pseudoplatanus* air-layered plants. The values presented in the table are mean and standard error with significant level at $p<0.05$.

Parameter	**Age (year)**		**F value**
	~27	**~140**	
A_{net} (μmol m^{-2} s^{-1})	10.82 ± 0.44	10.44 ± 0.48	0.33^{ns}
Ci (μmol mol^{-1})	174.43 ± 7.69	164.36 ± 8.19	0.80^{ns}
E (mmol m^{-2} s^{-1})	2.20 ± 0.12	2.06 ± 0.13	0.59^{ns}
G_{S} (mmol m^{-2} s^{-1})	133.70 ± 11.61	127.78 ± 12.49	0.12^{ns}
E_{L} (mmol m^{-2} s^{-1})	1.74 ± 0.22	1.66 ± 0.09	0.12^{ns}
Ψ_{predawn} (-MPa)	0.482 ± 0.04	0.476 ± 0.04	0.01^{ns}
Ψ_{midday} (-MPa)	0.755 ± 0.06	0.915 ± 0.09	2.16^{ns}
K_{L} (mmol m^{-2} s^{-1} MPa^{-1})	6.91 ± 1.09	4.70 ± 0.97	2.29^{ns}

Note: the '±' represents mean standard error. ns = not significant difference.

Regression analyses were conducted between morphological parameters and some physiological parameters to get a clearer picture of the results obtained previously. Regardless of plant age, negative relationships were observed between G_s and mean diameter and mean height of air-layered plants (Figure 5.9). These relationships were found significant at $p<0.01$ and $p<0.05$ respectively. Negative significant correlations ($p<0.05$) were also found between A_{net} and mean diameter and mean height of air-layered plants.

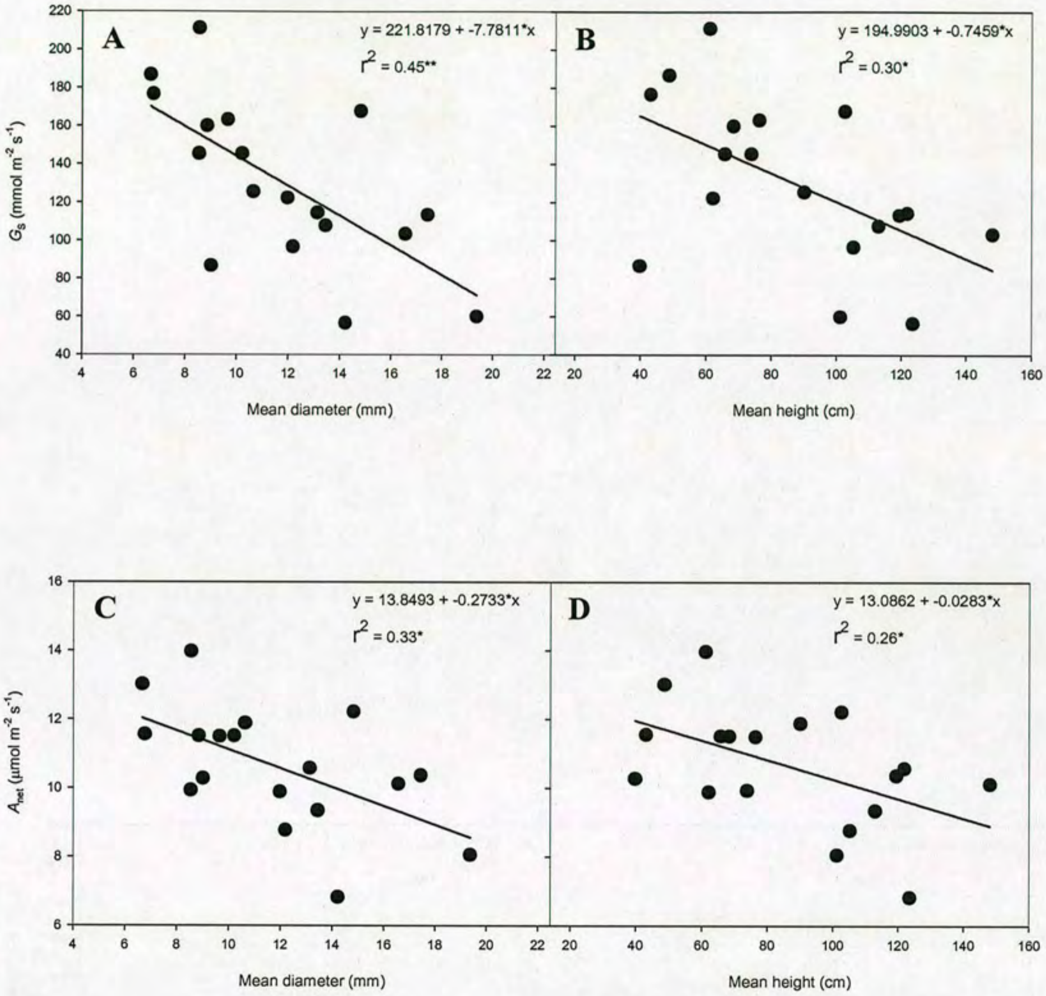

Figure 5.9: Relationship of stomatal conductance with mean diameter (A) and mean height (B), and net photosynthesis with mean diameter (C) and mean height (D) across two age classes. The '**' indicates significant correlation at $p<0.01$, and '*' indicates significant correlation at $p<0.05$.

The E_L and K_L of air-layered plants obtained from gravimetric methods were also correlated with their respective diameters and heights as shown in Figure 5.10. Significant negative correlations were found ($p<0.01$) for E_L with increasing diameter and height of the plants. Furthermore, higher negative correlations ($p<0.001$) were observed between K_L and plant diameter and height.

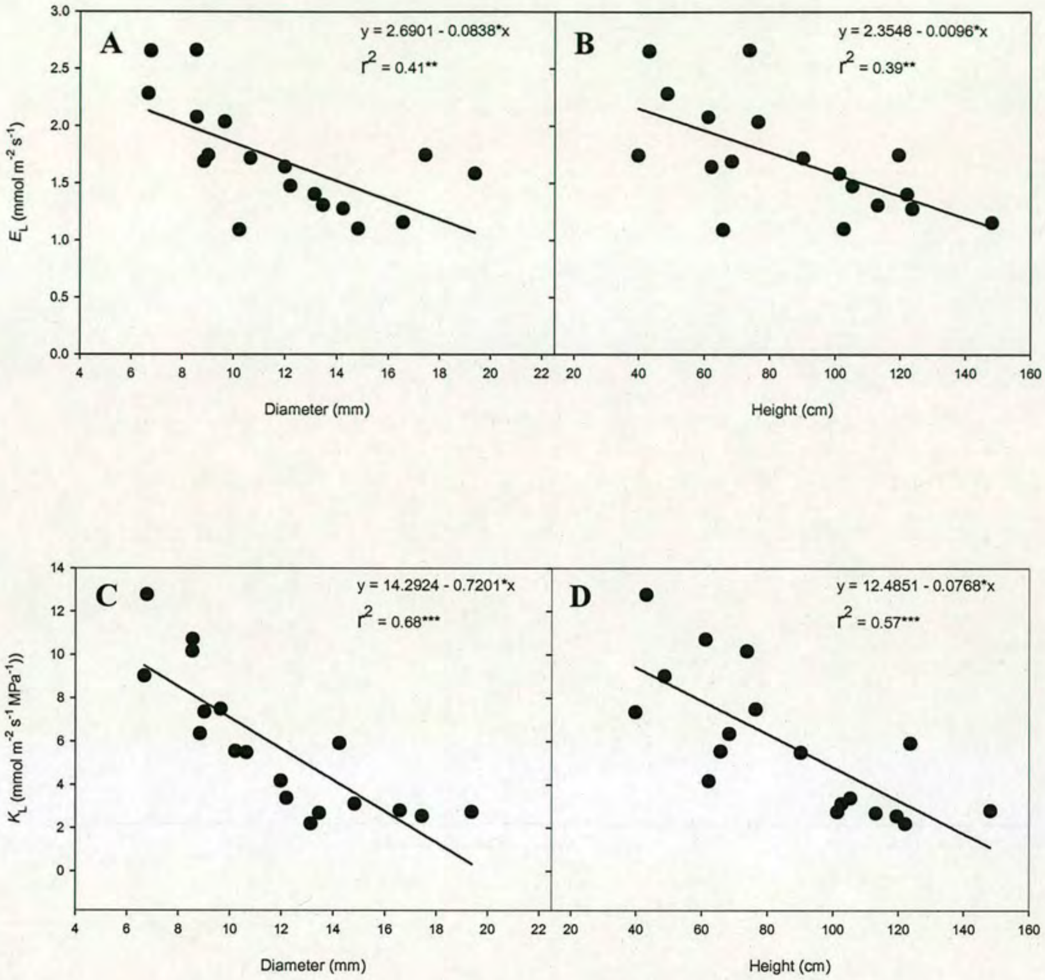

Figure 5.10: Relationship of transpiration rate with total diameter (A) and total height (B), and leaf-specific hydraulic conductance with total diameter (C) and total height (D) across two age classes. The '***' indicates significant correlation at $p<0.001$, and '**' indicates significant correlation at $p<0.01$.

Overall, the results obtained in this experiment showed that the difference in growth and physiological characteristics of air-layered seedlings were not due to the age of donor trees, instead, they were influenced by the size of the plants. The results also showed the effect of sink factor such as belowground root allocation and reproductive allocation, on lack of aboveground growth one year after air layering..

5.4 DISCUSSION

5.4.1 Comparisons between rootstock (RS), self-grafted (SG) and grafted seedling (GS)

Previous chapters provide evidence that the age-related decline trends are triggered by size rather than age *per se*. This argument is based on the comparison of the results obtained from the donor trees and the grafted seedlings. Despite the fact that grafted seedlings had been obtained from trees of different age, no age-related trends were observed in growth and physiological characteristics as well as leaf chemical compositions. Nevertheless, without proper evidence that grafting technique has no significant effect on scion growth, a strong conclusion cannot be established.

In this study, grafting success was found to be relatively high, especially in the case of *F. excelsior* that showed no evidence of graft incompatibility. Furthermore, the overall results obtained from growth characteristics and net photosynthesis from previous experiments can provide additional evidences that grafting technique did not affect the overall growth of grafted seedlings. For example, comparisons were established between rootstock (RS), self-grafted (SG) and a combination of grafted seedlings from four age classes (GS) with regard to these parameters. In *A. pseudoplatanus*, no significant difference was detected among RS, SG and GS in RGR_{mass} (Figure 5.11). After two growing seasons, the mean value of RGR_{mass} was found higher in RS rather than in SG and GS. Similar results were also observed in E_{G} (Figure 5.12). In contrast, Greenwood *et al.* (1989) found that grafted scions originating from the same rootstocks grew more than their intact counterpart, since the rootstock provided a considerably larger root system compared to rootstock itself. Their results were supported by the ones observed in *F. excelsior*. The potential reason explaining this lies in the stress experienced by RS in *A. pseudoplatanus* in the first growing season. In our observations, rootstocks from *A. pseudoplatanus* suffered some stress due to the attack from anthracnose fungus (refer to Plate 5 in Appendix). After RS overcome the stress, they grew much faster in the second growing season compared to SG and GS, which was the reason why RGR_{mass} and E_{G} were higher in RS. Figure 5.13 clearly shows the effect of stress on RS. In the first growing season (2003), net photosynthesis rate was much lower in

RS compared to SG and GS but increased tremendously in the second growing season (2004). These results also showed that upon recovery from the grafting shock in first growing season, net photosynthesis rates in *A. pseudoplatanus* increased substantially in second growing season. However, an opposite trend was observed in *F. excelsior* (Figure 5.14). Based on our observations, this could be due to the increase in leaf thickness over leaf area in *F. excelsior* that contribute to the decrease in net photosynthesis rate per unit mass in *F. excelsior*, whereas in *A. pseudoplatanus* the leaves tend to increase in area rather than thickness.

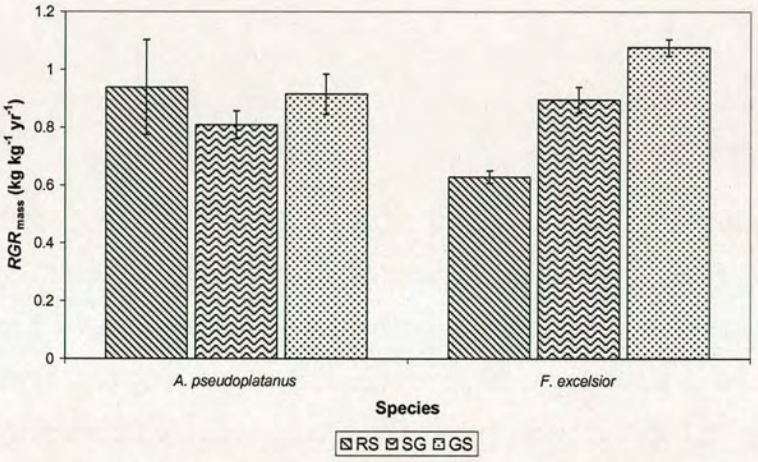

Figure 5.11: RGR_{mass} in rootstock (RS), self-grafted (SG) and combination of grafted seedlings from four age classes (GS) in both species.

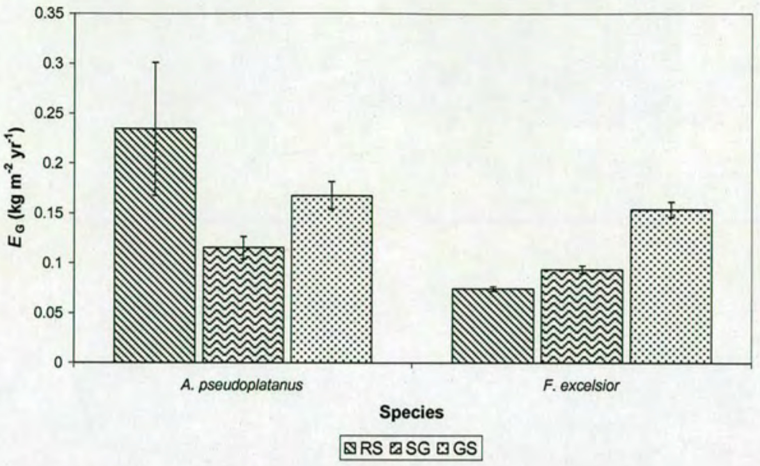

Figure 5.12: E_G in rootstock (RS), self-grafted (SG) and combination of grafted seedlings from four age classes (GS) in both species.

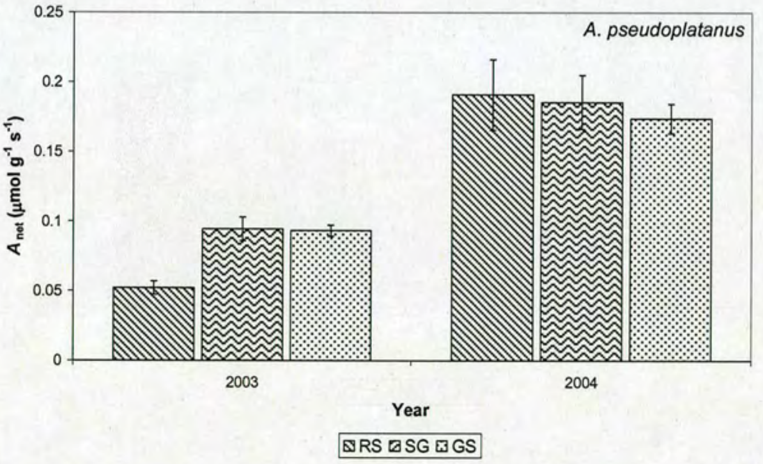

Figure 5.13: Net photosynthesis rates in rootstock (RS), self-grafted (SG) and combination of grafted seedlings from four age classes (GS) in *A. pseudoplatanus* over two growing seasons (2003 and 2004).

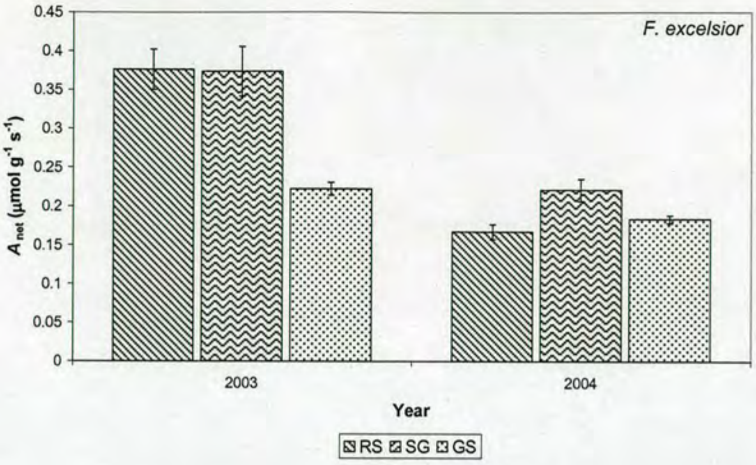

Figure 5.14: Net photosynthesis rates in rootstock (RS), self-grafted (SG) and combination of grafted seedlings from four age classes (GS) in *F. excelsior* over two growing seasons (2003 and 2004).

In synthesis, these comparisons did not show any evidence of grafting incompatibility in the grafted seedlings, despite some differences observed in growth characteristics and net photosynthesis rates in the first growing season. Grafted scions taken from four age classes in the field had either higher or similar growth and physiological behaviour as the self-grafted seedlings and the rootstock seedlings. We can then conclude that, upon recovery from the grafting shock, the differences in net photosynthesis rate disappeared and values became similar to the ones observed in control seedlings.

5.4.2 Hydraulic conductance of grafted seedling stems

It has been proposed that grafting can influence the total growth of trees, especially scions. There are numerous studies suggesting that rootstock and graft union play important roles in controlling scion growth. Those studies suggested that the vigour of rootstock onto which a scion was grafted influenced the scion hydraulic capacity. For example, low hydraulic conductance found in rootstock reduces scion vigour (Syvertsen and Graham 1985, Atkinson and Else 2001). When tissue become disorganised, particularly within the xylem, low hydraulic conductance was found in the graft union (Atkinson *et al.* 2001).

To cater for the problem that may exist from rootstock effects, we used one rootstock genotype with physical similarities. Some studies showed a reduction in vegetative growth of scions due to the use of dwarfing rootstocks (Beakbane and Thompson 1947, McKenzie 1961) and other reported increases in scion vigour with rootstock vigour (Syvertsen and Graham 1985). The use of compatible rootstock during grafting can eliminate the effect of rootstock on scion growth and lead to the conclusion that the differences occuring in grafted seedlings are mainly due to the differences among scions themselves. Our approach has been supported by Jayawickrama *et al.* (1991). They found that in most studies related to grafting in conifers, there were no large effects of rootstock on scion growth.

Results from previous chapters showed that no age-related trends were found in growth or even gas exchange and leaf chemical composition for the grafted seedlings, although statistical significant differences were sometimes detected for some of the parameters among scion ages. However, these results could not be confirmed without proper evidence related to grafting. The most important factor causing dwarfing effects by apparently restricting water flow between rootstocks and scions, is the graft union itself (Atkinson *et al.* 2001). Structural anomalies, specifically the graft union, can result in decreased water and nutrient status in the scion due to reduced transport volume. Hence, particular attention was focused on the effects of graft union instead of the effects of rootstock. Here, we show that graft union has had no effect on hydraulic conductance of grafted seedlings produced from physically and genetically similar rootstock in two different species with different xylem anatomy.

From the results obtained, we found that the hydraulic conductivity of entire stem segments (stems with graft union section) did not differ from that of scion segments (stems without graft union section) in either species, as shown in Figure 5.1 and Figure 5.2. In fact, the hydraulic resistances between these two segments were also not significantly different. Also, no significant differences were found among age classes in either species. These results suggest that the graft union did not significantly impact on water flow in the stem between rootstock and scion. Furthermore, Basile *et al.* (2003) found that rootstocks had no effect on hydraulic conductance through the scion or the graft union. Vanderklein *et al.* (2006) also found that hydraulic conductance through the graft union in Scots pine grafted seedlings no longer plays a role in water movement since their resistances were much lower than shoot and root resistances. Similar results were also found in our study. Hydraulic resistance observed in graft unions were substantially low compared with the ones observed in stem and scion segments for both species, suggesting that their relative importance is limited. The observed results may reflect the vascular development after grafting. In the most severe cases of abnormal graft union development, incompatibility can result from deterioration of vascular connections (Simons 1982) with building up of resistance in it that could reduce the flow of water, nutrients and carbohydrates through vascular tissues. Incompatibility occurring in grafting could lead to improper growth of trees, such as smaller scions and loss of vigour. Furthermore, the failure or deterioration of the graft union could possibly lead to tree death (Moore and Walker 1981). In our case, however, survival rates (refer to Chapter 2) in both species were considerably high, especially in *F. excelsior* suggesting that both rootstocks and scions are compatible with no restriction in transportation in graft union.

5.4.3 Growth and physiology of air-layered plants from two age classes

Our study has been extended by propagating branches through air-layering from donors identical to the ones used for grafting. Although we did not successfully propagate branches from *F. excelsior*, the ones obtained from *A. pseudoplatanus* provided useful information regarding age- and size-related trends. Furthermore, if there were any complications introduced by the presence of two different genotypes in grafting, air-layering study can act as a check to strengthen the results depicted by grafted seedlings in previous chapters. Given the small number of air-layered plants obtained, this kind of study should be considered as preliminary and further investigation is required to increase our understanding on age- and size-related trends.

Growth rates were generally very small, as shown in Figures 5.4 and 5.6. The absolute growth patterns were about levelled out over time (Figures 5.3 and 5.5) in both age classes. Slower growth suggests the partitioning of carbon or photosynthates into different sinks, such as belowground development (roots) especially during the initial establishment of air-layered plants, and fruit development. Another factor may possibly lie in meristem memory of donor trees that persist in air-layered plants and contributes to the slowing in growth. Furthermore, direct measurements on growth, gas exchange, whole plant transpiration and hydraulic conductance showed that air-layered plants did not differ between two age classes, despite the samples being taken from trees from two contrasting ages. Although only two age classes were used in this study, the findings support the results previously observed for grafted seedlings. Both grafted and air-layered plants did not show any age-related trends, despite all the studies were carried out on them. Meanwhile, when G_s, A_{net}, E_L and K_L were regressed against size of the plants, we found that the size-related decline trends occurred in plants ranging from 6.62 to 19.36 mm in diameter and 38.7 to 121.1 cm in height, with no regards to plant ages (Table 5.2). This is not surprising, because we believe that there is no alteration in xylem anatomy in air-layered plants at least initially, that contributes to increasing resistance with increasing length even over a few centimetres. This also supports the slower growth of air-layered plants observed above. Furthermore, the study conducted by Petit *et al.* (in prep) on branches sampled from the same trees showed that the hydraulic resistance of intact branches increased with increasing length of the branches from both donor trees in the field and from young trees in the field as well as grafted seedlings just within a meter length (Figure 5.15). He also found that the build-up of hydraulic resistance with distance from the tip was faster in old trees in the field compared to young trees and grafted seedlings, suggesting that build-up of hydraulic resistance in branches does not depend on age but on size.

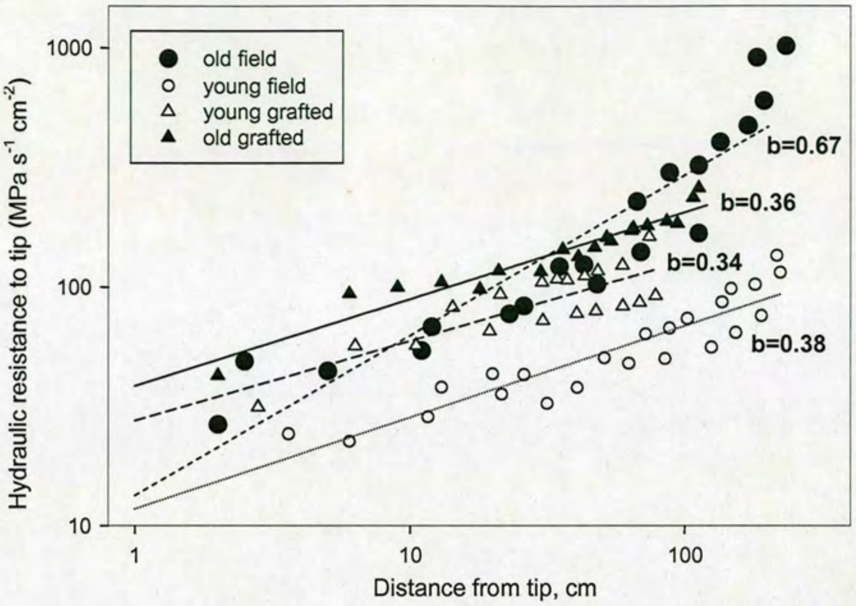

Figure 5.15: Relationship between hydraulic resistance to tip and distance from tip of branches taken from old and young trees in the field, and old and young grafted seedlings of *A. pseudoplatanus* (source: *Petit et al.* in prep)

In conclusion, there was no evidence that the graft union provided a hydraulic bottleneck to the scions. The proper contact between rootstocks and scions and compatible genotypes used for grafting may have contributed to this situation. Furthermore, air-layered plants once again supported the results obtained in the previous chapters. The growth and physiological changes in air-layered sycamore plants were independent of the differences in plant ages but simply controlled by differences in plant size.

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CHAPTER 6

GENERAL CONCLUSION

With two broadleaf species having different water transport anatomies selected in this study, we can finally come to the conclusion with regard to the effects of age (maturation) and size on growth, morphological and physiological changes in tree ageing. Despite numerous hypotheses presented in many journals, only two main hypotheses have been identified. The *hydraulic limitation hypothesis* proposes that the changes during tree ageing are mainly due to size limitations in transporting water and nutrients to the top of the tree. The reduction of water supply with increasing path length to the leaves will reduce stomatal conductance and reduce photosynthesis rate. Decreased photosynthesis may lower mass and height growth through lower photosynthetic capacity, whereas lower photosynthetic capacity is related to the decrease in leaf nutrition status. On the other hand, *the maturation-based hypothesis* refers to developmental changes in meristematic behaviour that occur as trees increase with age (meristem-intrinsic factors). These developmental changes are a form of differentiation and probably have a cellular basis, involving the meristematic cells themselves. As meristems become mature, they change by exhibiting lower growth potential, producing different types of foliage and branching patterns, exhibiting plagiotropic growth and reproductive competence.

The size of trees usually increases with increasing age. Hence, it is difficult to quantify which factor mainly controls growth and physiological characteristics of trees. Nevertheless, experimental manipulations such as grafting using scions from differently aged of trees onto similar rootstocks can separate the effects of size and age. If trees were genetically programmed, the characteristics of meristematic tissue would persist when scions from older trees are grafted onto young rootstocks. This technique provides the opportunity to have seedlings physically similar in terms of height or diameter but different in age.

In our study, we have separated a few experiments into chapters that focussed on growth, morphology and physiology of two species that grow in a mixed uneven-aged species woodland. This site was selected because it comprises trees from different age or size classes dominated by *A. pseudoplatanus* and *F. excelsior* that grow together in a loosely dense woodland avoiding serious resource competition effects. Comparisons between size and age effects has been established by propagating scions from selected trees onto similar rootstocks.

The growth and leaf characteristics were studied on both the donor trees and the grafted seedlings for both species and the results were presented in Chapter 2. In both species, the growth efficiency (E_G) declined with increasing age of donor trees in the field. The decline in E_G with ageing has been supported by the substantial reduction in relative mass growth rate (RGR_{mass}). The sharp decreases in RGR_{mass} with increasing tree age have been observed in both species suggesting that growth rates are getting slower in ageing trees. In contrast, AGR_{mass} increased with increasing age or size of the donor trees because it is a size-dependent parameter. It only describes the pattern of biomass accumulation through time not the rate at which a given unit of biomass contributes to tree growth. The *SLA* was also measured in this study since it is the most important determinant of *RGR*. Changes in *SLA* with increasing age were also observed in both species. The leaf area to leaf mass ratios got smaller with age, despite some older trees in *F. excelsior* showed broader leaves compared with younger trees. However, these results are still not enough to test whether the changes in growth or leaf characteristics were triggered by size or by age. Therefore, results from grafting experiments provided us with the answer to this phenomenon. Despite the fact that *SLA* and phenological characteristics persisted in scion meristems, grafted seedlings did not show any age-related trends in E_G and RGR_{mass} or even AGR_{mass}. These results suggest that the reduction in growth characteristics with tree age is lies in size not age *per se*.

Instead of growth measurements, gas exchange and leaf chemical composition measurements were also carried out on donor trees and grafted seedlings. These results may be important in explaining the decline in growth characteristics with tree ageing. The age-related decline trends were observed in net photosynthesis per unit leaf mass (A_{net}) for both species in the field as shown in

Chapter 3. The reduction in A_{net} with tree age has also been supported by leaf nitrogen content (N_{m}). The decrease in A_{net} of donor trees is triggered by lower photosynthetic capacity from decreased N_{m}. However, measurement of stomatal conductance (G_{s}) did not strongly support the reduction of A_{net} due to stomatal closure in tall or old trees through limitations in hydraulic conductance. The G_{s} trends were found to be similar to the trends from leaf water potential (Ψ_{leaf}), suggesting that stomata may have been regulated by Ψ_{leaf} at the time of measurements. Furthermore, the lack of support from one-time gas exchange measurements on donor trees, especially G_{s} is counter balanced by measuring carbon isotope discrimination ($\delta^{13}C$) on the same sampled leaves. Unlike G_{s}, $\delta^{13}C$ showed strong declining trends with increasing tree age in both species. These results support the reduction in G_{s} with ageing. Furthermore, our grafted seedlings did not show any age-related decline trends in gas exchange or even leaf chemical composition parameters, despite two series of measurements having been carried out during their first and second growing season. Nevertheless, those parameters regressed well with *SLA* and similar results were also observed in donor trees. Given these results, size had the greatest effects on tree growth as well as tree physiology rather than maturational processes.

The study of whole tree transpiration by sap flow measurements may provide further evidence that whole plant leaf-specific (Q_{L}) and whole plant leaf-specific hydraulic conductance (K_{L}) decreased with size or age. It is a shame that we could not calculate canopy stomatal conductance from Q_{L} data because net radiation data are not available. This canopy stomatal conductance may be useful to compare with the stomatal conductance values obtained from leaf level gas exchange measurements. Since there is a direct link between stomatal conductance and transpiration rate, any changes in stomatal response to fluctuating environmental conditions will also change the rate of transpiration. Hence, we can only use the Q_{L} parameter to explain the effect of age and size on water use of these two species. The results obtained by significant non-linear regression analyses in Chapter 4 showed that Q_{L} and K_{L} decreased with increasing age in both species. It is important to point out that Q_{L} reductions with tree age or size are fully coupled with hydraulic limitations to stomatal conductance. Comparing these results with those obtained from gravimetric methods for grafted seedlings in the glasshouse, no age-related trends were observed in E_L and K_L or even G_s in either species. Our results once again suggest that size alone is the dominant factor explaining differences in G_s, E_L and K_L between old and young trees.

As we were aware that grafting could create potential bias in the results observed for grafted seedlings, experiments regarding stem hydraulic conductance were carried out. Furthermore, air-layered seedlings were also introduced to cater for this problem, with regard to the potential genotypic complications that may arise from grafting. No significant effect of grafted union on stem hydraulic conductivity was found in either species. The hydraulic resistance differences between stem and scion were about 12.5 to 35.2% in *A. pseudoplatanus* and 1.44 to 2.85% in *F. excelsior*. The substantial differences in hydraulic resistance of the graft union between these two species are probably due to the differences in their anatomical structures. Apparently, these findings suggest that there was no restriction to water flow or other substances in the grafted seedlings. Furthermore, no significant differences were found among two age classes of air-layered *A. pseudoplatanus* plants with respect to growth, gas exchange parameters, E_L and K_L. The results also showed a reduced growth of air-layered plants, due to the sink effects to belowground growth and fruit development. In general, the differences in A_{net}, G_s, E_L and K_L among air-layered plants were triggered by size, since they varied in diameter and height or even in physical look such as single stem or multiple branches. This argument has been supported by the results from linear regression analysis between those parameters and diameter or height.

Overall, our results once again support the hydraulic limitation hypothesis, which is, that the reduction of growth of trees with ageing is triggered by size limiting factors rather than controlled by meristematic age. Since air-layering technique has the potential to propagate branches of trees from different ages and lengths, future studies should involve various tree ages and branch lengths to provide a clearer picture in age- and size-related research.

APPENDIX

Plate 1 : Edinburgh City Council woodland at Cramond (photo: Jordi Martinez-Vilalta).

Plate 2 : Grafted unions were transplanted in trays (photo: Jordi Martinez-Vilalta)

Plate 3 : All the grafted trees were placed in a well-ventilated plastic roof greenhouse (photo: Jordi Martinez-Vilalta)

Plate 4 : Grafted sycamore three months after grafting.

Plate 5 : Grafted sycamore six months after grafting (Note: sycamore rootstock leaves have serious anthracnose fungus infection).

Plate 6 : Grafted ash three months after grafting.

Plate 7 : Grafted ash six months after grafting.

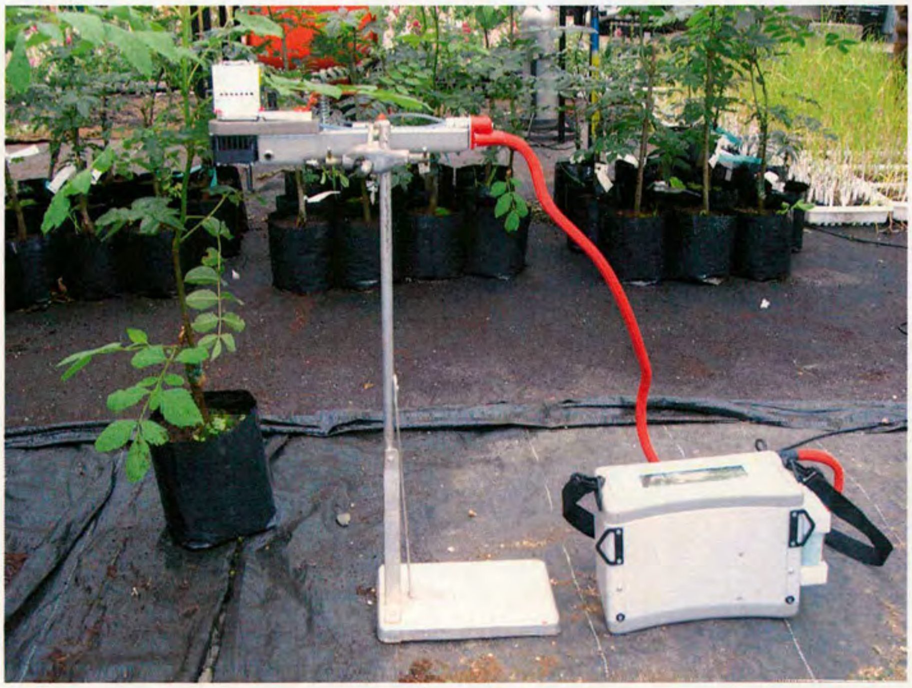

Plate 8 : LCPro portable photosynthesis system used in gas exchange measurements.

Plate 9 : Portable Plants Moisture System used for leaf water potential measurements.

Plate 10 : All grafted seedlings were left outside for hardening after first growing season.

Plate 11 : Grafted seedlings at the beginning of spring about 14 months after grafting.

Plate 12 : Grafted seedlings started to produce flowers in spring, especially from *F. excelsior*.

Plate 13 : Grafted seedlings from both species in second growing season.

Plate 14 : Grafted seedlings of *F. excelsior* in second growing season.

Plate 15 : Close up picture of *F. excelsior* pods.

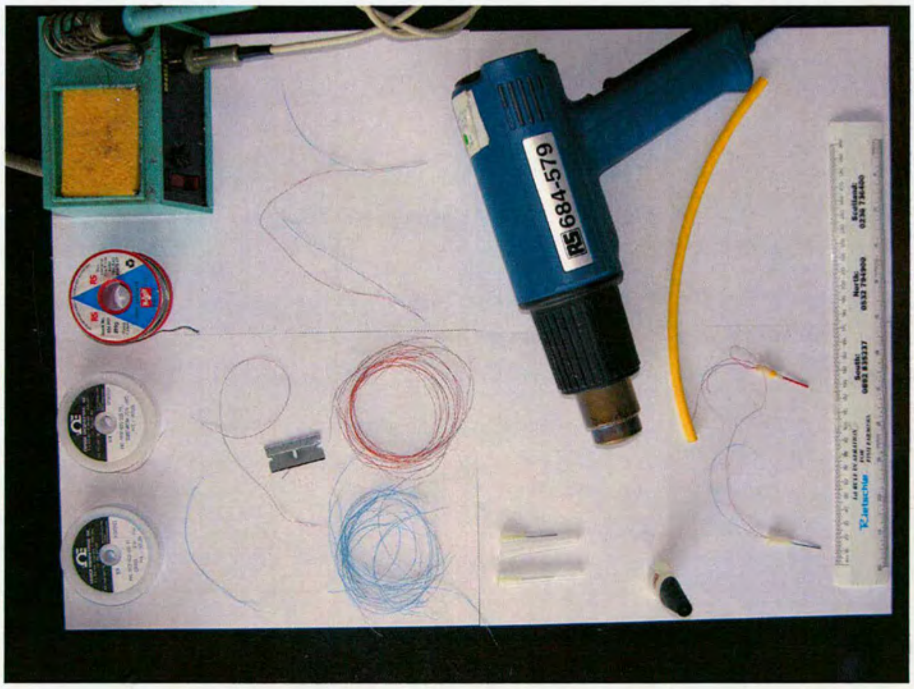

Plate 16 : Some of materials and equipments used in building Granier's sensors.

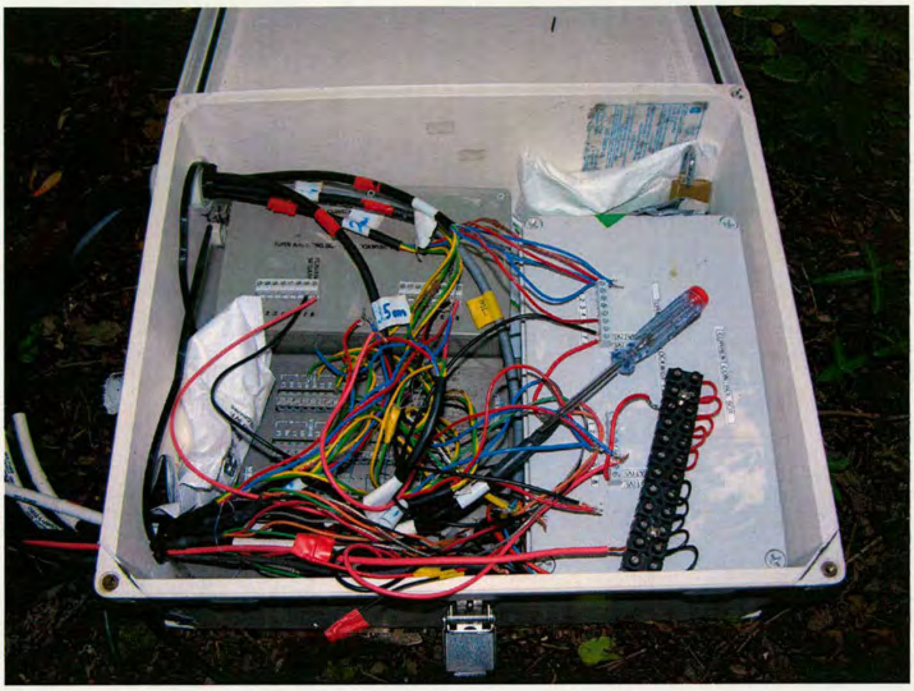

Plate 17 : Datalogger, multiplexer and heating box were kept in a secured box.

Plate 18 : Batteries to power the dataloggers and heating boxes were kept in this box to avoid exposure to rain etc.

Plate 19 : A climber was used to take branches from the top of big trees for gas exchange measurements.

Plate 20 : LCpro portable photosynthesis system, a bucket of water, tissues and black bags used in gas exchange measurements in the field.

Plate 21 : Checking the air-layered branches for any sign of rooting.

Plate 22 : Some of the air-layered branches are still intact on the donor trees.

Plate 23 : Sign of damage by birds.

Plate 24 : Serious damage on air-layered branch.

VITA

Hazandy Abdul Hamid was born as the third child in a family of five on 10th August 1973 in a small village located at the northern part of Malaysia called Changloon or 'Elephant fall'. He had his primary education at Dato' Wan Kemara National Primary School in the same town where he was born and secondary school at Jitra Secondary School, 20 km away from his town until 1991. In 1992, he applied for a tertiary education and was offered a Diploma in Forestry course in Putra University of Malaysia formerly known as Agriculture University of Malaysia at Serdang, located not far away from Kuala Lumpur, the capital city of Malaysia. After a year of study, he was offered a promotion to a Bachelor of Forestry Science and graduated from the course in 1997. Later in the same year, he was offered a scholarship to further his studies in Master Science in Forestry at the same university. In 2000, he was appointed as a tutor in the Department of Forest Production and got married in the same year. After two years of teaching in tree physiology and plantation silviculture subjects, he continued his study in PhD at the University of Edinburgh, United Kingdom.