

UNDERSTANDING PATTERNS AND REGULATORY PATHWAYS IN CONIFER ONTOGENY: THE ROLE OF THE PENOBSCOT EXPERIMENTAL FOREST

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Abstract.—Multi-cohort stands maintained by the U.S. Department of Agriculture, Forest Service on the Penobscot Experimental Forest (PEF) have played a central role in research into the mechanisms that regulate ontogenetic trends in forest conifers as they control effects due to population, climatic, and edaphic factors. These long-term silvicultural studies have permitted direct comparisons of trends in morphology and physiology across life-stages, and experimental reciprocal grafting research among juvenile, mid-aged and old-growth individuals has proved an important approach to discriminating between trends due to tree size and those related to life-stage. The results of this research have provided valuable insight into the physiological mechanisms that underlie age-related trends in growth and development. Two decades of study on the PEF have identified a surprising paradox of high photosynthetic rates and declining stemwood production that characterizes the transition from mid-aged to old-growth life-stages in spruce. Ultimately, this research has led to a novel explanation for that paradox in the ecologically stable strategy hypothesis, which integrates environment, intrinsic regulation of development, and life-stage specific challenges.

INTRODUCTION

For the past two decades the Penobscot Experimental Forest (PEF) has been a key asset for the University of Maine's research program in tree physiology and physiological ecology in the School of Forest Resources. A major focus of research by the school's plant physiology and physiological ecology group is how external factors, such as climate, and internal factors, such as life-stage (ontogeny), control tree productivity and how they relate to tree form (anatomy and structure) and function (physiology). In turn, we seek to understand how the influence of these factors relates to species' competitive abilities, range-limits, and response to changing climates. While working on the gas exchange physiology of red spruce (*Picea rubens* Sarg.) in the early 1990s, we were struck by substantial differences in morphological and physiological attributes between various tree age classes. The older age classes in multi-cohort stands differed from younger trees not only in overall form,

but also in branching patterns, needle morphology, and physiological properties such as stomatal sensitivity to atmospheric humidity and, potentially, in photosynthetic rates. It had been well established that stemwood production declines in forest trees after they reach roughly the midpoint of their normal lifespan (Assmann 1970). However, a physiological explanation for this and other observed age- and size-related trends was lacking.

The PEF stands that the U.S. Department of Agriculture (USDA), Forest Service has maintained under long-term selection silvicultural systems provided an ideal field laboratory to experimentally study age- and size-related phenomena in search of a physiological explanation for these trends. Stands in the Forest Service compartments on the PEF, managed for more than 50 years with an emphasis on red spruce, contain individuals from a common population ranging from germinants to old-growth trees, over a

century and a half in age, all growing on similar soil types. Moreover, by using adjacent stands managed under selection and shelterwood systems, individuals of both free-to-grow and shaded crown status of all age classes could be selected to control for the effects of light environment on form and function.

The decline in stemwood growth efficiency (production per unit foliage) from mid-age to old age is of particular importance to forest management and silvicultural decisions. The paradigm of decreasing productivity with tree age forms the basis for silvicultural decisions on harvesting by defining expected returns on tree growth. This model is particularly important when considering the value of old trees in uneven-aged silviculture, carbon sequestration, and biomaterials production, and can result in a tension between short- and long-rotation silvicultural approaches.

EARLY STUDIES

Our initial assessments showed that red spruce on the PEF did indeed decrease stemwood production efficiency approximately 50 percent between the mid-aged time of maximum production (60 yr) and the oldest individuals (Fig. 1) (Day et al. 2001). This trend was subsequently confirmed by Seymour and Kenefic (2002). In needle morphology, older trees produce foliage that is more massive (lower specific leaf area, SLA) irrespective of sun-foliage or shade-foliage status (Fig. 2), demonstrating the interplay of heteroblasty and heterophylly in foliar development (Greenwood et al. 2009). In addition, old conifers deviate from the pyramidal crown form of younger individuals and develop a characteristic flat-topped form with low rates of stem and branch elongation and increased rates of lateral branch initiation (Fig. 3).

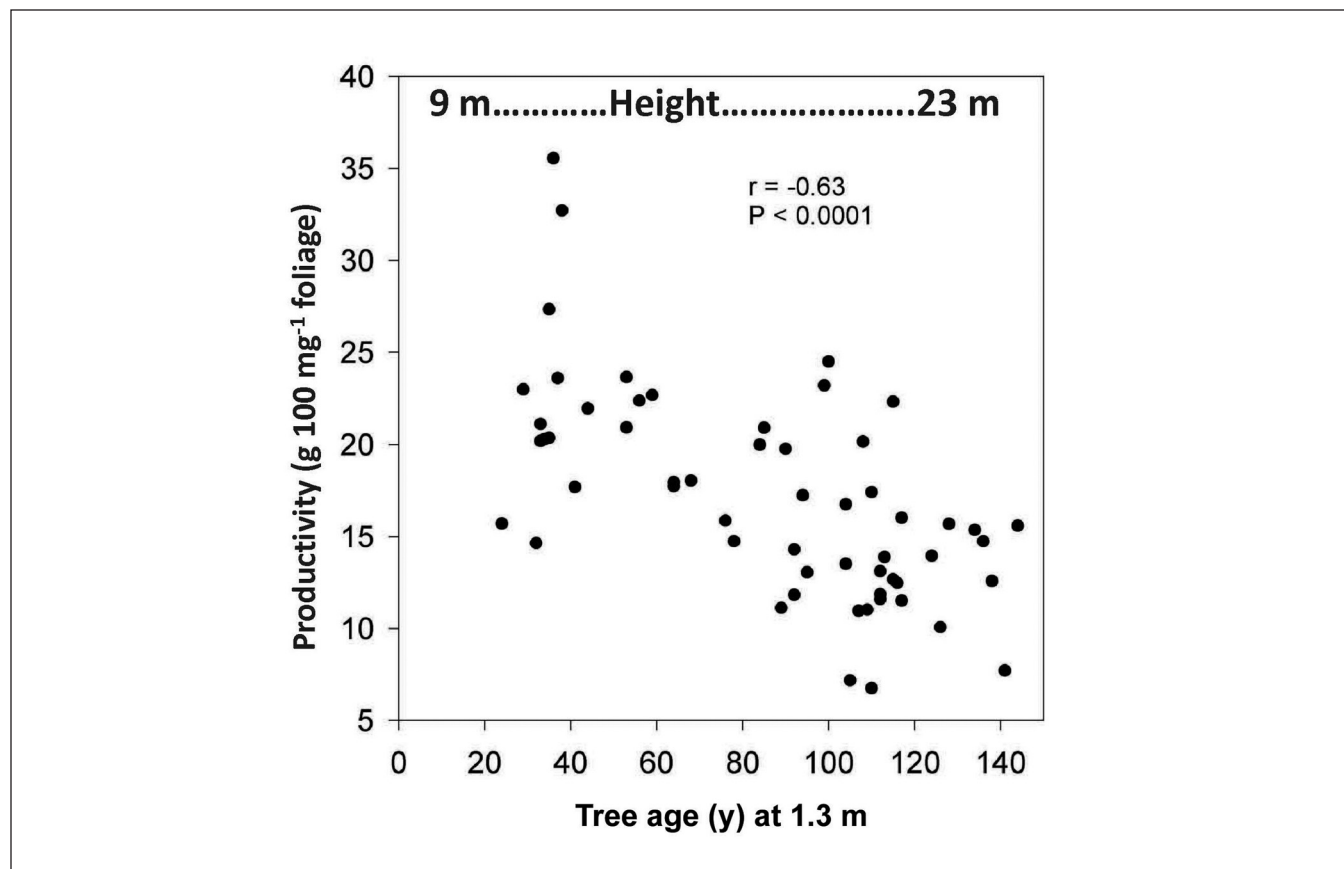


Figure 1.—Foliar efficiency in wood production for reproductively mature red spruce in the PEF (after Day et al. 2001).

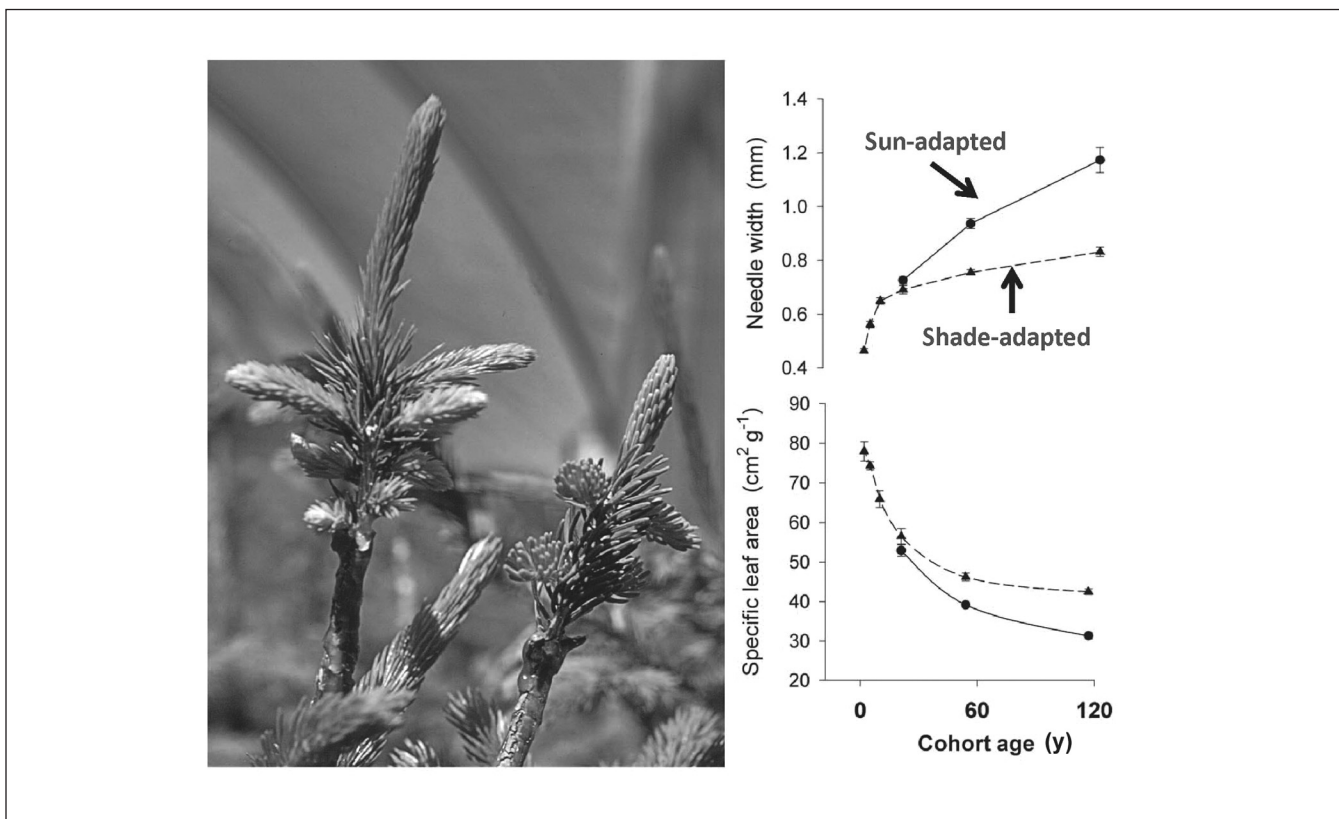


Figure 2.—Trends with tree age in specific leaf area for red spruce foliage from the PEF. Left, juvenile and old-growth foliage after grafting to common juvenile rootstock showing differences in overall needle morphology and robustness; right, independent trends associated with ontological heteroblasty and sun-shade heterophylly. (Means $\pm$ standard error.) (Data from Day et al. 2001). Photo courtesy of University of Maine.

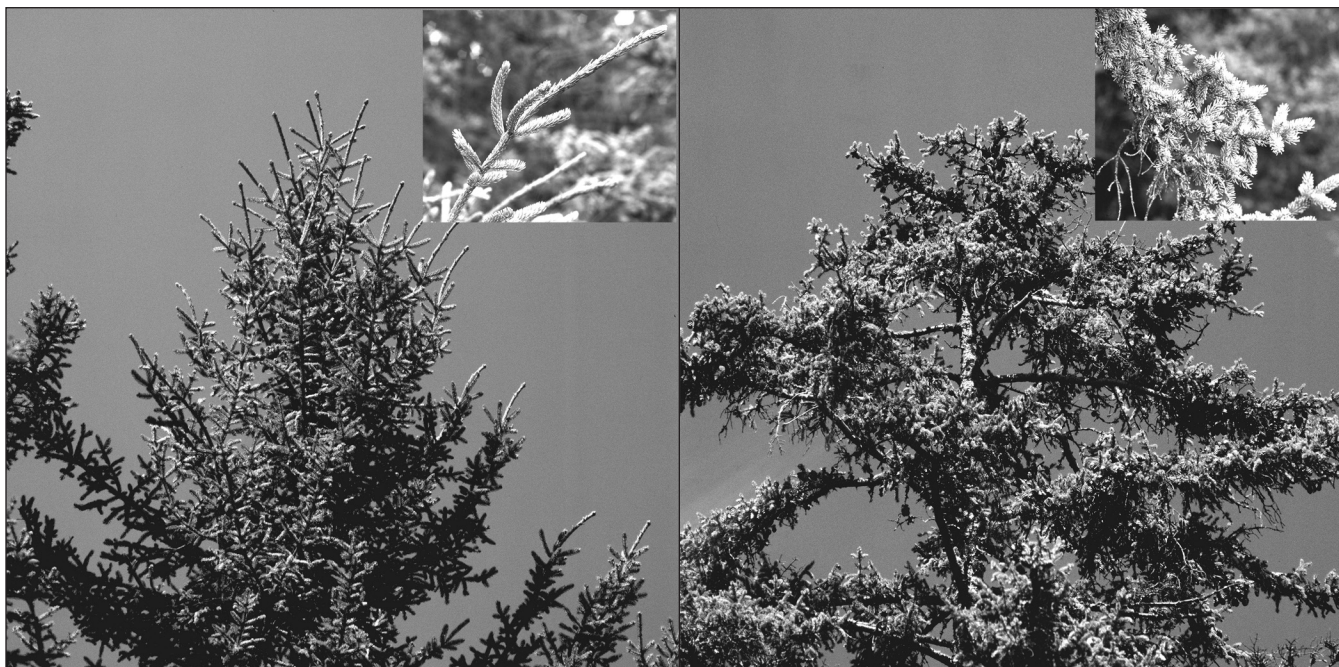


Figure 3.—Typical age- and size-related changes in crown structure, branch elongation, and branches per stem length for red spruce in the PEF with a mid-aged (60 yr) tree on the left and old-growth (150 yr) on the right. Insets detail upper crown lateral branches. Photos courtesy of University of Maine.

Studies in western North America during the 1990s suggested that observed trends in productivity and perhaps morphology were due to decreased photosynthetic rates and/or enhanced midday depression of photosynthesis in old trees because of the increased hydraulic stress associated with longer water transport pathways (Ryan and Yoder 1997, Yoder et al. 1994). Others suggested restricted availability of nutrients, as more became incorporated in living biomass and coarse woody material (e.g., Binkley et al. 1995). Research on redwoods (*Sequoia sempervirens*) (Koch et al. 2004) and Douglas-fir (*Pseudotsuga menziesii*) (Woodruff et al. 2008) further suggested that height gradients in needle SLA are correlated with increasing water stress with tree height through limitations on cell number and/or expansion growth of developing tissues. Because most studies on the issue of age- and size-related declines in productivity, foliar morphology, and crown form were based on very tall (60-100+ m) trees in the Pacific Northwest, a major thrust of our research has been the applicability of “tall tree” hypotheses to the 20- to 30-m-high species typical of the Acadian Forest of northeastern North America. In some of the tallest trees, the biophysical mechanisms that transport water from soil to foliage may reach their upper bounds (Koch et al. 2004). The influences of gravity and resistance of long transport pathways on water potential (approximately -0.02 MPa m^{-1}) are likely to play various roles such as limiting CO_2 uptake for photosynthesis, and limiting the turgor pressure within developing cells required for their expansion. But it also seems likely that biophysical restrictions on water transport and their effects on physiology and morphology are much diminished in species of shorter stature growing in mesic forests.

RESEARCH ON GRAFTING

Although age-related trends in productivity and outward morphology of forest trees and their organs are manifest, understanding the bases for these phenomena is complicated by the confounding effects of size, chronological age, and external environment on long-lived organisms that progress in biomass

through many orders of magnitude during their lifespan. One approach to addressing these potentially confounding factors is through grafting experiments in which scions from donors of various ages or sizes are grafted onto rootstock of a common age. A more comprehensive approach is reciprocally grafting scions from different ages onto rootstock representing the ages of the scion donors (Day et al. 2002). Earlier experiments using the former approach, where scions from older age classes are grafted onto juvenile rootstock, suggested that specific foliar traits, such as needle width, and growth habits such as plagiotropy are maintained in the genetic “memory” of scions from older donors (Day et al. 2001, Rebbeck et al. 1992).

In 2002 we initiated a reciprocal grafting study on the PEF between juvenile (< 10 yr, 1 m in height), mid-aged (60 yr, 10 m), and old (120+ yr, 20 m) red spruce, using multiple grafts in the crowns of eight trees in each of the two mature age-classes, and single grafts on juvenile rootstock. Three years into the study we made extensive in situ physiological and morphological measurements, and we completed a second series of measurements including destructive sampling when scions were harvested 7 years after grafting. Using this approach, we were able to separate the intrinsic and extrinsic mechanisms that regulate numerous morphological and physiological traits (Day and Greenwood 2011, Greenwood et al. 2010). In this regulatory scheme, intrinsic traits are those that are mainly influenced by mechanisms originating in shoot apical meristems (SAMs), the growth points for production of new cells that elongate shoots and branches. Intrinsic changes in SAM behavior in red spruce include age-related trends in rooting ability, decreased apical control of lateral buds, wider and more massive foliage, and increased reproductive competency. In contrast, extrinsic regulation results from influences outside of the SAM, and includes gas exchange behavior and resource allocation patterns. Other age- and size-related trends were explained by a complex interaction of intrinsic and extrinsic regulatory pathways (Fig. 4) (Day and Greenwood 2011). The influence of intrinsic and extrinsic factors

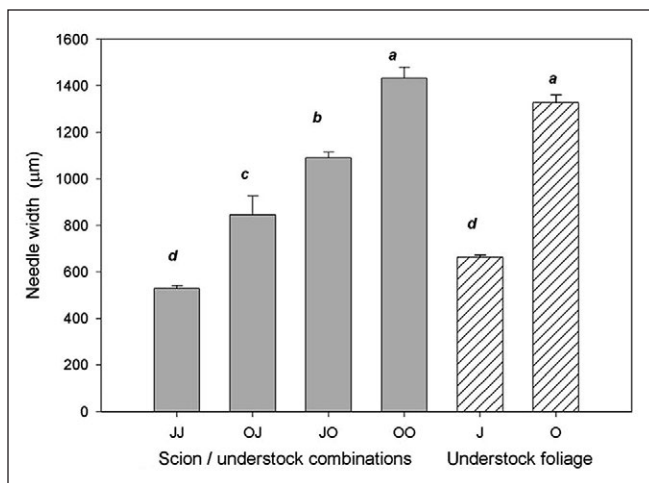


Figure 4.—Reciprocal grafting results showing complex intrinsic-extrinsic (relative to meristem) regulation of foliar morphology. Grafting combinations (left) are compared to understock foliage on the right. Scions from old donors maintained greater width than scions from juvenile donors on juvenile rootstock from intrinsic regulation, but were narrower than would be expected without extrinsic influence. Scions from juvenile donors grafted onto old rootstock maintained a narrower width of juvenile foliage, but were wider than “normal” juvenile needles. Dissimilar letters indicate significant differences ($\alpha = 0.05$; Tukey’s honestly significant difference).

in regulating branching patterns proved of particular significance in understanding age-related trends in crown form (Greenwood et al. 2010).

STUDIES ON RESOURCE SUPPLY LIMITATIONS

During the past decade our lab also tested the relevance of resource supply restrictions on the question of age- and size-related decreases in stemwood increment beyond mid-age in red spruce. Again, the PEF multicohort populations proved invaluable by providing trees that were free to grow on common soil types and with documented harvest histories extending over five decades. In addition, the PEF road and landing system allowed the use of a self-propelled hydraulic lift to reach upper crowns for nondestructive measurements. Our research has demonstrated that neither xylem conductivity, nutrient availability, photosynthetic capacity, nor diurnal trends in stomatal conductance and photosynthesis differ between mid-aged and old red spruce (Fig. 5) (Adams 2006, Day and Greenwood 2011, Greenwood

et al. 2008). In addition, a preliminary study of non-structural carbohydrates (NSC) in red spruce foliage suggested that net assimilation, the availability of photosynthetic products beyond the respiratory need of foliage, was not limiting in old trees (Fig. 6) (Day and Greenwood 2011). Subsequently, Spencer (2010) found no differences in soluble sugar concentrations between mid-aged and old trees across seasons and between two successive years. Similar trends in NSC concentrations have recently been reported for lodgepole pine (*Pinus contorta* Douglas ex Loudon) and redwoods in western North America and Australian eucalypts (*Eucalyptus* spp.) (Sala and Hoch 2009, Sillet et al. 2010).

Taken as a whole, the lack of evidence for resource limitations to observed age- and size-related decreases in tree productivity suggests that these trends may be regulated by demand-side (allocation and growth) pathways (Day et al. 2002). Further, Sillet et al. (2010) have suggested that the conventional use of stemwood production as a measure of tree productivity may suffer from a conceptual flaw stemming from a view of trees as wood production systems, as age- and size-related shifts in resource allocation patterns are not recognized. The results of our reciprocal grafting study supported continued high potential productivity in old red spruce (Greenwood et al. 2010). Despite less extension growth and greater branching of all age classes of scions grafted into the tops of old trees, all scions showed the same growth in total biomass after 7 years. Additionally, there were no differences in 7-year biomass increment between scions grafted on old rootstock and those on mid-aged rootstock, suggesting that growth potential was not influenced by age or size of rootstock.

STUDIES EXPLORING THE ECOLOGICALLY SUSTAINABLE STRATEGIES HYPOTHESIS

To address the adaptive significance of these ontological differences in growth habit, we have recently advanced the ecologically sustainable strategies (ESS) hypothesis (Day and Greenwood

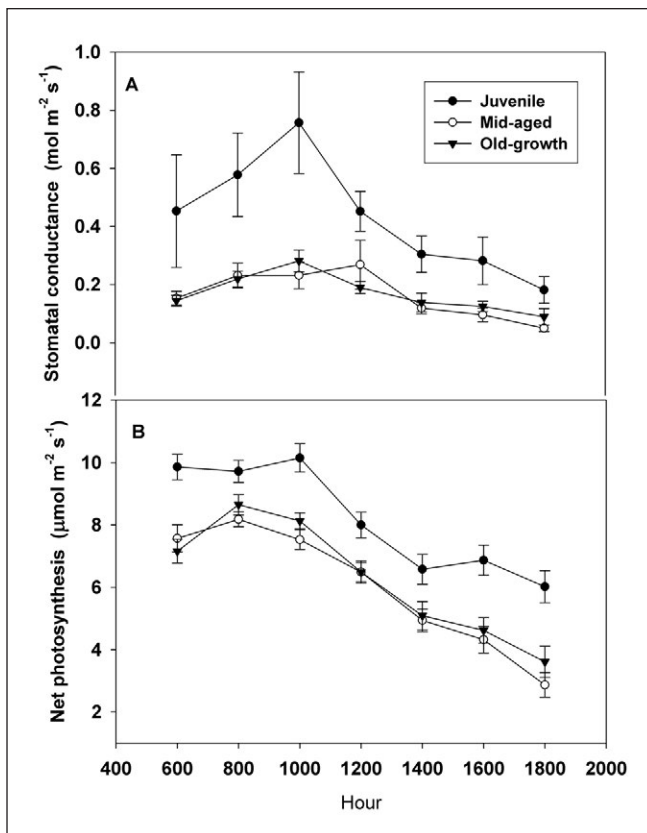


Figure 5.—Diurnal tracking of (A) stomatal conductance and (B) photosynthesis in the sun-adapted foliage of juvenile (10 yr, 1 m), mid-aged (60 yr, 10 m), and old (125+ yr, 20+ m) red spruce trees in the PEF. Juvenile trees exhibited higher net rates. Mid-aged and old trees showed the same rates and pattern of midday decrease in gas exchange. (Means $\pm$ standard error, $n=24$.) This pattern corresponds to those of electron transport rates (J_{max}) and carboxylation capacity ($V_{\text{c}_{\text{max}}}$) in the same trees (Greenwood et al. 2007).

2011). This hypothesis is built on the evolutionarily sustainable strategy concept from game theory (Vincent et al. 1996), where an individual (or, in our case, a tree species) evolves a life-strategy that permits it to indefinitely occupy a niche regardless of competition from other species. In long-lived tree species such a life-strategy not only requires a degree of plasticity, but will vary through ontogeny in a pattern adapted to confront the highly variable challenges faced by the tree as it progresses through its life-stages. In its overarching concept, the ESS hypothesis predicts that trees growing in regions with high seasonal variability in climate such as the Acadian Region and in stochastic disturbance events, such as downburst cells and extra-tropical or tropical

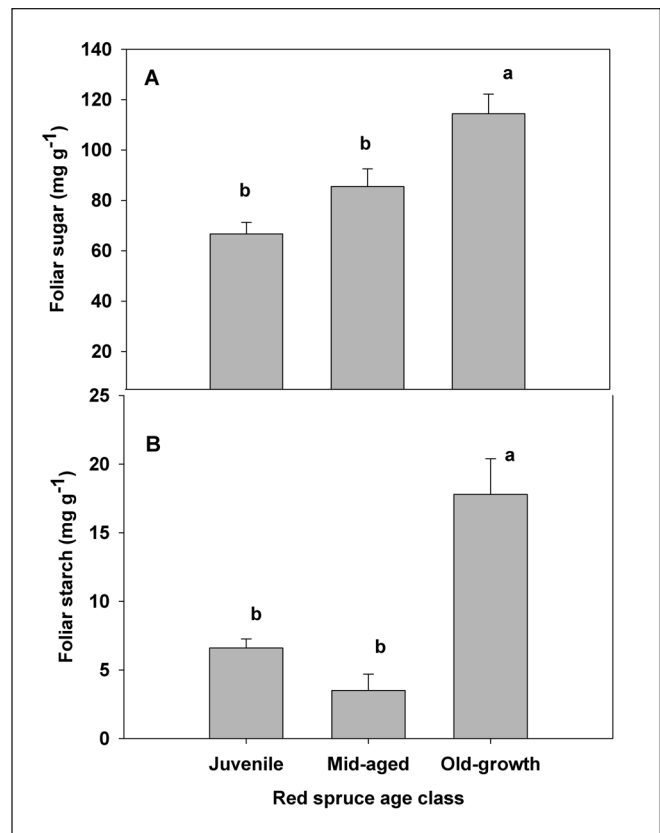


Figure 6.—Midday concentrations of the non-structural carbohydrates (A) sugar and (B) carbohydrates in red spruce foliage for juvenile (12 yr), mid-aged (60 yr), and old (120+ yr) red spruce in the PEF. The concentration of both soluble sugars and starch was greatest in the most rapidly growing age classes.

cyclones, will co-evolve a strategically uniform canopy. In these systems, emergent tree species would be evolutionarily penalized by increased risks from disturbance agents, a requirement for greater allocation of resources to stem diameter to counter dynamic loads, and increased likelihood of a shorter residence time in the upper canopy where reproductive effort has the greatest influence on population size. In contrast, maximum height growth resulting from positive-feedback “runaway” competition would be most likely in regions where relatively uniform climates and lower-intensity disturbances reduce the evolutionary costs of maximizing height growth and even provide an advantage to species that grow to their biophysical height limits.

Our continuing research based on the PEF provides support for the ESS hypothesis. We have shown that allocation patterns in conifer germinants are largely under the control of intrinsic mechanisms (Greenwood et al. 2008, Zazzaro 2009). At this life-stage, individuals have very limited capacity to assess their environment beyond a few centimeters' distance and any delay or resource/energy cost associated with sensing and responding to environmental cues decreases the individual's fitness to establish its roots in a water source and move towards a positive carbon balance. Therefore, intrinsically regulated allocation patterns that have been evolutionarily established as effective in previous generations are favored. Once established in the understory, seedlings tend to develop highly branched, flattened crowns and allocate minimal resources to extension growth of the main stem. When the seedling/sapling detects the presence of an overstory gap from changes in incident light quality and quantity, it begins its extension growth. During this phase of increasing stemwood productivity, allocation to the stem, apical down-regulation of lateral branching, and extension growth are maximized. Research on other conifer species suggests a role for a phytochrome-mediated response to altered red:far-red light wavelength composition resulting from gap formation or the presence of competitors in this ontogenetic stage (Hoddinott and Scott 1996). For PEF red spruce, Day and Greenwood (2011) provide evidence for a strong extrinsic control pathway in mid-aged individuals mediated by the external environment. Finally, once the tree reaches a position in the upper canopy, height growth is again decreased in favor of less apical control, increased branching, and more robust foliage, ultimately developing the spreading crown that characterizes the old life-stage. We believe this old-growth strategy minimizes risk and sustains long-term reproductive effort.

The ontogenetic pattern of growth allocation described above is supported by our long-term reciprocal grafting study. Scions from both old and juvenile donors showed the greatest tendency for branch production per centimeter of stem length and

those from mid-aged donors showed the greatest tendency for elongation growth on rootstock of all ages (Greenwood et al. 2010). Our research to date suggests that the reversion to higher branchiness in old trees is under a complex mix of extrinsic and intrinsic regulatory mechanisms, but we have yet to identify potential pathways that alter this and other old-tree allocation patterns.

Having established that the foliage of old trees is equal in photosynthetic assimilation to that of mid-aged individuals that show maximum accumulation of stemwood, our current research on the PEF red spruce population seeks to identify the old-tree sinks for the "missing" photosynthate. The more massive needles on old trees also have a 28 percent decrease in internal air space, resulting in a greater specific gravity (Greenwood et al. 2008), and may support thicker cuticles and increased lignin content, all of which may add to both resource cost and foliar longevity. Our preliminary data also suggest that the more conservative strategy in this ontogenetic stage includes substantially increased allocation of carbon to starch reserves in the stem. When reserves are standardized on a unit-foliage basis, old trees are holding more than 4.5 times as much carbon in stem reserves as are mid-aged spruce (Fig. 7), greatly enhancing their resiliency to external stresses.

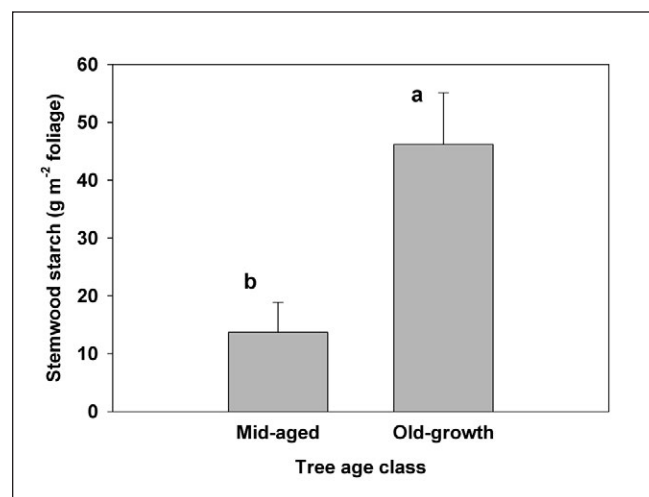


Figure 7.—Starch in stemwood reserves per unit foliage for mid-aged (65 yr) and old (120+ yr) red spruce age classes in the PEF. (Means $\pm$ standard errors, $P < 0.001$, $n=16$.)

CONCLUSIONS

The Forest Service long-term silvicultural experiment on the PEF has proven a critical resource in advancing our understanding of the complex and recalcitrant questions associated with age- and size-related trends in forest tree physiology and morphology. The answers to these questions will have substantial influence not only within the scientific understanding of tree ontogeny, but also practical application in defining paradigms for multicohort silviculture and the carbon economy of old trees. Our research group is continuing the study of age-related changes in temperate conifers with red spruce as our model species. Current projects include quantifying carbon dynamics and phenological cycles, variation in cell wall and foliar cuticular allocation, and the role of apical dominance in stage-specific crown attributes. The multicohort red spruce populations on the PEF continue to play a key role in our research.

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