

Developmental decline in height growth in Douglas-fir

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Summary The characteristic decline in height growth that occurs over a tree's lifespan is often called "age-related decline." But is the reduction in height growth in aging trees a function of age or of size? We grafted shoot tips across different ages and sizes of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees to determine whether the decline in height growth is mediated by tree size or by the age of the apical meristem. We also evaluated whether reduced carbon assimilation plays an important role in height growth decline. In one experiment we cut shoot tips from old-growth, young-mature and seedling trees and grafted them onto 2-year-old graft-compatible rootstock in a seed orchard in Lebanon, Oregon. In another experiment we performed reciprocal grafts between lateral branches of old-growth trees accessible from the canopy crane at Wind River, Washington and young-mature trees in a nearby plantation. We measured growth (diameter and elongation of the dominant new stem) and mortality annually for three years in the Seed Orchard experiment and for two years in the Reciprocal Graft experiment. In the Seed Orchard experiment we also measured photosynthetic capacity (determined from the response of net carbon assimilation to the intercellular CO₂ concentration of the leaf, or *A/C_i* curves), leaf mass per area (LMA) and carbon isotope composition ($\delta^{13}\text{C}$) of cellulose in 1-year-old foliage. Grafting caused changes in both growth and physiology of the grafted stems. Within two years after grafting, growth and physiology of all combinations of scions and rootstock exhibited characteristics of the rootstock. In some cases, the change in growth was dramatic—cuttings from old-growth trees showed a 10-fold increase in stem elongation rate within 2 years of grafting onto seedling rootstock. Similarly, carbon isotope discrimination of new foliage on shoots from old-growth trees increased by nearly 3‰ and 2‰ after grafting onto young-mature and seedling rootstock, respectively, whereas discrimination decreased by a similar magnitude in scions from young-mature trees after grafting on old-growth trees. Furthermore, differences in carbon assimilation estimated from carbon isotope discrimination and *A/C_i* relationships were small relative to growth differences. Our results confirm that size, not age, drives developmental changes in height growth in Douglas-fir. Reduced carbon assimilation

does not play an important role in height growth decline.

Keywords: age-related growth decline, grafting, hydraulic limitation, photosynthesis, *Pseudotsuga menziesii*.

Introduction

Perennial plants have predictable patterns of height growth over their life cycles, but the underlying causes of height growth decline in aging plants are poorly understood. As with growth in most other organisms as well as populations of organisms, the height of individual trees tends to follow a sigmoidal pattern over time (Weiner and Thomas 2001), and the age–height relationships in trees can be precisely modeled by logistic functions similar to those used to model population growth. However, these common dynamics do not necessarily imply similar controlling mechanisms.

To examine the potential mechanisms responsible for developmental decline in height growth, it is important to distinguish factors that limit maximum growth rates or total maximum height (e.g., Koch et al. 2004) from factors that regulate developmental decline in height growth. These phenomena are necessarily linked: if growth rates do not decline over a life cycle, total height will steadily increase until the organism dies. An important distinction, however, is that maximum growth rates and maximum size are strongly influenced by environment, whereas some of the characteristics of growth decline through development are relatively independent of site conditions.

Age–height relationships for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) provide an example of a site-independent developmental process. On high-quality sites, maximum height growth of Douglas-fir can exceed 1.5 m year⁻¹, and trees may achieve heights greater than 75 m, whereas maximum height growth and total maximum height of trees with similar genetic potential on poor sites can be a small fraction of these values (Figure 1a). Clearly, height growth is strongly influenced by environmental conditions. However, new insights emerge when height growth is viewed as a function of height (Figure 1b).

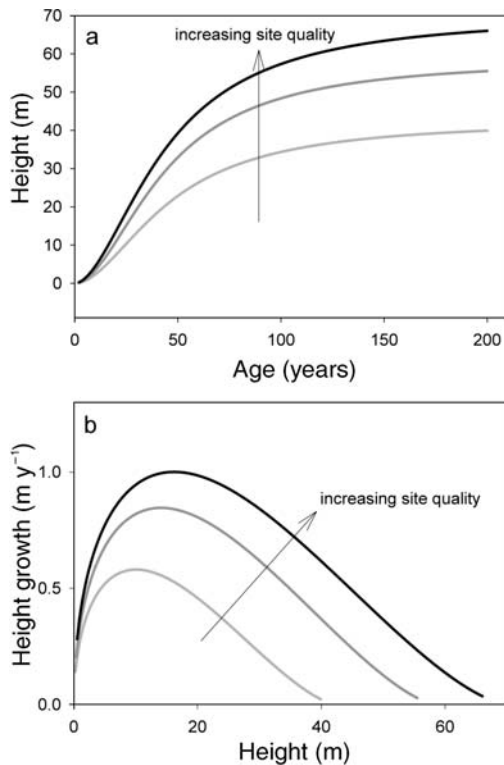


Figure 1. Age versus height relationships for Douglas-fir growing in low-, intermediate- and high-quality sites (a). The same data are shown in the lower panel (b), reconfigured to show height growth as a function of height. Adapted from Flewelling et al. 2001.

Under all site conditions, the maximum rate of height growth of trees occurs while they are relatively small; subsequently, growth declines as a linear function of height for more than a century. The slope of $\delta H/H$ is similar across differing site conditions, averaging a little less than $-0.02 \text{ m year}^{-1} \text{ m}^{-1}$. Thus, although site conditions strongly influence maximum growth rates of Douglas-fir, trees lose, on average, about 2 cm year^{-1} in height growth for each new meter of growth irrespective of site conditions after they reach their growth maximum.

The characteristic growth decline that occurs over a tree's lifespan is often called "age-related decline." But is the reduction in height growth in aging trees a function of age or of size? One of the objectives of our study was to determine whether this growth reduction, which we term "developmental decline in height growth" (DDHG) to avoid the implication of age as a causal factor, is mediated by tree size or by tree age. Our second objective was to determine whether reduced photosynthesis plays an important role in DDHG.

In animals and annual plants there is ample evidence that age-related growth decline is ontogenetic (i.e., genetically programmed). Height growth in humans and other vertebrates ceases irreversibly after puberty because of hormonal influences on bone elongation. In addition, animals face intrinsic aging processes after puberty and continuing into old age, owing in part to a species-specific limit to the number of mitotic divisions in any cell line (Hayflick and Moorhead 1961). This

limit is associated with the shortening of telomeres during cell differentiation and aging (Harley et al. 1990), and has led to the hypothesis that telomeres may serve as a "mitotic clock" (Allsop et al. 1992). There is a general correlation between an animal species' lifespan and the characteristic maximum number of cell divisions for that species, suggesting a cellular basis for organism aging after maturation in higher animals (Klarsfeld and Revah 2004).

There does not appear to be an intrinsic limit to cell division in plants, and telomere length does not change during plant growth and development (Riha et al. 1998). This does not rule out the possibility that another sort of "biological clock" slowly effects change in growth rate over time. For example, Day et al. (2001) proposed that genetically determined aging of meristems may be an important factor in age-related growth decline. A recent report by Mencuccini et al. (2005), however, casts doubt on the possibility of ontogenetic controls on developmental declines in growth.

Counter to the argument for ontogenetic control of tree height growth is the possibility that growth rate is regulated by size itself. The inverse relationship between tree size and growth rate (Figure 1b) strongly suggests such a possibility, and several hypotheses have been proposed to explain it. These include: (1) progressively greater substrate limitation to growth because of increased whole-tree respiration relative to photosynthetic production (e.g., Yoda et al. 1965); (2) increased water flow resistance between roots and leaves as a result of either increased transport distance (Maggs 1964) or impaired xylem function due to xylem cavitation (Zimmerman 1983); (3) reduced stomatal conductance and hence carbon assimilation rate because of increased hydraulic constraints (Yoder et al. 1994, Ryan and Yoder 1997); and (4) increased below- to aboveground biomass allocation ratio (Magnani et al. 2000).

Because trees generally increase in size as they age, it can be difficult to separate the effects of age from the effects of size on growth (Bond 2000, Peñuelas 2005). One way to get around this problem is through grafting: taking small cuttings from the tips of stems of one age or size class of tree and grafting them onto another age or size class of tree (Day et al. 2001, Mencuccini et al. 2005).

The use of grafting to separate the effects of age and size on growth is subject to some limitations. For example, the graft results in a wound, which must be repaired before normal growth can resume. To some extent the severity of the wounding response can be inferred from comparisons with self-grafts, in which the scion and rootstock are both of the same age or size class, but the rate of recovery from wounding may differ with scion and rootstock age. Also, because stem extension of large trees is less than that of younger trees, it may be impossible to find equivalent scions across different donor age classes for grafting onto a common rootstock. Scion length may be controlled, but not diameter or bud size. Thus, the initial conditions of the experiment may differ for different treatments. For these reasons, growth in the first year or so after grafting may not clearly differentiate the influence of the scion from that of the rootstock. Another requirement of grafting ex-

periments is the inclusion of adequate controls. To separate the effects of age from those of size, analyses should include ungrafted controls of both the rootstock and the scion donor.

Whether DDHG is controlled by age or size, a change in growth implies a change in carbon dynamics. Several of the hypotheses advanced to explain size-related controls over growth (e.g., nutrient limitations, hydraulic limitations) implicate reduced carbon assimilation as the ultimate causal factor, and ontogenetic controls could also directly impact carbon assimilation. It is also possible that growth inhibitions due to some other factor might result in a feedback inhibition of photosynthesis, or that carbon assimilation on a unit leaf basis remains unchanged but that altered growth patterns are reflected by shifts in allocation rather than assimilation. Our aim was to evaluate whether variations in height growth are accompanied by changes in carbon assimilation, and if so whether the magnitude of the change in assimilation could account for the altered growth.

Methods

Experimental design

We conducted two grafting studies with Douglas-fir. One, hereafter called the "Seed Orchard experiment," was a 3-year study in which scions from seedling (S), young-mature (YM), and old-growth (OG) donors were grafted on to seedling rootstock planted in an orchard. Growth was monitored each year, and in the third year we measured photosynthetic capacity (as determined from response functions of carbon assimilation to the intercellular CO₂ concentration of the leaf, or *A/C_i* curves), leaf mass per area (LMA) and stable carbon isotope composition ($\delta^{13}\text{C}$) of cellulose in 1-year-old foliage. The other experiment, hereafter called the "Reciprocal Graft experiment," was a 2-year study of growth in reciprocal grafts of shoot tips between sexually mature, young trees (YM) and old-growth trees (OG).

Terminology

The terminology used in grafting studies varies. Here the term "scion" denotes a small cutting from the tip of a stem that is grafted to another stem. The tree from which the scion is harvested is the "scion donor" and the tree that receives the graft is the "rootstock."

Grafting procedures

To prepare scions, shoot tips about 10 cm in length were cut from healthy lateral branches and stored in plastic bags over ice for up to two days. Before grafting, each scion was trimmed to about 5 cm, typically including only the most recent age class of wood and foliage, although scions from old-growth trees sometimes included 2-year-old wood because of the small size of the annual growth increments. Bark was stripped about 1 cm from the cut end, and thin strips were shaved from two sides of the stem to form a "V". When seedlings were used as rootstock, leaders were cut back by more than two thirds of their length and a vertical groove was cut

into the stem. Grafts were placed on lateral branches of YM and OG rootstock, which were not cut back before grafting. A scion was inserted into the groove so that the cambium of scion and rootstock matched on at least one side. The new grafts were wrapped with an elastic band and then with a strip of Parafilm. The protective covering generally decayed and fell off within 12 months; if it did not fall off, it was removed at the end of the first year.

Site characteristics and grafting experiments

The Seed Orchard experiment was conducted near Lebanon, Oregon (122°53' W, 44°36' N, elevation: 90 m) in a seed orchard owned and operated by the Roseburg Lumber Company. Soils are classified as Newberg sandy loam; mean annual rainfall is about 995 mm, with only 7% falling in the summer months of June–August. The orchard primarily produces Douglas-fir seed for use in reforestation. In 2000 Roseburg Lumber planted Douglas-fir seedlings selected for superior performance to serve as rootstock for grafting (Copes 1999) in an agricultural field at a spacing of about 1 × 1.5 m. The company donated a block of 160 seedlings for our experiment. From this block groups of 40 trees were selected at random to receive one of three grafting treatments, S, YM or OG, or to serve as an ungrafted control. Scions for S grafts were cut from five other healthy seedlings within the orchard; scions for YM grafts were cut from the upper half of the crowns of seed production trees on the seed orchard site; scions for OG grafts were cut from the upper third of five old-growth trees at the Wind River Canopy Crane Research Facility (see next section). Grafting was performed on March 26 and 27, 2002. Trees were maintained by Roseburg Lumber according to the normal seed orchard protocol. Weeds were controlled with an application of glyphosate and atrazine on October 25, 2002 and glyphosate and velpar on October 10, 2003. At the end of the first growing season, grafts were spray painted to facilitate identification and branches in the whorl beneath the graft were partially pruned to prevent their competing with the new grafted leader.

The Reciprocal Graft experiment was conducted in the Wind River basin, Carson, Washington. We selected five OG Douglas-fir trees from a stand surrounding the Wind River Canopy Crane (121°57' W, 45°49' N). Although we do not know the precise ages of the trees, the stand was initiated following a massive fire about 450 years ago; trees ranged in height from 50–60 m. The YM trees grew in a nearby site that was harvested in 1976 and planted the following year. The trees were approximately 18 m tall at the beginning of the study. A fixed tower of scaffolding provided access to the upper crowns of seven trees in the young-mature stand; we used the five largest trees for this study. Both of these stands have been used extensively for previous research and are described in previous publications (e.g., McDowell et al. 2002, Chen et al. 2004).

We first attempted the reciprocal graft experiment in 2002; however, none of the 80 grafts made in the upper canopy of OG trees survived. Grafting treatments were repeated in April

2003 with greater success using mid-crown positions for grafts in OG trees and upper canopy positions for grafts in YM trees. For each of the two age classes of rootstock, we performed 80 grafts, half with scions from YM trees and half with scions from OG trees (i.e., half of the cuttings were grafted back on to the same age class as a control to assess the impact of the grafting treatment). Grafts were distributed at random on healthy lateral branches among the five rootstock trees for each age class. Ungrafted stems were selected on the same branches as the grafts as additional controls.

Each of the grafts in the Seed Orchard experiment became the primary leader of the seedling rootstock, and all grafts in the Reciprocal Grafting experiment were made on lateral branches. We took scions from OG trees at Wind River for grafts in both experiments because this was the only location where we had access to canopies of large OG trees; thus, the same set of OG controls were used for both experiments, and the OG controls for the Seed Orchard study were lateral branches rather than leaders. In young Douglas-fir trees, the growth of leaders far exceeds that of laterals because of apical dominance, but in old trees these characteristics are reversed: new growth in the mid-canopy is greater than in the upper canopy, and there is no single leader. Thus, if we had used upper-canopy stems as OG controls for the Seed Orchard experiment, the differences in growth between grafts and OG controls would have been even greater than reported here. The YM trees for the two experiments were different; YM scions for the Seed Orchard grafts were harvested from on-site trees, and these trees also served as controls. We used lateral branches in these trees because it would have damaged the trees to remove leaders. In this case, the growth reported for YM controls is probably considerably less than the growth of the leaders on these trees.

Growth measurements

Growth and survival of grafts in the Seed Orchard experiment were measured in March 2003 (Year 1), May 2004 (Year 2), and March 2005 (Year 3), and for the Reciprocal Graft experiment the grafts were measured in September 2003 (Year 1) and April 2005 (Year 2). In each measurement period, we assessed mortality and measured the length and diameter of the most dominant new stem to emerge from each live graft. Diameter was measured with digital calipers to the nearest 0.1 mm at the base of the stem, about 1 cm above the previous year's bud. Stem length was measured with a measuring stick to the nearest 1 mm from the previous year's bud scar to the tip of the new bud.

$\delta^{13}\text{C}$ of foliar cellulose, A/C_i curves, and LMA

Foliage for carbon isotope analysis was harvested in March and April 2005 for both experiments. We collected 10–20 needles of the most recent age cohort from all live grafts and from 10 randomly selected controls for each age class. The needles were oven dried and milled to a fine powder, from which cellulose was extracted following the procedure of Wise et al. (1945). The cellulose extracts were analyzed by the Idaho Sta-

ble Isotope Laboratory (ISIL) for carbon isotope composition with a Finnigan-MAT, Delta+ isotope ratio mass spectrometer. Data are reported as $\delta^{13}\text{C}$ values relative to the Pee Dee Belemnite Standard (PDB).

We measured A/C_i curves on samples from five trees selected at random from each of the three treatments and on samples from the ungrafted controls in the Seed Orchard experiment. The A/C_i curves were measured with a Li-Cor LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE) equipped with a blue-red artificial light source. Cuvette irradiance was $1200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and temperature was 25°C . The A/C_i curves were generated by changing the cuvette CO_2 concentration in the following order: 350, 400, 600, 1200, 1600, 2000, 350, 300, 250, 150, 100, 50 and $25 \mu\text{mol mol}^{-1}$. After each exposure to a new CO_2 concentration, net CO_2 assimilation was allowed to reach steady state before measurement. The maximum catalytic activity of Rubisco (V_{cmax}) and maximum rate of electron transport (J_{max}) were calculated from the A/C_i curves by nonlinear, least-squares regression to fit the values of these parameters to the equations of the Farquhar et al. (1980) photosynthesis model (Harley et al. 1992). Parameters were temperature-corrected to 25°C using the equations of Leuning (1997), and the activation energies from Harley et al. (1992). Calculation of V_{cmax} was determined for the region of the response function when the slope was quasi-linear (Wullschleger 1993), which typically occurred when $C_i < 300 \mu\text{mol mol}^{-1}$.

Leaf area for gas exchange measurements was determined with a flatbed scanner operating at 300 dpi with backlighting. Scans were digitized with NIH-Image software for Macintosh to determine the aggregate one-sided silhouette leaf area of each sample. Leaf mass per area (LMA, g m^{-2} , based on one-sided silhouette areas of leaves and dry leaf mass) was determined for 8 to 10 needles of the same age and from the same twigs as those used for the gas exchange measurements. Needles were scanned as described above, then dried to constant mass and weighed.

Data analysis

In both experiments, our primary objective was to determine whether response variables (growth, $\delta^{13}\text{C}$, LMA and photosynthetic capacity) of grafts differed from either or both of two controls—ungrafted stems of the rootstock and of the scion donor. We performed 2-tailed *t*-tests for these comparisons with SAS version 8 software (1999; SAS Institute Inc., Cary, NC).

Results

Survival

First-year survival for commercial grafting operations in Douglas-fir seed orchards in Oregon is typically greater than 90% (Mike Albrecht, Roseburg Lumber Seed Orchard, personal communication); overall survival of grafts in both experiments of this study was considerably lower even though the grafts were performed by a professional grafter. According to

anecdotal evidence from workers in commercial seed orchards, grafting success was, for reasons unknown, poor throughout western Oregon in 2002. In the Seed Orchard experiment, 44% of the 120 grafts (all three scion age classes) survived. The poorest first-year survival (25%) was for S scions; i.e., for self-grafts. In contrast, 68% of the YM scions and 40% of the OG scion survived the first year. In the Reciprocal Graft experiment, the overall first-year survival was 20% on OG rootstock and 59% on YM rootstock. For the OG and YM scions, 10 and 30%, respectively, survived the first year after grafting onto OG rootstock, and 65 and 50% of the YM and OG scions survived one year after grafting onto YM rootstock.

Growth

Effects of wounding on growth We assessed wounding effects by comparing self-grafts (i.e., S scion on S rootstock in the Seed Orchard experiment, YM scion on YM rootstock and OG scion on OG rootstock in the Reciprocal Graft experiment) with uncut stems on the same branches. Despite the high mortality of S scions grafted onto S rootstocks, there was no evidence of a wounding effect on S scions in the Seed Orchard experiment (Figures 2d–f; Table 1). However, there was a strong effect of grafting on first-year elongation of YM scions ($P < 0.0001$) in the Reciprocal Graft experiment (Figure 3c; Table 2). Although there was no statistically significant wounding effect on elongation of OG scions because of the small sample size and high variance, the mean elongation of self-grafted OG scions was less than half that of OG control stems in the first year. Two years after grafting there were no statistically significant wound effects from the grafting treatments on diameter or stem elongation in either experiment.

Stem diameter growth and elongation In the Seed Orchard experiment, first-year elongation and diameter growth of the

scions were generally more similar to the age class of the scion donor trees than to the rootstock (Figure 2, Table 1). However, first-year diameter growth of OG scions was about midway between controls for the OG donor and the Y rootstock, and was significantly different from both of them. In subsequent years, elongation and diameter growth of all scion age classes was not significantly different from the rootstock, and both the YM and OG scions differed significantly from their donor controls.

In the Reciprocal Graft experiment, diameter growth did not vary significantly among any treatment combinations, or between control YM and OG stems (Figure 3, Table 2). This may be partly because of the characteristic thickened bark of OG stems, which could obscure potential differences in the diameter of the underlying stem wood. However, elongation appeared to be impaired by wounding from the graft treatment, and even the YM scion recovered poorly from grafting onto OG rootstock. Grafts on YM rootstock fared better. By the second year, there was no significant difference in the elongation of OG and YM scions grafted onto YM rootstock, or between either of the two scion age classes and the ungrafted YM controls.

$\delta^{13}\text{C}$ of foliar cellulose, LMA and A/C_i curves

For ungrafted stems in the two experiments there was enrichment of ^{13}C in foliar cellulose with increasing tree age, amounting to a difference of about 2‰ between Y and OG control stems. These results are similar to previous measurements of foliar carbon isotopes in a chronosequence at Wind River (McDowell et al. 2002).

Isotope composition of foliar cellulose was measured only in 2005, 3 years after grafting in the Seed Orchard Experiment and 2 years after grafting in the Reciprocal Grafting experiment. In general, the isotopic composition of new foliage on

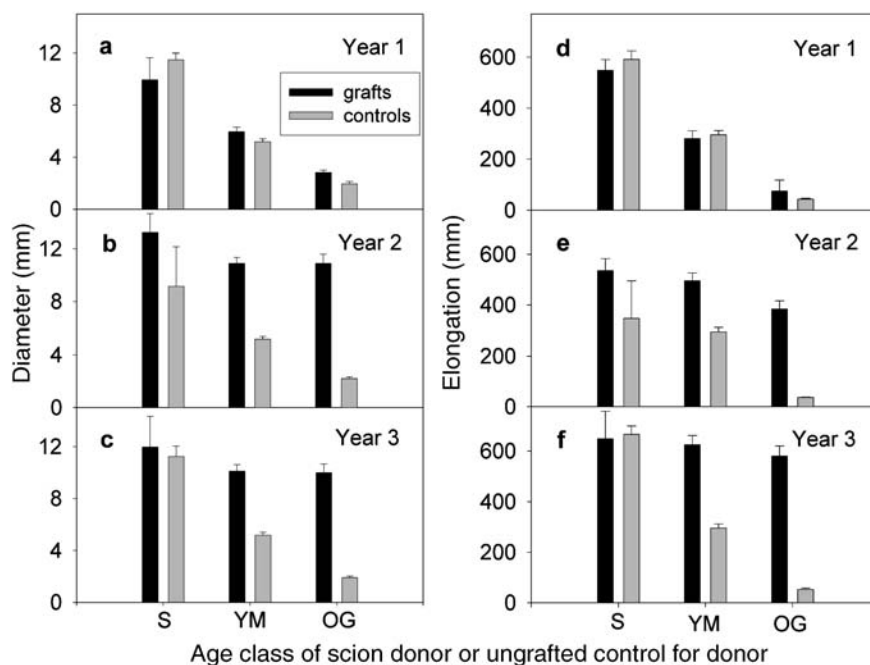


Figure 2. Diameter and elongation of the newest primary stem emerging each year for three years following grafting treatments in the Seed Orchard experiment. Scions from three tree age classes, seedling (S), young-mature (YM) and old-growth (OG), were grafted onto seedling rootstock. Dark bars show mean values for grafts, and light bars show mean values for ungrafted control stems of the scion donor. Error bars show standard errors of means. Sample sizes and results from statistical comparisons between grafts and controls are shown in Table 1.

Table 1. Results of two-tailed *t*-tests for pairwise comparisons of mean differences between grafts and controls for parameters measured in the Seed Orchard experiment. Sample sizes for the grafts and controls are indicated in parentheses. The diameter and elongation of the dominant new stem were measured each year for 3 years after grafting. Other parameters were measured only in Year 3. For each type of graft there are two controls. One control is the ungrafted stems of the scion donor. The other control is the rootstock, which in this experiment were in all cases seedling trees. Abbreviation: ns = not significant, OG = old-growth, YM = young-mature, S=seedling.

Graft (Donor/Rootstock)	Control	<i>P</i> > <i>t</i> Year 1	<i>P</i> > <i>t</i> Year 2	<i>P</i> > <i>t</i> Year 3
<i>Diameter</i>				
OG/S (12)	OG (14)	< 0.005	< 0.0001	< 0.0001
YM/S (17)	YM (22)	ns	< 0.0001	< 0.0001
S/S (5)	S (16)	ns	ns	ns
OG/S (12)	S (16)	< 0.0001	ns	ns
YM/S (17)	S (16)	< 0.0001	ns	ns
<i>Elongation</i>				
OG/S (12)	OG (14)	ns	< 0.0001	< 0.0001
YM/S (17)	YM (22)	ns	< 0.0001	< 0.0001
S/S (5)	S (16)	ns	ns	ns
OG/S (12)	S (16)	< 0.0001	ns	ns
YM/S (17)	S (16)	< 0.0001	ns	ns
$\delta^{13}C$				
OG/S (16)	OG (7)	–	–	< 0.0001
YM/S (12)	YM (9)	–	–	< 0.005
S/S (5)	S (11)	–	–	ns
OG/S (16)	S (11)	–	–	ns
YM/S (16)	S (11)	–	–	ns
<i>LMA</i>				
OG/S (5)	OG (3)	–	–	ns
YM/S (5)	YM (4)	–	–	< 0.05
S/S (4)	S (5)	–	–	ns
OG/S (5)	S (5)	–	–	< 0.05
YM/S (5)	S (5)	–	–	ns
<i>V_{cmax} (leaf area basis; $\mu\text{mol m}^{-2}\text{s}^{-1}$)</i>				
OG/S (5)	OG (3)	–	–	ns
YM/S (5)	YM (4)	–	–	ns
S/S (4)	S (5)	–	–	ns
OG/S (5)	S (5)	–	–	ns
YM/S (5)	S (5)	–	–	ns

grafts took on the characteristics of the rootstock, resulting in up to a 3‰ change in the isotopic signature of foliar carbon.

In the Seed Orchard experiment, $\delta^{13}C$ values were similar for scions of all ages, and the three age classes of scions did not differ significantly from control stems on the rootstock (Figure 4a, Table 1). Foliar $\delta^{13}C$ values of YM and OG scions were depleted in ^{13}C compared with uncut controls on the donor trees. There were more distinctions among treatments in the Reciprocal Grafting experiment. Scions tended to be depleted in ^{13}C irrespective of rootstock (Figure 4b, Table 2), and the isotopic composition of YM scions on OG rootstock was midway between the controls for the donor and the rootstock and significantly different from both of them. Old-growth scions grafted

onto YM rootstock were the most depleted in ^{13}C of all treatment and control categories in the Reciprocal Grafting experiment, including the YM controls; grafting onto YM rootstock resulted in a mean decrease in $\delta^{13}C$ of 3‰ for the OG scions.

In this and all other comparisons between OG controls and OG scions on seedling rootstock, it is possible that site effects confound age effects because all OG donor trees in this study grew in Wind River and all S trees were in Lebanon. The $\delta^{13}C$ values for YM trees at Wind River were significantly (*P* < 0.0001) heavier (less negative) than for YM trees at Lebanon, suggesting a difference either in site or the condition of the trees of the same age class at the two sites. If site is the cause of this difference, it implies that the isotope composition of OG trees might have been even more enriched if those trees were in Lebanon (the site of the Seed Orchard experiment) than Wind River (the site of the Reciprocal Graft experiment), and this would amplify conclusions made from comparisons of

Table 2. Results of two-tailed *t*-tests for pairwise comparisons of mean differences between grafts and controls for parameters measured in the Reciprocal Graft experiment. Sample sizes are indicated in parentheses. The diameter and elongation of the dominant new stem were measured each year for 2 years after grafting. Carbon isotope composition ($\delta^{13}C$) of foliar cellulose was measured only in Year 2. For each type of graft there are two controls—one control is the ungrafted stems of the scion donor, the other control is the rootstock. Abbreviation: ns = not significant.

Graft (Donor/Rootstock)	Control Year 1	<i>P</i> > <i>t</i> Year 2	<i>P</i> > <i>t</i>
<i>Diameter growth</i>			
OG/OG (3)	OG (13)	ns	ns
OG/OG (3)	YM (37)	ns	ns
YM/YM (22)	YM (37)	ns	ns
YM/YM (22)	OG (13)	ns	ns
OG/YM (18)	OG (13)	ns	ns
OG/YM (18)	YM (37)	ns	ns
YM/OG (11)	OG (13)	ns	ns
YM/OG (11)	YM (37)	ns	ns
<i>Elongation</i>			
OG/OG (3)	OG (13)	ns	ns
OG/OG (3)	YM (37)	< 0.0001	< 0.0001
YM/YM (22)	YM (37)	< 0.0001	ns
YM/YM (22)	OG (13)	ns	< 0.005
OG/YM (18)	OG (13)	ns	ns
OG/YM (18)	YM (37)	< 0.0001	ns
YM/OG (11)	OG (13)	< 0.0001	< 0.05
YM/OG (11)	YM (37)	< 0.0001	< 0.0001
$\delta^{13}C$			
OG/OG (2)	OG (10)	–	ns
OG/OG (2)	YM (26)	–	ns
YM/YM (17)	YM (26)	–	< 0.05
YM/YM (17)	OG (10)	–	< 0.0001
OG/YM (10)	OG (10)	–	< 0.0001
OG/YM (10)	YM (26)	–	< 0.005
YM/OG (10)	OG (10)	–	< 0.0001
YM/OG (10)	YM (26)	–	< 0.005

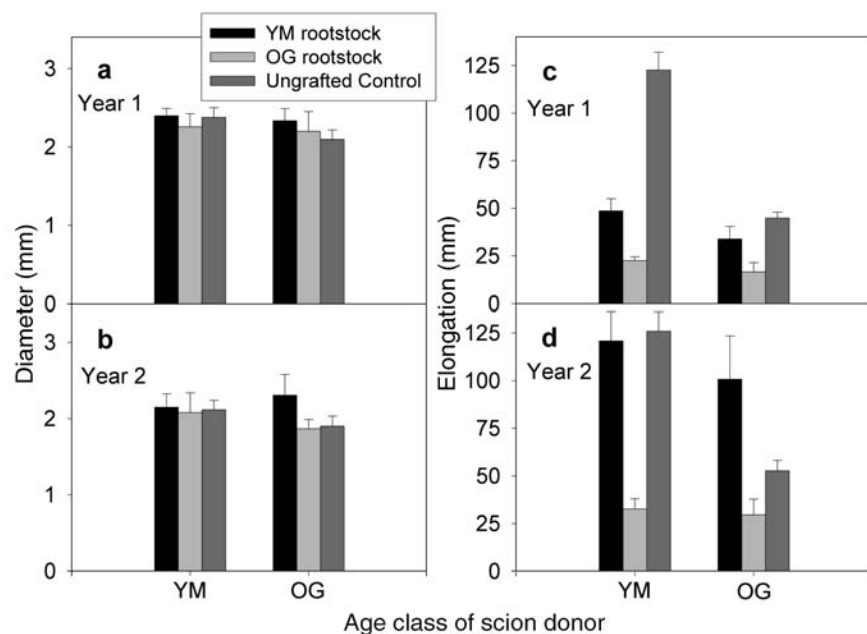


Figure 3. Diameter and elongation growth of the newest primary stem emerging for 2 years after grafting treatments in the Reciprocal Graft experiment. Dark bars show mean values for grafts; reciprocal grafts were performed between young-mature (YM) and old-growth (OG) trees. Light bars are mean values for ungrafted control stems of the scion donor. Error bars show standard errors of means. Sample sizes and results from statistical comparisons between grafts and controls are shown in Table 2.

OG controls at Wind River with OG scions grafted on seedling rootstock in the Seed Orchard experiment.

In the seed orchard experiment, we measured LMA and A/C_i curves of mature foliage from the most recent foliage age cohort (again, the OG controls were OG trees at Wind River, although the YM controls were on-site trees). Leaf mass per area was lower in control stems of S compared with YM and OG controls ($P < 0.0001$), although we found no significant difference in LMA between ungrafted control trees of YM and OG. In contrast to other measures, the LMA of OG scions generally maintained the characteristics of the scion donor, although the LMA of YM scions took on characteristics of the rootstock (Figure 5a, Table 1).

Both LMA and $\delta^{13}\text{C}$ increased with tree age and height in control stems (i.e., increased mass per unit area and reduced isotope discrimination in taller trees). When YM scions were grafted onto YM rootstock, both LMA and $\delta^{13}\text{C}$ decreased, suggesting that the differences in $\delta^{13}\text{C}$ could be at least partially a consequence of altered diffusive resistance between the stomatal cavity and the site of CO_2 fixation in chloroplasts (Vitousek et al. 1990). However, the overall relationship between LMA and $\delta^{13}\text{C}$ was only marginally significant among the 25 samples for which both measurements were made on the same stems ($r^2 = 0.15$; $P = 0.053$). Thus, we cannot rule out the possibility that some of the variation in $\delta^{13}\text{C}$ was caused by the change in LMA, but the large change in $\delta^{13}\text{C}$ of the OG scions coupled with the small change in LMA following grafting onto seedling rootstock suggests that altered leaf structure was not a dominant cause of the variation in isotope fractionation.

Photosynthetic potential, assessed as V_{cmax} and J_{max} on a leaf area basis, did not vary significantly among the three tree age classes (controls) or among any of the combinations of grafts, although photosynthetic potential of YM foliage tended to be higher than that of either S or OG foliage (results for V_{cmax}

shown in Figure 5; results for J_{max} were similar and are not shown). Because of the high LMA of OG trees, V_{cmax} and J_{max} of ungrafted stems on OG trees were about half that of ungrafted YM and S stems. However, because of high variance in these parameters, there were no significant differences between any combination of graft treatment and controls.

Discussion

Height growth of trees is both extremely variable and highly predictable. A tropical pioneer species like *Cecropia obtusifolia* may grow more than 2.5 m per year, whereas the height growth of other species growing nearby may be 20 cm or less (Clark and Clark 2001). Height growth of similar-aged trees of the same species in different sites may vary by more than an order of magnitude, but these differences are so predictable that foresters quantify variations in site quality based on differences in height of dominant individuals of a particular species at a particular age. Clearly, the potential for height growth is under strong genetic control, and the genetic potential is strongly moderated by the availability of resources, especially water, nutrients and sunlight. It is also well known that plant hormones such as IAA are important mediators of the influences of both genetics and environment on the growth of lateral and terminal stems (Salisbury and Ross 1992). Developmental decline in height growth does not exhibit the environmental sensitivity or variability that is evident in absolute height growth or maximum height (Figure 1); thus, DDHG is not necessarily controlled proximally by any of the factors that control overall height growth. Any potential explanation for DDHG must be consistent with observed dynamics: height growth peaks at an early age, usually before sexual maturity, and thereafter declines linearly with total height over decades or centuries.

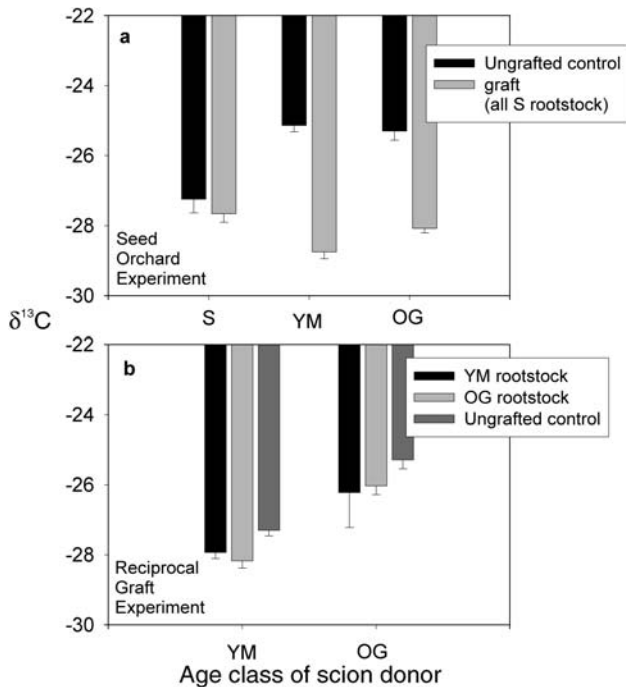


Figure 4. Carbon isotope composition of cellulose extracted from 1-year-old foliage harvested in spring 2005 (before bud break), from Lebanon (a) and Wind River (b). In (a), scions from three tree age classes, seedling (S), young-mature (YM) and old-growth (OG), were grafted onto seedling rootstock. In (b) reciprocal grafts were performed between young-mature (YM) and old-growth (OG) trees. Dark bars show mean values for grafts, and light bars show mean values for ungrafted control stems of the scion donor. Errors are standard errors of means. Sample sizes and results for statistical comparisons between grafts and controls are shown in Tables 1 and 2.

Is DDHG controlled ontogenetically?

There is strong evidence that in woody plants, as in many other organisms, the transition from juvenility to sexual maturity is genetically controlled (Greenwood and Hutchison 1993, Hackett 1985, Greenwood 1995). Although some juvenile characteristics are easily restored with grafting, it is difficult to induce complete rejuvenation in some species (e.g., Fraga et al. 2003), suggesting irreversible ontogenetic change. However, sexual maturation does not offer a good explanation for DDHG, even if it involves changes in photosynthesis or growth. This is because sexual maturation occurs over a relatively brief period of years, whereas DDHG involves a continuous change over many decades.

It is possible, however, that there is a yet-to-be-identified, post-maturational aging process in woody plants. Although there is no evidence for true senescence in perennial plants (Noodén and Guamét 1996), nor does there appear to be an ontogenetic limit to cell division in plants (Riha et al. 1998), evidence reported by Day et al. (2001) supports the notion that woody plants may have another type of “biological clock.” Day et al. (2001) grafted scions taken from juvenile, young-mature and old-growth red spruce (*Picea rubens* Sarg.) trees onto seedling rootstock. After three years, they found that the

physiological and structural characteristics of the parent trees were perpetuated in the grafted stems, and that growth, photosynthesis and stomatal conductance were progressively lower across the three age classes. Stem elongation and photosynthesis (leaf area basis) in scions from juveniles were approximately double that of scions from old-growth trees; growth, leaf structure and physiology of scions from young-mature trees were more similar to scions from juveniles than old-growth trees when grafted onto a common juvenile rootstock. Day et al. (2001) proposed that genetically determined aging of meristems may be an important factor in age-related growth decline. In contrast, Mencuccini et al. (2005) concluded from a study of grafts from mature individuals to seedling rootstock of three species (*Fraxinus excelsior* L., *Acer pseudoplatanus* L. and *Pinus sylvestris*), and rooted cuttings of a fourth species (*Populus balsamifera* L. ssp. *trichocarpa*) that size, rather than meristematic age, controlled growth.

In one respect, the results of our study were similar to those of Day et al. (2001). Even three years after grafting onto seedling rootstock, the mass per area ratio of new foliage on stems cut from old-growth trees maintained the characteristics of the scion donor rather than of the rootstock. However, this was not true for scions from young-mature trees grafted onto seedling rootstock. More importantly, within two years after grafting, the growth and physiology of all combinations of scions and

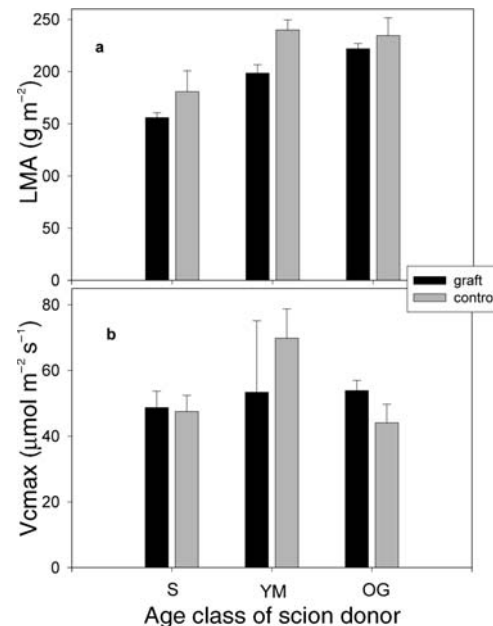


Figure 5. Leaf mass per projected foliage area (LMA) and V_{cmax} (determined from A/C_i curves) for the Seed Orchard experiment. Measurements were conducted on 1-year-old foliage in late spring 2005 (three years after scions from three tree age classes, seedling (S), young-mature (YM) and old-growth (OG) were grafted onto seedling rootstock). Dark bars show mean values for grafts and light bars show mean values for ungrafted control stems of the scion donor. Error bars show standard errors of means. Sample sizes and results for statistical comparisons between grafts and controls are shown in Table 1.

rootstock exhibited the characteristics of the rootstock. In some cases, the change in growth was dramatic—scions from old trees showed a 10-fold increase in stem elongation compared with controls from donor trees within two years of grafting onto seedling rootstock. Similarly, carbon isotope discrimination increased by nearly 3‰ and 2‰ after scions from old growth trees were grafted onto young-mature and seedling rootstock, respectively, whereas discrimination decreased by a similar magnitude in scions from young-mature trees grafted onto old-growth trees.

Mencuccini et al. (2005) commented that their results did not rule out the possibility that trees much older than the ones they sampled (maximum 269 years old) might undergo irreversible aging. The old-growth trees in our study were substantially older (about 450 years old) than those in the study of Mencuccini et al. (2005), but scions from our old-growth trees still responded promptly and dramatically to grafting onto both seedling and young-mature rootstocks. Our study also revealed that the impact of grafting is reciprocal—growth is inhibited when scions are grafted onto older, larger trees just as much as it is enhanced when old-growth scions are grafted onto smaller, younger trees. Furthermore, the growth response to grafting was continuous across the three age and size classes of trees we investigated—when old-growth scions were grafted onto young-mature trees, the growth enhancement was not as large as when old growth scions were grafted onto seedling rootstock.

To the extent that maturation is genetically determined, rejuvenation, the expression of juvenile characteristics in mature tissues, could be interpreted as the reversal of the expression of intrinsic aging factors (e.g., Robbins 1957, Hackett 1985, Greenwood 1987, Rodríguez et al. 1990). In the first year of our Seedling Orchard experiment, grafts involving scions from old-growth trees were more similar to the donor tree than to the rootstock. Is this a transient expression of genetic aging at the cellular level that is reversed in the second year? We think not. In Douglas-fir, vegetative buds are preformed in the previous year. The size and growth capacity of the bud is strongly determined by the environment in which the bud formed, resulting in a “carryover” effect of conditions in one year on growth in the following year (Harrington and Tappeiner 1991). Although all the cuttings used for grafting were of uniform length, it was impossible to select scions with similar-sized buds or stem diameters: stems on old growth trees always had smaller inside-bark diameters and buds compared with seedlings (bark tends to be thicker on stems of old growth trees, obscuring to some extent the diameter differences of stem wood). In addition, it is important to consider the potential wounding effect of the grafting treatment on growth. Young-mature and old-growth trees that were self-grafted (Reciprocal Graft experiment) experienced a significant loss of elongation in the first year of growth. The length of self-grafted stems was 55% less than that of control stems for both of these age classes in the first year. However, when cuttings from old-growth trees were grafted onto young-mature rootstock, elongation in the first year was significantly greater than that of cuttings from old-growth trees that were self-grafted (43 versus 16 mm).

One could argue, therefore, that being attached to a young-mature tree rather than an old-growth tree nearly tripled the stem elongation of cuttings from old-growth trees in the first year. Although there was typically a one-year carryover effect for slower-growing tissues to “catch up” with the growth of the rootstock, scions from young-mature trees in the Reciprocal Graft experiment showed a dramatic decrease in elongation in the first year after grafting onto old-growth rootstock, behaving more like the rootstock than the parent plant. Considering all of these findings, it is hard to argue that even transient ontogenetic change affects stem growth of Douglas-fir.

Reproduction It has been suggested that the metabolic costs of reproduction, which may be considered another consequence of maturation, contribute to age-related growth decline in trees (Weiner and Thomas 2001). Our study was not designed to analyze potential impacts of reproduction on DDHG, but we can evaluate likely impacts from published literature. In fruit trees, fruit production has a large impact on leaf physiology and tree growth, generally stimulating photosynthesis and inhibiting growth (reviewed in Forshey and Elfving 1989, Wünsche and Ferguson 2005). Compared with fruit trees, the impacts of reproduction on growth are less studied in forest trees, but they have been documented. In ponderosa pine, for example, there is an inverse relationship between production of female cones (but not male cones or pollen production) and diameter growth (Linhart and Mitton 1985). In dioecious species, males sometimes grow faster than females (e.g., Obeso et al. 1998), suggesting that the production of female flowers, fruits and seeds competes with vegetative growth for resources. However, the dynamics of reproduction are not consistent with the dynamics of DDHG. As ponderosa pine trees grow larger, for example, there is no significant change in fecundity in female cone production (Linhart and Mitton 1985), and even completely sterile plants undergo diminished height growth as they grow older, eventually reaching a maximum height. Thus, although reproduction may influence growth, it does not explain DDHG.

Direct control of height growth by height: potential mechanisms

Mencuccini et al. (2005; see also Peñuelas 2005, Pennisi 2005) concluded from their grafting studies that the so-called age-related decline in tree growth is controlled by size rather than age. Furthermore, observed growth patterns (Figure 1) strongly implicate a direct link between increasing tree size and decreasing growth. Several mechanisms have been proposed that might explain such a negative feedback, although generally they have not been evaluated in terms of consistency with the observed dynamics of DDHG.

Respiration At one time it was thought that respiration by non-photosynthetic tissues, especially sapwood, accounted for the decreasing rate of tree growth with increasing tree size (Yoda et al. 1965). This hypothesis is appealing because the ratio of photosynthetic to non-photosynthetic tissue decreases as overall size increases. However, as pointed out in many recent

papers (e.g., Ryan and Waring 1992, Mencuccini and Grace 1996, Ryan et al. 1997), direct measurements show that respiration does not explain age-related decline in overall productivity, and so is unlikely to explain DDHG. Furthermore, the ratio between sapwood volume and total tree volume does not change linearly with increasing tree height, nor does this ratio vary consistently within a species across a variety of site conditions. So it does not offer a good explanation for the dynamics of DDHG.

Roots and nutrients That roots influence shoot growth is incontrovertible. After all, dwarfism and semi-dwarfism in fruit trees is induced by grafting to an appropriate rootstock, and the growth of bonsai trees is manipulated largely by pruning roots and confining them to a small space. Likewise, early growth of coppice shoots (sprouts that emerge from stumps or roots after stems are cut or damaged) is typically much greater than that of seedlings because of their established root system (Rae et al. 2004). Just as growth is diminished in potted plants when they become root-bound, so could growth of field-grown trees be affected when roots have explored all available soil space. It is even possible that roots undergo a genetically mediated aging process that impacts shoot growth.

The physiological mechanisms by which roots control aboveground growth and maximum size are unknown (Sorce et al. 2002, Basile et al. 2003). Hypotheses include impaired water relations (Basile et al. 2003, Gonçalves et al. 2005), reduced capacity for nutrient uptake (Ebel et al. 2000, Rosati et al. 1997, Nielsen and Kappel 1996) and changes in the flux of hormones from roots to leaves (Lockard and Schneider 1981, Kamboj et al. 1999). Roots may also compete with shoots as a sink for carbohydrates, resulting in reduced aboveground growth when allocation to roots increases, or in cyclic patterns of growth that alternate between above- and belowground (Borchert 1991).

There is no obvious reason why root uptake of nutrients might be increasingly limited as a linear function of tree height, and recent studies have shown that decreased soil nutrient availability does not explain age-related growth decline (Ryan et al. 2004). However, it is conceivable that the delivery of nutrients to leaves is affected by decreased hydraulic conductance (see next section). On the other hand, if DDHG is caused by a decreased ability of roots or stems to deliver nutrients to leaves, reduced stem elongation should be associated with a reduction in photosynthetic capacity and increased carbon isotope discrimination. Our study showed no evidence for an association between decreased stem growth and photosynthetic capacity, as measured by V_{cmax} or J_{max} . Furthermore, carbon isotope discrimination was inversely, rather than directly, related to stem growth.

Hydraulic conductance As early as the 1960s, Maggs (1964) speculated that the distance between shoot apices and roots in large woody plants is too great to allow efficient transport between them, resulting in a decline in growth with increasing size. A variation on this hypothesis was proposed in the 1980s: the growth of large trees could be limited by the transport of

water and nutrients if xylem failure (due to cavitation) was not adequately remedied by refilling or by the production of new xylem (Zimmerman 1983). Yoder et al. (1994) suggested a mechanism linking large size to increased hydraulic resistance, which in turn would limit growth via reduced stomatal conductance and photosynthesis. This concept was later formalized as the hydraulic limitation hypothesis (Ryan and Yoder 1997).

If DDHG were primarily a function of stomatal limitations to photosynthesis, as predicted by the hydraulic limitation hypothesis (Ryan and Yoder 1997), we would expect an increase in carbon isotope discrimination with increased growth. We observed enrichment in ^{13}C of foliar cellulose with increasing tree size and decreasing stem elongation, both in grafted and non-grafted stems. This difference in isotope composition, combined with the observation that V_{cmax} was not significantly different among tree size classes, suggests greater stomatal limitation to photosynthesis in slower-growing stems. Our data do not permit detailed analysis of relationships between stomatal limitation and overall carbon gain, but given the huge differences in stem growth among treatments, we were able to assess whether reduced stomatal conductance offers a reasonable explanation for declining growth. First, we estimated the volume of dominant new stems in all grafting treatments and controls from the product of the square of the radius and the length. For 2005, this value was 10^3 times greater for ungrafted stems of seedlings compared with ungrafted stems of old-growth trees ($38,600 \pm 10,100 \text{ mm}^3$ for ungrafted seedling stems; $38.9 \pm 10.5 \text{ mm}^3$ for ungrafted old-growth controls). Because the new growth of grafted stems mirrored the growth potential of the rootstock, these values can be considered generally representative of new stem growth on grafts involving seedling and old-growth rootstocks.

Next, we used isotope composition and A/C_i curves to derive a rough, relative index of carbon assimilation. Discrimination of carbon isotopes during photosynthesis is a function of CO_2 concentration in air spaces within the leaf (C_i), which in turn is controlled both by stomatal conductance and the rate of photosynthesis (Farquhar et al. 1982):

$$\delta^{13}\text{C}_{\text{foliage}} = \delta^{13}\text{C}_{\text{atm}} + a - (b - a) \frac{C_i}{C_a}$$

where $\delta^{13}\text{C}_{\text{atm}}$ is the isotopic composition of CO_2 in ambient air, a is fractionation due to diffusion (4.4‰), b is fractionation by rubisco (27‰), and C_a is the CO_2 concentration of ambient air. Solving for C_i using $\delta^{13}\text{C}_{\text{atm}} = -8.0\text{‰}$, $a = 4.4\text{‰}$, $b = 27\text{‰}$, and $C_a = 360 \text{ mol mol}^{-1}$ yields mean values of 219 ± 8 and $202 \pm 5 \text{ mol mol}^{-1}$ for grafted seedlings and old-growth controls, respectively. We then used the A/C_i curves measured for each individual stem with the calculated C_i value for foliage from that stem, to estimate “average” net assimilation. This procedure yielded values of 10.9 ± 8 and $6.9 \pm 5 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for seedling and old-growth control stems, respectively, or about a 40% reduction. Although these values provide only a rough indication of differences in mean net assimilation, they show clearly that differences in unit-leaf carbon assimilation fall far

short of explaining differences in new stem growth. Thus, this study agrees with the conclusion of Ryan et al. (2006) that increased tree size is associated with both increased hydraulic resistance and reduced stomatal conductance, but the impact of reduced stomatal conductance on carbon assimilation is small relative to observed growth differences.

Allocation We did not measure allocation except for the amount of leaf mass allocated per unit leaf area (LMA). We found that LMA increased significantly with increasing tree size, as reported in many previous studies (McDowell et al. 2002, Marshall and Monserud 2003, Koch et al. 2004, Woodruff et al. 2004), although only two of the four species examined by Mencuccini et al. (2005) showed this trend. In our study, mean LMA was 181 ± 20 and $234 \pm 17 \text{ g m}^{-2}$ for seedling and old-growth control stems, respectively, indicating that for every gram of carbon allocated to foliage, the seedlings would have about 23% more leaf area. In combination with the greater rate of photosynthesis per unit leaf area in seedlings, this translates to approximately twice the foliar carbon-use efficiency (carbon assimilation per unit carbon investment in foliage) in seedlings compared with old growth stems—a large difference, but still far short of the three orders of magnitude difference in new stem biomass. In any event, the large growth enhancement of scions from old-growth trees after grafting onto seedling rootstock was unaccompanied by a significant change in LMA.

Size-mediated impacts on turgor and cell expansion Reduced photosynthesis is not the only mechanism by which size-mediated changes in water relations could impact growth. Hydraulic limitations to water flow as well as hydrostatic forces (gravity) may also induce changes in cell turgor (Marshall and Monserud 2003, Koch et al. 2004, Woodruff et al. 2004), which in turn may influence cell expansion. Woodruff et al. (2004) showed that, during the period of bud break and stem elongation in Douglas-fir, there was no osmotic regulation in expanding tissues to counter the hydrostatic gradient in water potential. Thus, nighttime turgor decreased with height at a rate close to the hydrostatic gradient, -0.01 MPa m^{-1} , during the period of leaf and stem elongation. During the day, the turgor gradient was slightly steeper. To the extent that cell expansion and tissue growth are controlled by turgor, it is plausible to consider that elongation of new stems as well as expansion of new leaves would also decrease as a linear function of the hydrostatic gradient. An appealing feature of this hypothesis is that it predicts a linear decline in cell elongation with increase in total tree height. It does not, however, explain why different species attain different maximum heights, or why maximum height and maximum height growth within a species differ at different sites.

As pointed out by Ryan et al. (2006), the turgor hypothesis requires rethinking in the context of a carbon-centric paradigm of growth limitations. It implies that rapidly-expanding stem tissues in small trees are a carbon sink; as a tree grows taller and there is less turgor for cell expansion, other tissues, such as roots, may be a stronger sink for carbon. Thus, the decline in

aboveground growth could be explained by greater allocation belowground, but the shift in allocation would itself be a consequence of reduced stem growth rather than a causal factor or an adaptive response to maintain hydraulic capacity as tree size increases (Magnani et al. 2000). Likewise, if the sink for carbon in both roots and shoots is diminished in older trees, one consequence could be a down regulation of photosynthetic capacity. Again, the change in carbon dynamics would be a consequence of lower growth rather than a cause.

In conclusion, we demonstrated that, although the dynamics of DDHG are similar to growth dynamics in animals and even populations of organisms, the underlying causes are different. We found that neither intrinsic aging nor photosynthetic reduction due to hydraulic constraints or other factors is a likely cause of DDHG.

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