

Age-related trends in red spruce foliar plasticity in relation to declining productivity

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Summary Phenotypic plasticity in needle morphology with increasing tree size and age was investigated by comparing four age classes of red spruce (*Picea rubens* Sarg.) ranging from juvenile (3–12 years old) to mature (over 100 years old). With increase in tree age there were significant increases in leaf mass per unit area (LMA), mesophyll and vascular bundle area as a percentage of total needle cross-sectional area, and stomatal density. Within the vascular bundle, both xylem cross-sectional area and tracheid lumen area increased significantly, whereas air space as a percentage of total cross-sectional area decreased. These morphological changes were associated with a significant decrease in photosynthetic capacity and stomatal conductance, and an increase in ¹³C enrichment. Although both photosynthetic capacity and whole-tree conductance decreased significantly between age classes 3 and 12 years, they did not differ between age classes 53 and 127 years, even though needle ¹³C enrichment was significantly greater in the 127-year age class. Thus there appear to be compensatory mechanisms that maintain photosynthetic capacity as trees increase in size and vascular complexity, which in red spruce and other species, may affect leaf hydraulic conductance. Although increased LMA may contribute to reduced photosynthetic capacity in red spruce, similar relationships are not seen in other conifers.

Keywords: age-related growth decline, foliar morphology, maturation, phenotypic plasticity, photosynthesis, *Picea rubens*.

Introduction

Most forest species, including red spruce (*Picea rubens* Sarg.), show a decline in growth efficiency (biomass production per unit foliar mass) beginning within the first half of their potential life span, referred to as age-related decline (Bond et al. 2007). Growth efficiency tends to increase until maximum leaf area is achieved, after which it declines gradually as the tree

ages (Ryan et al. 1997, Seymour and Kenefic 2002). Age-related decline is associated with reduced photosynthetic capacity in several species, such as ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), lodgepole pine (*Pinus contorta* Dougl. ex Loud.) and red spruce. In most trees, including red spruce and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), age-related decline in growth efficiency is accompanied by marked phenotypic changes in foliar morphology and physiology. The role of these morphological changes, if any, in adapting trees to increased size and complexity, and their contribution to age-related growth decline are at present unclear.

Plasticity in foliar morphology and physiology as a function of plant age and location within the plant is widespread among vascular plants and may facilitate adaptation to spatial and temporal variation in environmental conditions. The differentiation of sun and shade leaves is one of the best-known examples of foliar plasticity, but other types of plasticity, including some that are a function of plant development are also common. Historically, two categories of foliar plasticity have been recognized and documented (e.g., Sinnott 1960, Kozlowski 1971): heterophylly and heteroblasty.

Heterophylly is foliar plasticity in response to the immediate environment, as exemplified by the differentiation between sun and shade leaves or between aerial and aquatic foliage. In addition, heterophylly in foliar morphology produced at different times in the growing season occurs when leaves are neoformed (produced continuously; for example foliage on the long shoots of *Larix* and *Betula*).

Heteroblasty is foliar plasticity in response to the plant's developmental stage, as a result of maturation or phase change within the apical meristem, so that leaf primordia with different morphologies are produced as the plant ages and grows. Heteroblastic foliar plasticity as a function of changes in needle morphology with increasing age has been described for several spruce species, including *Picea abies* (L.) Karst. (Kull and Koppel 1987), *Picea sitchensis* (Bong.) Carr (Steele et al. 1989) and *Picea engelmanni* Parry × *Picea glauca* (Moench)

Voss \times *Picea sitchensis* (Richardson et al. 2000), pine species, including *Pinus taeda* L. (Greenwood 1984), *Pinus sylvestris* L. (Niinemets 2002), *Pinus monticola* Dougl. Ex D. Don and *Pinus ponderosa* (Marshall and Monserud 2003), eastern larch (*Larix laricina* (Du Roi) K. Koch; Greenwood et al. 1989) and Douglas-fir (*Pseudotsuga menziesii*; Bond 2000, Apple et al. 2002, Marshall and Monserud 2003). A common tendency among these conifers is for needles to increase in mass per unit area (LMA) with tree age. In *P. rubens*, LMA increases regardless of whether the foliage is sun- or shade-adapted (Day et al. 2001).

All other things being equal, increased LMA reduces water loss, because there is less leaf evaporative surface area per unit of foliar mass (Richardson et al. 2000). Leaf-specific hydraulic conductance may increase with increasing tree height (Yoder et al. 1994, Ryan and Yoder 1997, Bond 2000) to compensate for decreased conductance in the overall hydraulic system because of longer and more complex pathways (McDowell et al. 2002). Older hybrid Engelmann \times white \times Sitka spruce trees exhibit a larger ratio of vascular cylinder to needle cross-sectional area than younger trees (Richardson et al. 2000). Similarly, Apple et al. (2002) proposed that proportionally larger vascular cylinders found in old-growth *P. menziesii* increase water-transport efficiency. In conifers, the vascular bundle of the foliage is surrounded by an endodermis similar to that found in roots, but with relatively greater solute permeability than is found in roots (Wu et al. 2005).

Photosynthetic capacity may be affected by increased LMA, which has been associated with changes in the ratio of internal air space and mesophyll area to total area (e.g., Mediavilla et al. 2001, Apple et al. 2002). The effect of variation in internal anatomy on photosynthetic capacity is subject to varied and conflicting interpretations. For example, Mediavilla et al. (2001) suggested that increased mesophyll thickness provides a greater cell wall area available for CO₂ diffusion that contributes to decreased CO₂ liquid-phase resistance in mature trees. Alternatively, a more massive leaf may cause increased resistance to CO₂ diffusion through intercellular air spaces to carboxylation sites of chloroplasts because of a longer diffusion path from stomata to the surface of mesophyll cells (Parkhurst 1986, Syvertsen et al. 1995, Niinemets 2002).

In this paper, we describe heteroblastic age-related trends in the anatomy and morphology of *P. rubens* needles and their relationship to changes in photosynthetic capacity, and discuss how these changes may affect age-related decline in growth efficiency. Specifically, we sought to answer three questions. (1) Does the proportion of internal air space to total needle volume in needles increase progressively in older trees? For if it does not or if it declines, then efficiency of internal CO₂ diffusion may decline with increasing LMA, which will limit photosynthesis. (2) Does the greater LMA of older trees result in leaves with a lower proportion of photosynthetic to non-photosynthetic tissue, an effect that can be related to decreased photosynthesis? (3) How does conductive tissue area (e.g., total lumen cross-sectional area of all tracheids) within the vascular bundle vary with increased LMA as trees age? If increased LMA is unaccompanied by a proportional increase in

xylem area, then foliar hydraulic conductance will decrease, which may also decrease photosynthesis because leaf hydraulic conductance is a major bottleneck in the plant hydraulic pathway (Sack and Tyree 2005).

Materials and methods

Plant material and study site

The study site (described in detail by Day et al. 2001 and Seymour and Kenefic 2002) is located in the Penobscot Experimental Forest (PEF) in Bradley, ME (44°52'48" N, 68°39'12" W, 46 m a.s.l.), and consists of a 10-ha multi-species, multi-cohort stand (ranging from seedlings to 150-year-old trees) with a large component of *P. rubens*. The oldest trees probably originated from a disturbance, possibly harvesting, in the mid 1800s. The site has been managed under a selection system by the USDA Forest Service since the early 1950s with a 5-year cutting cycle (Seymour and Kenefic 2002). The upper canopy comprises primarily two distinct mature cohorts: mid-age (averaged at 53 years) and old (averaged at 127 years) (Table 1).

Open-growing juvenile trees (~12 years old) (Table 1) were selected from a shelterwood-regenerated stand adjacent to the multi-cohort stand described above. These individuals were in close proximity (about 200 m) to the *P. rubens* from the multi-cohort stand and exposed to direct sun for a large portion of the day, comparable with the foliage of the upper crowns of the mid-age and old trees. A younger group of juvenile trees (3 years old) established from seed collected from mature trees at the PEF study site (Table 1) were grown in pots at the University of Maine campus in Orono, ME. They were maintained at ± 4 °C in a greenhouse during the winter and under 30% interception shade cloth during the summer. The soil medium was 50% peat moss, 25% perlite and 25% vermiculite, supplemented with Osmocote fertilizer (N,P,K of 18,6,12) at a rate of 4 kg m⁻³.

Gas exchange measurements

Gas exchange measurements were made between 0800 and 1300 h in late July and August 2003 on current-year, fully expanded sun-exposed foliage with a Li-Cor LI-6400 portable photosynthesis system equipped with a 2 \times 3 cm cuvette (Li-Cor, Lincoln, NE). Shoots were exposed to a saturating irradiance of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with an LI-6400B red/blue

Table 1. Mean (\pm SD) age, diameter at breast height (DBH) and height for pot-grown juvenile, juvenile, mid-age and old trees of *Picea rubens*. Diameters of juvenile trees were measured 10 cm from the tree base.

| Age class | Age (years) | DBH (cm) | Height (m) |
|--------------------|--------------|---------------|-------------|
| Mature | 127 \pm 26 | 40 \pm 3 | 17 \pm 2 |
| Mid-age | 53 \pm 8 | 19 \pm 2 | 10 \pm 1 |
| Juvenile | 12 \pm 1 | 1.7 \pm 0.4 | 1 \pm 0.1 |
| Pot-grown juvenile | 3 | ~1 | ~0.5 |

LED light source. Photosynthesis was unrestricted by needle temperature (22–27 °C), or vapor pressure deficit (2.0 kPa) (Day 2000). Once conditions within the cuvette and photosynthetic rates stabilized, three gas exchange measurements were taken about 5 s apart and measurements were averaged. All shoots measured for photosynthesis were subsequently collected and transported to the laboratory while kept cool and moist. Gas exchange measurements including photosynthesis, stomatal conductance and internal CO₂ concentration were made on needles collected from six trees of each age class. Needles of each tree were sampled from three sun-exposed shoots on different branches on the south side of the upper third of the crown. Gas exchange measurements for each age class were made on separate days during the same time period to avoid diurnal extremes in light and temperature, which caused more variation in measured photosynthetic rates than measurements made on successive days at about the same time.

Morphology and anatomy

Needles used for gas exchange measurements were removed from the shoots and their projected area estimated with a high-resolution scanner equipped with WinSeedle software (Regent Instruments, Quebec, Canada), and photosynthetic rates were expressed on an area basis. Needle dry mass was recorded (after desiccation at 60 °C for 2 days), and specific leaf area (projected leaf area/leaf dry mass) was calculated.

A subsample of needles was killed and fixed in formalin:acetic acid:alcohol (FAA) as described by Berlyn and Miksche (1976). The ends of the needles were removed with a razor blade while the needles were immersed in FAA to allow the fixative to penetrate more readily. Needles in FAA were placed in a vacuum dessicator for about 10 min to remove air and promote subsequent infiltration, and then stored at room temperature.

The needles were dehydrated through a graded ethyl alcohol and tertiary butyl alcohol series and embedded in paraffin as described by Berlyn and Miksche (1976). After exposing the ends of the needles, the paraffin blocks were soaked overnight in water, glycerol, dimethyl sulfoxide and liquid detergent (88:10:1:1, v/v) at 35 °C to soften the cell walls for improved sectioning (Richardson et al. 2000). Needle cross sections near the mid-point of 18 needles were cut at 10 µm on a rotary microtome, and ribbons were mounted on slides with Haupt's adhesive (Berlyn and Miksche 1976). Slides were rehydrated and stained with Safranin O and Fast Green and cover slips attached with Permunt mounting medium.

Digital images of cross sections were made at 25, 100 and 400× with a SPOT RT color digital camera mounted on a Zeiss Axioscop microscope. We measured needle cross-sectional area, needle length and width, cross-sectional area of the vascular bundles, area of xylem and phloem within the vascular bundles, resin canal area and the perimeter of the entire needle cross section with the public domain Scion Image program (U.S. National Institutes of Health, <http://rsb.info.nih.gov/nih-image>). Percent mesophyll area was calculated by sub-

tracting the area of the vascular bundle and the area of the resin canals from the total cross-sectional area. The proportion of mesophyll was adjusted for internal air space for each age class (discussed below).

Four representative tracheids were selected across the width of the xylem bundle, and cross-sectional areas inside (lumen area) and outside the cell wall (including the wall of the adjoining tracheids) were measured on each tracheid. The area occupied by the cell wall in each tracheid was estimated as half the difference between lumen area and area outside the cell wall. Lumen diameter was estimated as the mean of two diameters measured perpendicular to one another.

Stomatal density was determined by counting stomata on the adaxial and abaxial sides of the needle at 50× magnification with the aid of a Wild MP5 dissecting microscope.

Internal air space

Internal air space of red spruce needles was determined in late July and August 2003 in current-year needles on 7- to 10-cm-long shoots collected from the trees sampled for gas exchange measurements. The total volume per needle was calculated by placing the shoot in a 10-ml graduated cylinder and filling the cylinder with water. The shoot was then removed and its volume estimated from the displaced volume of water. Next, all needles were removed from the shoot and the same method used to determine the volume of the twig. The difference between shoot and twig volume gave the volume of the needles.

Needle internal air space was estimated as described by Yokoi and Kishida (1985) and Koike (1988) for the evergreen woody plant *Aucuba japonica* Thunb.:

$$V_a = V_f - \frac{m_f - m_d}{\rho_w} - \frac{m_d}{\rho_d} \quad (1)$$

where V_a = air space in needles (ml), V_f = volume of fresh needles (ml), m_f = fresh mass (g); m_d = dry mass (g), ρ_w = density of water (g ml⁻¹) and ρ_d = density of dry matter (g ml⁻¹). Yokoi and Kishida (1985) determined that the density of foliar dry matter had a mean value of 1.45 g (range 1.4–1.5; Yokoi and Kishida 1985, Koike 1988).

Tree-level hydraulic conductance

Tree-level conductance (k_{tree}) was estimated on six trees from age classes 12, 53 and 127 years. Transpiration rates (E) were measured with an LI-6400 infrared gas analyzer, and water potential (Ψ) was measured at predawn and at noon with a pressure chamber (PMS Instrument Co., Albany, OR). Predawn water potential was used as a proxy for soil water potential. Although nighttime transpiration may result in an absolute difference between predawn foliar and soil water potentials, in this contiguous population, any bias should be uniform across age classes. In support of this assumption, gravity-corrected predawn water potentials were less than 0.1 MPa and not significantly different across age classes. Tree-level conductance was calculated as:

$$k_{\text{tree}} = \frac{E}{\Delta\Psi} \quad (2)$$

where E = transpiration ($\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) and $\Delta\Psi = \Psi_{\text{noon}} - \Psi_{\text{predawn}}$ corrected for gravity (0.01 MPa m^{-1}).

Stable carbon isotope analysis

Current-year needles were harvested in August 2004 from 10 trees in each age class, frozen in liquid nitrogen and stored at -20°C until dried at 60°C and ground to powder in a Wiley Mill. Cellulose was extracted by the Jayme-Wise method as described by Yoder et al. (1994). Carbon isotope ratios were measured with a Finnigan/MAT 251 isotope ratio mass spectrometer (Thermo Electron Corporation, Waltham MA). Ratios are presented in standard delta notation relative to those in Pee Dee Belemite limestone.

Statistical analysis

Three cross sections per slide from the mid-point of a single needle from each shoot were measured and the means used for analysis. This was done for all anatomical parameters except tracheid lumen diameter and cell wall thickness, where a subsample of four representative tracheids selected across the width of the xylem bundle was measured in each cross section.

The effects of age class on the physiological, morphological and anatomical variables were tested by single-factor analysis of variance (ANOVA). Means were separated by Tukey's Studentized Range (HSD) test, and homogeneity of variances was assessed by Levines test. To meet the assumptions of ANOVA, log-transformed data were used for needle cross section, width, xylem and the ratio of phloem area to mesophyll area, and square root transformations were used for percent mesophyll area and the ratio of xylem to mesophyll.

Results

Gas exchange, whole-tree hydraulic resistance and isotope discrimination

Gas exchange data obtained between 0800 and 1300 h (the period of peak daily photosynthesis) in late July and early August 2003 are shown in Table 2. At that time, photosynthesis declined with tree age, on both an area and mass basis. Trees in the 3- and 12-year age classes had similar photosynthetic rates

on an area basis. The field-grown trees, averaging 12 years in age, exhibited a slight but statistically significant decrease in photosynthetic rate on a mass basis compared with the 3-year-old pot-grown trees. Although the field-grown trees were 3 times older than the pot-grown, they were only about twice as tall. Both the 3- and 12-year age class trees exhibited much higher photosynthetic rates on both an area and a mass basis than trees in the 53- and 127-year age classes. However, differences in means were not statistically significant between the 53- and 127-year age classes. Variation in stomatal conductance was similar to that in photosynthetic rate among age classes. Therefore, in terms of gas exchange, two distinct tree groups were apparent. The first comprising the 3- and 12-year age class; the second comprising the 53- and 127-year age classes. There were few statistical differences in gas exchange characteristics within these groups. On a mass basis, net photosynthesis decreased by 59% between the first and second groups, whereas stomatal conductance decreased by 43%.

There was a highly significant increase in ^{13}C enrichment with increasing age amounting to a difference of 3.2‰ between the youngest and oldest age classes (Table 2), comparable with, but somewhat higher than, the trend with age reported for Douglas-fir (Bond et al. 2007). The significant increase in ^{13}C enrichment between the 53- and 127-year age classes measured in 2004 was not associated with a decrease in photosynthesis, but the photosynthesis measurements shown in Table 2 were made one year before the ^{13}C enrichment measurements. Photosynthesis was also measured in the summer of 2004 on the same trees, and the trends between age classes were the same as in 2003 (Adams 2006). Nonetheless ^{13}C enrichment is not only a function of current photosynthesis, but may reflect ratios of photosynthesis to stomatal conductance during the period of foliar expansion and incorporation of carbon fixed and stored in the previous year. Although trends in age class differences were the same in both years, photosynthetic rates were higher in 2003 than in 2004.

Whole-tree hydraulic conductance showed significant declines of 59 and 54% between the 12-year and the 53- and 127-year age classes, respectively. Conductance was slightly but not significantly greater in the oldest largest trees than in the 53-year-old trees.

Morphology and anatomy

Needle LMA increased steadily with age, and the means of all

Table 2. Mean (\pm SE) photosynthesis, stomatal conductance, whole-tree conductance (k_{tree}) and $\delta^{13}\text{C}$ of *Picea rubens* trees of different age classes. The significance of the effect of age was determined by univariate ANOVA. For each variable, means followed by different letters differ significantly ($P < 0.05$).

| Variable | 3 years | 12 years | 53 years | 127 years | <i>P</i> |
|--|-------------------|---------------------|---------------------|---------------------|----------|
| Net photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | 13.7 \pm 0.8 a | 13.0 \pm 0.5 a | 7.6 \pm 0.5 b | 8.6 \pm 0.6 b | 0.0001 |
| Net photosynthesis ($\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$) | 66.2 \pm 4.2 a | 55.8 \pm 1.6 b | 25.9 \pm 2.2 c | 23.8 \pm 1.7 c | 0.0001 |
| Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) | 0.25 \pm 0.02 a | 0.19 \pm 0.02 ab | 0.11 \pm 0.01 c | 0.14 \pm 1.5 bc | 0.0001 |
| k_{tree} ($\mu\text{mol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$) | – | 3.9 \pm 0.5 a | 1.6 \pm 0.2 b | 1.8 \pm 0.3 b | 0.0186 |
| $\delta^{13}\text{C}$ | – | –27.74 \pm 0.30 a | –25.61 \pm 0.27 b | –24.52 \pm 0.21 c | 0.0001 |

age classes differed significantly from one another. The increased LMA is in turn a function of needle length, width, perimeter and total cross-sectional area, all of which increased with tree age (Table 3, Figure 1). These characteristics showed the greatest change between the 12- to 53-year-old age classes. Needle width and cross-sectional area showed the most dramatic increases, 100 and 160%, respectively, between the 3- and 127-year-old age classes. Internal air space exhibited a significant steady decline with increasing age, decreasing from 29 to 21% of total cross-sectional area between the 3- and 127-year-old age classes. The proportion of the cross-sectional needle area occupied by mesophyll increased from 61 to 66% with increasing tree age (Figure 2).

The cross-sectional area of the vascular bundle increased steadily with increasing tree age, and the overall increase of 262% between the 3- and 127-year-old age classes was proportionally much greater than the increase in total needle cross-sectional area. Within the vascular bundle, phloem area increased 132% and xylem area increased 229%. The lumen diameter of individual tracheids increased by 19% with increasing tree age, resulting in a 270% increase in total tracheid lumen area within the bundle between the youngest and oldest age classes (Table 3). The differences between the two youngest and two oldest age classes were not significantly different. In addition, the ratio of xylem area to perimeter increased 94% between the 3- and 127-year-old trees (Table 3). The proportional relationships of the areas of the mesophyll, air space, resin canals and vascular bundle to total needle cross-sectional area are shown in Figure 2. These relationships exhibited minor but significant changes with increased tree age: the proportional area of the vascular bundle increased, air space area decreased, mesophyll area increased and the area of the resin canals remained constant.

Discussion

We have shown that, in a red spruce natural stand, the decline in photosynthesis with tree age is closely related to phenotypic plasticity in several foliar morphological parameters, includ-

ing LMA, needle width and cross-sectional area, and xylem cross-sectional/lumen diameter. These changes were accompanied by a decrease in whole-tree hydraulic conductance. Some of these changes may directly contribute to reduced photosynthetic capacity, whereas others may compensate for decreased hydraulic conductance.

Internal resistance to gas exchange

Not only does the total cross-sectional area of needle mesophyll tissue increase with increased LMA, but it increases as a percentage of cross-sectional needle area. Thus the path length from stomata to the innermost mesophyll cells is greater in more massive leaves, which combined with a decline in air space (as a proportion of total cross-sectional area) between these cells, must increase resistance to CO₂ diffusion within the needle. Decreased air space would also presumably decrease the surface area of mesophyll available for gas exchange. Given that resistance to gas exchange within the leaf can be described as $R_{\text{leaf}} = R_s + R_a + R_l$ (where R_s is stomatal resistance, R_a is resistance to diffusion from the stomata through intercellular air space and R_l is the liquid-phase resistance at the cell wall/plasma membrane), the anatomical changes described here may increase the contribution of all three components to overall gas exchange resistance. The slight but significant increase in mesophyll area as a percentage of needle cross-sectional area, plus the significant increase in stomatal density with age may be adaptations that partially offset decreased gas phase conductance caused by an overall increase in R_{leaf} with age.

When scions from mature red spruce trees are grafted on juvenile rootstock, they retain the increased LMA and reduced photosynthetic capacity of the donor trees for several years (Rebbeck et al. 1992, Day et al. 2001). In other conifer species, however, increased LMA is not always correlated with a decline in photosynthetic capacity. Even though older Douglas-fir trees exhibit increased LMA, photosynthetic rates do not decline (McDowell et al. 2002). Grafting studies on eastern larch (Hutchison et al. 1990) and Douglas-fir (Bond et al. 2007) demonstrate retention of increased LMA in scions

Table 3. Mean (\pm SE) morphological and anatomical characteristics of current-year needles of *Picea rubens* trees of different age classes. Internal anatomical features were measured from cross sections. Abbreviation: LMA = needle leaf mass per unit area. The significance of the affect of age was determined by univariate ANOVA. For each variable, means followed by different letters differ significantly ($P < 0.05$).

| Measurement | 3 years | 12 years | 53 years | 127 years | <i>P</i> |
|--|-------------------|-------------------|--------------------|--------------------|----------|
| LMA (g m ⁻²) | 207.9 \pm 3.9 a | 230.4 \pm 5.5 b | 300.3 \pm 5.46 c | 364.6 \pm 5.3 d | 0.0001 |
| Stomatal density (no. mm ⁻²) | 33.1 \pm 1.4 a | 33.0 \pm 1.6 a | 41.3 \pm 2.2 b | 43.6 \pm 3.1 b | 0.0002 |
| Width (μ m) | 663 \pm 10 d | 805 \pm 23 c | 1207 \pm 26 b | 1328 \pm 33 a | 0.0001 |
| Length (μ m) | 725 \pm 14 c | 852 \pm 25 b | 952 \pm 23 a | 997 \pm 29 a | 0.0001 |
| Perimeter (μ m) | 2083 \pm 25 d | 2496 \pm 56 c | 3248 \pm 515 b | 3520 \pm 64 a | 0.0001 |
| Phloem (μ m ²) | 1567 \pm 111 b | 1761 \pm 121 b | 3176 \pm 1456 a | 3636 \pm 174 a | 0.0001 |
| Xylem (μ m ²) | 1042 \pm 45 d | 1402 \pm 81 c | 2821 \pm 102 b | 3426 \pm 160 a | 0.0001 |
| Xylem/perimeter | 0.50 \pm 0.02 c | 0.56 \pm 0.02 c | 0.87 \pm 0.02 b | 0.97 \pm 0.04 a | 0.0001 |
| Tracheid lumen diameter (μ m) | 4.18 \pm 0.13 c | 4.77 \pm 0.08 b | 5.30 \pm 0.11 a | 4.99 \pm 0.09 ab | 0.0001 |
| Lumen area (μ m ²) | 387 \pm 20 b | 531 \pm 30 b | 996 \pm 42 a | 1121 \pm 62 a | 0.0001 |

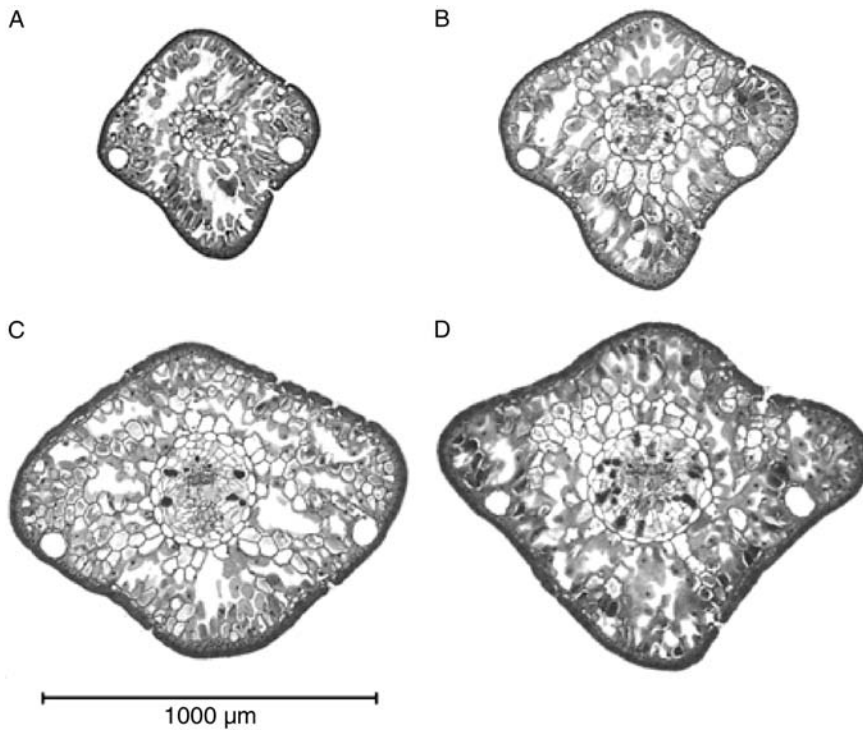


Figure 1. Needles cross sections of (A) 3-year, (B) 12-year, (C) 53-year and (D) 127-year age classes of *Picea rubens* trees. Note increasing cross-sectional areas of both needle and vascular bundle.

from older trees several years after grafting onto seedling rootstock, but reduced photosynthetic capacity was not observed. In larch, photosynthetic capacity increased with age, which in turn, was positively correlated with a significant increase in total chlorophyll concentration, but stomatal conductance did not vary with age. Therefore increasing chlorophyll concentration in larch foliage with age may have compensated for increased internal resistance to CO₂ movement in

more massive needles. There may be other forms of compensation as well. Marshall and Monserud (2003) proposed that increased LMA with increasing tree size is a function of a gravity-induced decrease in water potential with increased height, but this contention is not supported by the numerous grafting studies cited above, where the increase in LMA as a function of age is maintained for several years in scions grafted to juvenile rootstock.

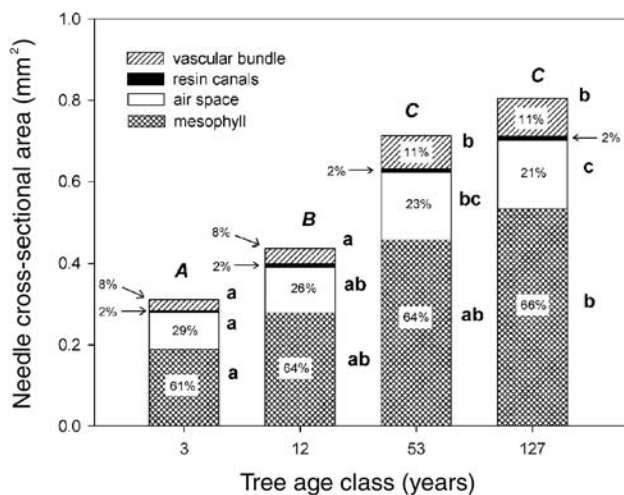


Figure 2. Amount and percentage of total needle cross-sectional area occupied by the vascular bundle, resin canals, air space and mesophyll from each age class of *Picea rubens*. Different uppercase letters indicate significant differences ($P < 0.05$) among age classes for total needle cross-sectional area, and different lowercase letters indicate significant differences ($P < 0.05$) among age classes for separate anatomical regions of the total cross section.

Hydraulic compensation

The effects of age-associated changes in foliar morphology on photosynthetic capacity are confounded with the effects of changes in hydraulic conductance due to the increased path length and complexity between roots and shoots as a tree increases in size. If hydraulic conductance is mainly a function of path length between roots and leaves, then a steady decrease would be expected with increasing age and size. In the 53-year age class, tree-level hydraulic conductance was less than half that in the 12-year age class. However, there was no significant difference in whole-tree hydraulic conductance (corrected for gravity) between the 53- and 127-year age classes, despite trees in the latter class being almost twice as tall as those in the former class (Table 1).

There was no significant difference in stomatal conductance or photosynthesis on an area basis between the 53- and 127-year age classes. One explanation for the similar photosynthetic capacity between these age classes could be hydraulic conductance compensation in the older age class relative to the younger, such as an increase in stem, branch or needle vascular conductance. Phillips et al. (2002) report similar results for Douglas-fir tree height classes of 15, 32 and 60 m. The first two height classes were similar in age to our first two age

classes, whereas the 60-m height class was more than three times as old as our 127-year age class. The Douglas-fir trees were much larger (more than three times as tall) across all height classes. Leaf-specific hydraulic conductance for the 15-m height class was twice that for the 32- and 60-m height classes, whereas the latter two were almost identical. From the data presented by Phillips et al. (2002), we calculated tree-level hydraulic conductances that exhibit trends comparable to those shown for red spruce in Table 2. Whole-tree conductance for the 32- and 60-m height classes was almost identical, but was slightly less than half the value for the 15-m size class. In both experiments, the oldest size class was twice as tall as the middle size class, but there was no difference in whole-tree conductance.

Even if measurements of predawn water potential reflect slight water stress, all trees would experience the same edaphic conditions so any underestimates of conductance would be similar across age classes. Also, even though accurate estimates of E may be difficult to obtain because of environmental variation and disruption of boundary layers during measurements, these factors would equally affect whole-tree conductance estimates for the 53- and 127-year age classes. The similarity between values for these age classes is the most important observation. Thus, our study and that of Phillips et al. (2002) present strong and complimentary evidence for compensatory increases in hydraulic conductance in large trees. But what is the nature of this compensation and where within the vascular system does it occur?

Mencuccini et al. (1997) and McDowell et al. (2002) examined stem hydraulic conductance in Scots pine and Douglas-fir, respectively, and neither found much evidence for hydraulic compensation in the main stem. In Scots pine, hydraulic conductance as a function of tracheid diameter increased significantly between age 7 and 15 years but remained constant thereafter, suggesting that hydraulic conducting capacity is redundant in the main stem, and that compensation in the form of increased hydraulic conductance occurs primarily in the upper parts of the tree. In support of this suggestion, Mencuccini et al. (1997) found that specific conductivity at the base of the live crown and in the upper crown increased steadily with age (7 to 60 years) in Scots pine. Tracheid length at the base of the live crown also increased with age, reducing the frequency of flow through bordered pits between tracheids. Further evidence for hydraulic compensation in the upper parts of the tree crown is provided by our observation that the enlargement of the vascular bundles in the needles of older age classes was accompanied by a significant, almost twofold, increase in lumen cross-sectional area of xylem tracheids. Takemoto and Greenwood (1993) reported an increase in vascular bundle area and xylem tracheid lumen area with increased age in long shoot foliage of grafted scions from four age classes of eastern larch trees. Given that the Hagen-Poiseuille law predicts that the rate of water flow is directly proportional to the fourth power of the diameter of the conducting element (Tyree et al. 1994), these findings represent strong evidence that increased leaf-specific hydraulic compensation may occur in needles of older trees.

Gas exchange and water-use efficiency

There was a steady significant increase in ^{13}C enrichment with tree age in the 12, 53 and 127-year age classes, even though stomatal conductance, stomatal density and photosynthesis did not differ between the two oldest age classes. Because our estimates of whole-tree conductance were corrected for gravity, the similar values for the 53- and 127-year age classes do not reflect the reduction in realized water potential gradients due to a gravitational potential of 0.01 MPa m^{-1} , that might reduce flux and increase water stress. Other factors affecting ^{13}C enrichment may involve the carbon fixation process itself. The similar photosynthetic rates of the 53- and 127-year age classes despite significantly higher ^{13}C enrichment in the latter may indicate a more conservative water-use strategy, for reasons that are as yet unclear. But it is also possible that ^{13}C enrichment occurred before or after the measurements of photosynthetic capacity reported here. Amundson et al. (1992) demonstrated a 32% decline in photosynthetic capacity over the growing season in first-year foliage of field-grown red spruce trees ranging from 15 to 150 years old. They also showed that photosynthetic capacity remains high in red spruce as late as December, so that with favorable temperatures some photosynthesis may occur while the ground is partially frozen, resulting in increased water stress leading to partial stomatal closure.

In conclusion, we have provided additional evidence of heteroblastic foliar plasticity in red spruce that allows foliage in the upper parts of increasingly large trees to adapt to an increasingly stressful environment. More massive leaves are more resistant to desiccation, and the increased area of conducting xylem compensates for reduced hydraulic conductance. Given that the estimated lumen volume of needle tracheids is only about 0.2% of total needle volume, increased water storage capacity within the xylem is probably negligible.

Although increased LMA may contribute, in part, to a decline in photosynthetic capacity in red spruce, we cannot conclude that the decrease in photosynthetic capacity in older age classes contributes directly to age-related decline in productivity. Although photosynthetic capacities of the 53- and 127-year age classes were identical, trees in the 53-year age class were the most productive per unit of foliar biomass of all three classes, whereas trees in the oldest age class exhibited only 50% of the productivity (based on foliar biomass) of those in the 53-year age class (Day et al. 2001). This strongly suggests that biomass production in older age classes is not primarily a function of photosynthetic capacity. Bond et al. (2007) questioned the significance of reduced photosynthetic capacity in age-related growth decline, because it may account for only a small proportion of the reduced growth in mature branches of Douglas-fir. Day et al. (2002) proposed that age-related growth decline may be ontogenetic, caused by an intrinsic, genetically-regulated decline in meristem vigor with increased size and age.

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