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Age and Size Related Changes in Growth of *Acer pseudoplatanus* and *Fraxinus excelsior* Species

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Abstract: The aim of this study is to investigate the effects of tree age and tree size on growth characteristics of two broadleaf species by conducting experiments in the field and the glasshouse. Growth characteristics, such as relative growth rate and growth efficiency were measured. 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.

Key words: Sycamore, ash, grafting, age-related properties, growth rates

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 *et al*., 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. Most of the findings discussed before did not separate the possible effect of age from height or size (Niinemets, 2002). Hence, which factors, 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

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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).

In this study, 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, the relative growth rate and the growth efficiency. The total specific leaf area and the ratio between total leaf area and total sapwood area 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* (c.f., Mencuccini *et al*., 2005).

MATERIALS AND METHODS

Field Study

Sampling

A. pseudoplatanus and *F. excelsior* were selected in this study since they are the dominant species in Cramond woodland, Almond Valley, West of Edinburgh (55°58'42" N, 3°16'09" W) and are represented by various sizes 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 (c.f., Mencuccini *et al*., 2007 for further details). 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 (Table 1).

Table 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 1.3 m (year)	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

Values are shown in mean±standard error. * Indicated that ages were estimated from bud scars on stem surface

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 estimating from bud scars was measured in each tree from the branches taken at the top of the tree. About 5-6 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 h. Leaf weights were measured afterward using a balance and the Specific Leaf Area (SLA) was calculated by dividing leaf area with leaf weight.

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. The equations used are as given below:

Crown biomass (DBH<7 cm):

$$W_{crown} = 0.00005122 * DBH^{2.06704428} * HT_{total}^{0.7321854} \quad (1)$$

Crown biomass (DBH>7 cm):

$$W_{crown} = 0.00729453 + 0.00003081 * DBH^{3.67047187} * HT_{total}^{-1.44028024} \quad (2)$$

Total leaf mass:

$$W_{leaf} = 0.06391085 - 0.06391085 * (0.17108421^{W_{crown}}) \quad (3)$$

$$\text{Total leaf area} = W_{leaf} * SLA \quad (4)$$

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 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.

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:

$$LnW_{branches+trunk} = -5.570499 + 2.529411 * LnDBH \text{ for } \textit{Acer pseudoplatanus} \quad (5)$$

$$LnW_{branches+trunk} = -5.234459 + 2.480921 * LnDBH \text{ for } \textit{Fraxinus excelsior} \quad (6)$$

Total aboveground biomass:

$$W_{total} = W_{leaf} + W_{branches+trunk} \quad (7)$$

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:

$$AGR = \frac{W_{total2} - W_{total1}}{T_2 - T_1} \quad (8)$$

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:

$$RGR = LAR * NAR \quad (9)$$

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:

$$LAR = LWR * SLA \quad (10)$$

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 Eq. 10 into 9, a more detailed definition of RGR is as follow:

$$RGR = LWR * SLA * NAR \tag{11}$$

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 earlier, 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:

$$RGR_{mass} = \frac{LnW_{total2} - LnW_{total1}}{\Delta T} \tag{12}$$

(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 this 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.

Grafting Study

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

Table 2: No. 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%

sizes (6-8 cm) from trees representing four diameter classes (Table 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 and 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 un-grafted) 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-L 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 (150 cm×624 cm×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). 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-L polyethylene bags.

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 7 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.

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

$\mathrm{Ln}W_{stem+branches} = 4.13956+1.00067*\mathrm{Ln}Diameter$, n = 5, $R^2 = 0.998$ for Age Class 1
$\mathrm{Ln}W_{stem+branches} = 3.34276+2.45623*\mathrm{Ln}Diameter$, n = 5, $R^2 = 0.991$ for Age Class 2
$\mathrm{Ln}W_{stem+branches} = 3.93661+0.07494*\mathrm{Ln}Diameter$, n = 3, $R^2 = 0.997$ for Age Class 3
$\mathrm{Ln}W_{stem+branches} = 2.87451+5.62113*\mathrm{Ln}Diameter$, n = 4, $R^2 = 0.998$ for Age Class 4

F. excelsior

$\mathrm{Ln}W_{stem+branches} = 3.71985+0.98988*\mathrm{Ln}Diameter$, n = 5, $R^2 = 0.999$ for Age Class 1
$\mathrm{Ln}W_{stem+branches} = 3.72016+0.98867*\mathrm{Ln}Diameter$, n = 5, $R^2 = 0.998$ for Age Class 2
$\mathrm{Ln}W_{stem+branches} = 3.64250+1.14150*\mathrm{Ln}Diameter$, n = 5, $R^2 = 0.905$ for Age Class 3
$\mathrm{Ln}W_{stem+branches} = 3.69586+1.26225*\mathrm{Ln}Diameter$, n = 5, $R^2 = 0.913$ for Age Class 4

Total aboveground biomass for both species:

$$W_{total} = W_{leaf} + W_{stem+branches} \tag{13}$$

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.

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., USA) 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., USA).

RESULTS

Growth Characteristics of the Donor Trees

Analysis of variance (Table 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 (Fig. 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 (Fig. 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 (Fig. 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 3: Summary of one-way analysis of variance on growth parameters of *A. pseudoplatanus* and *F. excelsior* donor trees

Growth parameters	*A. pseudoplatanus* F-value	*F. excelsior* 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 $year^{-1}$)	20.13***	8.18**
RGR_{mass} (kg kg^{-1} $year^{-1}$)	52.19***	104.27***
E_G (kg m^{-2} $year^{-1}$)	46.16***	7.84**

***Significantly different at $p<0.001$; **Significantly different at $p<0.01$; *Significantly different at $p<0.05$

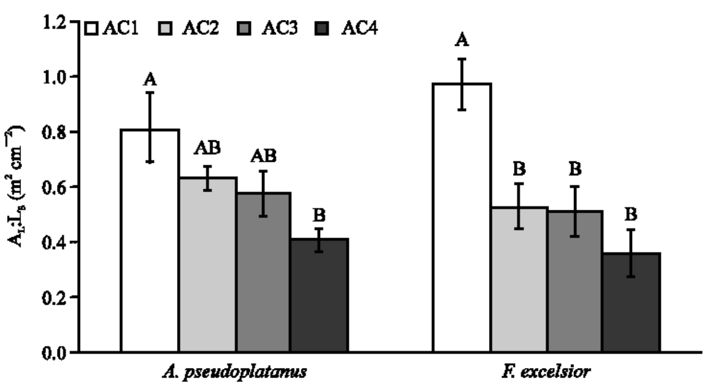

Fig. 1: Mean values of $A_L:A_S$ of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied

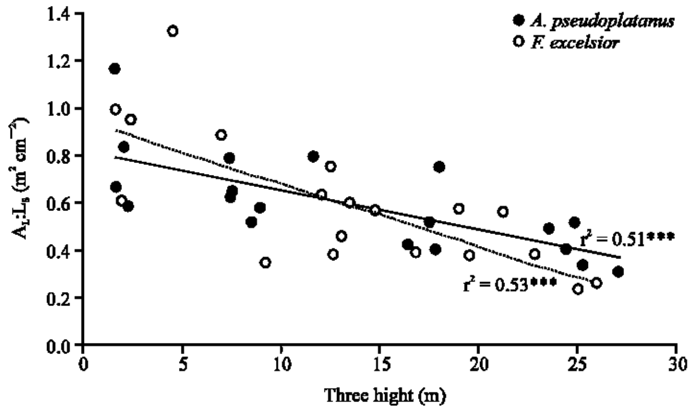

Fig. 2: Leaf area to sapwood area ratio (A_L:A_S) plotted against tree height for both species. The *** indicates p<0.001

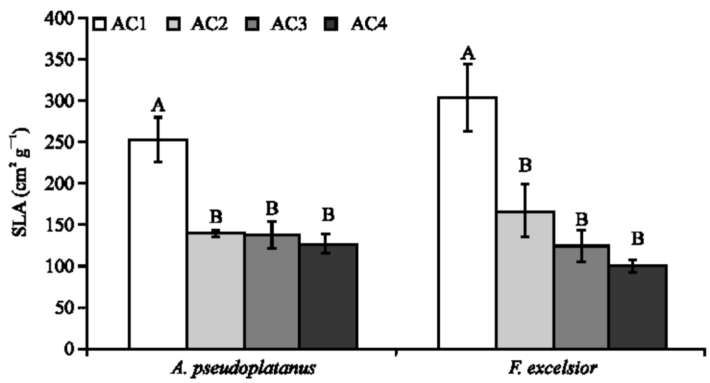

Fig. 3: Mean values of SLA of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences among age classes within species studied

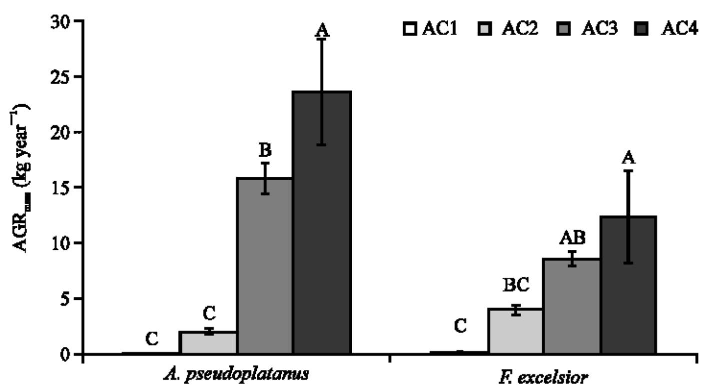

Fig. 4: Mean values of AGR_{mass} of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied

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 (Fig. 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 Fig. 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 (Fig. 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.

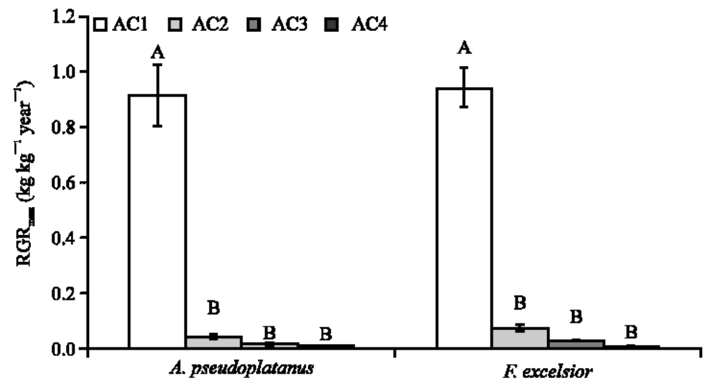

Fig. 5: Mean values of RGR_{mass} of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied

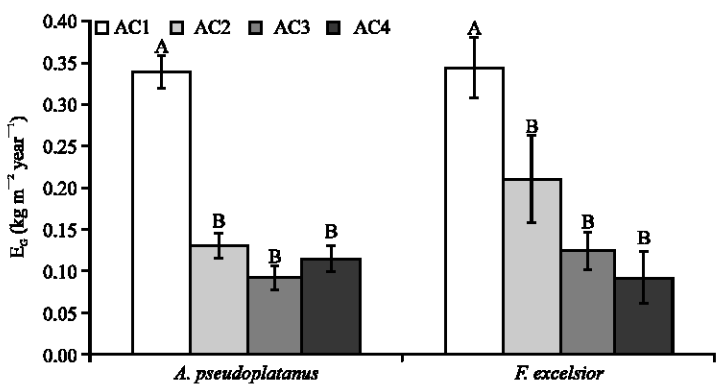

Fig. 6: Mean values of E_G of *A. pseudoplatanus* and *F. excelsior* across four age classes of donor trees. Different letters indicate significant differences between age classes within species studied

Growth Characteristics of the Grafted Seedlings

For study 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 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 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 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 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 year^{-1})	3.94**	8.89***
RGR_{mass} (kg kg^{-1} year^{-1})	1.67^{ns}	23.11***
E_G (kg m^{-2} year^{-1})	2.47*	17.51***
M_{Tot}	1.29^{ns}	7.26***
Rt:St (g g^{-1})	0.92^{ns}	1.60^{ns}

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

Table 5: Summary of growth characteristics of grafted seedling across age classes in both species after two growing seasons

Class	Scion age (Year)	A_L (m^2)	SLA ($m^2\ g^{-1}$)	L_N	AGR_{mass} (kg $year^{-1}$)
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}
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}
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}
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}
Self-grafted	3.0	0.49 ± 0.033^{A}	190.2 ± 14.40^{AB}	46.70 ± 3.74^{AB}	0.056 ± 0.003^{B}
Rootstock	3.0	0.56 ± 0.043^{A}	202.55 ± 14.40^{A}	50.90 ± 4.10^{A}	0.122 ± 0.032^{A}
F. excelsior					
1	5.0±0.4	0.48 ± 0.017^{C}	183.66 ± 9.82^{B}	27.40 ± 2.19^{BC}	0.056 ± 0.002^{A}
2	25.2±1.1	0.41 ± 0.031^{C}	159.84 ± 6.32^{C}	23.80 ± 2.79^{BCD}	0.054 ± 0.002^{AB}
3	40.4±0.9	0.24 ± 0.009^{D}	140.15 ± 7.95^{C}	21.50 ± 2.23^{CD}	0.046 ± 0.001^{C}
4	117.8±5.7	0.28 ± 0.041^{D}	145.07 ± 6.25^{C}	17.20 ± 1.50^{D}	0.040 ± 0.003^{D}
Self-grafted	3.0	0.56 ± 0.02^{B}	212.92 ± 4.65^{A}	32.90 ± 4.77^{B}	0.052 ± 0.002^{AB}
Rootstock	3.0	0.66 ± 0.03^{A}	219.35 ± 5.22^{A}	48.50 ± 4.73^{A}	0.049 ± 0.002^{BC}

Class	RGR_{mass} (kg kg^{-1} $year^{-1}$)	E_G (kg m^{-2} $year^{-1}$)	M_{tot} (g)	Rt:St (g g^{-1})
A. pseudoplatanus				
1	0.943 ± 0.111^{AB}	0.205 ± 0.028^{AB}	169.34 ± 14.97^{AB}	0.447 ± 0.030^{A}
2	0.663 ± 0.088^{B}	0.124 ± 0.018^{B}	146.18 ± 11.78^{AB}	0.467 ± 0.031^{A}
3	0.922 ± 0.201^{AB}	0.118 ± 0.018^{B}	113.53 ± 1.95^{B}	0.508 ± 0.027^{A}
4	1.158 ± 0.145^{A}	0.215 ± 0.027^{AB}	135.18 ± 8.21^{AB}	0.485 ± 0.038^{A}
Self-grafted	0.809 ± 0.049^{AB}	0.116 ± 0.011^{B}	150.08 ± 11.46^{AB}	0.462 ± 0.019^{A}
Rootstock	0.938 ± 0.164^{AB}	0.235 ± 0.066^{A}	225.04 ± 68.36^{A}	0.402 ± 0.054^{A}
F. excelsior				
1	1.047 ± 0.068^{B}	0.119 ± 0.006^{CD}	135.68 ± 6.53^{B}	0.567 ± 0.055^{AB}
2	1.157 ± 0.039^{AB}	0.138 ± 0.009^{BC}	126.24 ± 7.65^{BC}	0.590 ± 0.068^{AB}
3	1.190 ± 0.047^{A}	0.193 ± 0.007^{A}	110.30 ± 4.67^{C}	0.702 ± 0.081^{A}
4	0.911 ± 0.023^{C}	0.167 ± 0.022^{AB}	114.96 ± 9.43^{BC}	0.692 ± 0.063^{A}
Self-grafted	0.895 ± 0.044^{C}	0.094 ± 0.004^{DE}	133.94 ± 6.01^{B}	0.473 ± 0.044^{B}
Rootstock	0.629 ± 0.021^{D}	0.075 ± 0.002^{E}	165.68 ± 8.55^{A}	0.576 ± 0.086^{AB}

Values are show in mean±standard error, Different letters indicate significant differences between age classes within species

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 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 (Fig. 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 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 Fig. 9. However, when E_G was compared across scion ages in grafted seedlings, there was no age-related decline observed for either species.

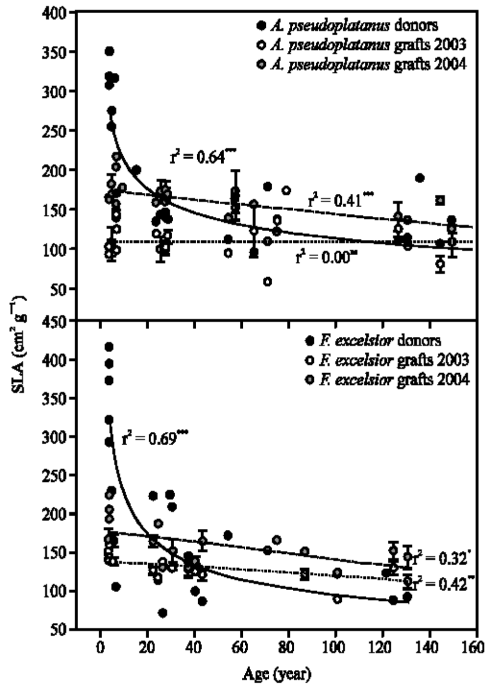

Fig. 7: Regression analyses between specific leaf area (SLA) and age of each individual donor tree and grafted scions. The bar indicates standard error, ns = not significant ($p>0.05$), * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$

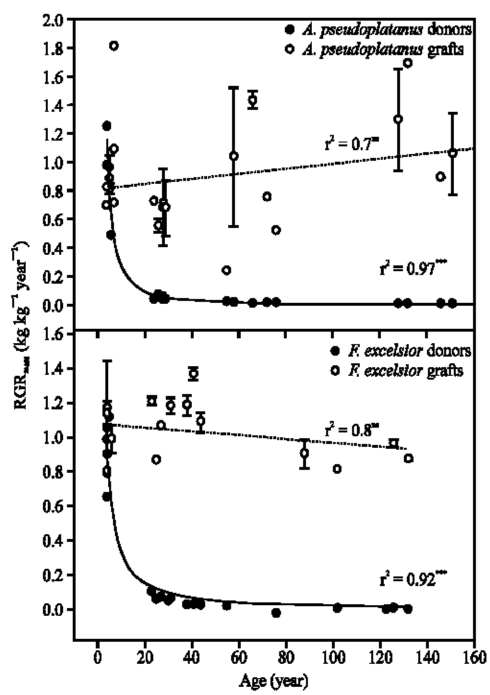

Fig. 8: Relationship between relative mass growth rate (RGR_{mass}) and age of each individual donor tree and grafted scions. The bar indicates standard error, ns = not significant and *** = $p<0.001$

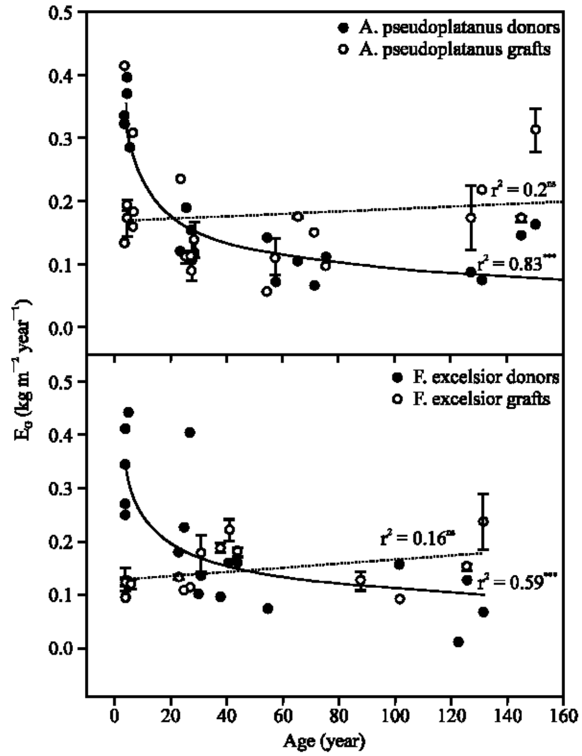

Fig. 9: Relationship between growth efficiency (E_G) and age of each individual donor tree and grafted scions. The bar indicates standard error, ns = not significant and *** = p<0.001

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* (Fig. 6) but similar trends were visible when the regression analyses were done for both species (Fig. 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 Fig. 5. The growth rates were more likely to level-out when the trees reached 40 year-old of age and over (Fig. 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 and Milburn (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. 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*. (2007). 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 differences, 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 study, 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 study 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.

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