

Evidence for age- and size-mediated controls of tree growth from grafting studies

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Summary Vegetative propagation techniques such as grafting can be used, in conjunction with field studies, to decouple the relative effects of age and size on tree metabolism and growth. Despite interest in this approach, little attention has been paid to the best metrics for assessing the growth performance of grafted plants over time. Based on an analysis of the grafting literature and our own data, we show that the choice of metrics to assess tree growth can entirely change the conclusions reached about the relative importance of age versus size. We recommend that absolute as well as relative rates of growth are calculated and that scion size be standardized as much as possible at the start of the experiment. Once proper metrics are chosen, all of the available published evidence is largely concordant with two concepts: (1) age-mediated controls of tree growth are likely to be important during the first few years of a tree's life (before phase change); and (2) after the first few years of a tree's life, size-mediated factors largely prevail over age-mediated factors in determining tree growth rates. We found no support for theories invoking age-mediated sink limitations in old trees.

Keywords: aging, age-related processes, grafting, hydraulic limitation hypothesis, relative growth rate, size-related processes.

Introduction

Investigations on age-related patterns in forest ecosystem structure and functioning have increased greatly in the last ten years. This interest has taken place at several spatial scales and has not been limited to trees. For example, processes associated with ecosystem productivity and respiration, stand primary productivity, soil development, and tree growth and metabolism have been examined, as have the effects of changes in stand structure on associated flora and fauna. Within this framework of general interest in age-related patterns, the “hydraulic limitation hypothesis” (Ryan and Yoder 1997) has triggered many studies, probably because of the attractiveness and

simplicity of the proposed mechanism; the basic idea, however, predates the current debate by at least 30 years and has periodically resurfaced (e.g., Maggs 1964, Zimmermann 1983, Friend 1993).

More recently, some of the research focus has shifted away from the demonstration that old and tall trees frequently have reduced metabolic rates (Bond 2000) to specific analyses of the effects that height (e.g., via the hydrostatic component of the water potential drop from soil to leaves) can have on tall trees (Woodruff et al. 2004, Koch et al. 2004) and, more broadly, to the study of the ecology of tall organisms (Koch et al. 2004, Burgess and Dawson 2004, Burgess et al. 2006, England and Attiwill 2006, but cf. Tobiessen et al. 1971, Rundel and Stecker 1977).

An additional line of research has examined the potential role of age on tree growth and metabolism (Day et al. 2001, Mencuccini et al. 2005, Bond et al. 2007). This idea is also not new (cf. Wangermann 1965), but has resurfaced within the context of the new interest in the ecology of old and tall organisms. In 1959, Wareing (1959) proposed that some of the changes associated with the adult habit result from stable changes in the apical meristems (called maturation or phase change) and superimposed on phase change are changes in the nutrient status of the plant resulting from the effects of its increasing size and complexity. These latter effects were referred to as aging changes. A current research question is whether this same paradigm can be applied to old trees, i.e., whether maturational processes leading to sexual competence gradually lead to senescence. Day et al. (2002) proposed that grafting allows discrimination between meristem-intrinsic and other processes affecting tree growth (i.e., stimulus–response, meristem–extrinsic and intrinsic–extrinsic processes). This distinction is more precise than the one based on the age–size dichotomy, because it makes no reference to whether the mechanisms have genetic or environmental causes. For instance, size effects could be mediated by a direct biophysical stimulus–response or other meristem-extrinsic mechanisms, but could also be mediated by changes in genetic expression

(e.g., DNA methylation, Fraga et al. 2002).

Separating age-mediated effects from size-mediated effects on tree growth and metabolism is usually achieved by propagation techniques such as grafting or rooting of cuttings. These techniques are based on the idea of obtaining vegetatively propagated plants that are of similar small size and are grown under identical conditions, while their tissues (aboveground for the grafts, above- plus belowground for rooted cuttings and air-layered plants) maintain the putative ages of the apical meristems of the original donor trees.

Despite the potential interest in this experimental approach, there is disagreement in the literature on the interpretation of results from grafting studies as well as on the best statistical techniques to apply to the data generated. This disagreement originates from the potential for artefacts inherent in the grafting approach, which need to be given proper consideration before valid conclusions can be obtained. As a result, authors of grafting studies appear to differ on the relative importance attributed to size-mediated versus age-mediated factors affecting tree growth.

The following terms are frequently employed in the grafting literature and are defined here. We call an *ortet* an individual donor tree from which twigs or scions are sampled for vegetative propagation by direct rooting, air-layering or grafting. Scions from a single ortet are called *ramets* and are therefore clonal replicates of the ortet.

In most cases, comparisons of ortet ages are carried out by analysis of variance of annual growth increments (although total heights and diameters have also been used). However Mencuccini et al. (2005), and before them Sweet and Wells (1974) and Foster et al. (1987), used relative growth rates (*R*) to compare ortets of different ages. Growth increments provide a direct comparison of the growth performance of ortets of various ages, particularly when scions are initially standardized by size (i.e., length). However if the initial performance of ortets of various ages differs greatly (for instance, because of a short-term “memory” effect linked to previous field performance, or because of the speed with which grafting shock is overcome), then size differences among ortets can quickly become established. Because of the geometric nature of tree growth, small initial size differences can be rapidly magnified, even though growth potentials during subsequent years are identical. In these circumstances, analysis of *R* can provide the best indication of whether inherent differences in growth potential exist across ortet ages, or whether the differences in absolute growth are carryover effects associated with differences in size. However, the use of *R* has its own limitations. Although *R* provides a measure of growth relative to initial size, it is commonly observed that *R* systematically declines with tree size (e.g., Burdon and Sweet 1976). This effect needs to be accounted for in any analysis employing *R*.

Here we review the grafting literature and present additional data from a recent study (Mencuccini et al. 2005). The goal of this contribution is to compare the different metrics used to assess tree growth. We demonstrate that an appropriate choice of metrics allows resolution of most of the discrepancies found in the literature. We also show that, based on all of the available

published evidence, (a) age-mediated controls of tree growth are likely to be important during the first few years of a tree's life (before phase change), and (b) after the first few years of a tree's life, size-mediated processes largely prevail over age-mediated processes in determining tree growth rates.

Materials and methods

Literature review

We selected published studies in which growth rates of scions from ortets of different ages were systematically compared. Because our primary objective was to compare different metrics used to assess age-mediated effects on growth rates, we chose only those publications in which at least two consecutive sets of measurements of plant size were available (to allow calculation of relative growth rate). This meant excluding some relevant literature on grafting and aging, such as an earlier study on red spruce (Rebbeck et al. 1992). In all the publications we examined, the metric employed to quantify size and growth was tree height (specifically, the vertical distance between the graft insertion point and the tip of the tree).

A study of Pinus radiata D. Don (Sweet 1973, Sweet and Wells 1974) This large field trial of radiate pine included experiments with both grafted plant and cuttings. The objective was to compare the field performance of 2-year-old seedlings with either grafted ramets taken from ortets of varying ages or with rooted cuttings taken from ortets of varying ages. The experiment was designed as a balanced incomplete block design with 25 blocks and nine units per block (Sweet 1973). Five ortet ages were represented with five ortets per ortet age and nine ramets per ortet. The ortet ages for the grafting experiment were 6, 15, 25, 43 and 66 years, whereas the ortet ages for the cuttings experiment were 6, 10, 19, 23 and 43 years. No attempt was made to standardize the seed source of the ortets. Two-year-old nursery-grown seedlings were also planted between the vegetative propagules as controls. As trees grew, thinnings were carried out to a specified spacing. Scions were selected from the upper part of the crown. Because of the variable ages of the ortets, there were differences in the size and nutritional status of the scions at grafting, which partially remained even after the grafts were regrafted onto a population of full-sib rootstock.

A study of Pseudotsuga menziesii (Mirb.) Franco (Ritchie and Keeley 1994) Of the two experiments conducted with grafted Douglas-fir, only Experiment 1 is considered here (Experiment 2 gave similar results). The experiment was designed as a completely randomized trial with a 1 × 1 m grid. Three ortet ages were represented, with 24 ortets of each age and one ramet per ortet. The experiment was replicated four times with four different seed lots (i.e., seed collected from specified seed zones). The ortet ages were 1, 4 and 9 years. Scion material was selected from the top third of the crown. Two-year-old seedlings from the same seed lots were used as rootstock.

A study of Larix laricina (Du Roi) K. Koch. (Greenwood et al. 1989, Hutchison et al. 1990) Four ortet ages (1, 5, 17 and 45 years) were represented in this experiment on eastern larch, with a total of 27 ortets for the three oldest age classes plus five half-sib families for the 1-year-old seedlings. Five to six ramets were taken from each ortet and family. Scions were sampled from the top third of the crown from a naturally regenerated stand where all ages were present. Rootstocks were obtained from uniform 2-year-old potted seedlings. All scions were pruned to 20 cm in length and the terminal bud was removed.

A study of Pinus taeda L. (Greenwood 1984, Foster et al. 1987) Of the two experiments conducted with loblolly pine cuttings, only Experiment 2 is considered here (Experiment 1 gave similar results). The experiment was designed as a completely randomized block trial, with two replications and row plots with three trees in each. The experiment compared 1-year-old seedlings with ramets from 4-year-old ortets. Fifteen half-sib families of ortets from two seed sources were represented, and there was one ramet from each ortet. The cuttings were collected following similar protocols to the previous studies. The 1-year-old seedlings were raised from seed taken from the same families.

Original data Original data were collected as part of a wider study on the effects of size-mediated changes on tree growth and leaf gas exchange in sycamore (*Acer pseudoplatanus* L.), ash (*Fraxinus excelsior* L.), Scots pine (*Pinus sylvestris* L.) and hybrid poplar (*Populus balsamifera* L. ssp. *trichocarpa* (Torr. & Gray ex Hook.) Brayshaw × *P. deltoides* Bartr. ex Marsh) (Mencuccini et al. 2005). A detailed explanation of the experimental protocols for the four species is given in Mencuccini et al. (2005) and is summarized here. Because the primary objective of the study was to understand the causes of reduced growth in relatively old trees, the range of ages sampled here was much larger than in any previous study.

For each of the four species, we selected between five and eight individuals for each of four (ash and sycamore) to eight (poplar) age classes. For all species except poplar, 10 ramets were taken from the top third of the crown of each ortet and were grafted on 2-year-old rootstocks of the same species. For poplar, the different-sized donor trees in the field were clonal, i.e., they were all derived from ramets from the same ortet (the original seedling). Therefore, for this species, only one ramet was selected from each of the genetically identical ramets of each “age” class. Ash and sycamore were sampled from the same mixed-species woodland, whereas Scots pine trees were sampled elsewhere (all in Scotland, U.K.). Both these woodlands were composed of trees of different ages, and the younger trees likely arose from seed from the same older parent trees. The poplar clone ‘Helix’ was sampled from planted material in Belgium. Scots pine, ash and sycamore were grown outdoors in pots, whereas the 20-cm-long poplar cuttings were grown in 10-l pots in Year 1 and later on were transplanted to our common garden. In all cases, a completely randomized design was employed. The sampled ortets were grouped into ortet age classes as follows: for Scots pine, six classes from 43 to 225 years; for sycamore, four classes from 5 to 140 years;

for ash, four age classes from 5 to 110 years; and for poplar, eight classes from 1 to 33 years (the age of the original seed-derived donor tree). The Scots pine sample did not entirely overlap with the sample used by Mencuccini et al. (2005), but we had a longer record of height growth for this subset.

For sycamore, ash and poplar, height was periodically measured (to the nearest cm) over two years to obtain estimates of absolute and relative growth rates. For Scots pine, yearly height growth estimates were obtained for the previous seven years by measuring internode lengths down to the grafting region. Measurements took place when poplar, ash and sycamore were in their first and second year after propagation, whereas Scots pine was measured seven years after grafting.

Graphical and statistical analyses

For each of the studies taken from the literature, data on height (and occasionally diameter) growth rates were extracted directly from published tables or by digitizing published figures. Mean scion R was calculated as:

$$R = \frac{\ln(H_2) - \ln(H_1)}{t_2 - t_1}, \quad (1)$$

where H_2 and H_1 are the measured heights at times t_2 and t_1 , respectively, and R is relative growth rate. Unless otherwise specified, the time interval for calculating R was one year.

Although R almost always systematically declined over time, this decline does not invalidate the use of R in assessing plant performance, it merely makes the first-order exponential growth model $\ln H = a + RT$ (where a is a parameter and H , T and R are plant height, time and relative growth rate, respectively) inadequate. There are several strategies to account for the time-dependent decline in R . If long-term data are available to describe the growth trajectory over time, R is frequently defined as a linear or curvilinear function of time and an exponential growth model of order greater than one is used. Hunt (1982) reviewed higher order models obtained by stepwise regression and gave several pages of examples. As explained below, a different strategy was followed here.

For analysis of data from the Mencuccini et al. (2005) study, raw data were available with which to conduct statistical tests. After calculating R by Equation 1, the effect of ortet age on R was assessed with a partly nested model with one covariate using a general linear model procedure (mixed linear model within SPSS v. 12; SPSS, Chicago, IL). The model structure was:

$$R_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + (\alpha\gamma)_{ik} + X + \alpha_i X + \gamma_k X + \epsilon_{ijk} \quad (2)$$

where α (age class) was entered as the fixed between-subject factor, β (individual ortets) were entered as a random factor nested within α , γ (year) was the fixed within-subject factor, and X (initial tree height between any two intervals) was used as a covariate, to account for the negative effect of increasing

size on R . For ash, sycamore and poplar only two repeated measures were available for R for each ortet within each age class, therefore the interaction term $\gamma_k X$ was dropped from Equation 2. No transformation was necessary to achieve homogeneity of variance. Ramets were averaged within each ortet.

The use of height as a covariate in the analysis of relative height growth rates is justified on the following grounds. First, the stem of the original rootstock was cut (in our experiments) almost immediately after grafting, leaving only the grafted shoot as aboveground biomass. Second, height was measured from the point of insertion of the graft, hence scion length and scion biomass are allometrically related and R represents the relative growth rate of the grafted scion only, not of the grafted plant as a whole. Sweet and Wells (1974) discuss the use of R as an index to assess the field performance of cuttings and grafted plants, and Burdon and Sweet (1976) provide an in-depth analysis of the potential problems in interpreting the inherent differences in tree growth after planting. The latter paper also provides a full statistical analysis of height as a covariate of R in planting trials, and compares this method with alternatives such as adjusting current heights based on initial heights and use of height increments (see also Hunt (1982) for an additional discussion of the use of covariates with R).

Results

Literature review

The radiata pine study Absolute height growth rates for the differently aged grafts were plotted in Figure 1A, and those for the cuttings in Figure 1B, and the insets give the same data plotted on a semi-log scale. Because R was obtained as the difference between two heights after logarithmic transformation, and because the time periods were constant, the slopes on a semi-log plot were effectively equivalent to R . Absolute heights demonstrated a clear trend associated with age for both the grafting and the cuttings study, i.e., scions from younger ortets grew more in absolute terms than scions from older ortets. The semi-log plots, however, made clear that, except for the 2-year-old seedlings, the slopes of all ortet age classes were essentially identical, i.e., growth relative to the current size was independent of age, and the ranking across ortet ages was a function of the different initial sizes, not the subsequent relative growth rates. However, because R typically declined with size (see changes in slopes over the years in insets), a comparison of the effects of ortet age required that account be taken of the different sizes achieved by each age class. This can be accomplished by covariance analysis and using, for example, the initial height to adjust for the time trends. In our case this was not possible, however, because the raw data were unavailable to us. We therefore conducted a more limited graphical analysis, i.e., we graphically explored whether the differences in R across ortet ages (but at constant size) were suggestive of an age-related trend. This analysis is shown for grafts and cuttings in Figures 2A and 2B, respectively, where values of R for all the

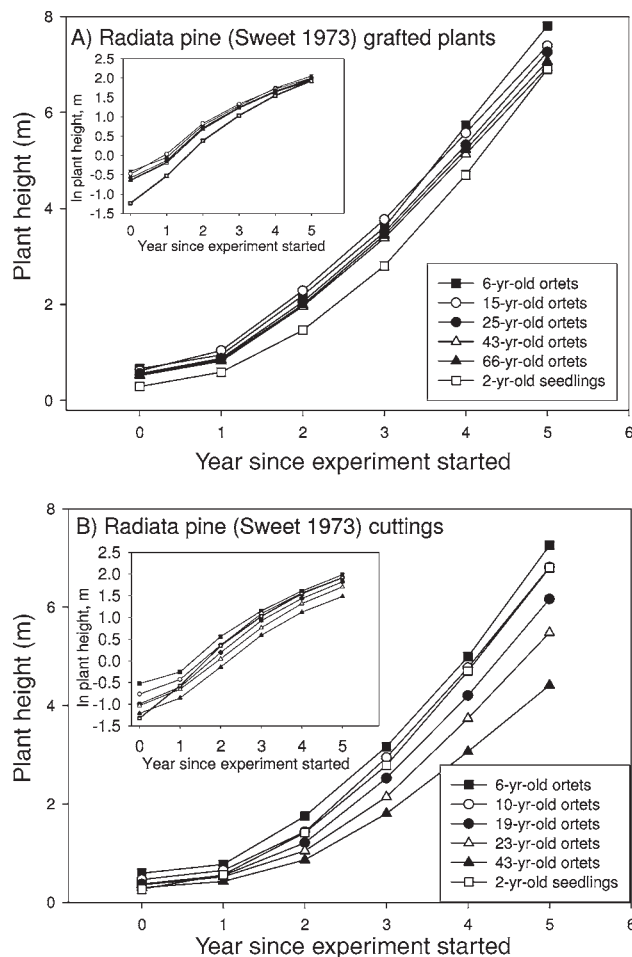


Figure 1. The Sweet (1973) experiment on radiata pine. (A) The grafting study. The main chart reports the absolute tree heights as a function of time since grafting for five ortet (i.e., donor tree) ages plus the ungrafted 2-year-old seedling controls. The absolute differences between ortet ages increase with time, and the ranking corresponds to the ortet ages. The inset shows the same data on a semi-log plot (i.e., equivalent to relative growth rates, R) to emphasize that slopes across ortet ages are similar and that differences across ortet ages are carried forward from Year 1. Note the higher R for the 2-year-old seedlings for almost the entire period. (B) The cuttings study. Significance of the main chart and of the inset is the same as in panel (A). Note the massive difference in final heights across ortet ages, and also that R slopes in the inset are essentially unchanged.

ortet age classes and the available periods are plotted against tree height. The graphs clearly illustrated that, even when size was accounted for, the values of R were essentially independent of scion age. The only exception to this rule was the 2-year-old seedlings, which showed substantially larger R values for the first two years. In the original publications, Sweet (1973) and Sweet and Wells (1974) reported a statistically insignificant effect of ortet age on R (except for the 2-year-old seedlings) based on a proper covariance analysis.

The Douglas-fir study The range of ortet ages examined in the Douglas-fir study was much smaller than in the radiata pine

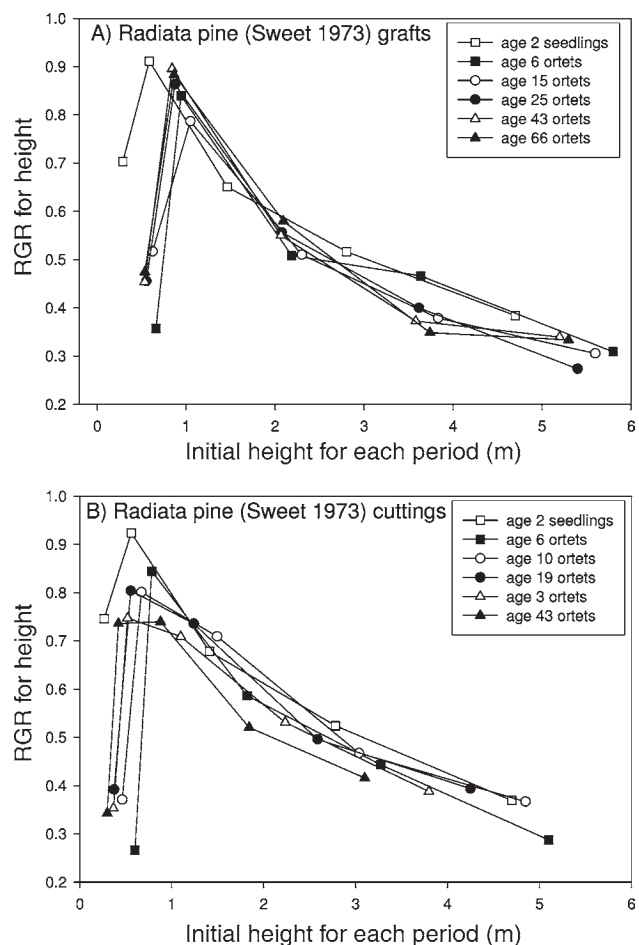


Figure 2. Plots of relative growth rates (R) against initial tree height during each period for the same experimental data used in Figure 1. (A) The grafting study. (B) The cuttings study. Values of R are plotted against initial tree height to account for the decline in R with increase in plant size in larger plants. Note that the ranking of ortets by age in Figure 1 has entirely disappeared here.

study. The plot of absolute heights against time (Figure 3A) showed a ranking somewhat based on ortet age, with the absolute differences increasing with time (i.e., annual increments were larger for ramets from 1-year-old ortets, although the ranking between the 4- and 9-year-old ortets was inverted). The inset on a semi-log scale showed essentially parallel lines with values converging over time. Again, because of the declining values of R with increasing size, we plotted R directly against mean tree height for each ortet age (Figure 3B) and it was apparent that, except for the ramets from the 1-year-old ortets in Year 1 and the 9-year-old ortets in Year 3, R values collapsed onto a single trend-line independent of ortet age. Although no covariance analysis was available from the original paper, it was evident from Figure 3B that the ranking in absolute heights among ortet ages (a difference of about 35 cm between 1- and 4-year-old seedlings at Year 6, Figure 3A) was largely a reflection of the larger R for the youngest age class during the initial year of establishment. Once those size differences were created, subsequent differences in absolute height increments

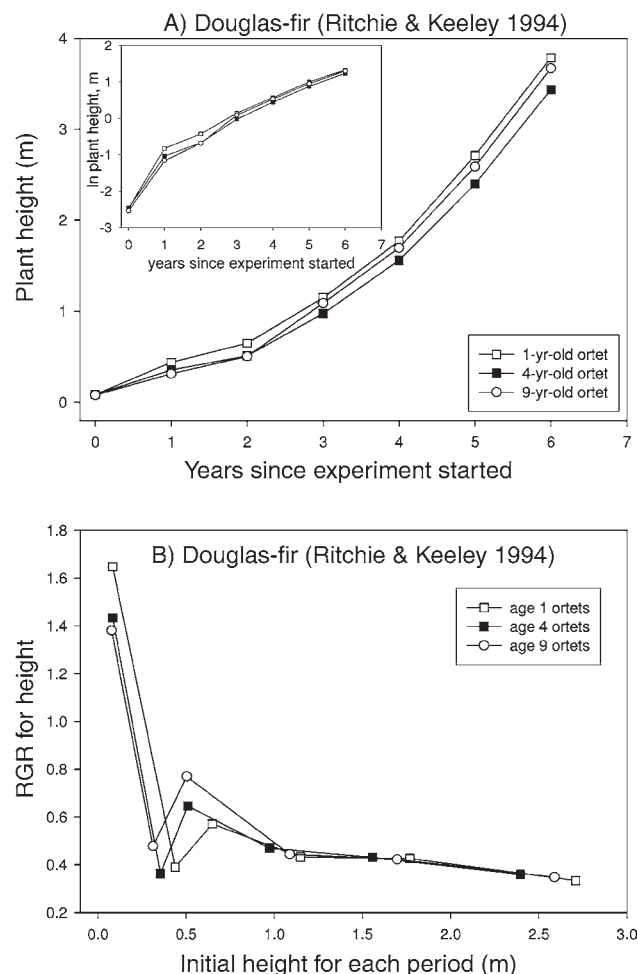


Figure 3. (A) The Ritchie and Keeley (1994) experiments on Douglas-fir. Absolute tree heights on a natural scale (main chart) and on a semi-log chart (inset). (B) Values of R plotted against initial tree height for the same study. Note that the ranking according to ortet ages visible in panel (A) has entirely disappeared in panel (B).

among ortet age classes were the result of the established size differences in Year 1 and not an inherent reflection of higher growth rates of the 1-year-old seedlings.

The eastern larch study Although growth data were given for both height and diameter in the original publication, only two time periods were available for analysis from the eastern larch study, so the data are presented in tabular form (Table 1). For the first time period (1986 growing season), growth rates (both annual increments and R values) segregated according to ortet ages. The difference in absolute growth rates (annual increments) across ortet ages was still visible during the second growing season. However, calculation of R for the second year showed that all classes behaved similarly, with a hint of larger values for the older ortet ages (probably a reflection of their smaller absolute sizes). It therefore appears that, similarly to the previous experiments, ramets from younger ortets grew faster during the initial period in Year 1, but the difference disappeared entirely during the second growing season. Hence the

Table 1. Data on size, and absolute and relative growth rates (*R*) of both diameter and height for grafted eastern larch from ortets of four ages (data extracted from Greenwood et al. 1989). Measurements in February 1986 were conducted before the start of the 1986 growing season, whereas measurements in November 1986 and November 1987 were conducted after the end of the 1986 and 1987 growing seasons, respectively.

Ortet age classes	1 year	5 years	17 years	45 years
Diameter (mm) in February 1986	2.2	2.2	2.2	2.2
Diameter (mm) in November 1986	7.8	6.1	4.1	3.9
Diameter (mm) in November 1987	19.6	15.6	14.1	10.4
Height (m) in February 1986	0.20	0.20	0.20	0.20
Height (m) in November 1986	0.64	0.54	0.41	0.24
Height (m) in November 1987	1.61	1.54	1.13	0.77
Diameter increment (mm) during 1986 growing season	5.6	3.9	1.9	1.7
Diameter increment (mm) during 1987 growing season	11.7	9.5	10.1	6.5
Height increment (m) during 1986 growing season	0.44	0.34	0.21	0.04
Height increment (m) during 1987 growing season	0.97	0.99	0.72	0.53
<i>R</i> in diameter (year ⁻¹) during 1986 growing season	1.27	1.02	0.63	0.58
<i>R</i> in diameter (year ⁻¹) during 1987 growing season	0.92	0.94	1.23	0.97
<i>R</i> in height (year ⁻¹) during 1986 growing season	1.16	1.00	0.73	0.17
<i>R</i> in height (year ⁻¹) during 1987 growing season	0.93	1.04	1.01	1.18

larger values of absolute increments for the younger ortets in Year 2 were the carryover effect of the size differences established during Year 1 and not evidence of an inherently higher growth rate.

The loblolly pine study Despite a smaller initial size, the 1-year-old seedlings displayed higher *R* during Year 1, which allowed them to overtop the ramets from the 4-year-old ortets (Figure 4A). After Year 1, differences in *R* between the two types of plant decreased, although the seedlings consistently displayed higher *R* values throughout the study period (Figure 4B).

Experimental data on Scots pine, sycamore, ash and hybrid poplar

In this study, raw data were available to us, and so statistical analyses were carried out to confirm the observed trends. The dataset on these four species spanned a much larger range of ortet ages than any of the published studies that we analyzed.

Scots pine Absolute heights ranked the 43-year-old age class at the top and the 184-year-old class at the bottom with a difference of up to 30 cm between the two and with ranks roughly reflecting ortet ages (Figure 5A). Seven years after grafting, absolute differences among ortet ages tended to decrease, although the ranking was still visible. A plot of *R* against mean tree height (Figure 5B) failed to reveal a consistent superiority of the younger age class over the others. This was confirmed by the mixed linear model analysis (Table 2A). Although significant differences were seen for different years, neither height, nor age class significantly affected yearly *R*, although there were some indications that the effects of year also depended on absolute height.

Sycamore and ash. Only two years of post-grafting growth data were available for these analyses (with two measurements per year, at the beginning and end of the growing season). The May 2003 measurements represent the initial scion lengths af-

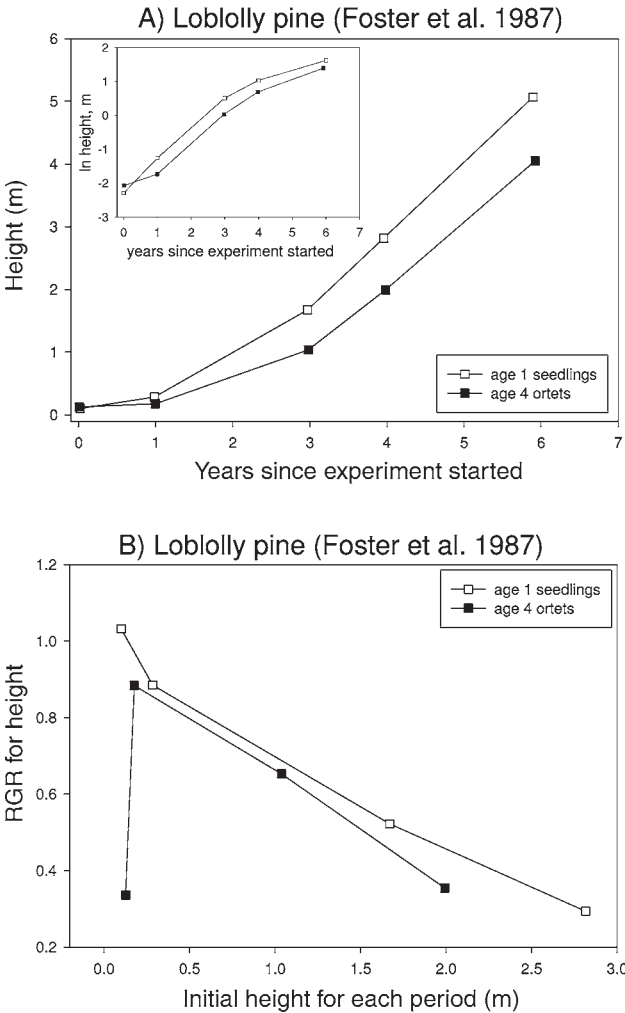


Figure 4. The Foster et al. (1987) experiment on loblolly pine. (A) Absolute tree heights on a natural scale (main chart) and on a semi-log chart (inset). (B) Relative growth rates (*R*) plotted against initial tree height for the same study.

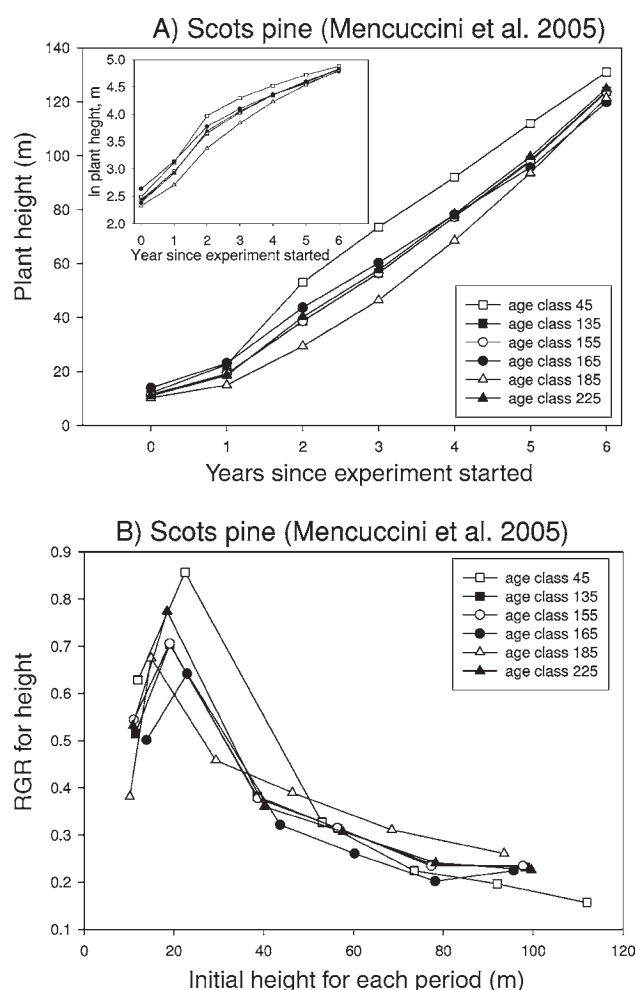


Figure 5. The Scots pine study (Mencuccini et al. 2005). (A) The main chart shows absolute values, and the inset shows the same data on a semi-log plot. Note the higher values for the youngest age class (43 years) as well as the lower values for the age class next to the oldest (183 years). (B) Values of R are plotted against initial tree height for the Scots pine study. A repeated-measures mixed linear model showed no significant difference across ortet ages.

ter grafting. The October measurements represent the final heights achieved after each growing season. Plots of absolute tree heights showed a systematic difference in size linked to ortet age, with smaller sizes for the ramets from older ortets (Figures 6 and 7), together with larger absolute values for the self-grafted and the ungrafted rootstock controls. This difference in height was established during 2003 when the ramets from the older ortets grew very little and it was maintained almost entirely during 2004. Based on the data from the two available years, plots of yearly R against tree height (insets in Figures 6 and 7) showed a complete recovery of R for the older ortets during 2004 for sycamore and an almost complete recovery for ash. The repeated-measures mixed linear model for the four ortet age classes (i.e., excluding the two controls of self-grafted material and ungrafted rootstock) confirmed the lack of significant differences across age classes for sycamore (Table 2B), although differences among ortet ages were only

Table 2. Results of the repeated-measures mixed linear model for relative growth rate (R). Initial height (H) was used as a covariate to account for differences in size over time and across age classes. The main between-subject factor was the ortet age class, with individual ortets nested within the age class, and year was the within-subject factor.

Source	df numerator	F	P
<i>Scots pine</i>			
H	1	1.528	0.231
Age	5	1.786	0.147
Year	5	7.587	0.000
Age \times year	25	1.486	0.157
Age $\times H$	5	2.292	0.069
Year $\times H$	5	2.346	0.080
<i>Sycamore</i>			
H	1	230.613	0.000
Age	3	0.507	0.680
Year	1	12.365	0.001
Age \times year	3	7.348	0.001
Age $\times H$	3	7.287	0.001
<i>Ash</i>			
H	1	32.312	0.000
Age	3	2.698	0.059
Year	1	18.712	0.000
Age \times year	3	5.886	0.002
Age $\times H$	3	3.350	0.031
<i>Poplar</i>			
H	1	0.100	0.755
Age	7	0.358	0.918
Year	1	156.653	0.000
Age \times year	7	0.667	0.698
Age $\times H$	7	0.471	0.846

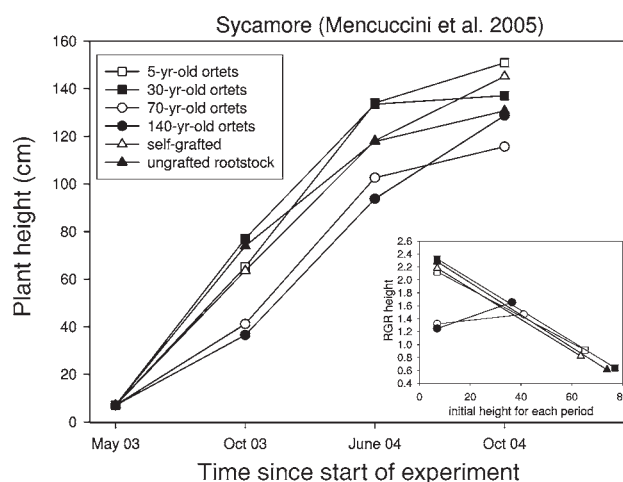


Figure 6. The sycamore study (Mencuccini et al. 2005). The main chart shows absolute tree heights for four periods during 2003 and 2004, and the inset shows values of yearly relative growth rates (R) plotted against initial tree height. A repeated-measures mixed linear model showed no significant difference across ortet ages.

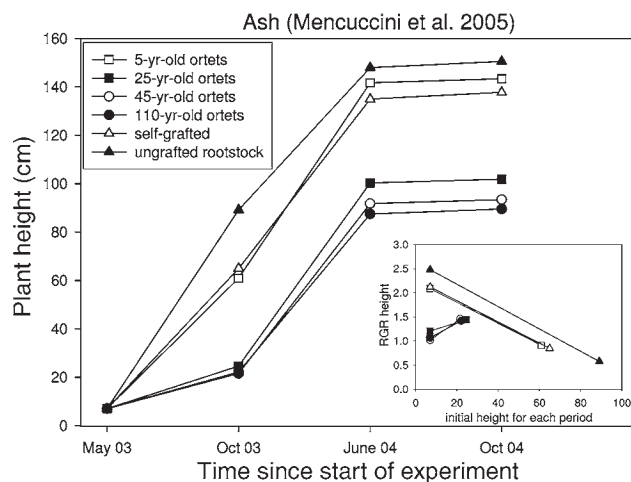


Figure 7. The ash study (Mencuccini et al. 2005). The main chart shows absolute tree heights for four periods during 2003 and 2004, and the inset shows values of yearly relative growth rates (R) plotted against initial tree height. A repeated-measures mixed linear model showed a marginal but not significant difference ($P > 0.05$) across ortet ages.

marginally nonsignificant for ash (Table 2C). There were significant interactions between age and year and between age and height, as evident also from the insets in Figures 6 and 7. The almost significant term for age class for ash likely indicates that the grafted plants of this species were still undergoing recovery from the initial post-grafting stage. Similar plots for diameter R showed a faster recovery from grafting shock than for height R for both species (data not shown).

Hybrid poplar clone. Ranking in absolute heights were created fairly rapidly after planting, again in accordance with donor tree's age (Figure 8A, the donor trees in the field from which scions were taken were themselves ramets from a single 33-year-old donor tree derived from seed). Plots of R against initial height showed a trend with the first two age classes (1- and 2-year-old donors) showing greater growth than older donors (Figure 8B), although this difference was nonsignificant based on a repeated-measures mixed linear model on the yearly R values for 2004 and 2005 (Table 2D). A significant effect was only seen across the two years for this species.

Discussion

Observed patterns and general interpretation

The combined analyses of the literature data and of the experiments of Mencuccini et al. (2005) revealed a broad across-study consistency in the observed patterns. In almost all cases, the studies document initial differences across age classes in either absolute size or R that appeared to be related to ortet age; however, these differences in R were not sustained for more than a few years. After this initial period, R was no longer related to ortet age, although absolute size and annual increments could still differ many years after grafting.

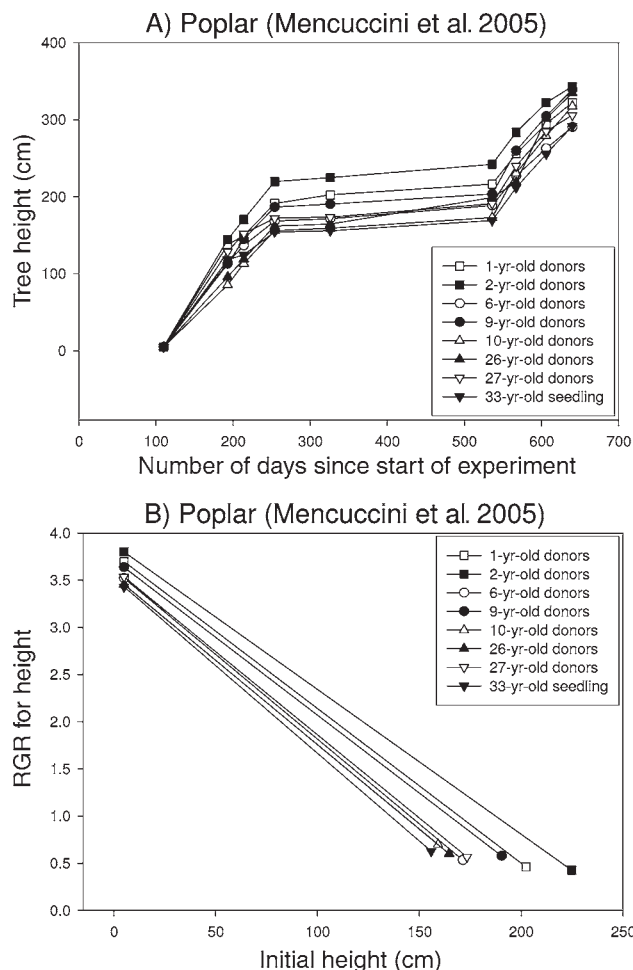


Figure 8. The poplar study (Mencuccini et al. 2005). (A) Absolute tree heights for the years 2004 and 2005. Ranking of heights closely followed ortet age, with the differences established fairly rapidly after planting. (B) Relative height growth rate (R) for the poplar study. Most of the differences apparent in (A) have now disappeared, and a repeated-measures mixed linear model showed no significant effect of age class.

The causes of the observed initial differences across ortet age classes are likely varied and, without additional evidence, we can only speculate. For the radiata pine study, the observed initial differences in size among ortet ages were related to pre-existing differences in size and perhaps nutritional status of the scions before grafting. The initial difference in R between the 2-year-old seedlings and the grafted scions of the same species was likely associated with size differences and with age-related genetic factors. Around age 3–4 from seed, radiata pine makes the transition from an open-growth juvenile shoot form to intermittent shoot growth (adolescent) where the bud undergoes resting phases several times a season (D. Smith, Metagenetics, Rotorua, New Zealand, personal communication). This is likely to elicit substantial changes in R . Similarly, loblolly pine undergoes several morphological changes during the initial few years of life, related, among other things, to the number of branches per unit of stem length (Greenwood 1984,

Foster et al. 1987). These genetically controlled processes likely account for the differences in R between seedlings and 4-year-old ortets in Figure 4B. Ontogenetic changes of this type are likely to be the cause of the differences in R one year after grafting between ramets from 1-year-old and older Douglas-fir ortets in Figure 3B, because scions from the younger ortets tended to produce more terminal bud flushes later in the growing season (lammas growth) than the scions from older ortets (Ritchie and Keeley 1994). All these changes are probably under genetic control and are part of the developmental transition or phase change known to occur in trees (Greenwood 1995). In other cases, it is possible that differential grafting success, susceptibility to grafting shock, or differences in nutrient or carbohydrate storage among ortet age classes affected the initial establishment and, in so doing, triggered the initial size differences that were then amplified over time. This might be the case for eastern larch, where clear and gradual differences in R were visible during Year 1 across all the age classes, although these differences entirely disappeared during Year 2. The sycamore and the ash examples from Mencuccini et al. (2005) may also belong to this category. We have documented this “memory” effect linked to grafting shock for our Scots pine study. Grafted Scots pine plants (Vanderklein et al. 2007) showed no evidence of changes in leaf photosynthesis, stomatal conductance or water-use efficiency related to ortet age. However, large inter-plant variability in these properties was apparent. The source of variability across individuals appeared linked to the speed of recovery from grafting shock. Although hydraulic resistance in the grafting region accounted for only about 8% of whole-plant hydraulic resistance at the time of measurement (i.e., six years after grafting), it was the best predictor of current plant leaf area, suggesting that plants that initially suffered severe grafting shock were unable to compete in leaf area production and growth with ungrafted plants (Vanderklein et al. 2007).

Whatever the cause or causes of the initial differences in R between young scions from young ortets and the rest of the ortet age classes in all of the examples studied, these differences completely disappeared or were even reversed over time. This suggests that, although probably under genetic control, the difference in R may last for only a few years and then rapidly disappear. Several other features are known to show a more gradual transition during the first few years of life of a plant (Greenwood 1995).

Unlike the ephemeral differences in R , absolute height differences were sustained over time across ortet ages (e.g., Figures 1A, 1B, 3A, 4A, 5A, 6, 7 and 8), and they often even increased with time. In some cases, this apparent discrepancy can be explained by the initial spurt of extra growth caused by the higher R in younger ortets. Once a greater initial size is attained, and because of the geometric nature of tree growth, absolute differences can increase and persist. This is exemplified in the Douglas-fir study, where the absolute height difference of about 0.35 m between 1-year-old and 4-year-old ortets six years after grafting still appears to be increasing. This is not a result of a continued difference in R across ortet ages, but

rather the result of the presence of additional ‘lammas growth’ in the younger trees and the consequent higher R in Year 1. In other cases, there were no apparent differences in R across ortet ages, but a difference was quickly established as a result of apparently minor initial differences in scion or cutting size across ortet ages. A good example is the radiata pine cuttings study, where a 3-m difference (and still increasing) was present five years after grafting between the youngest and the oldest ortet age classes. The R analysis showed that the difference was entirely due to the carryover effect from an initial small difference in size and not from an inherent difference in growth rates across ortet ages. For this species, cuttings from older donor trees frequently had finer branching and less foliage (i.e., indicative of less total photosynthesis) than twigs from seedlings, hence a slower progression of the geometric growth (M. Menzies, Forest Research, Rotorua, New Zealand, personal communication).

Because R varies inversely with tree size, the taller a plant becomes, the smaller its relative growth rate will be in the future. Hence, even for the radiata pine cuttings, it is predicted that, in time, the absolute differences will slowly decrease and may eventually disappear. This equalization will occur faster for some species than for others, depending on the species-specific R and its ontogenetic trajectory.

Ecological and physiological implications

Given the temporary nature of the accelerated growth rate in very young ortets compared with older ortets, and the disappearance of this difference after only a few years for all the species examined, we see no need to propose either inherently lower meristem growth potentials or sink limitations to explain growth reductions in ramets from old ortets (i.e., past the period of the phase change), as recently outlined by Day et al. (2002). The lower growth rates of ramets from older ortets can entirely be explained on the basis of allometric principles.

From the studies examined, however, it is clear that the situation is different in young plants. During the first few years of a tree’s life, there is substantial evidence that growth potential is inherently controlled by the shoot meristem. This raises the possibility that gas exchange as well as several other functional and structural elements (e.g. plant hydraulic conductance, cf. Ren and Sucoff 1995) could be genetically controlled and show direct age-mediated changes in young trees. An example where this may be relevant in the context of the hydraulic limitation hypothesis is the study by Ryan et al. (2004), where the growth and physiology of trees was followed during their first six years.

Another important consideration relates to the roles of molecular and genetic processes in size-mediated reductions in tree growth and metabolism. That size-mediated processes appear to be the major drivers of growth reductions in old trees, does not necessarily imply a stimulus–response mechanism, but rather that, whatever metabolic processes are involved, they respond to the reductions of whole-plant size. Day et al. (2002) reviewed a long list of molecular and genetic processes known to occur during plant development. Although that literature is largely focused on the initial stages of maturation, it

suggests that similar changes are likely to take place after phase change, possibly interacting with size changes. It is possible and likely that size-related processes are mediated by gene action. Investigations of the molecular pathways by which size affects tree metabolism and growth will likely help to clarify the significance of these results.

New questions and challenges for the future

Since 1997, when the hydraulic limitation theory was first formally presented (Ryan and Yoder 1997), much effort has been expended on characterizing the physiological ecology of old and tall trees (e.g., Koch et al. 2004, Körner et al. 2005, but also Pennisi 2005, Peñuelas 2005). Our knowledge of stand and ecosystem development is rapidly expanding and consensus has emerged in some areas. However, we are still not close to identifying the component or components that are most likely to determine the observed reductions in GPP and NPP with age. A recent modeling exercise based on the concept of optimal carbon allocation (Buckley and Roberts 2006) has highlighted how much work needs to be done to correctly interpret the significance of some of the observed patterns. Much of the progress thus far can be viewed as useful but marginal to the central question initially posed by Ryan and Waring (1992) (cf. Ryan et al. 2006).

On the positive side, however, the impetus provided by those initial studies has helped identify several research questions that had not been considered at the outset. For example, an important concept that has recently emerged is the demonstration that size exerts effects that are clearly independent of age (Mencuccini et al. 2005), a concept consistent with much earlier data. Much of the literature on aging in trees confused these two aspects and accepted that characters such as reduced ring width or height growth might be taken to indicate senescence processes (e.g., Finch 1990). One newly identified complexity of tree research, compared with animal research, is the current awareness that any symptom or biomarker of aging must be considered independently of the size-related effects that occur in large and tall trees. Other more fundamental questions remain unanswered.

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