

# Age-related changes in foliar morphology and physiology in red spruce and their influence on declining photosynthetic rates and productivity with tree age

MICHAEL E. DAY, MICHAEL S. GREENWOOD and ALAN S. WHITE

Department of Forest Ecosystem Science, University of Maine, 5755 Nutting Hall, Orono, Maine 04469-5755, USA

Received September 20, 2000

**Summary** The contribution of changes in meristem behavior to age-related decline in forest productivity is poorly understood. We studied age-related trends in needle morphology and gas exchange in a population of red spruce (*Picea rubens* Sarg.) growing in a multi-cohort stand where trees ranged from first-year germinants to trees over 150 years old, as well as in grafted scions from these trees. In the field study, age-related trends in foliar morphology were determined in six cohorts ranging in age from 2 to 120 years, and differences in gas exchange characteristics were compared between 60- and 120-year age classes. In a common-rootstock study, scions from trees representing 20-, 60-, and 120-year cohorts were grafted onto juvenile rootstock and maintained for three growing seasons, after which morphological and physiological foliar attributes were evaluated.

The field study revealed significant age-related trends in foliar morphology, including decreasing specific leaf area, and increasing needle width, projected area, and width/length ratio. Similar trends were apparent in foliage from the grafted scions. Both *in situ* foliage and shoots of grafted scions from the oldest cohort showed significantly lower photosynthetic rates than their counterparts from younger trees; however, differences in stomatal conductance and internal CO<sub>2</sub> concentrations were not significant. These results suggest that: (1) foliage of red spruce exhibits age-related trends in both morphology and physiology; (2) age-related decreases in photosynthetic rates contribute to declining productivity in old red spruce; (3) declines in photosynthetic rates result from non-stomatal limitations; and (4) age-related changes in morphology and physiology are inherent in meristems and persist for at least 3 years in scions grafted to juvenile rootstock.

*Key words:* carbon balance, gas exchange, maturation, ontogeny, sink strength.

## Introduction

The production of biomass by both individual trees and forest stands decreases substantially with age, and this decline commences well before trees reach the midpoint of their average lifespan (Assmann 1970). Age-related reduction in biomass

accumulation is an important consideration in mechanistic models that predict forest growth, and affects the capacity of forests as carbon sinks. With the current impetus toward silvicultural approaches that employ uneven-aged systems and extended rotations, an understanding of the physiological basis for age-related decline in the productivity of dominant species has become increasingly important. Additionally, understanding this component of productivity is crucial for quantifying and manipulating carbon fluxes in forest ecosystems and their influence on global CO<sub>2</sub> cycles.

Although both stand-level and tree-level factors may contribute to age-related decline in productivity, those operating at the scale of individual trees are likely to play a major, if not principal, role (Ryan et al. 1997). Several contrasting hypotheses have been advanced to establish a physiological basis for age-related declines in individual trees. Until the past decade, the textbook explanation centered on a declining ratio of photosynthesis to respiration (e.g., Waring and Schlesinger 1985). This hypothesis was questioned by Ryan and Waring (1992), and subsequent studies (e.g., Murty et al. 1996) indicated that differences in carbon allocation to maintenance respiration between mid-aged and old trees were insufficient to account for observed age-related declines in growth. However, Hunt et al. (1999) provided evidence that respiration plays a significant role in age-related decline in balsam fir (*Abies balsamea* (L.) Mill.) forests of eastern Canada. Other mechanisms based on carbon allocation (i.e., increased carbon allocation to fine roots, defensive compounds, and sexual reproduction) have been proposed, but little quantitative evidence has been advanced to support them (reviewed by Ryan et al. 1997).

An accumulating body of circumstantial evidence supports a model predicated on lower photosynthetic rates (or integrated photosynthesis) in older trees (Kull and Koppel 1987, Yoder et al. 1994, Hubbard et al. 1999, Bond 2000, Richardson et al. 2000). Yoder et al. (1994) proposed that lower photosynthetic rates in older trees are caused by reduced stomatal conductance, which, in turn, results from lower hydraulic conductivity in their longer (or more complex) hydraulic pathways. Although circumstantial evidence in support of the hydraulic limitation model has been advanced (Hubbard et al. 1999, Bond 2000), attempts to manipulate photosynthetic rates experimentally in younger trees by decreasing hydraulic

conductivity or to increase photosynthetic rates in older trees by reducing transpirational demand have often yielded ambiguous results (e.g., Hubbard et al. 1999, Brooks et al. 2000). Additionally, the universal applicability of the hydraulic limitation model to all tree species in all environments has been questioned on theoretical grounds (Becker et al. 2000) and on the basis of carbon balance modeling (Hunt et al. 1999). The current state of knowledge suggests that either age-related hydraulic limitations do not act on photosynthesis in a simple mechanistic way or that other factors play a key role in age-related decline in some species.

Little is known about the influence of genetically based maturational changes in tree meristems on age-related declines in productivity. Such changes could be purely maturational (related only to tree age) or mediated through physiological pathways related to tree size or external environmental cues. Dramatic changes in morphological and physiological attributes of foliage, including photosynthetic capacity, have been described for numerous species during their early development and have been attributed to different challenges to growth and survival between juvenile and reproductively mature life-stages, a transition referred to as phase-change (Greenwood 1984, Hackett 1985, Rebeck et al. 1992, Greenwood and Hutchison 1993). Hutchison et al. (1990), Woo et al. (1994), Emebiri et al. (1998) and others have implicated a genetic basis for ontogenetic changes by identifying differential patterns of gene expression related to juvenile and reproductively mature life-stages. Far less is known about trends in foliar attributes as trees age beyond reproductive maturity, or "mid-age," to "old-growth" status, and this transition is not made manifest by a rapidly appearing marker such as the onset of reproductive competence that indicates the juvenile to reproductively mature phase-change (Bond 2000). However, there is evidence that supports the concept of continuing change. Richardson et al. (2000) reported that changes in foliar morphology and gas exchange attributes continue past mid-age in hybrid Englemann  $\times$  white  $\times$  Sitka spruce (*Picea engelmanni* Parry ex Engelm.  $\times$  *glauca* (Moench) Voss  $\times$  *sitchensis* (Bong) Carrière). Similar trends in foliar attributes have been described for Norway spruce (*Picea abies* (L.) Karst) (Kull and Koppel 1987), and in needle morphology of Sitka spruce (Steele et al. 1989). However, none of these studies directly addressed ontogenetic changes in meristematic tissue as a contributing mechanism.

In this paper we have evaluated trends in foliar morphology and physiology that continue beyond mid-age in red spruce (*Picea rubens* Sarg.) and provide evidence that those trends are related to ontogenetic changes in meristems. Red spruce is a major component of several cover-types in forests of north-eastern North America, and its long lifespan (> 200 years) and propensity to occur naturally in multi-cohort stands make it ideally suited as a model species for investigation of long-term maturational change. A study in which scions from juvenile (2-year-old) and mature (~ 70-year-old) red spruce were grafted onto common rootstock (Rebeck et al. 1992) showed that maturation-related changes in meristems of red spruce persist for at least 2 years after grafting. Rebeck et al. (1992)

reported that scions from mature trees exhibited lower specific leaf area, stomatal conductance, and maximum photosynthetic rates than those from juvenile donors.

We conducted an investigation to evaluate a possible ontogenetic basis for age-related decline in productivity of red spruce by addressing four questions. (1) Is there evidence that productivity in red spruce growing in multi-cohort stands declines with tree age? (2) Is there a reduction in photosynthetic capacity associated with age-related decline in productivity? (3) Do trends in foliar morphological attributes continue beyond reproductive maturity or mid-age? (4) Are maturation-related changes in meristem behavior, suggesting differential gene expression, associated with age-related changes in foliar attributes?

## Methods and materials

### Study site and population

This study focused on a population of red spruce in a multi-cohort stand of approximately 10 ha (Compartment C16) at the Penobscot Experimental Forest (PEF), Penobscot County, Maine. Use of a multi-cohort population minimized site effects, including potential differences in nutrient availability related to stand age (Grier et al. 1981). The USDA Forest Service has managed C16 under a selection system with a 5-year cutting cycle since the early 1950s (Seymour and Kenefic 1998), and cohorts ranging from germinants to trees approximately 150 years old are present.

The upper canopy of C16 is dominated by two mature cohorts (Table 1a). The oldest originated following disturbance, perhaps harvesting, in the middle of the 19th century and has a mean age at breast height (1.3 m) of 117 years (nominal 120-year age class). The younger cohort, with a mean age of 54 years at breast height (nominal 60-year age class), was likely released by early Forest Service entries. Harvesting systems common when the oldest cohort was released were diameter-limit cuts that selectively removed the largest individuals

Table 1. Characteristics of the studied red spruce population. (a) Mean ( $\pm$  1 SE) age (measured at 1.3 m height), diameter at breast height (DBH), height and live crown ratio ( $n = 58$ ) for the two upper canopy cohorts from Penobscot Experimental Forest selection stand C16 used for field photosynthesis measurements, foliar morphology, and as scion donors. (b) Mean age, DBH, height and live crown ratio ( $n = 32$ ) for the subset of the two upper canopy cohorts selected for field gas exchange measurements, which excluded trees between 25 and 30 cm DBH.

Age (years)	DBH (cm)	Height (m)	Live crown ratio
<i>(a) Field population</i>			
53.8 $\pm$ 26.4	15.4 $\pm$ 4.6	10.6 $\pm$ 3.2	0.56 $\pm$ 0.15
117.1 $\pm$ 13.2	39.7 $\pm$ 2.6	20.3 $\pm$ 2.8	0.55 $\pm$ 0.15
<i>(b) Gas exchange sampling population</i>			
57.7 $\pm$ 21.3	16.5 $\pm$ 3.0	11.5 $\pm$ 2.3	0.57 $\pm$ 0.14
116.5 $\pm$ 14.7	37.5 $\pm$ 2.7	20.7 $\pm$ 1.2	0.53 $\pm$ 0.13

(Seymour 1992), resulting in disturbance patterns that would closely resemble those of the single-tree to small group selection system that released younger cohorts. Thus, it is unlikely that the earlier harvest favored regeneration of trees with different growth characteristics or foliar attributes to those of more recently released cohorts.

#### *Growth efficiency*

Growth efficiency was evaluated on a random sample ( $n = 58$ ) of upper canopy individuals (defined as those trees with > 60% of south-facing crown exposed to direct sunlight for at least 6 hours per day during summer). Measurements of diameter at breast height (DBH), height and crown length were made on sample trees in the summer of 1995. Two opposing increment cores (from N and S aspects) were taken at breast height and the extent of sapwood marked on the fresh cores by observing the change in transparency. Ages, radial growth increments and sapwood radii were determined with an optical core reading system (Measuchron, Bangor, ME) coupled to a computer using the PJKv5DOS software (P.J. Krusic, Lamont-Doherty Geological Observatory, Palisades, NY). Total leaf mass was calculated from regressions of leaf mass on sapwood area at breast height developed by Maguire et al. (1998) for red spruce growing in the PEF. For individual trees, total aboveground biomass for 1989 and 1994 was calculated from locally developed equations for biomass versus diameter given in Young et al. (1980). Aboveground annual net production was determined as the mean annual change in aboveground biomass for the 5-year period 1989–1994, and foliar efficiency (Waring 1983) as the mean annual aboveground biomass production per unit foliar mass. This model assumes that leaf mass remained the same over that period. The assumption is supported by the relatively consistent longevity of red spruce foliage (Blum 1990) and the lack of reports of defoliating disturbances during that period in the PEF (USDA Forest Service records). Correlations between productivity and age were assessed using Pearson  $r$ -values.

#### *Field gas exchange and water potential*

In the summer of 1995, 16 trees from each of the two dominant canopy cohorts were randomly selected for gas exchange measurements (Table 1b). A small number of trees with DBH between 25 and 30 cm were eliminated from the sampling population to better separate the upper canopy age classes. Two trees from each cohort were randomly assigned without replacement to each of eight sampling dates (Julian dates 172, 173, 201, 206, 208, 233, 235 and 237). On each sampling date, the order of sampling was randomized, and the sequence repeated twice between 1000 and 1200 h EST, providing two subsamples from each tree. Samples consisting of a terminal branch shoot from the southern aspect of the upper third of the crown were harvested with a shotgun and taken to a portable lab within 1 min. A section of shoot with 1-year-old foliage was excised for gas exchange measurements, and the remaining sample was wrapped in plastic film and placed on ice in an insulated box for subsequent water potential measurements.

Gas exchange measurements were made with a Li-Cor 6200 closed-type photosynthesis system with a 0.25-l cuvette (Li-Cor, Inc., Lincoln, NE). Calibrations for flow meter, IRGA zero points, and CO<sub>2</sub> span values were made before each measurement series. Light from a halogen lamp was filtered through water to reduce long-wave infrared input, while maintaining saturating irradiance (1000–1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Day 2000). Needle temperatures in the cuvette were maintained near 25 °C, and at a vapor pressure deficit (VPD) of < 1.5 kPa, within the optimum range for photosynthesis for this species (Day 2000). Three sequential measurements were made within 1–2 min, and the mean calculated. Preliminary measurements indicated that excision of branches did not affect photosynthetic rates for at least 6–10 min. All gas exchange samples were returned to the laboratory, dried at 65 °C for at least 72 h and weighed. Area-based photosynthetic rates were calculated based on specific leaf areas for individual leaf age classes. Xylem water potential was measured within 10 min of harvest by the pressure bomb technique (Koide et al. 1989). Pressurization was with N<sub>2</sub> and endpoints were observed with a stereoscope. For each sample, three trials were made and the mean calculated. Dawn water potential measurements were made on all trees, except on Day 233, as early as light conditions permitted. Samples of 1-year-old foliage from five trees of each cohort, five subsamples from each tree, were tested for total N content. After drying to constant mass, samples were ground in a Wiley mill to pass a 20-mesh screen and analyzed with a Leco C-N Analyzer (Leco Corp., St. Joseph, MI) at the Maine Testing Laboratory, University of Maine.

Results were evaluated by analysis of variance with the SAS statistical software package (SAS Institute, Cary, NC). A randomized block design was employed, with date as the blocking factor, to account for variance due to phenological and climatic factors. Concordance of analyses with the assumptions of ANOVA was evaluated with the Durban-Watson statistic for normality and Levene's test for heteroscedasticity (Snedecor and Corcoran 1989). As a result, log-transformed data were used for the ANOVA of stomatal conductance.

#### *Foliar morphology*

To minimize confounding effects of foliar adaptation to the light environment, separate analyses were carried out on shade- and sun-adapted foliage. Shade foliage was collected from 15 randomly selected individuals in each of six nominal cohorts (2, 5, 10, 20, 60 and 120 years). Ages of the first four classes were determined by counting whorls, and those of the older classes by cores taken at breast height. The two oldest classes are the equivalent of the mature cohorts used for field gas exchange measurements. Because light adaptation is a fluence response (Chabot et al. 1979), sun-adapted foliage was collected from only the three oldest classes. Trees in the younger classes are growing in canopy gaps and likely received substantially less integrated light flux than upper canopy trees.

Current-year foliage was collected during the autumn. For each tree, three subsamples of approximately 3 cm twig length were analyzed as previously described. Age-related trends in morphological attributes were analyzed with polynomial contrasts.

### Grafting study

Eighteen trees from each of three maturational states—juvenile, young-mature and old-growth—were randomly selected as scion donors. The young-mature and old-growth classes were roughly equivalent to the two oldest cohorts of the field study (mean ages of 54 and 117 years, respectively). The juvenile-class scions were harvested from pre-reproductive individuals that were 1–2 m in height. Scions for the grafting experiment originated as terminal branch shoots from canopy positions with sun-adapted foliage. Scions were collected during the last 2 weeks of February 1996, and held dormant under refrigeration until grafting.

Three-year-old potted rootstocks were brought from outdoors into a heated greenhouse in mid-February, and grafts were made shortly after rootstock transpiration rates stabilized. Thus dormant scions were grafted onto nondormant rootstocks, which promoted establishment of vascular connections before scion bud break. Scions, consisting of a terminal bud and approximately 4 cm of subtending stem, were cleft-grafted onto leader shoots.

Grafted trees were maintained in a shadehouse (35% interception) for three growing seasons, resulting in four foliar flushes. (Trees flushed twice in 1996 because of the extended growing season initiated in the greenhouse.) Trees were transferred to 25-l pots at the beginning of the second season after grafting (1998), potted with peat, vermiculite and sand (2:1:1), and supplied with time-release fertilizer (Osmocote 18:6:12 (N,P,K), Sierra Chemical Co., Milpitas, CA) at a rate of 0.124 kg m<sup>-2</sup>. Uppermost branches were pruned from the rootstocks to prevent the development of competitive leading shoots (Figure 1).

To test for potential differences in hydraulic conductivity across graft unions related to scion age class, grafts were excised from trees, recut and planed under water, and coupled to a gravity-flow conductivity apparatus with a 1-m hydraulic head. After allowing 45 min for flow stabilization, three 30-min flux measurements were made over the next 1.5 h. The excised grafts were then placed in a beaker containing 0.1% rhodamine dye and a vacuum applied to the scion end to draw

dye through the section. Three cross sections were cut from the center of each graft union, and the cross-sectional area of the active xylem was determined by high-resolution scanning and color image analysis (WinSeedle Version 5.1a, Regent Instruments, Quebec, QC). Specific conductivity was determined with Darcy's law (Gartner 1995).

### Foliar attributes of scions

In the summer of 1999, four trees from each age class were selected for sampling, including all four surviving trees with scions from the 120-year cohort, and individuals randomly selected from the other two age classes. Gas exchange measurements were made on three shoot tips from the uppermost whorl of each scion. Control measurements were made on upper crown shoot tips from each rootstock. Measurements were taken on fully expanded current-year foliage during the first 2 weeks of August. The standard 2 × 3-cm cuvette from an LI-6400 (Li-Cor, Inc.) open-type photosynthesis system was clamped on the center portion of a sample shoot and the foliage acclimated for 30 min to a photosynthetic photon flux density (PPFD) of 2000 μmol m<sup>-2</sup> s<sup>-1</sup> from an LI-6400-02B light source. Gas exchange measurements were made successively at a PPFD of 2000, 1500, 1000, 750, 500, 350, 200, 120, 90, 60, 40, 25, 10 and 0 μmol m<sup>-2</sup> s<sup>-1</sup>. Following each change in irradiance there was a 3-min adjustment period, followed by a test for stability (<0.5% maximum CV for ΔCO<sub>2</sub>), and acclimation periods were repeated if necessary. Infrared gas analyzers were matched before each measurement, zero values were calibrated daily, and span values were checked weekly. Measurements were made on one tree per day with trees measured in random order. On each day, sampling began at 0800 h EST and was completed in approximately 3 h. Needle temperatures within the cuvette were maintained at 25 °C and VPD at < 1.5 kPa. After sampling, the shoot sections in the cuvette were severed for morphological measurements as previously described. Apparent quantum efficiencies were calculated as the slope of the linear portion of the photosynthetic light-response curve (Leverenz 1988).

Results were evaluated by analysis of variance (ANOVA)



Figure 1. Grafted trees after four growing seasons. Typical trees with scions from juvenile, young-mature and old-growth donors are shown from left to right. The white arrows indicate the position of graft union, and foliage on branches below arrows originated from the rootstock. The vertical bar is a 10 cm ruler.

of the subsample means in a simple random design. To meet the assumptions of ANOVA, analyses of photosynthetic and stomatal conductance data were log-transformed.

## Results

Foliar efficiency index (biomass production per unit foliar mass) was inversely related to tree age (Figure 2), suggesting either that older trees produced less photosynthate per unit foliar mass or that a smaller proportion of production is allocated to accretion of aboveground biomass. Differences in percent foliar N concentration and dawn and midday twig xylem water potentials between cohorts were not significant (Table 2). Small differences in mean xylem water potentials between cohorts were close to those predicted by differences in mean tree height (Table 1b) and gravitational potential. Therefore, the upper canopy cohorts appear not to have had access to different pools of belowground resources. This would be expected for a shallow-rooted species growing in a multi-cohort stand.

Red spruce exhibited age-related trends in several aspects of foliar morphology (Table 3). Specific leaf area (SLA; cm<sup>2</sup> projected leaf area per g dry mass) declined with age in both sun- and shade-adapted foliage (Figure 3a). The most pronounced decreases were across age classes < 40 years, with the rate of change lessening across the mature cohorts. Needle width (Figure 3b), needle area (Figure 3c), and the needle width/length ratio (Figure 3d) showed a positive relationship to tree age that was consistent across foliar types (sun- or shade-adapted). The increasingly steep slope of the needle width/length trend between the 60- and 120-year age classes compared to the slope between the 20- and 60-year cohorts (Figure 3d) may indicate that the rate of change in needle shape accelerates as red spruce attains the old-growth maturational states. Additionally, foliage showed a tendency toward greater density on the shoots

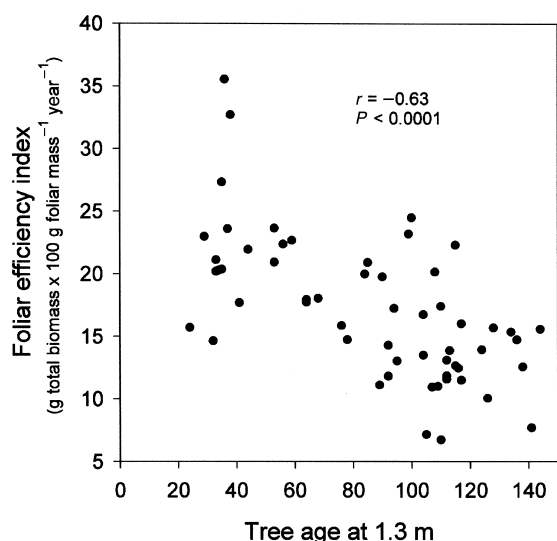


Figure 2. Relationship between tree age and foliar efficiency index for upper canopy red spruce in C16 ( $n = 58$ ). Foliar efficiency index values represent aboveground annual net biomass production (g) per 100 g foliar biomass.

Table 2. Gas exchange and water potential ( $\Psi$ ) measurements on the two upper canopy cohorts of red spruce in PEF Compartment 16. All values are means  $\pm$  standard error,  $n = 32$ . Abbreviations and symbols are defined in the text, and mensurational data for the two cohorts are summarized in Table 1a.

Attribute	Cohort		$P > F$
	60-year	120-year	
Net photosynthesis ( $\mu\text{mol kg}^{-1} \text{s}^{-1}$ )	$14.4 \pm 0.86$	$11.7 \pm 0.84$	< 0.01
( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$3.6 \pm 0.34$	$3.2 \pm 0.31$	0.14
Stomatal conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	$87.5 \pm 6.58$	$92.1 \pm 6.63$	0.28
Internal CO <sub>2</sub> concentration ( $\mu\text{l l}^{-1}$ )	$261 \pm 5.01$	$276 \pm 5.08$	0.06
Total N concentration (%)	$1.01 \pm 0.032$	$1.11 \pm 0.058$	0.30
$\Psi_{\text{midday}}$ (MPa)	$-1.42 \pm 0.046$	$-1.52 \pm 0.043$	0.12
$\Psi_{\text{dawn}}$ (MPa)	$-0.41 \pm 0.053$	$-0.45 \pm 0.053$	0.24

of older trees, with significant trends in foliar area per cm of shoot length for both foliar types (Table 3).

The analysis of variance showed that late-morning photosynthetic rates in the 120-year upper canopy cohort were significantly lower than rates in the 60-year cohort when calculated on a leaf mass basis (Table 2). When expressed on a projected area basis, the mean rate for the 120-year cohort was lower but not significantly so, because of the greater SLA of foliage on the old trees. Stomatal conductance ( $g_s$ ) and internal CO<sub>2</sub> concentration ( $C_i$ ) were not significantly different between age classes. Mass-based foliar N concentrations did not differ significantly between age classes, although values were higher in old-growth foliage (Table 2). Because of the greater SLA of foliage from the 60-year cohort, N concentration was significantly higher in the old-growth cohort on a projected area basis. However, the relationship between foliar N concentration and photosynthesis is inconsistent in red spruce (Schaberg et al. 1997), which may exhibit retention of excess N, possibly as an adaptation to nutrient-poor sites.

Of the 18 initial scions from each age class, eight, 12 and seven grafts from the juvenile (J), young-mature (YM), and old-growth (OG) age classes, respectively, were successful, showing no age-related trend in initial graft success. Caliper of scions from YM donors more closely matched those of rootstock, which may account for the greater success in grafting the YM scions. Six, eight and four trees from the J, YM and OG scion classes, respectively, survived to the third growing season. Losses after the first growing season were from whole-tree mortality (rootstock death). Typically, foliage of both rootstock and graft turned brown in spring, suggesting that losses resulted from winter freezing damage. Again, there was no clear relationship between mortality and maturation states of scion donors. Scions from OG donors showed significantly less growth than those from the J and YM donors (Figure 1, Table 4), whereas growth differences between the J and YM scions were nonsignificant.

Table 3. Trends in foliar morphological attributes for sun- and shade-foliage of red spruce growing in a selection stand (C16) at the Penobscot Experimental Forest, Penobscot County, Maine. Polynomial contrasts were performed using a model series ( $Y = b_1\text{Age}$ ,  $Y = b_1\text{Age} + b_2\text{Age}^2$ ,  $Y = b_1\text{Age} + b_2\text{Age}^2 + b_3\text{Age}^3, \dots$ ) where the highest order model testable =  $df - 1$ , or quadratic for three classes of sun-foliage and quintic for six classes of shade-foliage. Coefficients and  $P > F$  for the highest order model are given. No models of greater than third order were significant. For each age class, foliage was sampled from 15 trees, three subsamples per tree. Trends are represented graphically in Figure 3.

Attribute	Foliar type	df	Highest significant polynomial form	Polynomial coefficients ( $b_1, b_2, b_3$ )			$P > F$
				Age	Age <sup>2</sup>	Age <sup>3</sup>	
Specific leaf area	Sun	2	Quadratic	-0.5997	0.0028	-	< 0.001
	Shade	5	Cubic	0.0066	-0.2424	0.0135	< 0.001
Projected needle area	Sun	2	Quadratic	0.1648	-0.0008	-	< 0.001
	Shade	5	Cubic	0.1712	0.0296	-0.0023	< 0.001
Needle width	Sun	2	Linear	0.0084	ns	-	< 0.001
	Shade	5	Cubic	0.0522	-0.0033	0.0001	< 0.001
Needle length	Sun	2	Quadratic	0.1060	-0.0007	-	< 0.001
	Shade	5	Cubic	-0.3057	0.0815	-0.0046	0.04
Needle width/length ratio	Sun	2	Linear	0.0002	ns	-	< 0.001
	Shade	5	Cubic	0.0071	-0.0008	0.00003	0.004
Foliage area per cm shoot length	Sun	2	Quadratic	4.4524	-0.0226	-	< 0.001
	Shade	5	Quadratic	-5.2458	0.9617	ns	0.005

Juvenile scions exhibited the greatest specific conductivity ( $k_s$ ) across graft unions, followed by the OG and then YM classes (Table 4). However, high variance within classes led to nonsignificant differences among scion age classes. Although high variance indicated substantial differences in water conductivity among grafts, trends related to scion donor class

were absent. Whereas  $g_s$  was strongly correlated with net photosynthesis ( $P_n$ ;  $r = 0.93$ ,  $P < 0.001$ ), correlation analyses of  $k_s$  with  $P_n$  and  $g_s$  were nonsignificant ( $P = 0.88$  and  $0.28$ , respectively), indicating that  $k_s$  was a poor predictor of both photosynthetic performance and foliar resistance to gas exchange.

For the grafted scions, ANOVA of foliar morphological

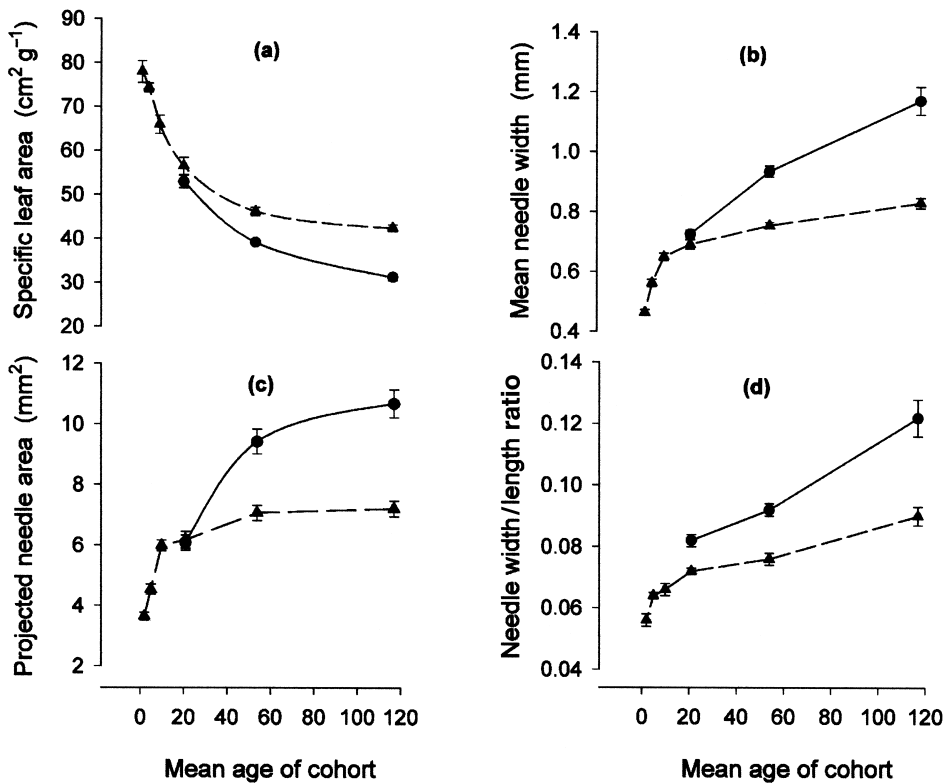


Figure 3. Trends in foliar morphological attributes with tree age in the multi-cohort red spruce field population: (a) specific leaf area; (b) mean needle width; (c) mean projected needle area; and (d) needle width/length ratio. Means were calculated from 15 trees of each age class, three subsamples per tree. Bars represent one standard error. Sun foliage trends are shown with solid lines and shade foliage with dashed lines. Results of trend analysis with polynomial contrast are presented in Table 3.

Table 4. Gas exchange attributes, growth and conductivity of graft unions after three growing seasons for scions from donors of juvenile, young-mature, and old-growth maturation states. Measurements of physiological attributes of the rootstock are provided for comparison. Means with different letters are significantly different at  $\alpha = 0.05$ , determined by Duncan's New Multiple Range Test.

Attribute	Maturation state of scion donor			
	Rootstock	Juvenile	Young-mature	Old-growth
<i>Foliar morphology</i>				
Specific leaf area ( $\text{cm}^2 \text{g}^{-1}$ )	–	41.4 a	41.9 a	40.5 a
Projected needle area ( $\text{mm}^2$ )	–	7.58 b	8.84 b	11.23 a
Needle width (mm)	–	0.017 b	0.014 b	0.029 a
Needle width/length ratio	–	0.050 ab	0.048 b	0.052 a
<i>Physiological attributes</i>				
Net photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	10.0 a	10.2 a	9.1 a	5.0 b
( $\mu\text{mol kg}^{-1} \text{s}^{-1}$ )	48.1 a	42.4 a	38.3 a	20.2 b
Stomatal conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	123.8 b	154.8 a	118.7 b	49.6 c
Internal $\text{CO}_2$ concentration ( $\mu\text{mol mol}^{-1}$ )	207.8 b	238.2 a	211.6 b	198.8 b
Apparent quantum efficiency ( $\mu\text{mol mmol}^{-1}$ )	0.048 a	0.041 a	0.039 a	0.027 b
Scion growth ( $\text{cm year}^{-1}$ )	n/a	16.1 a	14.1 a	8.8 b
Specific conductivity of graft union ( $10^3 \text{ cm}^2 \text{ s}^{-1} \text{ kPa}^{-1}$ )	n/a	2.99 a	1.26 a	1.78 a

attributes showed age-related trends in mean needle width, width/length ratio and area similar to those observed in the field population (Table 4). Projected needle area was significantly different because of trends in needle width and length, but differences in SLA, which showed a declining trend with scion donor age, were not significant (Table 4).

Net photosynthesis ( $P_n$ ) exhibited a decreasing trend across age classes, but only differences between OG scions and those of J and YM donors were significant (Table 4). Stomatal conductance showed a strong declining trend with maturation states of scion donors. Internal  $\text{CO}_2$  concentration also decreased with scion class; however, the mean for the OG class was only 6% lower than that for the YM scions, and differences between these groups were nonsignificant. Apparent quantum efficiency (AQE) exhibited a decreasing trend with scion age class, with AQE of OG scions only about 60% of that of scions from J donors.

## Discussion

In the upper canopy cohorts, foliar efficiency (biomass production per unit foliar mass) declined with tree age (Figure 2). Further, the trend in Figure 2 indicates that the decline in foliar efficiency with age began soon after reproductive maturity. Similar patterns have been reported for other species (reviewed in Ryan et al. 1997). Thus either (1) productivity per unit leaf mass is substantially lower in older trees, or (2) a greater proportion of primary production is allocated to sinks other than that associated with aboveground biomass accretion. For example, Grier et al. (1981) speculated that increasing nutrient limitations in older stands can result in greater proportional allocation to root systems. However, in this multi-

cohort stand, where individuals of contrasting age classes were growing adjacent to one another, differential nutrient availability are an unlikely cause of differing foliar efficiencies. This is supported by the lack of difference in foliar mass-based N concentrations between upper canopy cohorts found in this study (Table 2).

Morphological changes between juvenile and mature foliage (Figure 3) correspond with those reported for red spruce by Rebbeck et al. (1994) and Sitka spruce by Steele et al. (1989). In red spruce growing in non-suppressed canopy positions, reproductive maturity usually occurs at about 25 years (Blum 1990). Thus, this study shows that age-related trends in foliar morphology continue well beyond reproductive maturity in both *in situ* shade- and sun-adapted foliage (Figure 3), and in foliage of grafted scions growing on rootstock of common age (Table 4). Richardson et al. (2000) reported similar trends for hybrid Englemann  $\times$  white  $\times$  Sitka spruce, although they found that age-related morphological trends are complicated by canopy position (shade–sun adaptation). Richardson et al. (2000) also reported that foliar plasticity (defined as the relative difference between sun- and shade-adapted foliage) decreased with tree age. This is in contrast to the morphological differences found in this study where older age classes showed greater morphological variation between sun- and shade-adapted foliage in all attributes tested (Figure 3). This incongruity may reflect true interspecific differences or merely the greater interception of photosynthetically active radiation by taller age classes in multi-cohort stands.

Environmental effects may explain the inconsistency in SLA trends between *in situ* foliage and that of the grafting experiment. Hutchison et al. (1990) found that foliage from larch scions from donors of a continuum of age classes grafted to

juvenile rootstock showed slightly diminished trends in age-related morphological attributes in trees growing in the greenhouse compared to those outside. Alternatively, if development of more massive needles is related to needle age, this ambiguity may be an artifact of comparisons using 1-year-old foliage in the field study and newly expanded foliage in the grafting experiment. A decrease in SLA with needle age has been described for related conifers (Hatcher 1990, Gilmore et al. 1995).

Although photosynthetic rates based on projected needle area in the field population were lower for the old trees than the young-mature cohort, the difference was not statistically significant. However, on a mass basis the old cohort showed substantially and significantly lower photosynthetic rates. This inconsistency resulted from the lower SLA of needles from the old age class. When SLA of foliage from the two cohorts was similar (the grafting study), the foliage from the old cohort exhibited significantly lower photosynthetic rates on both projected area and mass bases. Changes in needle form across maturational states, suggested by the significant trends in foliar width/length (Table 3), make comparisons on a projected area basis problematic. In this study, comparisons of photosynthetic rates on a mass basis controlled for the confounding effects of differences in SLA and provide a clearer picture of the return on investment in photosynthetic tissue and of the light-use efficiency of needles on complex shoot structures in their natural, multidirectional light environment. Smith et al. (1991) and Stenberg et al. (1995) explored issues associated with various bases for reporting photosynthetic rates in conifers. They concluded that the shoot structure of many conifers dictates that light-capture and photosynthesis are best expressed using different metrics, with projected area measurements (needle or shoot silhouette) most appropriate for reporting light-capture, and metrics such as total needle area, mass, chlorophyll content, or N content most appropriate for reporting photosynthetic rates.

Although N content, which was similar across age classes in this study, is generally correlated with photosynthetic capacity (Evans 1989), Stenberg et al. (1997) have shown that foliar N content is a poor predictor of photosynthesis in mature red spruce. Although not directly addressed in this study, these results are consistent with a poor relationship between N content and photosynthetic capacity in this species.

Differences in absolute gas exchange rates between the field population and grafted scions across all age classes are likely the result of measurements being made on 1-year-old foliage in the field study but on newly expanded current-year foliage in the grafting study. Similar differences in photosynthetic rates and  $g_s$  between current- and previous-year foliage have been reported for red spruce (Day 2000).

The grafting study showed little consistent influence of scion donor age on grafting success, grafted tree survival, or hydraulic properties of the graft union. Grafts made with scions from both the juvenile and old donor age classes had substantially poorer success rates than those from the young-mature class. This is likely because of a better match in stem diameter between scions from young-mature donors and root-

stock. Although survival to the third post-grafting season was lowest for the old group, survival showed no consistent relationship with donor age. The bulk of foliage on grafted trees originated from the rootstock (Figure 1), and almost all losses involved death of both rootstock and scion. Trends in specific conductivity across the graft union were also equivocal, with the lowest values in grafts made with young-mature scions, and high variability in grafts with scions of all age classes. M.S. Greenwood (unpublished data) found a similar lack of age-related patterns in hydraulic conductivity for grafts of juvenile and mature larch. These observations suggest that the effects of the grafting treatments were consistent across age classes and showed no obvious bias that would confound interpretation of gas exchange results in this study.

The lower photosynthetic rates of the old-growth cohort, both in the field and in grafted scions, supports the hypothesis that photosynthetic production decreases with tree age (Ryan and Waring 1992, Yoder et al. 1994). The lack of significant differences in  $g_s$  and  $C_i$  in the field study and the small differences in  $C_i$  between young-mature and old-growth scions in the grafting experiment do not support the hypothesis that lower photosynthetic rates are caused primarily by stomatal limitations to gas exchange (Yoder et al. 1994). It has been proposed that increased stomatal limitation to gas exchange in old trees results from decreased hydraulic conductivity in water transport pathways (Yoder et al. 1994, Ryan and Yoder 1997), and there is substantial evidence to support this hypothesis from measurements on field-grown ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) and other species (Hubbard et al. 1999). However, in the grafting study, there was no correlation between  $k_s$  and  $g_s$  in red spruce ( $P = 0.88$ ). This may indicate that, in red spruce,  $k_s$  and stomatal closure are poorly coupled, or that graft union  $k_s$  did not restrict water flow to the extent required to elicit stomatal responses.

Alternatively, our results could be explained by a model where  $g_s$  is in balance with lower inherent photosynthetic rates (Sharkey 1985). In turn, lower photosynthetic rates may be related to larger scale (branch-level or whole tree) carbon balance. In this model, investment in foliar photosynthetic capacity would be determined by demand for photosynthate or sink strength (Farrar 1992). Age- or size-related changes in meristematic activity could result in decreased sink strength in shoots of older trees (or grafted scions harvested from older trees), ultimately reflected in changes to foliar gas exchange attributes. The growth of trees in the field population (Figure 2) and grafted scions (Table 4) suggest that red spruce exhibits an age-related decline in growth. Similar inverse relationships between inherent growth potential of scions and donor age have been reported for loblolly pine (*Pinus taeda* L.) by Greenwood (1984) and for eastern larch (*Larix laricina* (Du Roi) K. Koch) by Greenwood et al. (1989). In eastern larch, growth potential of grafted scions declines with donor age despite higher photosynthetic rates and foliar chlorophyll content (Hutchison et al. 1990).

A decreasing ability of grafted scions to compete with rootstock foliage for available resources may also be related to differences in growth of scions from donors of different ages.



Observing that lateral meristems of larch scions from older donors produce tracheids of smaller radial diameter than younger counterparts when grafted on common rootstock, Takemoto and Greenwood (1993) proposed that resource acquisition of older scions may be limited by competitively weaker sink strengths. As scions in this study competed with a substantial amount of rootstock foliage, such a scenario may be relevant to our results. However, the design of our study did not permit the direct analysis of this possibility, or the separation of cause and effect relative to resource acquisition by scions, i.e., are older scions poorer sinks due to low growth potential, or does low growth potential result from decreased ability to compete with juvenile rootstock shoots for resources?

A maturation-based decline in inherent photosynthetic rates was indicated in our study by equivalent  $C_i$  values in young-mature trees, which exhibited high photosynthetic rates and  $g_s$ , and old trees with lower values for both attributes, suggesting that  $g_s$  is in balance with inherent photosynthetic capacity. This relationship was consistent for both field (Table 3) and grafted scion (Table 4) studies. Further evidence of decreasing inherent photosynthetic capacity with tree age is the lower AQE of the older scion classes (Table 4). The high-to-low PPF protocol used in developing the light-response curves did not provide sufficient response times for  $g_s$  to equilibrate with photosynthesis at lower irradiance (20–30 min; M. Day, unpublished data). Thus, except at saturating irradiances,  $g_s$  was higher than would normally be the case for prevailing PPF, and  $C_i$  would presumably not be limiting over the range of PPFs ( $10\text{--}60\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ ) used to determine AQE.

After three growing seasons, grafted scions exhibited age-related trends in foliar morphology and physiology corresponding to those found in the field population, indicating that age-related characteristics are retained for at least several growing seasons by scions grafted to juvenile rootstocks. Thus, red spruce meristems from older trees appear to retain a “memory” with respect to the attributes of foliage they produce. Such a scenario may explain why some experiments designed to alter  $g_s$  or  $P_n$  by manipulating leaf-specific conductivity of branches have produced equivocal results (e.g., Hubbard et al. 1999, Brooks et al. 2000). It is unclear whether this meristematic memory is age-related in the strict sense (deterministically related to an internal timing mechanism) or is induced by signals related to tree size that cause changes in meristematic gene expression. In the latter model, attributes would ultimately be determined by such factors as whole-tree conductivity or carbon sink strength, and presumably, meristems of old trees would produce foliage better adapted to survive the challenges faced by larger trees. “Rejuvenation” of old meristems grafted to young rootstock has been described for numerous fruit-bearing trees (Hackett 1985), but this process is rarely observed in conifers. Reversibility of attributes that show age-related change would provide indirect evidence that gene expression is nondeterministic and induced by factors external to meristems. Long-term studies will be required to achieve an understanding of the implications of reversibility.

It is possible, if not likely, that age-related changes of vari-

ous attributes occur independently. Therefore, the induction factors for, and trajectories of, change in different attributes may vary. For example, Greenwood et al. (1989) found that, in eastern larch, maturational changes in foliar morphology (needle size and SLA) were uncoupled from changes in net photosynthesis and chlorophyll content. Although studies across phylogenetically diverse groups of plants have found that species with low SLA tend to have low photosynthetic rates (e.g., Chabot and Hicks 1982), it is unknown if these attributes are mechanistically coupled in age sequences of single species. It is possible that age-related changes in attributes that appear correlated (such as SLA and photosynthetic rates) are responses to different challenges and thus may follow independent trajectories. In either case, these results imply that gene expression in meristems changes as trees age or grow larger beyond reproductive maturity or mid-age.

#### Acknowledgments

The authors thank J. Gaoutte, S. Gourde, M. Perrera, and R. Soucy for their assistance in the field and laboratory, and the Northeastern Forest Experiment Station, USDA Forest Service for use of research sites on the Penobscot Experimental Forest. Support for this research was provided, in part, by a McIntire-Stennis grant to the Forest Ecosystem Research Program, administered through the Maine Forest and Agriculture Experiment Station (MAFES), University of Maine. This paper is MAFES Publication Number 2491.

#### References

- Assmann, E. 1970. The principles of forest yield study. Pergamon Press, Oxford, 506 p.
- Becker, P., F.C. Meinzer and S.D. Wullschlegel. 2000. Hydraulic limitation of tree height: a critique. *Funct. Ecol.* 14:4–11.
- Blum, B.M. 1990. *Picea rubens* Sarg., red spruce. In *Silvics of North America*. Vol 1. Conifers. Eds. R. Burns and B.H. Honkala. USDA Agricultural Handbook 654. USDA, Washington, DC, pp 250–259.
- Bond, B.J. 2000. Age-related changes in photosynthesis of woody plants. *Trends Plant Sci.* 5:349–353.
- Brooks, J.R., B.J. Bond, R. Coulombe, J.C. Domec, T.M. Hinkley, N. McDowel, N. Phillips and P.J. Schulte. 2000. The effects of transient reductions in functional leaf area on stomatal conductance: branch level experiments on young and old trees. In *ESA Abstracts, The Ecological Society of America 85th Annual Meeting*. Ecological Society of America, Washington, DC, 62 p.
- Chabot, B.F. and D.J. Hicks. 1982. The ecology of leaf life spans. *Annu. Rev. Ecol. Syst.* 13:229–259.
- Chabot, B.F., T.W. Jurik and J.F. Chabot. 1979. Influence of instantaneous and integrated light-flux density of leaf anatomy and photosynthesis. *Am. J. Bot.* 66:940–945.
- Davis, W.C. 1991. The role of advance regeneration of red spruce and balsam fir in east central Maine. In *Proceedings of the Conference on Natural Regeneration Management*. Ed. C.M. Simpson. Forestry Canada Maritimes Region, Fredericton, NB, pp 157–168.
- Day, M.E. 2000. Influence of temperature and leaf-to-air vapor pressure deficit on net photosynthesis and stomatal conductance in red spruce (*Picea rubens*). *Tree Physiol.* 20:47–53.
- Emebiri, L.C., M.E. Devey, A.C. Matheson and M.U. Slee. 1998. Age-related changes in the expression of QTLs for growth in radiata pine seedlings. *Theor. Appl. Genet.* 97:1053–1061.
- Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of  $C_3$  plants. *Oecologia* 78:1–19.

- Farrar, J.F. 1992. The whole plant: carbon partitioning during development. *In* Carbon Partitioning Within and Between Organisms. Eds. C.J. Pollock, J.F. Farrar and A.J. Gordon. Bios Press, Oxford, pp 163–179.
- Gartner, B.L. 1995. Patterns of xylem variation within a tree and their hydraulic and mechanical consequences. *In* Plant Stems: Physiology and Functional Morphology. Ed. B.L. Gartner. Academic Press, San Diego, pp 125–149.
- Gilmore, D.W., R.S. Seymour, W.A. Halteman and M.G. Greenwood. 1995. Canopy dynamics and the morphological development of *Abies balsamea*: effects of foliage age on specific leaf area and secondary vascular development. *Tree Physiol.* 15:47–55.
- Greenwood, M.S. 1984. Phase change in loblolly pine: Shoot development as a function of age. *Physiol. Plant.* 61:518–522.
- Greenwood, M.S. and K.W. Hutchison. 1993. Maturation as a developmental process. *In* Colonial Forestry I: Genetics and Biotechnology. Eds. M.R. Ahuja and W.J. Libby. Springer-Verlag, New York, pp 14–33.
- Greenwood, M.S., C.A. Hooper and K.W. Hutchison. 1989. Maturation in larch. I. Effect of age on shoot growth, foliar characteristics, and DNA methylation. *Plant Physiol.* 90:406–412.
- Grier, C.C., K.A. Vogt, M.R. Keyes and R.L. Edmonds. 1981. Biomass distribution and above- and below-ground production in young and mature *Abies amabilis* zone ecosystems of the Washington Cascades. *Can. J. For. Res.* 11:155–167.
- Hackett, W.P. 1985. Juvenility, maturation, and rejuvenation in woody plants. *Hortic. Rev.* 7:109–155.
- Hatcher, P.E. 1990. Seasonal and age-related variation in the needle quality of five conifer species. *Oecologia* 85:200–212.
- Hunt, E.R., Jr., M.B. Lavigne and S.B. Franklin. 1999. Factors controlling the decline of net primary production with stand age for balsam fir in Newfoundland assessed using an ecosystem simulation model. *Ecol. Model.* 122:151–164.
- Hutchison, K.W., C.D. Sherman, J. Weber, S.S. Smith, P.B. Singer and M.S. Greenwood. 1990. Maturation in larch. II. Effects of age on photosynthesis and gene expression in developing foliage. *Plant Physiol.* 94:1308–1315.
- Hubbard, R.M., B.J. Bond and M.G. Ryan. 1999. Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. *Tree Physiol.* 19:165–172.
- Keyes, M.R. and C.C. Grier. 1981. Above- and below-ground net production in 40-year-old Douglas-fir stands on high and low productivity sites. *Can. J. For. Res.* 11:599–605.
- Koide, R.T., R.H. Robichaux, S.R. Morse and C.M. Smith. 1989. Plant water status, hydraulic resistance and capacitance. *In* Plant Physiological Ecology: Field Methods and Instrumentation. Eds. R.W. Pearcy, J.R. Ehleringer, H.A. Mooney and P.W. Rundal. Chapman and Hall, New York, pp 161–183.
- Kull, O. and A. Koppel. 1987. Net photosynthetic response to light intensity of shoots from different crown positions and age in *Picea abies* (L.) Karst. *Scand. J. For. Res.* 2:157–166.
- Leverenz, J.W. 1988. The effects of illumination sequence, CO<sub>2</sub> concentration, temperature and acclimation on the convexity of the photosynthetic light response curve. *Physiol. Plant.* 74:332–341.
- Maguire, D.A., J.C. Brisette and L. Gu. 1998. Crown structure and growth efficiency of red spruce in uneven-aged, mixed-species stands in Maine. *Can. J. For. Res.* 28:1233–1240.
- Murty, D., R.E. McMurtrie and M.G. Ryan. 1996. Declining forest productivity in aging forest stands: a modeling analysis of alternative hypotheses. *Tree Physiol.* 16:187–200.
- Rebbeck, J., K.F. Jensen and M.S. Greenwood. 1992. Ozone effects on grafted mature and juvenile red spruce: Photosynthesis, stomatal conductance, and chlorophyll concentration. *Can. J. For. Res.* 23:450–456.
- Richardson, A.D., G.P. Berlyn, P.M.S. Ashton, R. Thadani and I.R. Cameron. 2000. Foliar plasticity of hybrid spruce in relation to crown position and stand age. *Can. J. Bot.* 78:305–317.
- Ryan, M.G. and R.H. Waring. 1992. Maintenance respiration and stand development in a subalpine lodgepole pine forest. *Ecology* 73:2100–2108.
- Ryan, M.J. and B.J. Yoder. 1997. Hydraulic limits to tree height and tree growth. *Bioscience* 47:235–242.
- Ryan, M.G., D. Binkley and J.H. Fownes. 1997. Age-related decline in forest productivity: Patterns and process. *Adv. Ecol. Res.* 27: 213–256.
- Salisbury, F.B. and C.W. Ross. 1992. *Plant physiology*. 4th Edn. Wadsworth Publishing, Belmont, CA, 682 p.
- Seymour, R.S. 1992. The red spruce–balsam fir forest of Maine: Evolution of silvicultural practice in response to stand development patterns and disturbances. *In* The Ecology and Silviculture of Mixed-Species Forests: A Festschrift for David M. Smith. Eds. M.J. Kelty, B.C. Larson and C.D. Oliver. Kluwer Academic Publishers, Boston, pp 217–244.
- Seymour, R.S. and L.S. Kenefic. 1998. Balance and sustainability in multiaged stands: a northern conifer case study. *J. For.* 96:12–17.
- Schaberg, P.G., T.D. Perkins and S.G. McNulty. 1997. Effects of chronic low-level N additions on foliar elemental concentrations, morphology, and gas exchange of mature montane red spruce. *Can. J. For. Res.* 27:1622–1629.
- Sharkey, T.D. 1985. Photosynthesis in intact leaves of C<sub>3</sub> plants: physics, physiology and rate limitations. *Bot. Rev.* 51:53–106.
- Snedecor, G.W. and W.G. Cochran. 1989. *Statistical methods*. 8th Edn. Iowa State Univ. Press, Ames, IA, 503 p.
- Sperry, J.S., and M.T. Tyree. 1990. Water-stress-induced xylem embolism in three species of conifers. *Plant Cell Environ.* 13:427–436.
- Steele, M.J., M.P. Coutts and M.M. Yeoman. 1989. Developmental changes in Sitka spruce as indices of physiological age. I. Changes in needle morphology. *New Phytol.* 113:367–375.
- Stenberg, P., E.H. DeLucia, A.W. Schoettle and H. Smolander. 1995. Photosynthetic light capture and processing from cell to canopy. *In* Resource Physiology of Conifers: Acquisition, Allocation, and Utilization. Eds. W.K. Smith and T.M. Hinckley. Academic Press, San Diego, pp 3–32.
- Smith, W.K., A.W. Schoettle and M. Cui. 1991. Importance of the method of leaf area measurement to the interpretation of gas exchange of complex shoots. *Tree Physiol.* 8:121–127.
- Stitt, M. and W.P. Quick. 1989. Photosynthetic carbon partitioning, its regulation and possibilities for manipulation. *Physiol. Plant.* 77: 633–641.
- Takemoto, Y. and M.S. Greenwood. 1993. Maturation in larch: age-related changes in xylem development in the long-shoot foliage and the main stem. *Tree Physiol.* 13:253–262.
- Waring, R.H. 1983. Estimating forest growth and efficiency in relation to canopy leaf area. *Adv. Ecol. Res.* 13:327–354.
- Waring, R.H. and W.H. Schlesinger. 1985. *Forest Ecosystems: Concepts and management*. Academic Press, Orlando, FL, 340 p.
- Woo, H.H., W.P. Hackett and A. Das. 1994. Differential expression of a chlorophyll a/b binding protein gene and a proline rich protein gene in juvenile and mature phase English ivy (*Hedera helix*). *Physiol. Plant.* 92:69–78.
- Yoder, B.J., M.G. Ryan, R.H. Waring, A.W. Schoettle and M.R. Kaufmann. 1994. Evidence of reduced photosynthetic rates in old trees. *For. Sci.* 40:513–527.
- Young, H.E., J.H. Ribe and K. Wainwright. 1980. Weight tables for tree and shrub species in Maine. Misc. Report 230. Life Sciences and Agriculture Experiment Station, University of Maine, Orono, ME, 84 p.