

Age- and size-related changes in physiological characteristics and chemical composition of *Acer pseudoplatanus* and *Fraxinus excelsior* trees

HAZANDY ABDUL-HAMID^{1,2} and MAURIZIO MENCUCCINI³

¹ Department of Forest Production, Faculty of Forestry, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

² Corresponding author (hazandy@putra.upm.edu.my)

³ School of GeoSciences, University of Edinburgh, Edinburgh EH9 3JN, UK

Received March 22, 2008; accepted August 24, 2008; published online December 3, 2008

Summary Forest growth is an important factor both economically and ecologically, and it follows a predictable trend with age. Generally, growth accelerates as canopies develop in young forests and declines substantially soon after maximum leaf area is attained. The causes of this decline are multiple and may be linked to age- or size-related processes, or both. Our objective was to determine the relative effects of tree age and tree size on the physiological attributes of two broadleaf species. As age and size are normally coupled during growth, an approach based on grafting techniques to separate the effects of size from those of age was adopted. Genetically identical grafted seedlings were produced from scions taken from trees of four age classes, ranging from 4 to 162 years. We found that leaf-level net photosynthetic rate per unit of leaf mass and some other leaf structural and biochemical characteristics had decreased substantially with increasing size of the donor trees in the field, whereas other gas exchange parameters expressed on a leaf area basis did not. In contrast, these parameters remained almost constant in grafted seedlings, i.e., scions taken from donor trees with different meristematic ages show no age-related trend after they were grafted onto young rootstocks. In general, the results suggested that size-related limitations triggered the declines in photosynthate production and tree growth, whereas less evidence was found to support a role of meristematic age.

Keywords: age-related properties, ash, gas exchange, grafting, sycamore, water relations.

Introduction

About 90% of a plant's dry mass originates from the products fixed in photosynthesis (Poorter et al. 1990) and this value reaches 100% in trees. It is therefore not surprising that photosynthesis has been the subject of many studies examining the basis of variation in tree growth. Although

photosynthesis is responsible for tree growth, the links between these two processes are still not well understood. Growth declines when photosynthesis is impaired; however, there appears to be a wide range of photosynthetic rates observed for any given rate of growth, and there may be situations where growth appears to control photosynthesis. This dynamic relationship between photosynthesis and growth is vital to studies of the so-called age-related decline in forest productivity. As trees get older or grow taller, photosynthetic rates appear to decrease, leading to the hypothesis of hydraulic limitations to growth (Ryan and Yoder 1997). However, the reverse could also be happening, with reductions in the inherent sink strength of growing organs driving the decline in leaf-level photosynthetic rates. This opposite view has led to the maturation hypothesis (Wareing 1959, Day et al. 2002) that invokes the occurrence of stable changes in plant meristems to explain the age-related declines in growth potential.

Grafting techniques can help disentangle these alternative hypotheses, as they enable experimental separation of the effects of size from those of age, by producing vegetatively propagated plants that are of similar size, whereas their aboveground tissues maintain the putative ages of the apical meristems of the original donor trees. Studies of tree aging based on grafting techniques have shown that physiological traits such as net photosynthetic rate and stomatal conductance can decline with tree age, irrespective of differences in tree size (Rebbeck et al. 1993, Day et al. 2001). However, the evidence for growth reductions being directly driven by age is more controversial. Height and diameter growth with branch numbers have been found to decrease with increasing age of scions in Douglas-fir (Ritchie and Keeley 1994), eastern larch (Greenwood et al. 1989) and radiata pine (Sweet 1973). However, a recent reanalysis of the published literature (Mencuccini et al. 2007) showed that earlier conclusions were affected by the choice of the growth metrics employed (absolute versus relative growth rates) and that age-mediated controls of tree growth were only likely to be important during the first few years of a

tree's life (i.e., well before sexual competence). Hence, these grafting studies primarily demonstrated that phase change or maturation during the first few years of a tree's life results in changes in the growth habit of the apical meristem that persist when the mature meristem is reexposed to physiological conditions associated with a young immature plant; i.e., the juvenile rootstock (Hackett 1985, Greenwood 1995). In contrast, net photosynthetic rates of *Hedera helix* L. (Bauer and Bauer 1980) and *Larix laricina* (Du Roi) K. Koch (Hutchison et al. 1990) have been found to increase with increasing scion age. Therefore, for these species, the maturation hypothesis seems unlikely.

Several studies examining size-related changes have shown that photosynthetic rates can be reduced in tall trees, because of the limitation of hydraulic transport (Mencuccini and Grace 1996a). It has been suggested that a size-related reduction in leaf-specific hydraulic conductance is the main mechanism constraining stomatal conductance of tall trees, and this consequently underlies the reductions in photosynthetic rates and primary productivity (Yoder et al. 1994, Ryan and Yoder 1997). Leaf-specific hydraulic conductance may decrease with tree size as a result of the greater path length from soil to stomata, causing reductions in stomatal conductance and photosynthetic rate that directly affect tree growth. In addition, height directly affects the hydrostatic component of the water potential, possibly reducing leaf turgor and growth (e.g., Woodruff et al. 2004). A recent reanalysis of the published literature, however, failed to support the hypothesis that the observed reductions in leaf-level photosynthetic rates are sufficient to explain the growth reductions (Ryan et al. 2006).

Considering the obvious differences in growth trends of field trees of different sizes (Mencuccini et al. 2005), one would expect strong differences in carbon assimilation as well. Do these differences in age- or size-related growth imply similar differences in net carbon gain? To answer this question, physiological characteristics and chemical compositions at the leaf level were measured in the field and compared with those of grafted scion seedlings obtained from the same donor trees. The aim was to link the reduction

in tree growth during aging with changes in leaf-level physiological properties.

Materials and methods

Field site and mature tree sampling

The grafting material was taken at Cramond, Almond Valley, west of Edinburgh (55°58'42" N and 3°16'09" W). The mixed woodland contains several naturally regenerated age classes of sycamore (*Acer pseudoplatanus* L.) and ash (*Fraxinus excelsior* L.), together with other species. The woodland is located at a fertile site on deep alluvial soil in a sheltered location at sea level. The selected trees varied in age from 3 to 162 years and were clearly grouped into four sizes and age classes (Table 1, see Mencuccini et al. 2005, 2007 for further details). Ten trees were selected in the youngest class of both species, and five trees were selected in each of the second, third and fourth age classes.

Field gas exchange, water potential and leaf properties

Gas exchange was measured in the field in summer 2004 with an LCpro Portable Photosynthesis System (ADC, Inc., Lincoln, UK) equipped with a standard 2.5 × 2.5 cm broadleaf cuvette. Four sampling dates were chosen (days of year (DOY) 175, 176, 177 and 178) and 8–10 trees from each age class of both species were selected randomly on each day. The measurements were made between 1030 and 1430 h GMT with an ambient irradiance ranging between 1100 and 2800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (data from Edinburgh Gogarbank Meteorological Station) and an ambient temperature of around 22–26 °C (except DOY 175 when the day was overcast).

Before the gas exchange measurements, calibrations for flow meter and CO₂ zero values were made. To avoid the effects of fluctuating environmental conditions, cuvette irradiance was set at 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (saturating irradiance) for both species based on trial measurements where photosynthetic rate saturated at > 1000 μmol

Table 1. Characteristics of the *A. pseudoplatanus* and *F. excelsior* donor trees used in this study. Mean attributes are shown for each age class in each species. Symbols and abbreviations: \pm denotes the mean standard error; an asterisk (*) indicates that ages were estimated from bud scars on stem surface; and DBH is diameter at breast height.

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

photons $\text{m}^{-2} \text{s}^{-1}$ provided by the external light unit using a red/blue LED array. Cuvette CO_2 concentration (C_a), temperature and relative humidity were set at 360 ppm CO_2 , 25 °C and 40%, respectively. Branches of 2–4 m long were cut with a pole pruner from the top third of the donor tree crowns by a climber. Within 3 min of branch excision, 4–6 attached leaves were measured directly. Trials on excised branches from smaller trees conducted before making the actual measurements showed no deleterious effects on photosynthetic rates for the first 5–8 min after branch excision.

The measured leaves were then excised from the branch, placed in a black bag with wet tissues to minimize evaporation and transported to the laboratory where leaf water potential (Ψ_{leaf}) was measured with a portable Plant Moisture System (Skye Instruments Ltd, Powys, UK) pressure chamber with N_2 . Leaf areas of the samples were measured with an LI-3100 leaf area meter (Li-Cor, Lincoln, NE), and specific leaf area (SLA) was calculated after the leaves were oven-dried at 60 °C for about 48 h. The same samples were used to determine leaf nitrogen concentration on a mass basis (N_m) and leaf $\delta^{13}\text{C}$ isotope composition. These samples were oven-dried at 60 °C, ground in a freezer mill with liquid nitrogen and sent to the Cornell University stable isotope laboratory (Ithaca, NY) for analysis. The $\delta^{13}\text{C}$ isotopes were measured with a Finnigan MAT Delta Plus mass spectrometer interfaced to a Carlo Erba NC2500 elemental analyzer. The $\delta^{13}\text{C}$ values were measured against the PDB standard.

Grafting technique

Scions originating as terminal branch shoots with relatively uniform sizes (6–8 cm diameter) were collected from the top third of the canopies of the selected trees during the last two weeks of February 2003. Two hundred seedlings (rootstocks) of each species were used for grafting (10 scions \times 5 trees \times 4 age classes (AC1–AC4)) and 50 seedlings were used as controls (25 ungrafted rootstocks and 25 self-grafted seedlings, i.e., juvenile twigs grafted onto different,

but still juvenile, individuals). All the grafted seedlings and rootstocks were grown in 3-l polyethylene bags containing a 2:1:1 v/v sphagnum peat:sand:vermiculite mix, and supplied with slow-release fertilizer. The potted trees were placed in open-top glass frames in the nursery at the University of Edinburgh. Survival was about 80% for ash, but much lower for sycamore (about 40%), particularly in age class 3 (AC3). In early 2004, all of the grafted seedlings were transferred to 10-l pots. Further details are described by Mencuccini et al. (2005, 2007), who reported other data for these same plants.

Grafted seedling gas exchange, Ψ_{leaf} , N_m , SLA and $\delta^{13}\text{C}$

Gas exchange was compared among the four age classes (AC1–AC4) of the grafted trees together with two controls (i.e., self-grafted seedlings (SG) and rootstock material (RS)) in the first and the second growing seasons after grafting. In both growing seasons, 10 trees from each age class of the two species were randomly selected for gas exchange measurements. This included all seven surviving trees in AC3 of *A. pseudoplatanus*. The main characteristics of the grafted seedlings used in this study are presented in Table 2. The selected seedlings were placed in a greenhouse before gas exchange measurements. Two sampling periods, i.e., June and August, were selected in both growing seasons on the basis of seasonal changes in foliar attributes. As the number of trees was high, the measurements were staggered over different days, by making sure that trees from all age classes were equally sampled during each day. The gas exchange measurements were made on fully expanded leaves from the uppermost part of each selected grafted scion. A LCpro Portable Photosynthesis System (ADC, Lincoln, UK) was used, and the cuvette climate was set as described for the field gas exchange campaigns. Three sequential measurements were made within 1–4 min, and mean values were used for analyses. After sampling, the leaves used for gas exchange were severed and their Ψ_{leaf} was measured. In addition, predawn Ψ_{leaf} measurements

Table 2. Characteristics of *A. pseudoplatanus* and *F. 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. Symbols and abbreviations: \pm denotes the mean standard error; and SLA is specific leaf area.

Class	2003			2004		
	Diameter (cm)	Height (cm)	SLA ($\text{cm}^2 \text{g}^{-1}$)	Diameter (cm)	Height (cm)	SLA ($\text{cm}^2 \text{g}^{-1}$)
<i>A. pseudoplatanus</i>						
1	0.94 \pm 0.06	90.2 \pm 9.6	108.3 \pm 7.3	1.55 \pm 0.05	175.9 \pm 12.3	178.9 \pm 6.9
2	0.95 \pm 0.06	102.0 \pm 8.7	107.0 \pm 4.7	1.45 \pm 0.05	162.2 \pm 6.4	169.4 \pm 4.7
3	0.81 \pm 0.07	66.1 \pm 12.4	119.8 \pm 15.0	1.38 \pm 0.07	140.6 \pm 8.8	147.0 \pm 10.9
4	0.79 \pm 0.07	61.5 \pm 9.6	110.5 \pm 7.3	1.32 \pm 0.05	153.7 \pm 6.5	139.7 \pm 6.4
<i>F. excelsior</i>						
1	1.03 \pm 0.07	65.9 \pm 7.6	148.5 \pm 4.2	1.49 \pm 0.07	148.3 \pm 8.6	183.7 \pm 9.8
2	0.84 \pm 0.04	29.6 \pm 2.3	126.1 \pm 3.2	1.31 \pm 0.05	106.9 \pm 7.8	159.8 \pm 6.3
3	0.75 \pm 0.03	26.6 \pm 3.3	123.5 \pm 3.8	1.19 \pm 0.04	98.4 \pm 13.2	140.2 \pm 7.9
4	0.93 \pm 0.04	27.1 \pm 3.0	120.8 \pm 4.8	1.18 \pm 0.04	94.5 \pm 10.5	145.1 \pm 6.3

were made on all selected seedlings between 0230 and 0530 h. The leaf area was also measured on the leaves taken for Ψ_{leaf} measurements.

Leaves of six of the 10 grafted seedlings in each age class used for gas exchange and Ψ_{leaf} measurements were randomly selected to determine leaf N_m and $\delta^{13}\text{C}$. The procedures for N_m and $\delta^{13}\text{C}$ analyses were similar to those applied to the donor tree leaves.

Photosynthetic efficiency and capacity of grafted seedlings

Photosynthetic efficiency was assessed by determining the response of net photosynthetic rate (A_{net}) of grafted seedlings to increasing photosynthetic active radiation (Q). The A_{net}/Q response curves were generated by maintaining cuvette CO_2 concentration (C_a) at $360 \mu\text{mol mol}^{-1}$ at a constant temperature under high and stable humidity conditions while increasing Q from 530 to 760, 950 and $1190 \mu\text{mol m}^{-2} \text{s}^{-1}$ until complete light saturation was reached and then decreasing Q in five steps, i.e., 330, 150, 100, 50 and $0 \mu\text{mol m}^{-2} \text{s}^{-1}$. The response of A_{net} to irradiance (Q) was modeled with a non-rectangular hyperbola where the initial slope was the apparent quantum efficiency (Φ), the light compensation point and apparent respiration were estimated from axis intercepts, and the light-saturated maximum photosynthetic rate (A_{max}) was the upper asymptote. An additional parameter k (convexity) was required to describe the progressive rate of bending between the linear gradient and maximum value. All these parameters were determined by fitting data to the model function expressed as a quadratic equation by Prioul and Chartier (1977).

Photosynthetic capacity was assessed by determining the response of A_{net} in grafted seedlings to increasing intercellular CO_2 concentration (C_i). The A_{net}/C_i response curve was generated by measuring A and intercellular CO_2 (C_i) at a series of CO_2 concentrations (C_a) (cf. Ainsworth et al. 2002). The protocol used was to measure A and C_i as C_a was decreased from ambient C_a in steps to 300, 250, 200, 150, 100 and $50 \mu\text{mol mol}^{-1}$ and then increased to 370, 450, 550, 650, 800 and $1000 \mu\text{mol mol}^{-1}$ (Long and Bernacchi 2003). Maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) were calculated from the A_{net}/C_i curves by nonlinear least squares regression to give the best fit to the equations of Farquhar et al. (1980) and Farquhar and von Caemmerer (1982) photosynthesis model (see also, Harley et al. 1992). Photosyn Assistant Software package (Dundee Scientific, Dundee, UK) was used for calculations and data interpretation for both A_{net}/C_i and A_{net}/Q curves.

Data analyses

Data obtained from multiple leaves and times were averaged for each individual tree before statistical analysis. If necessary, data transformations were applied to stabilize error variance. The data were then analyzed by one-way analysis of variance (ANOVA) and the general linear model

for balanced and unbalanced designs among age classes. The mean values obtained were compared among age classes by Duncan multiple range test (DMRT). All statistical analyses were performed with Statistical Analysis System Version 9.0 (SAS Institute, Cary, NC), and the significance level was set at 0.05. Linear regression analyses were carried out with Sigma Plot Version 9.0 (Systat Software, Richmond, CA). Differences among the slopes of the regression equations were tested by analysis of covariance (ANCOVA) in SPSS Version 12.0 (SPSS, Chicago, IL).

Results

Leaf-level gas exchange, Ψ_{leaf} , SLA, N_m and $\delta^{13}\text{C}$ of the donor trees

Analysis of variance showed significant effects of age class on physiological parameters such as mass-based A_{net} , C_i (*A. pseudoplatanus* only), Ψ_{leaf} (*A. pseudoplatanus* only), and leaf characteristics such as SLA and $\delta^{13}\text{C}$ (Table 3). Values of mass-based A_{net} differed significantly between age classes in *A. pseudoplatanus* ($P < 0.01$) and in *F. excelsior* ($P < 0.05$, Table 3, Figure 1A); however, area-based A_{net} , that ranged from 7.19 to $8.76 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *A. pseudoplatanus* and from 6.48 to $9.51 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *F. excelsior*, did not differ significantly across age classes. There was a significant difference ($P < 0.01$) in C_i between age classes in *A. pseudoplatanus* but not in *F. excelsior* (Table 3). Leaf C_i was higher in the youngest class in *A. pseudoplatanus*, followed by AC4, AC2 and AC3. Similar trends, albeit insignificant, were observed in *F. excelsior* (Figure 1B). Stomatal conductance (G_s) did not differ significantly between age classes in either species (Table 3). There were highly significant differences in Ψ_{leaf} between

Table 3. Summary of analysis of variance (ANOVA) of leaf-level gas exchange, Ψ_{leaf} and leaf characters in *A. pseudoplatanus* and *F. excelsior* donor trees. Significance values: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$ and ns, not significant.

Parameters	<i>A. pseudoplatanus</i>	<i>F. excelsior</i>
	F value	F value
Net assimilation rate, A_{net} ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	5.20**	2.57*
Internal CO_2 , C_i ($\mu\text{mol mol}^{-1}$)	5.67**	1.66 ns
Stomatal conductance, G_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	0.85 ns	0.34 ns
Leaf water potential, Ψ_{leaf} (–MPa)	9.84***	0.02 ns
Specific leaf area (SLA) ($\text{cm}^2 \text{g}^{-1}$)	8.96***	8.71**
Nitrogen concentration, N_m (% mass)	0.99 ns	2.91 ns
Carbon isotope discrimination, $\delta^{13}\text{C}$	7.79**	11.25***

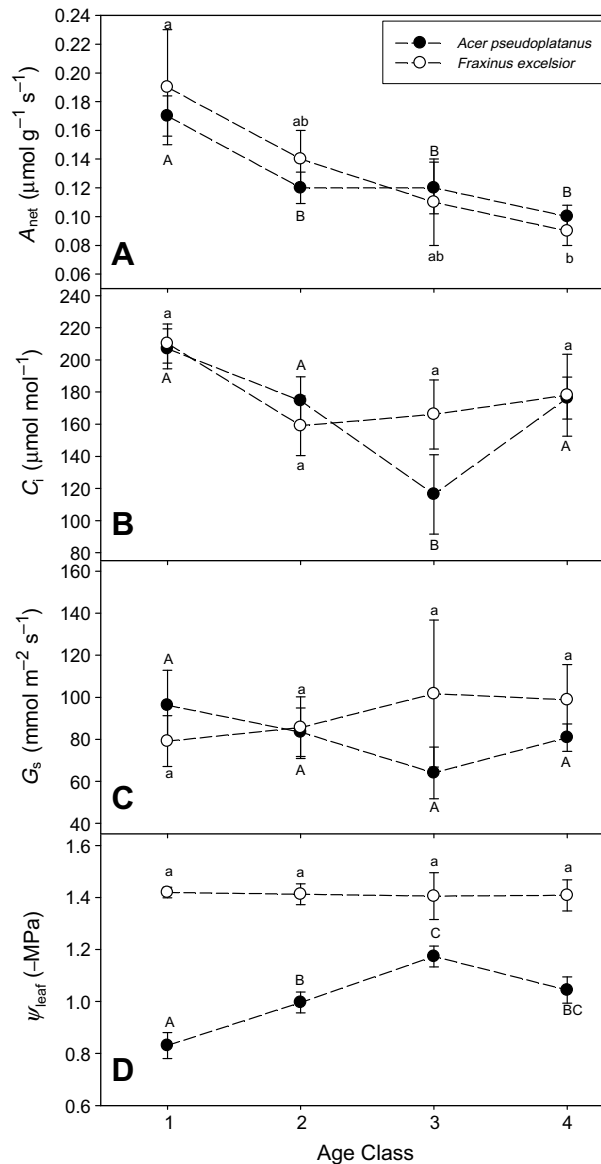


Figure 1. Mean values of net photosynthetic rate A_{net} (A), internal CO_2 concentration C_i (B), stomatal conductance G_s (C), and leaf water potential Ψ_{leaf} (D) for *A. pseudoplatanus* and *F. excelsior*. The different letters represent statistically significant differences across age classes. Black circles refer to *A. pseudoplatanus* and white circles to *F. excelsior*. Age classes 1–4 are defined in Table 1.

age classes in *A. pseudoplatanus* ($P < 0.001$), but not in *F. excelsior* (Table 3). For *A. pseudoplatanus*, the highest Ψ_{leaf} value was in AC1 followed by AC2, AC4 and AC3 (Figure 1D). The trends in Ψ_{leaf} across age classes paralleled those in G_s in both species. Specific leaf area differed significantly (at least $P < 0.01$, Table 3) between age classes in both species (Figure 2A), with large reductions in SLA between AC1 and AC4 (a factor of about 3 and 2 for *A. pseudoplatanus* and *F. excelsior*, respectively). Although no significant differences were detected in N_m between age classes in either species (Table 3), an age-related trend

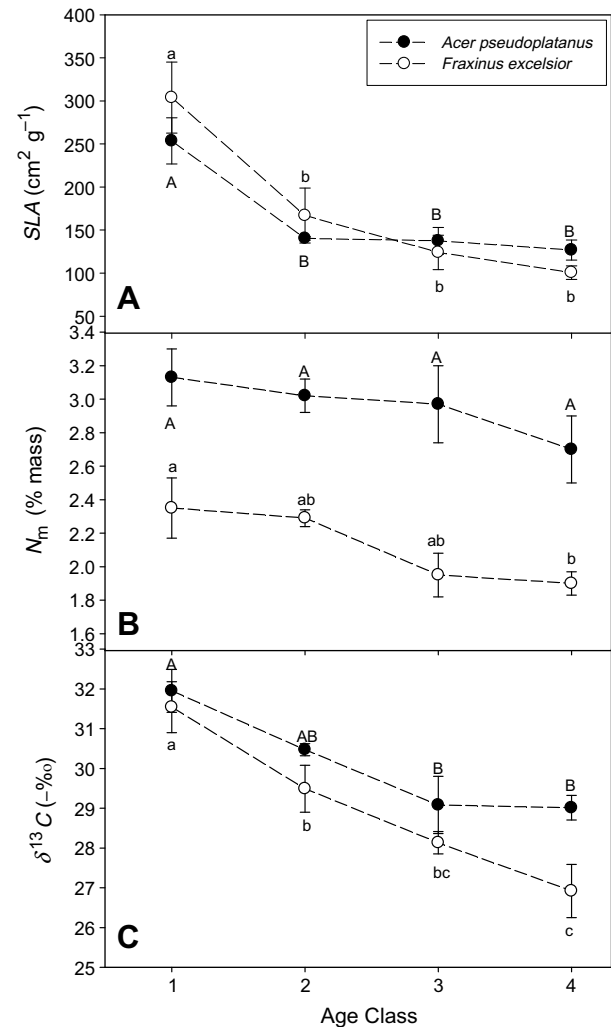


Figure 2. Mean values of specific leaf area SLA (A), nitrogen concentration N_m (B) and carbon isotope composition $\delta^{13}C$ (C) for *A. pseudoplatanus* and *F. excelsior*. The different letters represent statistically significant differences across age classes. Black circles refer to *A. pseudoplatanus* and white circles to *F. excelsior*.

was revealed by DMRT which showed different groupings for *F. excelsior* (Figure 2B). Leaf $\delta^{13}C$ differed significantly between age classes in *A. pseudoplatanus* ($P < 0.01$) and *F. excelsior* (at least $P < 0.01$, Table 3), with strong age-related patterns in both species (Figure 2C).

Leaf-level gas exchange, Ψ_{leaf} , SLA, N_m and $\delta^{13}C$ of the grafted seedlings

Leaf-level gas exchange, Ψ_{leaf} , and leaf structural and biochemical characters were determined on grafted seedlings from the four classes of scion age (AC1–AC4) and two controls (i.e., RS and SG) in the first and the second growing seasons after grafting. Mass-based A_{net} differed significantly between plant groups for both *A. pseudoplatanus* and *F. excelsior*, and for both growing seasons (Table 4). In the first growing season, a highly significant difference

Table 4. Summary of analysis of variance (ANOVA) of leaf-level gas exchanges, Ψ_{leaf} and leaf characters in *A. pseudoplatanus* and *F. excelsior* grafted seedlings. Significance values: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$ and ns, not significant.

Parameters	<i>A. pseudoplatanus</i>		<i>F. excelsior</i>	
	F value		F value	
	2003	2004	2003	2004
Net assimilation rate, A_{net} ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	5.18***	2.85*	21.21***	4.64***
Internal CO_2 , $\ln C_i$ ($\mu\text{mol mol}^{-1}$)	2.13 ns	0.52 ns	4.62**	0.83 ns
Stomatal conductance, $\ln G_s$ ($\text{mmol m}^{-2} \text{s}^{-1}$)	3.58**	14.7***	5.49***	9.11***
Leaf water potential, Ψ_{leaf} (MPa)	0.73 ns	2.89*	3.31*	17.18***
Specific leaf area (SLA) ($\text{cm}^2 \text{g}^{-1}$)	–	5.47***	–	24.21***
Nitrogen concentration, N_m (% mass)	4.47**	0.95 ns	4.37**	2.27 ns
Carbon isotope discrimination, $\delta^{13}\text{C}$	8.41***	2.71*	9.35***	17.37***

Table 5. Summary of leaf level gas exchange parameters, Ψ_{leaf} , and leaf characters of grafted seedlings and controls in *A. pseudoplatanus* and *F. excelsior*. Symbol: \pm denotes the mean standard error. Different letters indicate significant differences between age classes within species.

Class	A_{net} ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	$\ln C_i$ ($\mu\text{mol mol}^{-1}$)	$\ln G_s$ ($\text{mmol m}^{-2} \text{s}^{-1}$)	Ψ_{leaf} (MPa)	N_m (%)	$\delta^{13}\text{C}$
<i>A. pseudoplatanus</i> – 2003						
1	0.087 \pm 0.008 a	2.460 \pm 0.006 a	2.440 \pm 0.048 a	–0.396 \pm 0.04 a	2.30 \pm 0.13 c	–29.55 \pm 0.48 b
2	0.099 \pm 0.006 a	2.398 \pm 0.012 a	2.344 \pm 0.041 ab	–0.369 \pm 0.03 a	2.64 \pm 0.04 bc	–28.91 \pm 0.30 b
3	0.098 \pm 0.012 a	2.380 \pm 0.017 ab	2.159 \pm 0.058 b	–0.408 \pm 0.02 a	3.09 \pm 0.06 a	–29.54 \pm 0.19 b
4	0.091 \pm 0.008 a	2.408 \pm 0.013 a	2.232 \pm 0.052 b	–0.391 \pm 0.03 a	2.55 \pm 0.18 bc	–28.97 \pm 0.48 b
SG	0.095 \pm 0.009 a	2.399 \pm 0.006 a	2.200 \pm 0.047 b	–0.396 \pm 0.03 a	2.49 \pm 0.15 bc	–27.73 \pm 0.38 a
RS	0.052 \pm 0.005 b	2.214 \pm 0.135 b	1.769 \pm 0.101 c	–0.450 \pm 0.03 a	2.89 \pm 0.17 ab	–26.92 \pm 0.25 a
<i>A. pseudoplatanus</i> – 2004						
1	0.223 \pm 0.022 a	2.254 \pm 0.024 a	2.162 \pm 0.061 ab	–0.598 \pm 0.03 b	2.32 \pm 0.26 a	–29.78 \pm 0.53 b
2	0.150 \pm 0.018 bc	2.256 \pm 0.034 a	1.935 \pm 0.078 c	–0.580 \pm 0.02 b	2.42 \pm 0.04 a	–28.98 \pm 0.47 ab
3	0.123 \pm 0.018 c	2.301 \pm 0.016 a	2.010 \pm 0.069 bc	–0.478 \pm 0.02 a	2.35 \pm 0.17 a	–28.42 \pm 0.61 ab
4	0.185 \pm 0.010 ab	2.265 \pm 0.016 a	2.235 \pm 0.029 a	–0.465 \pm 0.03 a	2.10 \pm 0.18 a	–28.83 \pm 0.72 ab
SG	0.186 \pm 0.019 ab	2.263 \pm 0.015 a	2.047 \pm 0.064 bc	–0.530 \pm 0.04 ab	2.49 \pm 0.13 a	–27.83 \pm 0.31 a
RS	0.191 \pm 0.025 ab	2.258 \pm 0.015 a	2.007 \pm 0.049 bc	–0.501 \pm 0.04 ab	2.58 \pm 0.15 a	–27.42 \pm 0.33 a
<i>F. excelsior</i> – 2003						
1	0.289 \pm 0.012 b	2.358 \pm 0.009 ab	2.693 \pm 0.05 ab	–0.346 \pm 0.02 ab	2.87 \pm 0.09 c	–27.64 \pm 0.32 b
2	0.179 \pm 0.013 c	2.350 \pm 0.008 b	2.439 \pm 0.069 c	–0.374 \pm 0.02 a	3.35 \pm 0.06 a	–26.93 \pm 0.06 a
3	0.219 \pm 0.008 c	2.375 \pm 0.008 a	2.683 \pm 0.048 ab	–0.297 \pm 0.02 bc	3.21 \pm 0.08 ab	–28.14 \pm 0.17 b
4	0.204 \pm 0.008 c	2.351 \pm 0.005 b	2.584 \pm 0.048 b	–0.355 \pm 0.03 ab	3.30 \pm 0.05 a	–27.64 \pm 0.14 b
SG	0.374 \pm 0.032 a	2.350 \pm 0.006 b	2.786 \pm 0.040 a	–0.261 \pm 0.02 c	3.28 \pm 0.12 a	–27.82 \pm 0.06 b
RS	0.377 \pm 0.026 a	2.329 \pm 0.004 c	2.621 \pm 0.039 b	–0.306 \pm 0.03 abc	2.98 \pm 0.12 bc	–26.84 \pm 0.08 a
<i>F. excelsior</i> – 2004						
1	0.159 \pm 0.014 c	2.112 \pm 0.064 a	1.886 \pm 0.072 cd	–0.706 \pm 0.03 b	2.27 \pm 0.04 a	–26.36 \pm 0.40 a
2	0.191 \pm 0.008 abc	2.187 \pm 0.042 a	2.119 \pm 0.057 ab	–0.624 \pm 0.02 c	2.61 \pm 0.12 a	–27.59 \pm 0.16 b
3	0.189 \pm 0.003 bc	2.194 \pm 0.037 a	2.197 \pm 0.032 a	–0.565 \pm 0.02 c	2.61 \pm 0.16 a	–29.02 \pm 0.17 c
4	0.196 \pm 0.007 ab	2.226 \pm 0.021 a	2.205 \pm 0.025 a	–0.581 \pm 0.03 c	2.24 \pm 0.05 a	–28.34 \pm 0.32 c
SG	0.221 \pm 0.014 a	2.129 \pm 0.060 a	1.986 \pm 0.063 bc	–0.705 \pm 0.01 b	2.25 \pm 0.14 a	–26.85 \pm 0.11 a
RS	0.167 \pm 0.010 bc	2.146 \pm 0.041 a	1.825 \pm 0.051 d	–0.868 \pm 0.03 a	2.21 \pm 0.17 a	–26.97 \pm 0.13 ab

($P < 0.001$) in mass-based A_{net} in *A. pseudoplatanus* between plant groups was a result of low values in RS, whereas during the second growing season mass-based A_{net} varied among the age classes of the grafted seedlings but with no clear age-related trends (i.e., AC4 had significantly higher values than AC3, but did not differ from either AC1 or AC2) (Table 5). In *F. excelsior*, mass-based A_{net} was highest in RS followed by SG and AC1 during the first

growing season. During the second growing season, however, SG plants had the highest values, and differences across age classes actually showed an opposite age-related trend, with highest values in AC4 (Table 5). Values of C_i did not differ significantly among scion ages and controls in *A. pseudoplatanus* in either season (Table 4), whereas C_i differed only in the RS plants in the first growing season for *F. excelsior* (Table 5). In both growing seasons, G_s

values largely followed the trends for A_{net} (Table 4). In *A. pseudoplatanus*, there was evidence of an age-related decline in 2003 but it disappeared in 2004. In *F. excelsior*, the differences across age classes did not show any age-related trend (2003) or showed an opposite trend (2004) (Table 5). Although Ψ_{leaf} did not differ significantly between age classes in the first growing season in *A. pseudoplatanus* (Table 4), Ψ_{leaf} values became less negative in the older age classes in both species during the second growing season (Table 5). During 2003, SLA differed between age classes only for *F. excelsior*, but the differences became significant in both species in 2004 (Tables 2 and 4). In *A. pseudoplatanus*, SLA declined by about 22% from AC1 to AC4, compared with a 24% decline from AC1 to AC4 in *F. excelsior*. This decline represented only a fraction of the observed reductions in the donor trees, where SLA declined by about 50 and 67%, respectively, between AC1 and AC4. Scion ages and controls differed significantly in N_{m} in both species during the first growing season but no significant effect was found in either species during the second growing season (Tables 4 and 5). Leaf $\delta^{13}\text{C}$ varied across plant groups during both growing seasons (Table 4), although the values for the four age classes of grafted seedlings either remained constant during both years (*A. pseudoplatanus*) or showed more negative values in the older age classes in both years (*F. excelsior*) (Table 4).

Photosynthetic capacity and efficiency

Data on photosynthetic capacity and efficiency obtained over two growing seasons are summarized in Figure 3. Mean values of apparent quantum efficiency (Φ) observed in 2003 and 2004 were not significantly different among scion ages and controls for either species, and no age-related trend was observed in either season for either species.

Mean values of A_{max} did not differ among scion ages and controls in either the first or the second growing season in *A. pseudoplatanus*, although the absolute values were higher in the second season than in the first growing season. In *F. excelsior*, A_{max} showed an age-related trend during the first season, but values were similar across the age classes during the second growing season.

Maximum carboxylation rate (V_{cmax}) was the highest in AC1 in *A. pseudoplatanus* and AC2 in *F. excelsior* during the first growing season. In the second growing season, however, only the SG showed higher values of V_{cmax} in *A. pseudoplatanus*, whereas V_{cmax} showed an inverse age-related trend in *F. excelsior*. A similar behavior was seen for J_{max} in both species and for both growing seasons. Overall, these results showed that there was no age-related declining trend in V_{cmax} in the grafted seedlings.

Relationships between physiological parameters and leaf properties in donor trees and grafted seedlings

As age-related declining in SLA were observed for both donor trees and grafted seedlings, SLA was regressed

against A_{net} and N_{m} for the donor trees, and A_{net} , A_{max} , V_{cmax} and N_{m} for the grafted seedlings. Similar analyses were also carried out between A_{net} and N_{m} in both donor trees and grafted seedlings for the two species.

Figure 4 shows the relationships between SLA and mass-based A_{net} 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. Strong correlations ($P < 0.001$) were also found between SLA and A_{net} in *F. excelsior* in the donor trees and the grafted seedlings in the first growing season, but no significant correlation was observed in the grafted seedlings in the second growing season. When SLA was held constant, the ANCOVA revealed significant differences ($P < 0.001$) in mass-based A_{net} between donor trees and grafted seedlings of both species, with higher values in the seedlings.

Nitrogen concentrations were positively correlated with A_{net} in donor trees of both species (Figure 5). For the grafted seedlings, however, a significant positive correlation was only found in *A. pseudoplatanus* in the second growing season. In 2004, higher A_{net} values were found in grafted seedlings than in donor trees of *A. pseudoplatanus*, whereas the evidence was more ambiguous for *F. excelsior* because of the lack of a relationship between A_{net} and N_{m} .

Discussion

Generally, our gas exchange results did not reflect the large declines in growth rates observed in the donor trees by Mencuccini et al. (2005), confirming that the measurements of leaf-level photosynthetic rates do not readily allow for conclusions to be reached about tree growth (e.g., Küppers and Küppers 2003). Although area-based A_{net} did not show age-related trends, these values were within the range recorded by Morecroft and Roberts (1999) for *A. pseudoplatanus* in an unmanaged old woodland and by Hölscher (2004) for *F. excelsior* in an old-growth forest of broad-leaved deciduous tree species. In contrast, we did observe age-related declines in mass-based A_{net} in both species (Figure 1A). Similarly, Day et al. (2001) had found trends of mass-based A_{net} in red spruce, but they had also found that other parameters such as C_i and G_s were reduced in older trees compared with younger trees. Lower G_s of large trees compared with small trees would be consistent with the hydraulic limitation hypothesis and the effect of the gravitational hydrostatic gradient (Ryan and Yoder 1997, Ryan et al. 1997, Bond and Ryan 2000, Woodruff et al. 2004); however, in our study, C_i , G_s and area-based A_{net} did not show any significant age-related trends in either *A. pseudoplatanus* or *F. excelsior* (Figure 1). Our study site was a mixed uneven-aged species woodland, with individuals of all ages coexisting in the same plot. Attention was paid to selection of individuals to avoid suppressed trees and direct

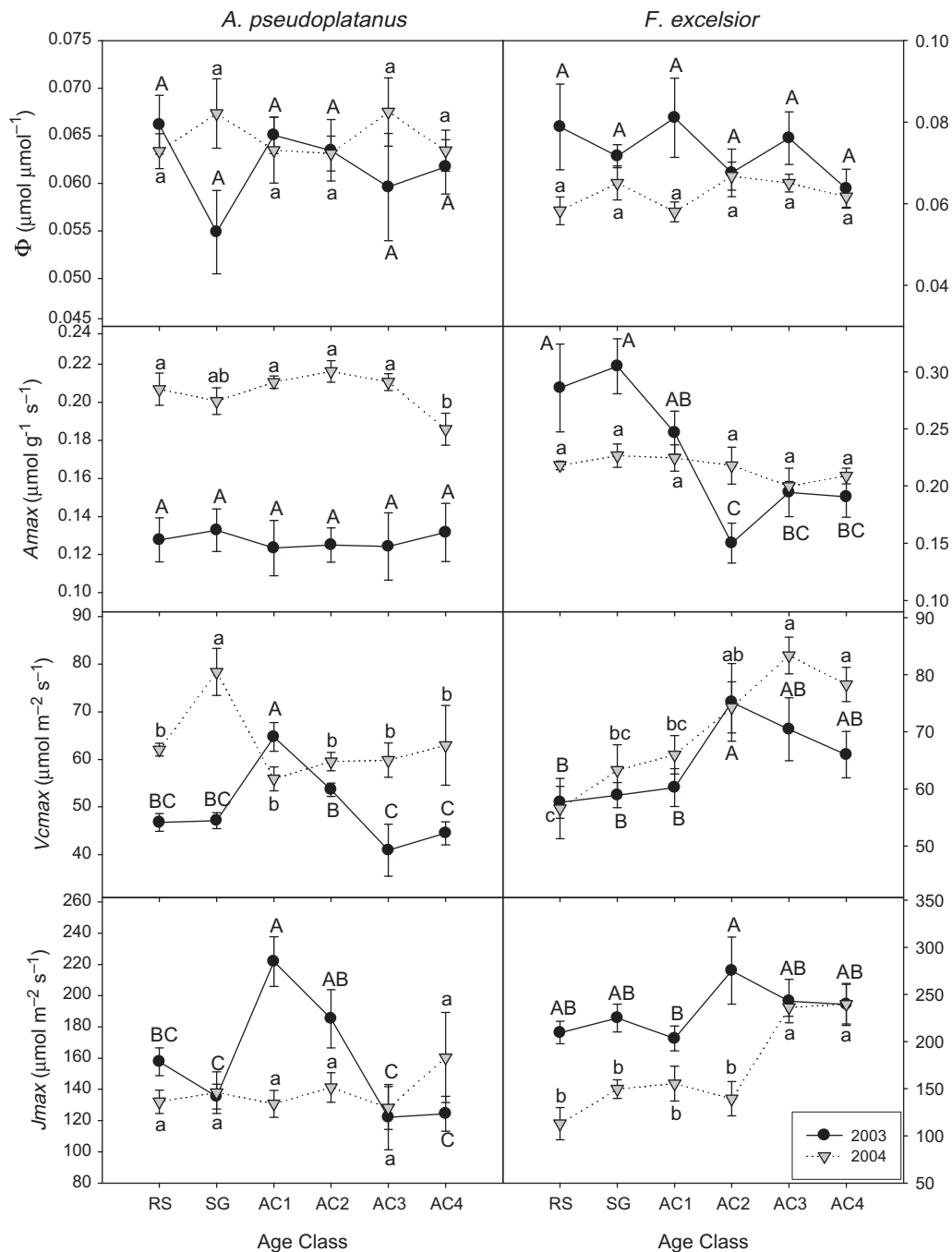


Figure 3. Mean values of various parameters of photosynthetic efficiency and capacity for *A. pseudoplatanus* and *F. excelsior* over two growing seasons. The different letters represent statistically significant differences across age classes (AC1–AC4) and control groups (RS, rootstock; SG, self-grafted). Black symbols refer to measurements taken in the first growing season (2003), gray symbols to those taken during the second growing season (2004). Age classes 1–4 are defined in Table 1.

vertical shading; but a degree of lateral shading from the emergent oldest trees was unavoidable. As partially shaded seedlings and saplings often have lower G_s than unshaded dominant trees (Köstner et al. 1992, Fredericksen et al. 1995, Martin et al. 1997, Samuelson and Kelly 1997), the results may be partially affected. However, it is unlikely that shading can explain all the differences we observed across

age classes, because we measured A_{net} and G_s in saturating light, and mass-based A_{net} of the small saplings was almost twice as that of the large dominant trees. Other potentially confounding factors may be that (1) the gas exchange measurements were not carried out at the same time on all trees because of difficulties accessing the trees and (2) the Ψ_{leaf} values at the time of measurements showed a relationship

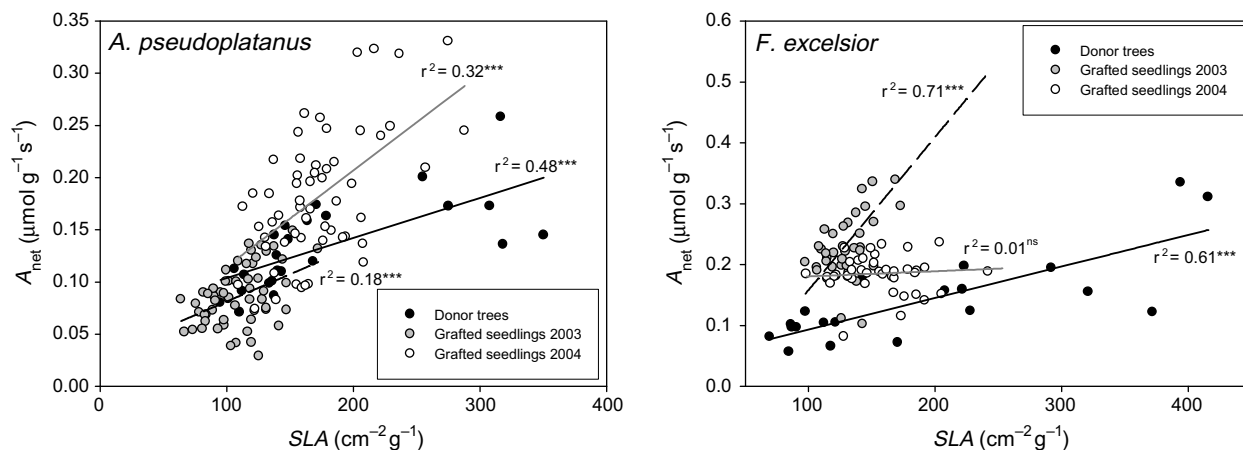


Figure 4. Relationship between leaf-level net photosynthetic rate A_{net} and specific leaf area SLA across age classes in *A. pseudoplatanus* and *F. excelsior* donor trees and grafted seedlings. Black circles refer to donor trees, gray circles to grafted plants measured in 2003, and white circles to grafted plants measured in 2004.

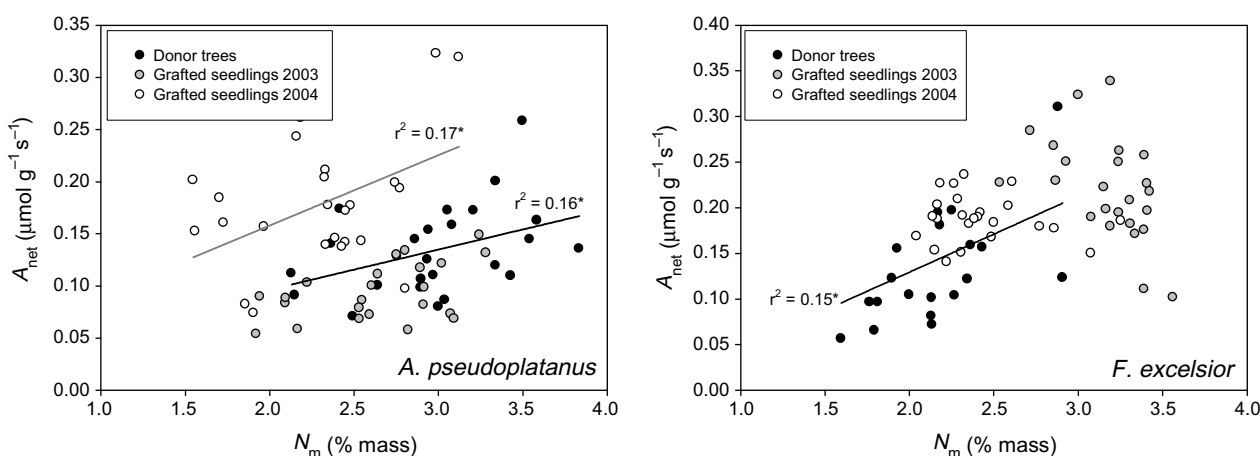


Figure 5. Relationship between leaf-level nitrogen concentration N_m and mass-based net photosynthetic rate A_{net} in *A. pseudoplatanus* and *F. excelsior* donor trees and grafted seedlings. Black circles refer to donor trees, gray circles refer to grafted plants measured in 2003, and white circles to grafted plants measured in 2004.

with G_s in both species as indicated in Figure 1, suggesting that stomatal closure played an important role in determining G_s at the time of sampling. It is well known that stomatal closure is associated with reduced soil to leaf hydraulic conductance (Kolb and Stone 2000) and differences in Ψ_{leaf} (Hubbard et al. 1999).

Because of the difficulties associated with measuring gas exchange, leaf characters such as N_m , SLA and $\delta^{13}\text{C}$ may be more useful for assessing age-related trends. Leaf nitrogen concentration has generally been shown to be a good predictor of A_{net} and G_s (Field and Mooney 1986, Reich et al. 1994, Samuelson and Kelly 1997). Leaf N_m tended to decrease with increasing age of the trees in both species, although the DMRT showed significant groupings only for *F. excelsior* (Figure 2B). In addition, significant but weak relationships were found between N_m and mass-based A_{net}

for the donor trees (Figure 5). Kull and Koppel (1987) observed declines in leaf nitrogen and photosynthetic capacity as trees aged. Furthermore, Schoettle (1994) found that leaf nitrogen concentrations were lower in old *Pinus aristata* trees than in young trees. In contrast, other studies have shown no changes in nitrogen concentration or photosynthetic capacity with tree age (e.g., Schoettle 1994, Mencuccini and Grace 1996b, Hubbard et al. 1999, McDowell et al. 2002, Barnard and Ryan 2003). We observed marked declines in SLA in the field, with values for AC4 being 33 to 50% of those of AC1 (Figure 2). In addition, much stronger relationships were found between mass-based A_{net} and SLA for the donor trees than between mass-based A_{net} and N_m (Figure 4). Taken together, these results suggest that the lower A_{net} in the taller trees resulted from declines in SLA, possibly as a result of the difference in the

hydrostatic potential gradient between the 3-m-tall and the 23–25-m-tall trees in AC1 and AC4, respectively, as also seen in much taller trees (e.g., Marshall and Monserud 2003, Woodruff et al. 2004).

Leaf $\delta^{13}\text{C}$ can provide an independent test of the hypothesis that G_s declines with increasing height, because area-based A_{net} did not change and mass-based A_{net} declined with tree size (McDowell et al. 2002). Leaf $\delta^{13}\text{C}$ showed age-related trends in both species in the field with the values becoming less negative with increasing tree age (Figure 2C), suggesting increasing stomatal closure in old trees. Hence, the trends in $\delta^{13}\text{C}$ differed from the trends in measured G_s . McDowell et al. (2002) also found different trends between cuvette-based G_s and G_s inferred from $\delta^{13}\text{C}$ of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*), and suggested that hydraulic limitations to gas exchange occur during spring but not during summer drought, a factor unlikely to play a role in our case. It is possible that our protocol of cutting 2–4-m-long branches before measuring G_s temporarily rehydrated the leaves by tension release or by the use of capacitance water from cavitation, or by both (e.g., Lo Gullo and Salleo 1992). Bauerle et al. (1999) concluded that cuvette-based G_s was a poor measure compared with $\delta^{13}\text{C}$ data because of the limited temporal integration of gas exchange measurements. Based on the studies cited, we believe that our $\delta^{13}\text{C}$ results provide evidence that G_s (or an internal conductance; Greenwood et al. 2008) likely decreased with increasing age or size of the donor trees.

Generally, none of the parameters (i.e., A_{net} , C_i , Ψ_{midday} , G_s , N_m or $\delta^{13}\text{C}$) monitored in the grafted plants during two growing seasons showed clear trends that were interpretable in terms of an age-related decline. For some variables (e.g., G_s for sycamore, A_{net} for ash), significant declines from the youngest to the oldest age class were detected during Year 1; however, these trends (as well as other non-age-related differences among classes) either entirely disappeared during Year 2 or were sometimes reversed, particularly for ash. In this species, significant age-related increases during Year 2 were detected in A_{net} , G_s , Ψ_{midday} and $\delta^{13}\text{C}$. The parameters derived from the A/Q and the A/C_i response curves showed a similar behavior, with differences in Year 1 disappearing or changing direction in Year 2 for ash. Therefore, we conclude from these data that there is no clear dependency of gas exchange properties on tree age in either species. If the age-related declines observed in the field for both species were directly controlled by age (as opposed to size or environment), then the same differences should persist in the grafted seedlings and should not disappear or change direction. A second conclusion from this dataset is that one year of gas exchange data is insufficient to characterize the response of these species to grafting. It would appear that both species underwent an acclimation period in the first year after grafting. Every parameter we measured showed much clearer and more consistent patterns during Year 2 compared with Year 1, leading us to conclude that the differences among age classes in physiological characteristics and leaf

characteristics during Year 1 were strongly influenced by grafting shock. This conclusion is supported by the growth patterns during both years reported for the same plants by Mencuccini et al. (2007).

The SLA changed across age classes of the grafted seedlings, during both growing seasons, suggesting a direct age-mediated control for this characteristic; however, longer monitoring periods are required to test this hypothesis. Beside the previous observations that many other physiological characters appeared to undergo a period of recovery following the grafting shock, we found that only a minor fraction of the differences seen across age classes in the field were retained in the differences in SLA across the age classes of the grafted seedlings in 2004. The regression analyses conducted on some physiological parameters such as A_{net} , A_{max} , V_{cmax} and leaf N_m against SLA of the grafted seedlings or the donor trees showed that SLA correlated well with A_{net} in the first and in the second growing season for both species (with the exception of the *F. excelsior* grafted seedlings in the second growing season, Figure 4) and the ANCOVA showed that, at constant SLA, light-saturated A_{net} was higher in the grafted seedlings than in the donor trees.

Our results supported the general correlations between SLA, A_{net} and N_m for six biomes and different plant life forms reported by Reich et al. (1999). Mass-based A_{net} was positively correlated with SLA and N_m (Poorter et al. 1990, Reich et al. 1994), and these patterns appear to be common to many species (Reich et al. 1999). 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 in which trees in younger classes tended to have higher relative growth rates compared to older trees (Mencuccini et al. 2005).

The gas exchange results only partially supported the observed size-related changes in growth parameters (Mencuccini et al. 2005), but they clearly disproved the hypothesis that the changes were caused by age-related changes, in agreement with several recent papers (Matsuzaki et al. 2005, Mencuccini et al. 2005, 2007, Bond et al. 2007). In the field, clearly declining trends were observed for mass-based A_{net} , SLA and $\delta^{13}\text{C}$ for both species. Overall, our results showed that the changes in A_{net} and leaf characteristics were primarily triggered by size and not age. Further investigations have to be carried out, especially involving stomatal response and hydraulic conductance.

Acknowledgments

The authors thank Jordi Martinez-Vilalta, Dirk Vanderklein, Johanna Pulli, Evi Korakaki, Chris Kettle, Rosa Maria Roman Cuesta, Manuel e. Lucas Borja, Pedro Polo and Jamie Gardiner for field and laboratory assistance, and Bob Astles and Bill Adams for looking after the plants. This research was funded by NERC (UK) Competitive Grant NER/A/S/2001/01193 to M.M. H.A.-H. was supported by a studentship from the Malaysian Government. The authors also thank the Edinburgh City Council for the permission to work on their land.

References

- Ainsworth, E.A., P.A. Davey, C.J. Bernacchi et al. 2002. A meta-analysis of elevated [CO₂] effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biol.* 8:695–709.
- Barnard, H.R. and M.G. Ryan. 2003. A test of the hydraulic limitation hypothesis in fast-growing *Eucalyptus saligna*. *Plant Cell Environ.* 26:1235–1245.
- Bauer, H. and U. Bauer. 1980. Photosynthesis in leaves of the juvenile and adult phase of ivy (*Hedera helix*). *Physiol. Plant.* 49:366–372.
- Bauerle, W.L., T.M. Hinckley, J. Cermak and J. Kucera. 1999. The canopy water relations of old growth Douglas-fir trees. *Trees* 13:211–217.
- Bond, B.J. and M.G. Ryan. 2000. Comment on 'Hydraulic limitation of tree height: a critique' by Becker, Meinzer and Wullschlegel. *Funct. Ecol.* 14:135–140.
- Bond, B.J., N.M. Czarnomski, C. Cooper, M.E. Day and M.S. Greenwood. 2007. Developmental decline in height growth in Douglas fir. *Tree Physiol.* 27:441–453.
- Day, M.E., M.S. Greenwood and A.S. White. 2001. Age-related changes in foliar morphology and physiology in red spruce and their influence on declining photosynthetic rates and productivity with tree age. *Tree Physiol.* 21:1195–1204.
- Day, M.E., M.S. Greenwood and C. Diaz-Sala. 2002. Age- and size-related trends in woody plant shoot development: regulatory pathways and evidence for genetic control. *Tree Physiol.* 22:507–513.
- Farquhar, G.D. and S. von Caemmerer. 1982. Modelling of photosynthetic response to environmental conditions. In *Encyclopedia of Plant Physiology, New Series*. Eds. O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler. *Physiol. Plant Ecol.* 12:549–587.
- Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149:78–90.
- Field, C. and H.A. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. In *On the Economy of Plant Form and Function*. Ed. T.J. Givnish. Cambridge University Press, Cambridge, England, 255 p.
- Fredericksen, T.S., B.J. Joyce, J.M. Skelly, K.C. Steiner, T.E. Kolb, K.B. Kouterick, J.E. Savage and K.R. Snyder. 1995. Physiology, morphology, and ozone uptake of black cherry seedlings, saplings, and canopy trees. *Environ. Pollut.* 89:273–283.
- Greenwood, M.S. 1995. Juvenility and maturation in conifers: current concepts. *Tree Physiol.* 15:433–438.
- Greenwood, M.S., C.A. Hooper and K.W. Hutchison. 1989. Maturation in larch. I. Effect of age on shoot growth, foliar characteristics, and DNA methylation. *Plant Physiol.* 90:406–412.
- Greenwood, M.S., M.H. Ward, M.E. Day, S.L. Adams and B.J. Bond. 2008. Age-related trends in red spruce foliar plasticity in relation to declining productivity. *Tree Physiol.* 28:225–232.
- Hackett, W.P. 1985. Juvenility, maturation, and rejuvenation in woody plants. *Hortic. Rev.* 7:109–155.
- Harley, P.C., R.B. Thomas, J.F. Reynolds and B.R. Strain. 1992. Modelling photosynthesis of cotton grown in elevated CO₂. *Plant Cell Environ.* 15:271–282.
- Hölscher, D. 2004. Leaf traits and photosynthetic parameters of saplings and adult trees of co-existing species in a temperate broad-leaved forest. *Basic Appl. Ecol.* 5:163–172.
- Hubbard, R.M., B.J. Bond and M.G. Ryan. 1999. Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. *Tree Physiol.* 19:165–172.
- Hunt, R. and J.H.C. Cornelissen. 1997. Components of relative growth rate and their interrelations in 59 temperate plant species. *New Phytol.* 135:395–417.
- Hutchison, K.W., C.D. Sherman, J. Weber, S.S. Smith, P.B. Singer and M.S. Greenwood. 1990. Maturation in larch. II. Effects of age on photosynthesis and gene expression in developing foliage. *Plant Physiol.* 94:1308–1315.
- Kolb, T.E. and J.E. Stone. 2000. Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine-oak forest. *Tree Physiol.* 20:1–12.
- Köstner, B.M.M., E.D. Schulze, F.M. Kelliher, D.I. Hollinger, J.N. Byers, J.E. Hunt, T.M. McSeveny, R. Meserth and P.I. Weir. 1992. Transpiration and canopy conductance in a pristine broad leaved forest of *Nothofagus*: an analysis of xylem sap flow and eddy correlation measurements. *Oecologia* 91:350–359.
- Kull, O. and A. Koppel. 1987. Net photosynthetic response to light intensity of shoots from different crown positions and age in *Picea abies* (L.) Karst. *Scand. J. For. Res.* 2:157–166.
- Küppers, B.I.L. and M. Küppers. 2003. Diverse responses of photosynthesis and tree growth in competing eucalypts result in niche separation. *Lyonia* 4(2),151–156.
- Lo Gullo, M.A. and S. Salleo. 1992. Water storage in the wood and xylem cavitation in 1-year-old twigs of *Populus deltoides* Bartr. *Plant Cell Environ.* 15:431–438.
- Long, S.P. and C.J. Bernacchi. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J. Exp. Bot.* 54:2393–2401.
- Marshall, J.D. and R.A. Monserud. 2003. Foliage height influences specific leaf area of three conifer species. *Can. J. For. Res.* 33:164–170.
- Martin, T.A., K.J. Brown, J. Čermák, R. Ceulemans, J. Kucera, F.C. Meinzer, J.S. Rombold, D.G. Sprugel and T.M. Hinckley. 1997. Crown conductance and tree and stand transpiration in a second growth *Abies amabilis* forest. *Can. J. For. Res.* 27:797–808.
- Matsuzaki, J., M. Norisada, J. Kodaira, M. Suzuki and T. Tange. 2005. Shoots grafted into the upper crowns of tall Japanese cedar (*Cryptomeria japonica* D. Don) show foliar gas exchange characteristics similar to those of intact shoots. *Trees* 19:198–203.
- McDowell, N.G., N. Phillips, C. Lunch, B.J. Bond and M.G. Ryan. 2002. An investigation of hydraulic limitation and compensation in large, old Douglas-fir trees. *Tree Physiol.* 22:763–774.
- Mencuccini, M. and J. Grace. 1996a. Developmental patterns of aboveground hydraulic conductance in a Scots pine (*Pinus sylvestris* L.) age sequence. *Plant Cell Environ.* 19:939–948.
- Mencuccini, M. and J. Grace. 1996b. Hydraulic conductance, light interception and needle nutrient concentration in Scots pine stands and their relation with net primary productivity. *Tree Physiol.* 16:459–468.
- Mencuccini, M., J. Martínez-Vilalta, D. Vanderklein, H.A. Hamid, E. Korakaki, S. Lee and B. Michiels. 2005. Size-mediated ageing reduces vigour in tall trees. *Ecol. Lett.* 8:1183–1190.
- Mencuccini, M., J. Martínez-Vilalta, H.A. Hamid, E. Korakaki and D. Vanderklein. 2007. Evidence for age- and size-mediated controls of tree growth from grafting studies. *Tree Physiol.* 27:463–473.

- Morecroft, M.D. and J.M. Roberts. 1999. Photosynthesis and stomatal conductance of mature canopy oak (*Quercus robur*) and sycamore (*Acer pseudoplatanus*) trees throughout the growing season. *Funct. Ecol.* 13:332–342.
- Poorter, H., C. Remkes and H. Lambers. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiol.* 94:621–627.
- Prioul, J.L. and P. Chartier. 1977. Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO₂ fixation: a critical analysis of the methods used. *Ann. Bot.* 41:789–800.
- Rebbeck, J., K.F. Jensen and M.S. Greenwood. 1993. Ozone effects on grafted mature and juvenile red spruce: photosynthesis, stomatal conductance, and chlorophyll concentration. *Can. J. For. Res.* 23:450–456.
- Reich, P.B., M.B. Walters, D.S. Ellsworth and C. Uhl. 1994. Photosynthesis-nitrogen relations in Amazonian tree species. I. Patterns among species and communities. *Oecologia* 97:62–72.
- Reich, P.B., D.S. Ellsworth, M.B. Walters, J.M. Vose, C. Gresham, J.C. Volin and W.D. Bowman. 1999. Generality of leaf trait relationships: a test across six biomes. *Ecology* 80:1955–1969.
- Ritchie, G.A. and J.W. Keeley. 1994. Maturation in Douglas-fir: I. Changes in stem, branch and foliage characteristics associated with ontogenetic aging. *Tree Physiol.* 14:1245–1259.
- Ryan, M.G. and B.J. Yoder. 1997. Hydraulic limits to tree height and tree growth. *Bioscience* 47:235–242.
- Ryan, M.G., D. Binkley and J.H. Fownes. 1997. Age-related decline in forest productivity: patterns and process. *Adv. Ecol. Res.* 27:213–256.
- Ryan, M.G., N. Phillips and B.J. Bond. 2006. The hydraulic limitation hypothesis revisited. *Plant Cell Environ.* 29:367–381.
- Samuelson, L.J. and J.M. Kelly. 1997. Ozone uptake in *Prunus serotina*, *Acer rubrum* and *Quercus rubra* forest trees of different sizes. *New Phytol.* 136:255–264.
- Schoettle, A.W. 1994. Influence of tree size on shoot structure and physiology of *Pinus contorta* and *Pinus aristata*. *Tree Physiol.* 14:1055–1068.
- Sweet, G.B. 1973. The effect of maturation on the growth and form of vegetative propagules of radiata pine. *N. Z. J. For. Sci.* 3:191–210.
- Yoder, B.J., M.G. Ryan, R.H. Waring, A.W. Schoettle and M.R. Kaufmann. 1994. Evidence of reduced photosynthetic rates in old trees. *For. Sci.* 40:513–527.
- Wareing, P.F. 1959. Problems of juvenility and flowering in trees. *J. Linn. Soc. Bot.* 56:282–289.
- Woodruff, D.R., B.J. Bond and F.C. Meinzer. 2004. Does turgor limit growth in tall trees? *Plant Cell Environ.* 27: 229–236.