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Johathan William Hagen
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To the Graduate Council:

I am submitting herewith a thesis written by Johathan William Hagen entitled "Physiologic Acclimation of Southern Appalachian Red Spruce to Simulated Climatic Warming." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

Jennifer A. Franklin, Major Professor

We have read this thesis and recommend its acceptance:

Jake Weltzin, Dave Buckley

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Dave Buckley

Acceptance for the Council:

Anne Mayhew
Vice Chancellor and
Dean of Graduate Studies

(Official signatures are on file with official student records.)

Physiologic Acclimation of Southern Appalachian Red Spruce
to Simulated Climatic Warming

A Thesis
presented for the
Master of Science
Degree
The University of Tennessee

Jonathan William Hagen
May 2006

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Abstract

Global air temperatures are predicted to rise 1 ° to 4.5 °C by the year 2100 and perhaps greater at high latitudes. In order to correctly identify the effects of climate change on tree species, the present thesis is an assessment of the sensitivity, response and acclimation potential of southern Appalachian red spruce (*Picea rubens* Sarg.) to the impact of warming. This objective was approached through examinations of synthesis (instantaneous and short-term acclimation rates of photosynthesis and respiration) as well as investment (biomass accumulation and partitioning, growth rate, and carbohydrate allocation) of carbon assimilates.

Red spruce from two southern Appalachian provenances were germinated and grown in two separate growth chambers set at day/night temperatures of 19.9/12.9 °C and 23.9/16.9 °C (means of 17 ° and 21 °C). At weeks 8, 12 and 16 after germination, concurrent analyses of carbon gas exchange and biomass accumulation were conducted. Seedlings grown at 21 °C had low photosynthetic rates at week 8, but fully acclimated by week 16 to rates equal to seedlings grown at 17 °C. Daytime dark respiration and Q_{10} values remained near equal throughout the study duration and showed minimal dependence on treatment temperature. Despite equivalent carbon assimilation, seedlings grown at the low temperature had significantly lower whole plant mass at every measurement date. Throughout the study duration, plants grown at 17 °C allocated more mass to roots, while seedlings in our elevated temperature partitioned more to foliage. Relative growth rate was 154% greater for plants grown at 21 °C than for plants at 17 °C. As supply of photosynthate surpassed growth demand for seedlings grown at low temperature conditions, these seedlings had higher concentrations of starch in both roots and shoots.

We conclude that carbon assimilation in southern Appalachian red spruce has the potential for physiological acclimation to temperature. As the cool adapted spruce seedlings performed better at 21 °C rather than at 17 °C, as displayed by increased growth rate and dry mass accumulation, we question the temperature limitations of the deciduous-boreal ecotone as it relates to this species. Interspecific variations to warmer temperatures leading to modifications in interspecific competition may be a greater determinate of a displacement of this ecotone.

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Part 1.

Introduction

Introduction

One of the most challenging questions confronting foresters and ecologists is the extent to which the future health of forest ecosystems can be predicted in a reliable manner on time scales of biological, economical and societal relevance. Concern with the management of forest ecosystems has been amplified by recent awareness of global climate change. Climate change presents a major challenge to forest ecologists studying the dynamics of natural and managed forest ecosystems. Not only must these ecologists contend with changes that all forests are constantly undergoing, but they must try to separate this natural variation from changes induced by climate. Many forests have an added complexity of management activities that result in further alterations to the ecosystem. Thus, determining the effects of climate change on these highly dynamic systems is a complex and daunting task.

The foundation of most forest ecological processes is the carbon budgets of trees, at individual, community and ecosystem levels. An enormous body of literature has developed concerning factors that directly or indirectly control carbon budgets of trees and one can conclude that climate affects forests in a variety of ways. Stand and leaf level mechanisms may interact to cause deviations in carbon assimilation, thus net primary production is the result of the interaction of extrinsic (environmental) and intrinsic (physiological) factors. Extrinsic factors are associated with the physical environment, which is often modified by structure or competitive processes associated with biotic communities. Intrinsic factors are related to inherent abilities of tree species or individuals to use available resources for growth. Thus, the intrinsic adaptedness of populations and plasticity of individuals to their extrinsic environment is a primary condition for the stability of any natural ecosystem.

Adaptability of species is a display of the genetic variation and phenotypic plasticity at all levels of biological scaling, from molecular to community. Genetic variation is fundamental for the permanence of a species as it provides the necessary short term acclimation potential (plasticity) of an individual to the current biotic and abiotic conditions, while on the long term it enables changes in the genetic composition to react with changes in the environment (van Delden 1994). If the reproductive and/or genetic structure within a species or population is jeopardized due to population reduction and isolation, then the stability of the forest ecosystem in response to a

changing environment may also be compromised (DeHayes et al. 2000). The fossil record documents both successes and failures of species to persist, evolve, and spread in response to climatic change. Successes are the result of evolutionary adaptation of species and phenotypic acclimation of individuals to enable them to survive the changes in climate and temperature extremes that occur over their lifetime.

As most extant slow-migrating tree species have survived past climatic changes of moderate intensity, there must be sufficient plasticity in individuals (or genetic variability of species) to tolerate such changes. But because there have been substantial changes in landscapes (management leading to fragmentation for example), ecological and economic systems may not be able to adapt to robust changes of spatial or temporal magnitude. Genetic constraints due to reduced gene flow are likely to reduce the level of heterozygosity (which may be used as a measure of evolutionary adaptability) to levels below what is needed to match environmental changes. Long-lived and relatively immobile ecosystems, such as mature forests are likely to be especially sensitive to climate change, and vulnerable to abrupt change (Alley et al. 2003).

The coniferous forests of mid- and high-latitudes may be particularly affected by global environmental change (Smith and Hinckley 1995, Körner 2003). These cool-adapted forest types are often considered ecological specialists and as such, the probability for adaptive improvement declines with an increase in severity of climate change. The deciduous-boreal ecotone is the transition zone between lower elevation (or latitude) northern hardwood forests and upper elevation (or latitude) boreal-type communities. The deciduous-boreal ecotone has been suggested as a possible monitoring location for the effects of global environmental change (Noble 1993, Kupfer and Cairns 1996) because plant communities are often at a dynamic equilibrium with climate at these transitory sites. As such, changes in major community types with changing climate are predicted to occur earliest at their margins, or at least be the most visible. Because ecotones are the boundaries between such community types, they are a logical place to begin looking for the effects of climate change.

Within the scientific and popular press, there is a growing interest in the declining growth rate and increased mortality of red spruce (*Picea rubens* Sarg.) in the eastern United States. In the Great Smoky Mountains National Park of the southern Appalachians, 80% of high elevation red spruce exhibit slow growth, canopy dieback and needle thinning (Peine 1986). Red spruce live basal area has steadily dropped from

26.0 m²/ha in 1951 to 18.7 m²/ha in 1990, and to 15.4 m²/ha in 2000; -41% over 50 years (Oostings and Billings 1951, Mancusi 2004). Beyond natural montane forest dynamics, suspected causes of this decline have been air pollution (Eager and Adams 1992, DeHayes et al. 1989), high soil aluminum concentrations (McLaughlin et al. 1990, Borer et al. 2004) and direct and indirect effects of the balsam woolly adelgid (*Adelges piceae* Ratz.) (Eager 1983) on the associated Fraser fir (*Abies fraseri* Pursh). The recently available evidence of global climate change presents an additional, but not yet quantified, potential factor in forest decline.

A climate change may manifest itself by a shift in the altitudinal range of alpine ecotonal species. The combination of changing climate (warming) and disturbance regimes potentially could result in the replacement of many native tree species by more competitively superior tree species. As such, the deciduous-boreal ecotone of the southern Appalachians (red spruce / Fraser fir forests abutted with hardwood species such as yellow birch) may be displaced upwards in elevation by 10s to 100s of meters (Delcourt and Delcourt 1998). The present thesis is designed to assess the sensitivity, response and acclimation potential of select natural populations of southern Appalachian red spruce to the impact of warming. Through examinations of synthesis (instantaneous and short-term acclimation rates of photosynthesis and respiration) as well as investment (biomass accumulation and partitioning, growth rates, and carbon allocation) of carbon assimilates, this thesis will identify the effects of increased temperature on upland southern Appalachian red spruce.

Spruce-Fir Forests

All natural systems exist in time and space; there can be no atemporal systems any more than there can be a non-spatial process. As climate change is continuous, and not finite, vegetation may be considered in disequilibrium with its climate on scales of time and space (Davis 1986, Webb et al. 1993) and rapid changes in climate create disturbances with which vegetation may not be able to successfully respond. Present day communities are often regarded as “transitory combinations of taxa that respond (or have been responding) individualistically to continual (or abrupt) climate change” (Ritchie 1987), and as individual taxa respond to change so does the community. Since ecosystems are not homogenous and pass through a continuous development cycle,

their state variables vary in time and space. Ecosystems can however be characterized by mean values of state variables (e.g. species composition) and their variances in space and time.

Pre-historical development and distribution

Historic temporal patterns of distribution lend themselves readily to the interpretation that glacial ice separated many species of the same genus into north-south or east-west populations during the long glaciations, probably via refugia. These populations developed distinct morphologic, physiologic and genetic characteristics by which they can be differentiated. Such adaptations to site specific microclimates have arisen to support the argument that genus speciation is largely a function of evolutionary adaptations to their environment, and genetic divergences among populations are the response by many species in accommodating or adapting to spatial variation. For example, Auckland and colleagues (2001) reviewed monoterpene and nuclear DNA content of balsam fir (*Abies balsamea* L. Mill.), Canaan fir (*A. balsamea* var *phanerleptos* Fern.) and Fraser fir (*A. fraseri*) and concluded that these three species probably arose from a single species and individualized less than 7000 years from present. This conclusion is in agreement with results stemming from monoterpene relationships (Zavarin and Snajberk 1971) and cone scale / bract ratio data (Myers and Bormann 1963).

The distribution and composition of eastern North American spruce forests have continuously changed in response to the past 20,000 years of changing climates, and more recently in response to direct and indirect human impact (Delcourt and Delcourt 1991). Historically, the major climate controls have included change in insolation, glaciations, atmospheric concentrations and oceanic circulation and temperature. The expanding and contracting ice sheets, not always in synchrony with changing climate, contribute to the difficulty in determining generalizations about the full-glacial vegetation south of the ice front (Ritchie 1987). Climatic alteration in concert with a receding or advancing glacial ice sheet has produced a relatively uninterrupted geographic shift of suitable habitats for a species' distribution. Instead of physiological adaptation to new conditions, local populations may die away and new populations establish in distant locations where conditions are closer to the species optimum (Davis 1986).

The conclusion to be drawn as to the response of vegetation to these events is that genera, and sometimes species, demonstrate a sensitivity to temperature range, and perhaps other physical aspects of the physical environment. During the Eocene and Miocene, some species were able to survive in northern ice-free areas, whereas others migrated south before the edge of the advancing ice along the north - south Appalachian mountain chain (Larson 1989). For example, the boundary of spruce trees in northeastern North America spread north around 8 thousand years before present (kybp) into the area formerly occupied by ice sheets, and has since shifted south in recent millennia coinciding with the Little Ice Age (1550 – 1850 AD; Huntley 1997).

Although competition and disturbance (mainly wind) affect the abundance and distribution of this species, it is primarily influenced by the changing climate occurring at the decade-, century- and millennia-level. As the distribution of spruce is closely linked to climate, the warming beginning ca. 14-12 kybp allowed *Picea* to follow the retreating Laurentide ice sheet northward (Ritchie 1988). Spruce was the first to migrate on the newly exposed land surfaces and thereby assumed dominance due to the lack of competitors. Progressive climate change towards longer, warmer growing seasons and milder winters subsequently enabled temperate taxa to move north. By 10-9 kybp, the spruce woodlands in the northern sites were being replaced by various mixtures of pine and deciduous tree species. The southern Appalachian extension of spruce became established by 6 kybp, and became completely disjunct from the full expanse by 2 kybp. A more thorough investigation of the changing distribution of *Picea* is presented elsewhere (Delcourt and Delcourt 1987, Delcourt and Delcourt 1991).

Red spruce remains boreal in distribution, with the center of the continuous range limited to northern New England and the Atlantic provinces of Canada. The western and southernmost extensions occur as disjunct and highly fragmented metapopulations in Ontario and the southern Appalachians respectively. Water relations are a strong determinate of local tree distribution in these forests, with red spruce occupying more mesic, closed canopy locations. Because of geomorphic instability imparted both by present-day slope processes and past periglacial activity, soils in the high-elevation spruce-fir forest zone of the southern Appalachians are often shallow (as little as 50 cm deep) Haplumbrepts and lack significant accumulation of clay minerals (Feldman and Zelazny 1987). These soils are undergoing early stages of podzolization and are more similar to northern Appalachian Spodosols than they are to adjacent low-elevation soils

of the southern mountains (Feldman et al 1991). Natural plant uptake and acidic deposition are both imposing negative effects on these soils, leading to nutrient depletion and leaching. Spruce-fir nutrient budgets indicate that current Ca and Mg leaching loss rates are of the same magnitude as losses to whole tree harvest removals spread out over a 50-yr rotation (Joslin et al. 1992). This Ca leaching is associated with a variety of physiological impairments in red spruce, including reductions in membrane stability, stomatal responsiveness and cold hardiness, among others (Schaberg et al. 2000, Borer et al. 2005)

Red spruce currently extend along the Appalachian Mountains of eastern North America from 35° to 49°N (figure 1.1). Southern Appalachian populations (below 38°N) are limited to high elevation sites, as a result of a retreat during the last post-glacial era to the coolest and most moist sites available (Cogbill and White 1991). Within the Holocene, red spruce and balsam fir of the central and northern Appalachians (> 38°N) migrated to higher latitudes, while red spruce and Fraser fir of the southern Appalachians (38 – 35°N) migrated to higher altitudes, generally above 1370m. This elevation limitation declines inversely with latitude with suitable sites at 1650m at 35°N to 150m at 49°N (Oosting and Billings 1951, Whittaker 1956, Cogbill and White 1991). The altitudinal transition of the deciduous-boreal ecotone is very gradual, and with the exception of stream margins, red spruce is rarely found below 1190m (Whittaker 1956, Schofield 1960). This site-specific occurrence implies that climate is a driving force in the distribution of montane red spruce.

Inter-mountain differences in microclimate, however, contribute to the difficulty in generalizing climate-vegetation relationships, even within a region (Richardson et al. 2004). Climatic controls that can be extrapolated however, include annual and seasonal temperatures ranging from -18°C in January to 24°C in July (average annual of 9°C at 1525m), growing season duration of 90 – 150 frost free days, seasonal distribution and annual amounts of precipitation totaling up to 250 cm, desiccating winds, high humidity, inconsistent snow cover (often greater than 100 days), cloud base elevation and rime ice associated, and all of the above as they relate to the frequency of natural disturbances (Siccama 1974, Larsen 1989, Burns and Honkala 1990, Cogbill and White 1991, Thompson et al. 2000, Richardson et al. 2003).

The transition of such climatic factors is often abrupt at certain altitudes, thereby determining the position of ecotones (Noble 1993). Temperature-based parameters are

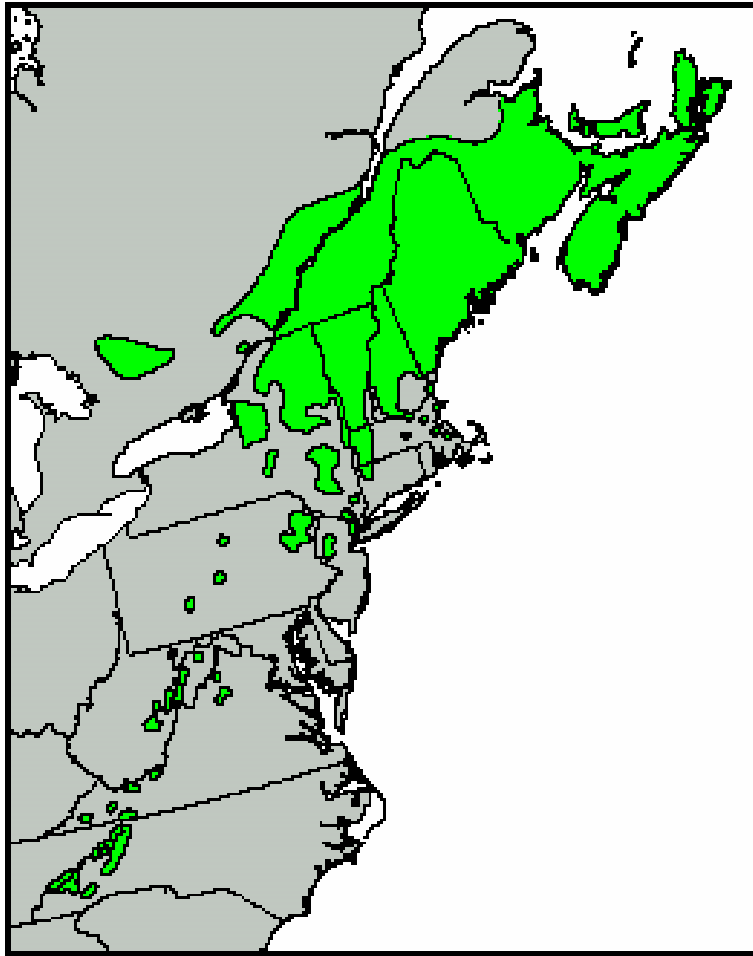


Figure 1.1. Natural distribution of *Picea rubens* of eastern North America.

perhaps the most consistent, thus often cited as the most important climatic parameter for vegetation ecotones. Throughout the natural range (35 – 47°N), the ecotone between spruce-fir forests and adjacent deciduous forest vegetation has been found to coincide with a mean July temperature of 17°C (Cogbill and White 1991). The temperature limit of upper treeline was found to coincide with 13°C, although no treeline is evident in the southern Appalachians, due to the paucity of high peaks available to accommodate such a bioclimatic threshold.

Below the lower edge of the montane spruce-fir zone (as defined by the climatic and topographic requirements above) lie deciduous northern hardwoods. Species of this forest type include yellow birch (*Betula alleghaniensis* Britton, *B. lutea* Michx.), red maple (*Acer rubrum* L.), mountain maple (*A. spicatum* Lam.), mountain ash (*Sorbus Americana* Marsh.), serviceberry (*Amelanchier arborea* Michx. f.), mountain laurel (*Kalmia latifolia* L.), black cherry (*Prunus serotina* Ehrh.) and to a lesser importance American beech (*Fagus grandifolia* Ehrh.), eastern hemlock (*Tsuga canadensis* L.) and other cove forest species (Whittaker 1956, Bailey and Ware 1990, Wisser 1998).

Stand dynamics

“The meaning of any natural thing or event can not be fully grasped or explained until we discover its relations to the components of the orderly flow of process”.

- C. Judson Herrick

The product of space fixed and time changing, forest succession may be defined as a sequential change in relative structure, kind and relative abundance of the dominant species. Ecosystem change and forest succession never end, so as opposed to defining succession as the movement towards a chosen climax, a more suitable phrase may be change away from a stochastically determined beginning. Unlike Aristotle’s *telos*, which centers on a predestined final goal, the primary focus of succession is on intervals between disturbances. Ecosystem distribution and species composition vary across landscape scales and are strongly influenced by site and environmental variables, such as disturbance history, including time since last disturbance and stand age. Successional vegetation change over the last 1000 years has been for the most part a

result of natural forcing, however, judicious use of chronosequences can provide only approximations of succession at a particular site (Jackson et al. 1988).

Unlike that of Europe or China, the vegetation of eastern North America has not been extensively disturbed or modified by human activities over a time scale of millennia (Jackson et al. 1988, Liu 1988). Changes in vegetation occurring within the last 150 years undoubtedly have resulted from activities following European settlement of the region. Disturbances within the spruce-fir forests of the southern Appalachians strongly affect stand composition, structure and dynamics. While topographic and geologic factors of high-elevation spruce forests offered little to farmers and herders, the major disturbance in this forest type prior to the 1930's was logging and subsequent slash fires (Pyle 1984).

Logging began in the 1880's and diminished around 1930 (Shafer 1984) with the initial stages of regional National Park development. The Forney Creek watershed of the Great Smoky Mountains National Park (GSMNP) currently contains 809 ha of spruce-fir forest out of a total of 12,696 ha prior to logging activities (Pyle 1988). Of this logged area, 10% was considered to have been heavy cut, 10% was light cut, and 80% was cut and burned. Fire following logging was so common that, according to Korstian (1937), "practically all cut-over spruce lands have been burned one or more times", leading to a loss of spruce due to destruction of soil organic matter and encroachment by hardwoods. As natural fires in the GSMNP are infrequent and small due to high humidity and fuel moisture, they create an unusually abrupt transition from spruce-fir to hardwoods, because spruce and fir do not regenerate well (if at all) in burned cut-over areas (Pyle 1988).

The southern Appalachian upland spruce-fir forest is presently being directly and indirectly damaged by the Balsam wooly adelgid (BWA). The BWA has eliminated 95% of the mature Fraser fir from the forest and the residual fir individuals rarely reach greater than 40 years of age (Eager 1983). As the insect reduces canopy closure and leads to fir mortality, gaps are created in the forest canopy thereby increasing susceptibility of spruce to damage by other factors. Due to these gaps, spruce, which characteristically has a shallow root system, is affected by increased wind exposure. Busing and Pauley (1994) observed that 94% of red spruce mortality in BWA infested stands was caused by wind-induced snapping and/or uprooting. Additionally, Moore et al. (2005) found that between 1993 and 1998, overstory live biomass decreased at a

rate of 1028 kg/ha/yr, mostly due to windthrow of large stems. Conversely, some plant pests allow red spruce to thrive. For example, in the Blue Ridge Mountains of North Carolina, the percentage of spruce increased considerably in 1935 as the Chestnut Blight (*Cryphonectria parasitica*) led to a decrease in the percent density of American chestnut (*Castanea dentata*) (Shafer 1984).

The high degree of deterioration in the overstory caused by the BWA has also led to a positive response in understory growth (Busing 1985, Busing and Pauley 1994, Mancusi 2004) resulting in a structure resembling a two-aged forest. Under this disturbance regime, the old-growth and second-growth forests studied herein appear to be close to compositional equilibrium. However, synergistic disturbances are operating and canopy gaps are changing, more specifically, expanding. Environmental severity (e.g. low temperatures, high wind speeds, and frequent ice storms) and air pollutant disturbances found at high elevation sites are only recently being quantified. Sunlight exposure, which reflects overstory canopy structure, has been shown to be an important factor influencing the survival, establishment and distribution of newly emerged Fraser fir (Johnson and Smith 2005).

Natural disturbance in the southern Appalachian spruce-fir forest is almost solely in the form of small (<0.02 ha) gaps that are often colonized by highly successful spruce and fir saplings. Larger, non-expanding gaps, involving a few canopy trees, allow the regeneration of mid-tolerant tree species, such as yellow birch, which can not continue under a continuous canopy (Worrall et al. 2005). Trees that have been exposed to gap initiation and expansion suffer crown damage due to wind and deterioration as decay fungi invade damaged branches. Eventually, a new conifer cohort, spruce and fir already present in the understory, will dominate, and as they increase in size and age, will be set up for another disturbance / gap. Auclair (2005) has recently identified coherent, cyclical patterns of disturbance in the mixed species forests of the northeastern United States, including red spruce, sugar maple (*Acer saccharum* Marsh.) and *Betula* spp. By addressing issues of spatial scale and long-term population dynamics, the author identified 'predictable' characteristics of forest dieback between 1950 and 1995, including: abrupt and cyclical episodic events enduring 13.6 years on average, with a frequency of 22.3 years between recurrences (Auclair 2005).

Development has also compromised the health of the spruce-fir forests of eastern North America. The Blue Ridge Parkway as well as the road to Clingman's

Dome (GSMNP) has been responsible for the presence of potential stress factors not to be expected with such frequency elsewhere in these high-elevation sites. Such stresses include erosion and soil modification, auto exhaust, increased windthrow and a distribution outlet for the BWA (Pyle and Schafale 1985). Development, along with erosion, fire, and logging, has created disturbances that may have major impacts on ecosystem resilience during stress.

During the intervals without disturbances, competitive strategies among species result in the “partitioning of realized niches”, and as a consequence, biological interactions become a major factor in structuring plant communities (Delcourt and Delcourt 1991). Consequently, there are strong feedbacks between succession, species dominance, resource competition, disturbance regime and trophic structure. The species with the lowest density in the understory (birch) has the fastest growth rate in open gaps, the species with the highest density in the understory (fir) will not reach full maturity due to the BWA, and the species with intermediate density (spruce) lives the longest and has the best survivorship. Indeed, Mancusi (2004) observed an increase in understory spruce sapling (>1.37 m in ht) density since 1990 on high elevation sites in the GSMNP.

Collectively from these data, one can postulate that the inherited competitive traits of these species are important at different successional stages. Huston and Smith (1987) state that “facilitation, tolerance, and inhibition describe processes and properties that are relative, not absolute” (p. 190), and these processes and properties can, and often do, occur simultaneously during every successional sequence. Hence, as a result of the severe limitations of fir due to the BWA, in concert with documentation that spruce can remain idle in the understory for many (50+) years (Landis and Peart 2005) suggests that local spruce-fir populations may never obtain the old-growth un-even-aged structure that existed prior to BWA infestation.

The dictating factors of forests (or any ecosystem) are important at different space and time scales. The spatial and temporal distribution of disturbances result not only from stochastic events such as high winds, ice storms, and unusually cold winters (Peart et al. 1992 and sources within), but also from specific host agents of disturbance and their tendency to attack certain age classes of trees attacking at gap-phase cycles nested within long-term, landscape-level cycles (Worrall et al. 2005). Given the variety of processes that control a forests’ structure (e.g. climate cycles, soil modification, fuel loading), and the complex interaction of these processes, even similar sites will not

necessarily result in the development of similar forests. There can be no question that natural stand dynamics are influencing the spruce-fir forests and the deciduous-boreal ecotone; all natural ecosystems undergo temporal patterns of disturbance. However the processes that control patterns can change in importance, just as the scales of the pattern are changed. Ignoring the effects of stand dynamics is hazardous, while insisting that such processes explain all ecosystem patterns is dangerously simplistic. Within the southern Appalachian spruce-fir forests, one can assume that, despite the unsightly dead fir trees, nothing beyond the scope of natural disturbances are at play. However, these natural disturbances are making the ecotone between the spruce-fir and northern hardwoods vulnerable to serious successional or forced change.

Temperature Effects on Whole-Plant Carbon Balance

Photosynthesis is the basis of ecosystem processes, including those which human society depends on, therefore photosynthetic adaptation to microclimate is more than an academic interest. Different genera, and more often different species within each genus, have different combinations of adaptation as expressed via shade tolerance, water requirements, and temperature optimum for photosynthesis. For tree species, a physiological range of existence, a physiological optimum of best growth and an existence optimum (range in which they can compete) can be distinguished (Puhe and Ulrich 2001). Those individuals with the greatest net carbon gain (amount of carbon fixed through P exceeds the amount of carbon lost to respiration) under a specific set of environmental conditions are often the best competitors at that site. Additional internal ecosystem developments, environmental influences and management practices can all act as destabilizing or stabilizing effects on carbon gain. It is through this diversity of functional characteristics that ecosystem properties are intimately linked to population and community dynamics.

As mentioned earlier in the text, climate is not static and due to the immobile status of plant individuals, they are generally the last to migrate in response to environmental change. Ecotone adjustments may occur rapidly or they may lag significantly behind biological or environmental change. Ecotones can change in both their position and strength along environmental gradients, depending upon changes in both magnitude and rapidity of changing climate. Understanding the nature of

temperature adjustments in forest trees (both short-term physiological acclimation potential of individuals and population-level adaptations to climatic regimes) is important for predicting whole-forest responses.

As temperature not only affects the distribution of plant species, it is also an important determinant of how a plant performs within its natural range. In order to understand the processes that may alter plant distribution, an insight into how plant performance is affected by temperature is required. For example, some conifers are able to adapt to sub-optimal environmental conditions such as sufficient P and tissue production on sites that have otherwise suppressive microclimates. In general, however, evergreen trees generally have $\frac{1}{2}$ to $< \frac{1}{4}$ the photosynthetic capacities of co-occurring deciduous species (Waring 1991), due in part to high weight per unit surface area and low foliar nutrient levels. Thus, specific analyses on the adaptability and efficiency of conifer carbon balance are valid in any context, and must include the processes involved in carbon synthesis: photosynthesis and respiration.

Temperature effects on photosynthesis

The temperature response of photosynthesis (P) is complex, reflecting the temperature dependencies of several interacting physical and biochemical processes. Temperature can influence carbon gain and utilization through several physiologic pathways. High temperatures can result in denaturation of enzymes critical to photosynthetic light harvesting and carboxylation pathways, and further result in carbon losses to maintenance respiration. Temperature is a critical variable in determining relative activity of Rubisco in carbon fixation (photosynthetic pathway) as opposed to oxygen fixation (photorespiratory pathway). In addition, temperature determines the saturation vapor pressure in the boundary layers of air at foliar surfaces, and thus lead to air vapor pressure deficit (VPD, defined as the water vapor pressure of air at saturation minus actual vapor pressure).

Several factors suggest that red spruce is sensitive to high air temperatures and low humidity. Optimum conditions for regeneration of red spruce have been associated with partially closed, or fully closed, canopies (Seymour 1992), indicating that environmental conditions associated with open, or absent, canopy covers may inhibit early growth. The diurnal temperature regime experienced by a plant is closely

associated with sunlight and sky exposure. Germino et al. (2002) found that microsite facilitation by surrounding vegetation (reduced sky exposure) was critical for survival of alpine silver fir (*Abies lasiocarpa* Hook. (Nutt)) and Englemann spruce (*Picea englemanni* Parry ex Engelm.) seedlings in the Rocky Mountains of western North America. Similarly, Johnson and Smith (2005) have found that Fraser fir seedlings in open canopy sites of the southern Appalachians were estimated to assimilate 3.3 – 4.5 times more carbon than seedlings at closed sites. However, excessive gap sizes, or open sites, were found to limit seedling establishment due to excessive sky exposure.

Although recent observations of gas exchange in red spruce at low temperatures have been conducted (DeHayes et al. 1989, Schaberg et al. 1995, Schaberg et al. 1998), few have explored the relative importance of high temperatures in influencing *P*. Those studies concentrating on high temperature effects have been conducted solely in the northern portions of red spruce range (Vann et al. 1994, Schwarz et al. 1997, Day 2000), and have included only instantaneous response, rather than acclimation potential. No systematic examination of southern red spruce physiological response to elevated growth temperatures has been reported.

A study conducted in the northern Appalachians (Vermont), indicated that net *P* of understory red spruce generally remains near a maximum between 15°C and 20°C, which is at or below average growing season temperatures of this region. This peak in *P* was followed by a decline to 47% at 30°C (Alexander et al. 1995). This optimum temperature (T_{opt}) for *P* coincides with the differential selectivity of Rubisco for current ambient CO₂ (300 -350 μmol CO₂ mol⁻¹ air) and O₂ (21%) and the effects of temperature on water solubility of the two gases (Ku and Edwards 1977). However, as temperature increases, the affinity of Rubisco for CO₂ falls, as does the solubility of CO₂, the affinity of Rubisco for O₂ also falls, but not as fast, so the proportion of oxygenation reaction increases. As carboxylation and oxygenation are in competition with one another, an increase in oxygenation may make Rubisco activity for carbamylation or carboxylation less efficient, thereby decreasing *P*.

Red spruce needles of Whiteface Mountain, NY displayed visible chlorosis and thermal inhibition occurring between 32 and 40°C (Vann et al. 1994), which is lower than the range reported for heat shock in temperate plants (Salisbury and Ross 1978). This suggests the needle tissue of trees sampled may have been sensitized to additional stress, possibly resulting in a deterioration of phospholipid membranes or accumulating

active oxygen species within photosystem I or II. This elevated temperature did not surpass the temperature threshold (generally $> 40^{\circ}\text{C}$) that would lead to the disruption of one or more components of the photosynthetic carboxylation pathway, as this temperature is lower than the temperature generally associated with disruption of enzymatic and membrane systems (Ku and Edwards 1977, Salisbury and Ross 1978).

The role of temperature in concert with leaf-to-air vapor pressure deficit (VPD) in regulating P of red spruce was recently investigated in a field and greenhouse study in central Maine (Day 2000). VPD calculation is an improvement over relative humidity measurement alone, because VPD considers the effect of temperature on water holding capacity of the air, which roughly doubles with every 11°C increase in temperature (Prenger and Ling 2001). The author noted strong influences of increasing temperatures and VPD on P and stomatal conductance, and stomatal response (resistance) provided significant control over gas exchange. Four-yr-old trees grown in highly variable environments with daytime temperatures of 15 to 38°C displayed a relatively flat response to temperatures between 16 and 32°C , followed by a drop at 36°C (Day 2000).

Temperature effects on respiration

While the effects of elevated temperature on P have received considerable attention, much less is known about the response of respiration. Respiration (R) in plants may consume 30-80% of the CO_2 fixed by P (Farrar 1985, DeLucia et al. 2005), and subsequently contributes up to 65% of the total CO_2 released into the atmosphere at the ecosystem level; with the remaining CO_2 being derived from heterotrophic soil activity (Xu et al. 2001, Atkin et al. 2005). This efflux is thus important for the whole-plant, ecosystem and global carbon balance. Most studies that have investigated acclimation of R to temperature have shown some degree of population-level adaptation, and even less acclimation of individuals, to temperature. Such studies have reported that when measured at a standard temperature, plants acclimated to low temperatures have greater rates of R than plants acclimated to higher temperatures (Tjoelker et al. 1999, Gunderson et al. 2000, Bolstad et al. 2003), but the results are not universal.

Increasing foliar temperature can have substantial influence on carbon gain by increasing losses to maintenance respiration. Maintenance R is in essence carbon loss due to routine metabolic activity, and is a relatively complex function of temperature,

substrate availability and other allogenic influences (Tjoelker et al. 1999, Bolstad et al. 2003). A major sink of maintenance R is related to the changes in morphology and physiology of trees as they age. Older trees generally have an inherently reduced carboxylation activity (reduced P rate), possibly resulting from greater R , lower investments in photosynthetic systems, or reduced chlorophyll concentrations (Adams et al. 2005). In addition to its role as a catalyst in the carboxylation of ribulose-1,5-bisphosphate in the C3 cycle, the enzyme Rubisco also acts as a catalyst for oxygenation in the C2, or photorespiratory, cycle.

Mass- and area- specific R rates typically increase exponentially with temperature, at least when this change in temperature occurs over a period of 1 hour or less (Bolstad et al. 2003). This exponential increase in physiologic (enzymatic) reactions with an increase in temperature is defined as the Q_{10} function. Q_{10} is often assumed to be 2.0 (doubling with a 10°C increase); however the Q_{10} value is not static, it declines near linearly with a rise in temperature (Atkin and Tjoelker 2003). Being that Q_{10} is an increase in R of near 100% for 10°C, then even a 1°C increase in growing temperature may elevate R loss by 10%.

Temperate conifers generally have Q_{10} values between 1.8 and 2.4, while no Q_{10} values specific to red spruce have been published. The shade-adapted red spruce may allocate a greater proportion of photosynthetically fixed carbon to maintenance R due to lower photosynthetic rates (Alexander et al. 1995). Further, acclimation might involve changes in Q_{10} of R with cold-grown plants being more temperature sensitive than their warm counterparts (Atkin et al. 2000). However, an earlier study on sugar maple (*Acer saccharum* Marsh.) reported no effect of growth temperature on Q_{10} values, but the authors did find a seed source effect with cold-adapted trees from Michigan having higher Q_{10} values than warm-adapted trees from Tennessee (Gunderson et al. 2000).

Intraspecific Variation Along Altitudinal Gradients

As one may intuitively expect, phytomass increases as one goes south along the global ecotypes of the northern hemisphere (Schultz 1995). This north-south gradient reflects the improvement in climatic growing conditions with an increased distance from the pole, more specifically an elevated temperature and a longer growing season. Plant responses to altitude follow many of the assumptions of plant-latitude relationships. For

example the climatic tolerance of extremes increases with latitude as with altitude, thus so does the range of the species (Stevens 1992). Thus, plant production increases with a decrease in altitude along with latitude as a response to environmental gradients.

Plant dry matter production is the net result of uptake and loss of carbon, and photosynthetic *capacity* does not tend to differ with elevation. Photosynthetic capacity is defined here, after Körner 2003a, as the highest rate of P that can be measured under optimum temperature, light and moisture condition. Based on analyses of the ecological and climatic factors associated with alpine forests, it has been suggested that photosynthetic carbon gain may not be limiting at high-elevation sites or tree line, but rather trees are restricted in their ability to process acquired carbon (i.e. growth limitation hypothesis; Hoch and Körner 2003, Smith et al. 2003, Körner 2003a, 2003b). As trees become carbon saturated, they may not always respond to increased CO_2 , unless the increase is paralleled with increased nutrient availability or more favorable temperatures for growth during the growing season (Kirschbaum 2000, Sigurdsson et al. 2001). At cooler temperatures photosynthate remains in the form of sugar and starch rather than transforming to constructive cellulose. Such increases in carbon storage have been found with increasing altitude, thereby further supporting a 'growth limitation' hypothesis (Johnson et al. 2004). This type of carbon storage may in fact be a display of adaptation for survival in a harsh, high-stress environment by serving as a reserve for future biosynthesis during particularly stressful and/or low resource seasons.

Although the photosynthetic capacity of a plant is relatively static with elevation R is not, and rates at high altitudes will almost always be greater than rates at lower altitudes, regardless of time or temperature (Tranquillini 1979). If P remains stable while R increases, net carbon accumulation decreases and the potential growth rate is limited (Berninger et al. 2004). Therefore, trees growing in a valley can fix more carbon than those growing at treeline, and in this sense be more productive. McLaughlin et al. (1990) found a 30% increase in R at high elevation sites in the GSMNP thereby not allowing high altitude growth rates to match rates achieved at low altitudes. An explanation for this seems to be a combination of a greater number of mitochondria per cell at high elevations, as suggested by Miroslavov et al. (1991) and a higher oxidative activity per mitochondria (Klikoff 1968).

At alpine sites, light conditions for P are less favorable than might be expected due to timberlines generally following contours on steep slopes where elevated horizons

screen the site from possible insolation (Tranquillini 1979). As adequate light wavelengths and amounts are required, yet variable, for P , so is atmospheric $[\text{CO}_2]$. Ambient CO_2 is 380 ppm at sea level, which corresponds to a partial pressure of 380 μbar (36 Pa), and declines to 360 μbar at 600m and 290 μbar at 2600m. Reduced partial pressure of CO_2 on P at high altitudes and its direct influence on plants may be affected by 1) oxygen partial pressure declining, and hence R is reduced; 2) 'thinner' air allows CO_2 to diffuse through stomata faster; and 3) air temperatures drop, as does leaf temperature, which may counteract 2 and enhance 1. The overall negative influence of harsh montane conditions such as low temperatures, strong wind and low CO_2 concentrations on productivity of trees at or near treeline is however slightly ameliorated by higher humidity.

Phenotypic adaptations do exist to these harsh sites as a higher altitude seed source often equates to a lower temperature optimum (T_{opt}) for P . Fryer and Ledig (1972) monitored photosynthetic rates of balsam fir from an elevational gradient in the White Mountains of New Hampshire and found that individuals from higher altitudes required a lower T_{opt} for a common P . They found a T_{opt} lapse rate of 2.7°C for 300m in altitude, which corresponds to the lapse rate of mean temperature for summer months for their site. McLaughlin and colleagues (1990) found that red spruce saplings at high elevation sites in the GSMNP had a lower P rate than saplings at low elevations when measured *in situ* and expressed as mass-based rates. The authors suggest that this finding was related with greater heavy metal concentrations in the upper elevation soils.

As with photosynthetic T_{opt} , intraspecific growth and partitioning parameters are likely not a genotypic adaptation of local populations, but rather a phenotypic acclimation driven by environmental conditions, or a balance of both. However, analyses among genera and species suggest that both phenotypic plasticity and genotypic variation contribute to the effects of elevation and latitude on anatomy and physiology. Previous research using the common garden approach indicates that Norway spruce (*Picea abies* L. Karst) populations originating from higher altitudes display higher nitrogen concentrations in needles and overall higher needle retention than those in low altitudes. Subsequently, chlorophyll and carotene concentrations increased with seed source elevation, as did P and R (Reich et al. 1996, Olesksyn et al. 1998). Along with higher rates of P , high-altitude populations displayed lower above-ground growth rates, higher

proportional biomass partitioning in roots, and overall higher mortality than populations originating from low altitudes (Modrzyński 1995, Oleksyn et al. 1998).

The short-term response to *in situ* environmental conditions is considered the classic example of phenotypic plasticity in plants. From gap to understory, or upper canopy to lower canopy, plants experience an environment that varies in intensity over several orders of magnitude. As a response to such gradients, variation in leaf structure and function can occur not only among plants in different environments, but also within an individual. Richardson et al. (2001) and Richardson (2004) found reduced plasticity of red spruce and balsam fir along an elevation and canopy light gradient as a response to harsh environments. The authors indicate that while foliar nutrient investments (N, P, K, and Cu) were relatively constant along a 400m altitudinal transect (1070 – 1460m asl), fiber and cellulose content, spectral reflectance (chlorophyll content), and photochemical reflectance index (measure of photosynthetic radiation use efficiency) decreased sharply at higher elevations.

Needle morphology also varies with elevation with a general decrease in length, width and projected area with an increase in elevation, though thickness may be constant or greater (Tranquillini 1979, Körner 2003a). Richardson et al. (2001) found such results on morphological characteristics with balsam fir and red spruce along elevational gradients within the Green and White Mountains of New England. Projected needle area was lower at high elevations than at low elevations for both species, and needle length and mass tended to be smaller at high elevations rather than low elevations. Results are similar in the GSMNP and Black Mountains of the southern Appalachians for Fraser fir and red spruce with needle length and leaf area index (LAI; m^2/m^2) decreasing with an increase in elevation. Red spruce needle length decreased from 12.02mm at 1525m to 11.02mm at 1980m (-8.4%) and LAI fell from 13.66 m^2/m^2 at 1525m to 7.08 m^2/m^2 at 1980m (-48%)(Nicholas 1992). Incidentally, LAI and stand live basal area for three spruce-fir study sites decreased for those sites with a history of logging (Nicholas 1992).

The experimental field studies cited above generally present greater *P* and mobile carbon compounds at high-elevations, yet greater size at low-elevations. These findings support the 'growth limitation hypothesis', because a growth reduction due to insufficient photosynthetic carbon fixation ('carbon limitation hypothesis') should lead to diminished mobile carbon pools at higher sites, rather than carbon saturation (Körner

2003a, Hoch and Körner 2003). Low air and / or soil temperatures may limit growth processes more so than photosynthetic processes, irrespective of carbon supply. These findings further suggest that trees along high-elevation sites are limited more so by variations in mesotopography and microsite such as photosynthetically active radiation (PAR), minimum temperatures, sky exposure, drought stress and any combinations, rather than specific limitations due directly to altitude.

Evidence is accumulating that meristematic activity in Pinaceae ceases at higher temperatures than P activity. The formation of new cells is, however, much more sensitive to low temperatures than P . Cell doubling time, for instance, approaches infinity at +1 to +2°C, whereas the rate of P may still be at 30% of full capacity at such temperatures (Tranquillini 1979). Kontunen-Soppela et al. (2002) reported no shoot growth, but a significant increase in starch concentrations in needles of Scots pine (*Pinus sylvestris* L.) in a 14-day 4°C treatment. Counter to expectation, tissue density, non-structural carbohydrate and lipid concentrations increase with elevation on both a dry mass and volume basis (Körner 2003b). Similar elevational trends are found in needles, branches and roots, regardless of latitude (Hoch and Körner 2003). A steady, high non-structural carbohydrate concentration (starch in chloroplasts) is likely to indicate excessive photosynthate supply over demand, arguing against a 'carbon limitation' hypothesis and for a 'growth limitation' hypothesis of depressed growth at high elevations.

Individual traits exhibit both genotypic and phenotypic variation in response to micrometeorological factors and environmental gradients. What is interesting though, is that some traits exhibit greater genotypic variation (among populations) than others. Likewise, other traits are comparatively plastic and as such are more strongly influenced by the environment. Genotype x environment interactions are also an outcome and both can be adaptive. In the Norway spruce study, shoot growth phenology and biomass partitioning in favor of roots seemed to be under strong genetic control, such that despite the higher P of the high-elevation seed sources (also genetically influenced), plant sizes of the high-elevation seed sources were reduced in the common garden compared to the lower elevation populations (M. Tjoelker pers. comm.). The challenge, of course, for physiological ecologists, is sorting this out in the context of integrated plant function.

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Part 2.

Physiological response of southern Appalachian red spruce to experimental warming

Introduction

There is now considerable evidence of ongoing climatic change (Luterbacher et al. 2004) and observed trends (e.g. an increase of 1°C on average since the Industrial Revolution) are predicted to increase over the next century (IPCC 2001). Together with habitat destruction and biological invasions (due to global change), climate change now represents one of the major threats to species, ecosystems and biodiversity (Root et al. 2003). The direct focus of immediate stability of small populations of rare and endangered species diverts attention from the probability that regional and global environmental changes in the near future may compound other, more local threats to continued existence of species that are narrowly adapted to specific environments. For many species with confining temperature niches, a temperature increase of 4°C could change the environment from being optimal to unsuitable (Shugart et al. 2003, Day et al. 2005). In response to forecasted accelerated summer warming, forest succession models predict that high-elevation red spruce (*Picea rubens* Sarg.) may be forced to migrate to a climate suitable for their survival (Davis and Botkin 1985, Solomon 1986). Even with less drastic changes in climate however, the rapidity of the change may cause restricted ranges of boreal conifers, or local extinctions, because the species may not be able to disperse northward at a sufficient rate to keep up with shifts in climate (Davis and Shaw 2001, Alley et al. 2003).

Along the Appalachian Mountains (35 to 47°N), the lower limit of red spruce occurrence has been found to correspond with a mean July temperature of 17°C (Cogbill and White 1991). Due to biogeographical history (Delcourt and Delcourt 1987), red spruce provenances from the southern isolated region have been shown to be unique from each other and from all other red spruce provenances, possibly due to founder effects and inbreeding (Hawley and DeHayes 1994). It is unknown whether these genetic differences represent adaptations to higher temperatures, or any differences in plasticity. An earlier study conducted in northern Appalachian provenances have indicated that net photosynthesis (P) of understory red spruce generally remains near maximum between 15°C and 20°C. A peak in P near 20°C was followed by a decline to 47% at 30°C, when grown at mean daytime temperatures of 22°C (Alexander et al. 1995). Temperature-related adaptations due to geography have been previously

demonstrated in this genus (Qamaruddin et al. 1993, Bigras 2000), however the photosynthetic optimum of southern provenances is unknown.

The deciduous-boreal ecotone of the southern Appalachian Mountain region is currently showing signs of declining health of high-elevation red spruce and Fraser fir (*Abies fraseri* (Pursh) Poir.). Patterns of reduced vigor and mortality of red spruce have long been linked to acidic deposition (Eager and Adams 1992), high soil aluminum concentrations (McLaughlin et al. 1990, Borer et al. 2005) nitrogen deposition (Boggs et al. 2005), and direct and indirect effects of the exotic balsam wooly adelgid (*Adelges piceae* Ratz.) (Eager 1983, Smith and Nicholas 2000). This decline, in concert with a predicted (and highly documented) increase in productivity of deciduous hardwoods with elevated temperatures (Tjoelker et al. 1998, Gunderson et al 2000, Wan et al. 2004, C. Gunderson unpublished data) may stimulate any displacement of the deciduous / boreal ecotone of the southern Appalachian Mountains. However, no process studies have been conducted on the response of red spruce grown at elevated temperatures, and the significance of the response must be assessed in terms of whole-plant responses and overall carbon balance.

The thermal stability of Rubisco activase represents a major biochemical factor limiting the ability of plants to photosynthesize at increasing temperatures. By limiting *P* activity, activase may ultimately affect plant growth in a particular thermal environment, the geographic distribution of higher plants, and their ability to respond to changing climates (Salisbury and Ross 1978, Salvucci and Crafts-Brander 2004). There is evidence, however, of short-term physiologic acclimation to changes in temperature over time, through the adjustment of temperature related responses of metabolism. For example, inductions of heat shock proteins are involved in acclimation to higher growth temperatures over periods of hours, affording protection to the various components of the photosynthetic apparatus (Bigras 2000). The accumulation of transient cryoprotective carbohydrates in response to cold temperatures is an example of acclimation over the period of days (Schaberg et al. 2000). Four-yr-old red spruce trees grown in greenhouse temperatures of 15 to 38°C displayed a relatively flat response to temperatures up to 32°C, followed by a significant drop at 36°C (Day 2000), suggesting an acclimation over the period of weeks. The magnitude and variability of such responses are dependent upon species (Larigauderie and Körner 1995, Tjoelker et al. 1998, 1999), provenance (Bigras 2000, Gunderson et al. 2000), elevation (Fryer and

Ledig 1971, Tranquillini 1979), metabolic status (Salvucci and Crafts-Brander 2004), and growth temperature (Atkin et al. 2000), among others.

The objective of this research was to assess the sensitivity, response and acclimation potential of southern Appalachian provenances of red spruce to the impact of a warming climate. Focus was on the effects of temperature on whole-plant carbon balance and allocation strategies as they relate to storage and growth. As red spruce is a cool-adapted boreal species, the specific hypothesis to be addressed was that a higher growth temperature will negatively affect carbon assimilation and investment when measured at growth temperature. We predicted that seedlings exposed to elevated growth temperatures will exhibit a decline in net carbon assimilation and show a greater sensitivity to pulse measurement temperatures, when compared to ambient growth temperatures. Limitations on P will result in limitations on photosynthate supply for growth and / storage, therefore we predicted restricted growth for plants grown at elevated temperatures. We further hypothesized that effects of elevated growth temperature will be greater for seedlings from high elevation seed sources (cooler temperatures). To address the hypotheses above, as they relate to distributional limits, we used montane red spruce from the southern Appalachians. Seedlings were grown at an ambient mean temperature of 17°C and an elevated (+4°C) mean temperature of 21°C. Carbon synthesis and investment were evaluated over 3 dates to quantify any aberrations due to an elevated temperature.

Materials and Methods

Plant material

Red spruce seeds were collected from 2 stands from within two provenances with microclimates that are moderately cool and moist (mean July temperature $\leq 17^\circ\text{C}$, mean annual ppt ≥ 180 cm). Richland Balsam, within the Balsam Mountains of North Carolina (35° 22' N, 82° 59'W; summit of 1954m) and Clingman's Dome, within the Great Smoky Mountain National Park of NC / TN (35° 34'N, 83° 30'W; 2025m) provided our seed source of southern Appalachian red spruce (figure 2.1). At both provenances, two stands were selected for seed cone collection delineated by elevations of 1650 and 1950m. From within these two collection stands, 2 randomly located 20m (diameter)



Figure 2.1. Natural range and collection sites of *Picea rubens* from within the southern Appalachians. Modified map from Kevin Potter, NC State University, used with permission.

circular plots were established during the summer of 2004, for a total of 8 plots (2 provenances X 2 stand elevations X 2 plots). At elevations of cone collection, spruce-fir forests were the dominant forest type at 1950m; spruce forests with scattered yellow birch (*Betula alleghaniensis* Britton, *B. lutea* Michx.) were dominant at 1650m.

General seed collections from previous studies (Agmata and Bonner 1988, Nicholas et al. 1992, and preliminary results) suggest that red spruce from high elevations produce very low viable seed yields (< 5%). For this reason, 90 fallen, mature spruce seed cones were collected from the forest floor from within the 8 plots during October 2004. Collections from the two plots per elevation were then pooled for a total of 180 cones per elevation per provenance. Cones were placed in individually labeled paper bags for ease of transport, then placed in an incubator where dry, warm conditions prompted cone opening. Cones were then air-dried for approximately seventy-two hours, and bags shaken periodically to promote seed release, after which the bracts were removed and large, well-formed seeds were selected and de-winged. The seeds from each source remained separated, placed in labeled glass containers, and stored at 4°C until further processing as red spruce require no pretreatment (Wilson-Griffin 1965). Seed from all sources were germinated under uniform conditions at 20/14 °C for four weeks in a 1:1 commercial sand / vermiculite medium, as this temperature regime allows successful germination (CFRU 2002).

Temperature treatments

Four weeks after sowing (after cotyledon expansion), established seedlings were transplanted to a 2:1:1 (v/v/v) peat, vermiculite and sand mixture in 25cm deep by 6cm diameter black cylindrical root trainers. Seedlings (26 from each provenance x elevation combination) were assigned and divided into two groups, and moved to two matched 0.5m³ reach-in environmentally controlled growth chambers (Precision 818, Winchester VA, US). Fifty-two seedlings were maintained at a day/night temperature of 19.9/12.9° C (mean of 17°C), and 52 seedlings of corresponding seed source combination were maintained at 23.9/16.9° C (mean of 21°C), with an abrupt change of less than 1 hour. These temperatures are based on the day/night equation of:

$$\frac{T(a) + (T-7)(24-a)}{24} = x$$

assuming a 7°C difference in day/night, a = daylength (hrs) and x = daily mean temperature (°C).

Both chambers provided a 14-h photoperiod, with supplemental light provided by 6 DULUX® EL 27 watt twist compact fluorescent lamps (Osram Sylvania Inc.). Lamps have an average rated lamp life of 8000 hrs while collectively providing a maximum of about 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (photosynthetic photon flux density, PPF) irradiance at canopy height. This light irradiance level is based on the literature and closed canopy measurements collected on site during plot establishment during July 2004 (180 – 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at breast height; 24 at the forest floor; 600 – 800 at sunflecks). Photosynthetic rates for sympatric understory Fraser fir have been found to light-saturate at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Johnson and Smith 2005), while understory red spruce light saturation has been documented at 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Alexander et al. 1995).

Seedlings were watered to excess thrice weekly with de-ionized water. Root trainers were periodically repositioned within the chamber to prevent shading and randomize any effect of position. Temperature treatments were applied for a total of 16 weeks, which is equivalent to the average length of the high-elevation southern Appalachian growing season. An independent sensor was periodically used to verify both temperature and light intensity.

Gas exchange measurements

Light saturated photosynthesis and dark mitochondrial respiration rates were determined with an infrared gas exchange system (LI-COR 6400, Lincoln, NE), with a 7.5 cm conifer chamber (LI-COR 6400-05) throughout the study duration. This system permitted accurate control of cuvette temperature and humidity as the cuvette enclosed the entire aboveground stem. Sampling order was alternated between chambers to limit time of day effects. Calibrations for flow meter and IRGA zero points occurred at weeks 8, 12 and 16 prior to process measurement. Needle temperatures in the cuvette were maintained at the applied growth temperature, and at a vapor pressure deficit (VPD) of < 2.0 kPa (Day 2000). VPD, relative humidity (RH), transpiration and time of day were recorded at each measurement. The measurements occurred without removal from the controlled environment chamber at light irradiances at which the plants were grown.

Beginning at week 8 of temperature treatments, two sequential readings 5 minutes apart were taken for the determination of P calculated on a leaf area basis ($\mu\text{mol m}^{-2} \text{s}^{-1}$). These data were averaged to obtain an instantaneous photosynthetic rate for each seedling. Short ($\pm 5^\circ\text{C}$) temperature response curves were then constructed on a random sample of individuals to examine whether rates of P differed between treatments. Five $\pm 2.5^\circ\text{C}$ intervals ($-5, -2.5, 0, +2.5, +5^\circ\text{C}$) from low to high temperature were applied by adjusting the internal block conifer chamber temperature. Gas exchange measurements were observed until readings stabilized, typically 3 – 10 minutes.

As leaf respiration (R) can only be measured in the absence of carbon fixation (illuminated leaves), the growth chamber was then darkened to obtain R . McLaughlin et al. (1990) suggest that steady state R can be attained within 1 minute after light blockage so after a delay of one minute, two R readings were obtained, then averaged. Temperature coefficient (Q_{10}) measurements were evaluated by monitoring R rates at week 8, 12 and 16 by exposing randomly selected seedlings from both treatments to controlled temperatures of $\pm 5^\circ\text{C}$ from ambient. Upon completion of gas exchange measurements, leaf areas were determined by stripping all needles from the shoot and scanning at 0.1mm^2 resolution with a LI-COR 3100 Leaf Area Meter (LI-COR, Lincoln, NE). These needle areas were then used to calculate area-based gas exchange readings ($\mu\text{mol m}^{-2} \text{s}^{-1}$) with the standard algorithms of Li-Cor's Open 5.1 operating system.

Both P and R values were used to construct carbon use efficiency values (CUE, the ratio between the amount of carbon incorporated into dry matter and the amount of carbon fixed in gross photosynthesis or net P / gross P). The specific R of plants grown and measured at their respective growth temperature was also used to calculate a long-term acclimation ratio. This is defined as the ratio of R from plants grown and measured at a given temperature to R from plants grown and measured at a lower temperature. The short-term Q_{10} values were then compared to the long-term acclimation values to assess the degree of acclimation of R to thermal environment, as defined by Larigauderie and Körner 1995.

Biomass and growth analysis

To quantify growth and partitioning, leaf area (cm²), root length (cm), and dry biomass (g) were assessed every four weeks beginning at week 8 after germination. Plant dry mass was destructively determined at weeks 8, 12 and 16 by removing seedlings from the soil, rinsing the roots and oven drying at 60°C for 72 hours. Needle, stem, root and whole-plant dry mass were then determined. Week 16 samples were freeze dried for later carbohydrate analysis. These data were used to determine growth parameters including: specific leaf area (SLA, m² leaf / g leaf); specific root length (SRL, cm root / g root); leaf mass ratio (LMR, g leaf / g plant); and root mass ratio (RMR, g root / g plant). These relative parameters were used to avoid solely comparing plants of different sizes.

Changes in mean dry mass over time for each treatment were analyzed using a classical approach to plant growth analysis (Evans 1972) as follows:

$$\text{RGR} = (\ln w_2 - \ln w_1) / (t_2 - t_1)$$

where $\ln w$ is the natural log (\log_e) of dry biomass at time 1 (t_1) and time 2 (t_2). Each harvest constituted a small independent sample of the population and RGR could only be compared as mean values of treatment by week with no statistical test for difference (Poorter and Lewis 1986).

Carbohydrate analysis

At the conclusion of the 16 week temperature treatment and after dry-weight determination, seedlings were separated by tissue components: needles and roots. Tissues were washed with distilled water and then homogenized into < 1 g subsamples by grinding with liquid nitrogen in a mortar. Along with a glucose standard, tissues were extracted twice with 85% ethanol at 90° for 10 min. and centrifuged. This extract was dried, redissolved in water and sugars measured by the anthrone method (Ashwell 1957). Following addition of anthrone reagent, soluble sugar extract were determined spectrophotometrically at 625 nm. The residual tissue was dried, and starch converted to sugars using the enzymatic digestion method described by Hendrix (1993), with soluble

starch (Fisher Scientific S-516) as a standard. Starch was gelled and converted to glucose by α -amylase and amyloglucosidase from *Aspergillus niger* (Sigma, St. Louis, MO). Glucose concentrations were then quantified spectrophotometrically using anthrone reagent.

Data analysis

The design is set as a randomized complete block with four fixed independent variables of temperature treatment, provenance, elevation and week. A replication of the full study block was run consecutively following the first and was considered a random variable. Alternating chamber assignments of temperature treatments between block 1 and block 2 minimized any chamber effects. Data were tested for statistical assumptions of normality and equality of variance. When necessary, individual variable data were log-transformed to satisfy test assumptions and back-transformed for display.

Instantaneous gas exchange, growth and carbohydrate data were analyzed by using analysis of variance (ANOVA) for the main effects of growth temperature, provenance, and elevation and interactions between these factors. These variables were analyzed at individual weeks. Differences between weeks were characterized by LSD post-hoc tests, as we have unequal samples sizes between weeks. Block was analyzed as a main effect only. Because the same trees were measured at different temperatures to obtain a pulse temperature response, curves were analyzed with a repeated measures ANOVA. The ANOVA tested between-subject factors consisting of growth temperature, provenance, elevation, and interactions between such factors. As ANOVAs are 2-tailed tests, p -values were considered significant at 0.10, to obtain 1-tailed α of 0.05.

Results

Instantaneous gas exchange

When measured at respective growth temperatures, mean rates of net photosynthesis (P) on a leaf area basis were higher in seedlings grown at 17°C than seedlings grown at 21°C at measurement weeks 8 and 12 (figure 2.2, table 2.1). P for

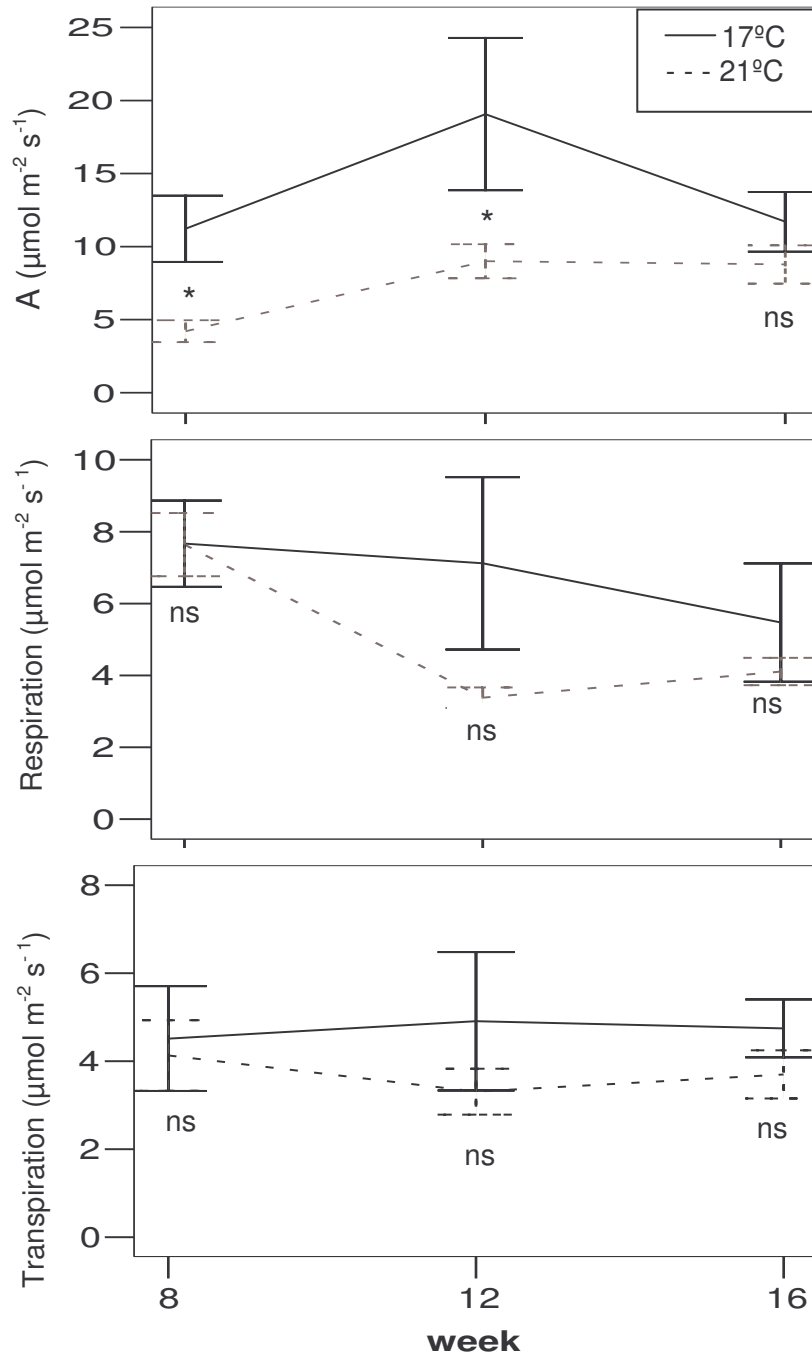


Figure 2.2. Net photosynthesis (A), respiration and transpiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$) values over time for red spruce seedlings grown for 16 weeks in ambient (17°C) and elevated (21°C) temperature treatments. Values represent means \pm SE of $n = 11-19$ for all weeks. Asterisks represent a significant difference between temperatures ($p < 0.10$); ns = not significant.

Table 2.1. Sources of variation p-values from ANOVA for gas exchange parameters of red spruce seedlings grown at 17°C and 21°C at weeks 8, 12 and 16.

<u>week 8</u>				
	Photosynthesis	Respiration	Transpiration	Q10
Temperature	0.019	0.498	0.735	0.013
Provenance	0.397	0.372	0.001	0.786
Elevation	0.673	0.372	0.821	0.675
T x P	0.900	0.335	0.644	0.319
T x E	0.267	0.972	0.401	0.398
P x E	0.862	0.923	0.250	0.557
T x P x E	0.924	0.262	0.241	0.601
Block	0.207	0.002	0.139	0.035
<u>week 12</u>				
	Photosynthesis	Respiration	Transpiration	Q10
Temperature	0.063	0.229	0.225	0.053
Provenance	0.019	0.262	0.011	0.131
Elevation	0.293	0.162	0.764	0.349
T x P	0.077	0.413	0.194	0.620
T x E	0.201	0.277	0.433	0.148
P x E	0.135	0.721	0.734	0.267
T x P x E	0.095	0.676	0.575	0.129
<u>week 16</u>				
	Photosynthesis	Respiration	Transpiration	Q10
Temperature	0.236	0.425	0.245	0.362
Provenance	0.106	0.958	0.214	0.746
Elevation	0.153	0.693	0.708	0.611
T x P	0.823	0.131	0.114	0.975
T x E	0.744	0.663	0.589	0.576
P x E	0.734	0.365	0.395	0.644
T x P x E	0.503	0.610	0.171	0.394
Block	0.000	0.001	0.001	0.001

seedlings from the 21°C temperature treatment was less than half the rate of P of seedlings grown at 17°C at week 8. These seedlings however, produced gas exchange rates at week 8 significantly different than weeks 12 and 16. Least square differences (LSD) post- hoc tests of plants grown at 21°C resulted in a difference of P at $p = 0.017$. No change in P over time was exhibited for seedlings grown at 17°C. Mean P of seedlings grown at 17°C at week 12 are skewed by two high readings of 56.8 and 45.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$, otherwise the slope for P over time would be relatively flat at around 11.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$. By week 12, seedlings grown at 21°C had an increase in P , followed by a plateau near 8.75 $\mu\text{mol m}^{-2} \text{s}^{-1}$. At week 16, mean P for both sets of seedlings was not significantly different.

Rates of dark respiration (R) did not exhibit changes associated with growth temperature. Over all weeks, plants grown at 17°C displayed rates of R near equal to plants grown at 21°C. As P is shown to adjust with time, so did CUE. At week 8, CUE for red spruce seedlings grown at 17°C and 21°C was 59 and 35% respectively. However, these values increased and matched over time to 73% at week 12 and 68% at week 16.

There was no consistent difference in the way seedlings from the two geographic sources or elevations responded to growth temperature (table 2.1). Thus, for both Richland Balsam (RB) and Clingman's Dome (CD) seedlings from both 1650 and 1950m, rates of P and R were equal for temperature conditions native to the parent tree, and at a +4°C temperature. No relationship was found between growth chamber time of day and P ($r^2 = 0.102$ for both treatment temperatures), or growth chamber time of day and R ($r^2 = 0.028$ for both treatment temperatures). A significant block effect was observed with block 1 displaying greater rates of both P and R than block 2.

Despite higher VPD and lower RH at the higher growth temperature, due to the natural water holding differences in air at higher temperatures, there were similar trends in photosynthetic response to VPD and RH between seedlings from the different growth temperatures. Seedlings from both temperature treatments responded negatively to an increase in VPD by substantially lowering rates of P (figure 2.3). This is what we would expect as a previous study identified a significant deficit of P at VPD above 2 kPa (Day 2000). Across all measurement dates, transpiration rates did not vary with growth temperature (figure 2.2). A provenance effect on transpiration however, was evident at weeks 8 and 12 but was lost at week 16. Seedlings from the RB provenance exhibited higher rates of transpiration, irrespective of growth temperature, than seedlings

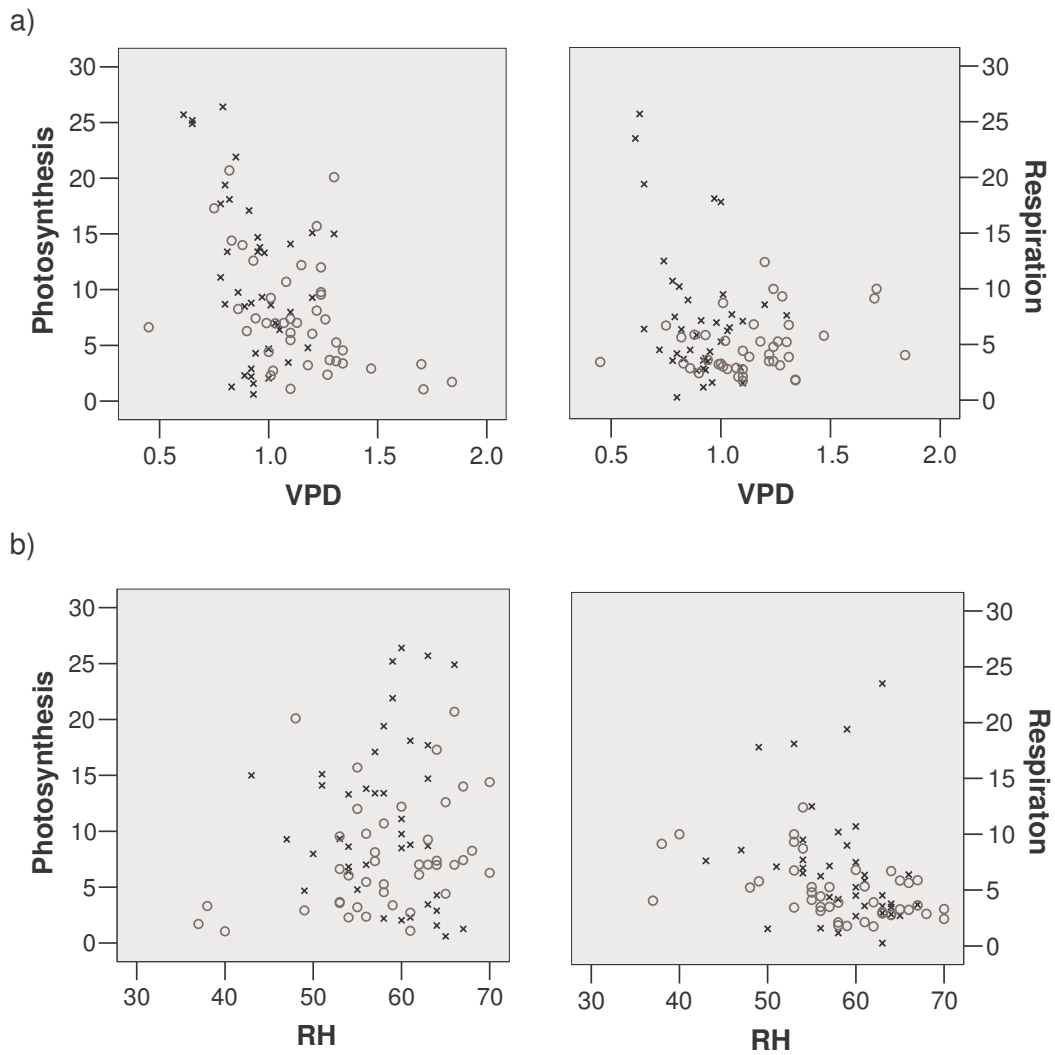


Figure 2.3. Scatterplots of red spruce photosynthesis and respiration ($\mu\text{mol}/\text{m}^2/\text{s}$) with a) vapor pressure deficit (VPD in kPa) and b) relative humidity (RH in %). Gray circles represent seedlings grown at 21°C and black x's represent seedlings grown at 17°C for 16 weeks.

from CD (table 2.1).

Temperature response values

For seedlings grown at both 17 and 21°C, the highest P was recorded at the lowest measurement temperature of -5°C from growth temperature, and then decreased with increasing measurement temperature (figures 2.4, 2.5 and 2.6). Both sets of seedlings responded to the +5°C measurement temperature with a significant decline in observed P . The pattern of response of P to measurement temperature depended on growth temperature, i.e. at week 16 the slope of the response curve was greater for trees grown at 17°C over those grown at 21°C. Output from the repeated measures ANOVA for growth temperature between-subject effects at week 16 was significant both for P at $p = 0.005$, and for R at $p = 0.001$. The degree of respiratory response calculated from $\pm 5^\circ\text{C}$ from growth temperatures varied with treatment temperature, although, regardless of growth temperature, foliar R increased with measurement temperature (figures 2.4, 2.5 and 2.6). The intercept of the relationships did not vary among growth temperature, but slopes did.

At week 8, plants grown at 17°C had a Q_{10} of 2.30, whereas the mean Q_{10} value of plants grown at 21°C was 1.69 (significantly different at $p = 0.013$). However, the degree of difference lessened over time, with Q_{10} values for plants grown at 17°C and 21°C of 2.29 and 1.90 respectively at week 16 (not significantly different at $p = 0.362$, table 2.1). Shoot R exhibited lower proportional long-term respiration increases with increased growth temperatures in comparison to short-term temperature responses. The long-term respiration acclimation ratio of shoot respiration of plants grown and measured in dark periods of high (21°C) and low (17°C) at week 16 was 1.33, which was less than the mean Q_{10} value for both sets of seedlings. That the long-term acclimation ratio was less than the value of instantaneous Q_{10} , but greater than 1.0, suggests a partial acclimation to thermal environment. Instantaneous Q_{10} values at week 16 and the long-term were consistent among collection sites.

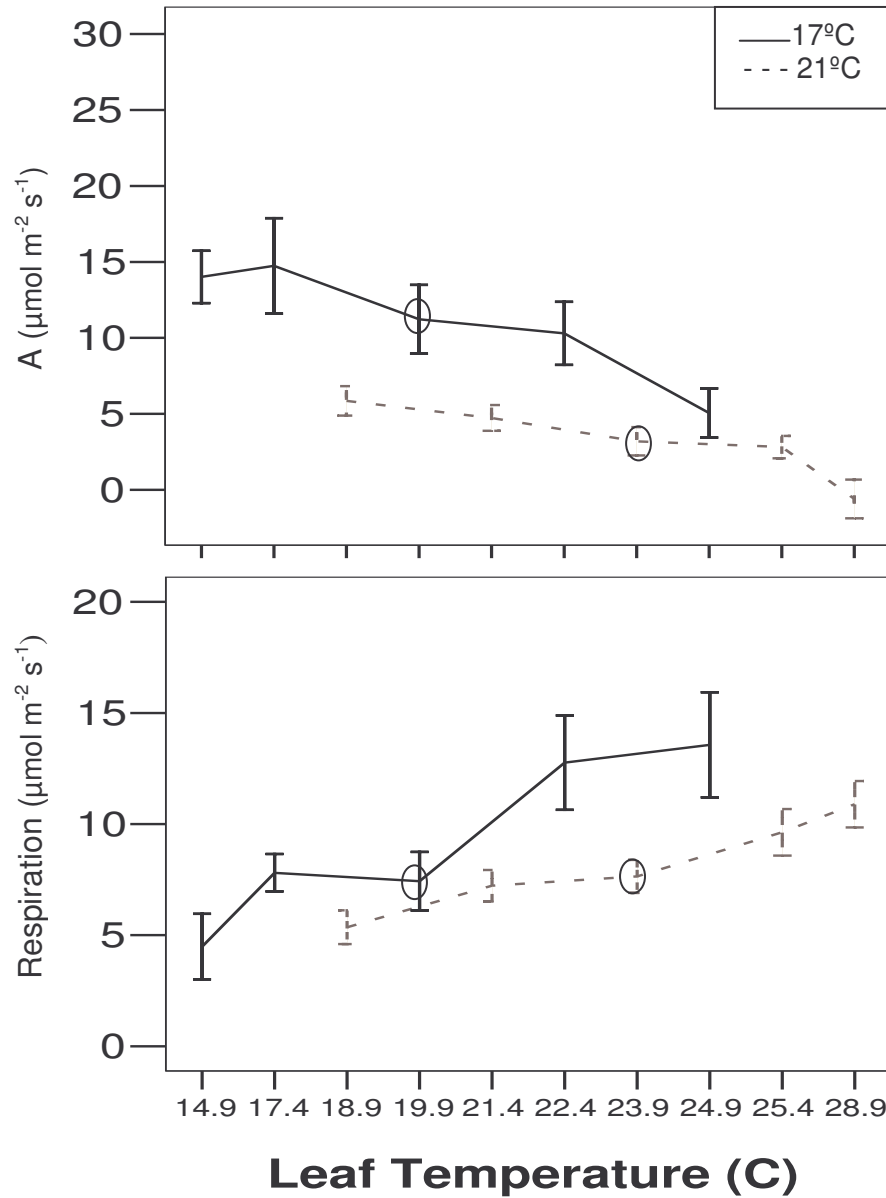


Figure 2.4. Week 8 temperature response curves of net photosynthesis (A) and respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$) for red spruce seedlings grown at 17 and 21 °C. Lines represent means \pm 1 SE of 13 seedlings per treatment temperature; open ovals represent measurement temperature equals growth temperature. X-axis represents temperatures \pm 5°C from daytime growth temperatures of 19.9 and 23.9°C.

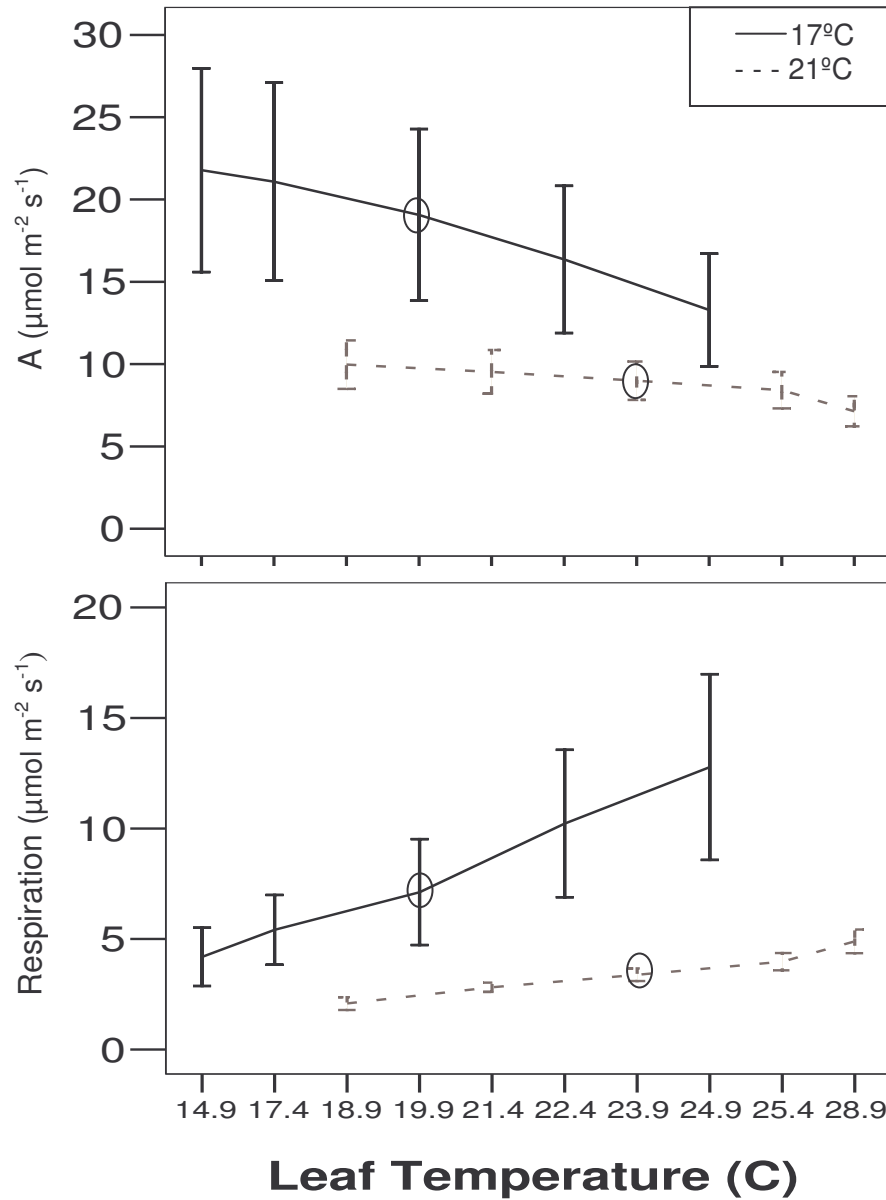


Figure 2.5. Week 12 temperature response curves of net photosynthesis (A) and respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$) for red spruce seedlings grown at 17 and 21 °C. Lines represent means \pm 1 SE of 9 - 12 seedlings per treatment temperature; open ovals represent measurement temperature equals growth temperature. X-axis represents temperatures \pm 5°C from daytime growth temperatures of 19.9 and 23.9°C.

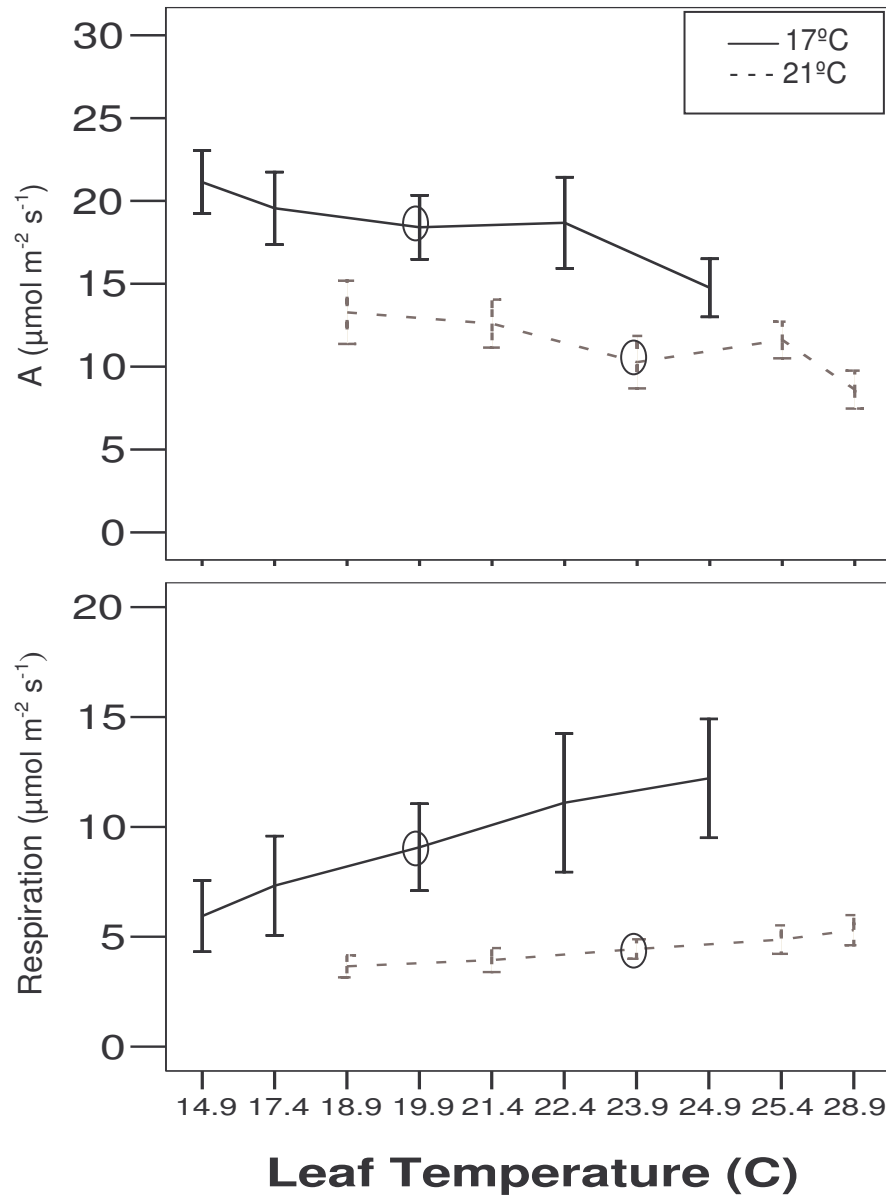


Figure 2.6. Week 16 temperature response curves of net photosynthesis (A) and respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$) for red spruce seedlings grown at 17 and 21 °C. Lines represent means \pm 1 SE of 10 -14 seedlings per treatment temperature; open ovals represent measurement temperature equals growth temperature. X-axis represents temperatures \pm 5°C from daytime growth temperatures of 19.9 and 23.9°C.

Growth response to temperature

As observed via plant dry mass accumulation over time, RGR was higher for spruce seedlings grown at our elevated temperature. From week 8 to 12, seedlings grown at 21°C had a RGR of 0.0415 mg/g dw/day, as compared to those grown at 17°C with a rate of 0.0270 mg/g dw/d. While this trend was maintained over time, the RGR declined with increasing age, and/or increasing plant mass. From week 12 to 16, the RGR was 0.0320 mg/g/d for plants grown at 21°C and 0.0225 mg/g/d for plants grown at 17°C. For the full study duration, week 8 to 16 RGR was 0.0368 mg/g dw/d (21°C) versus 0.0247 mg/g dw/d (17°C) which represented a 49% difference in RGR between temperature treatments.

The constant temperature treatments had a consistent effect on red spruce seedling biomass. Across all weeks measured, total and aboveground biomass were significantly higher in the 21°C treatment than in the 17°C treatment (tables 2.2 and 2.3). Elevated temperatures increased total plant biomass by 214% at the end of the study duration, which was mainly comprised of aboveground tissue. At week 16, stem mass of seedlings grown at 21°C was nearly triple (3x) and leaf mass was nearly six times (6x) the mass of these components of seedlings grown at 17°C. SLA, a measure of leaf area relative to whole plant mass, was near equal at week 16 between temperature treatments, whereas the difference of absolute leaf area was the greatest at this measurement date. LMR, a comparison of mass proportions, was however highly dependent upon treatment temperature, with seedlings grown at 21°C having consistently higher values of LMR over seedlings grown at 17°C.

Belowground biomass was equivalent for seedlings grown at 21°C and at 17°C, except at week 16 (tables 2.2 and 2.3). SRL showed no response to elevated temperatures at any week. RMR values were significantly greater for seedlings grown at 17°C across all weeks measured, representing more mass being deferred to root structure over shoot structure.

There existed a very significant difference among blocks. Seedlings of trial 2 were smaller and lighter for all growth values irrespective of temperature treatment. The trends across temperatures, however, remained constant between blocks. No consistent provenance or elevation main effect was observed throughout the three measurement dates for any growth parameter. At week 16, several temperature by provenance by

Table 2.2. Growth analysis of *Picea rubens* seedlings after 16 weeks of treatment in ambient (17°C) and elevated (21°C) temperatures. Values are means (\pm SE) of n = 31 at week 8; n = 23 at week 12; and n = 46 at week 16.

	Week 8		Week 12		Week 16	
	17	21	17	21	17	21
Leaf Area (cm ²)	0.39 (.08)	0.86 (.14)	1.05 (.18)	3.83 (.60)	0.45 (.06)	3.85 (.85)
Leaf Mass (g)	0.0148 (.002)	0.0227 (.003)	0.0356 (.002)	0.082 (.013)	0.0295 (.005)	0.1263 (.025)
Stem Mass (g)	0.0024 (.000)	0.0036 (.000)	0.0067 (.000)	0.0142 (.003)	0.0056 (.001)	0.0157 (.003)
Root Length (cm)	10 (.69)	8.1 (.46)	13.1 (1.09)	15.1 (1.24)	22.4 (1.32)	27.5 (.87)
Root Mass (g)	0.0067 (.001)	0.006 (.001)	0.0156 (.002)	0.0218 (.003)	0.0385 (.001)	0.0663 (.009)
Plant Mass (g)	0.024 (.003)	0.0323 (.003)	0.0578 (.004)	0.1242 (.018)	0.0735 (.011)	0.2083 (.035)
Specific Leaf Area (m ² / g)	31.94 (6.35)	43.01 (5.33)	28.02 (4.25)	42.73 (3.53)	35.35 (7.01)	35.94 (3.51)
Specific Root Length (cm / g)	17.72 (1.99)	14.92 (1.18)	9.22 (.92)	8.01 (1.01)	9.82 (1.53)	6.77 (1.04)
Leaf Mass Ratio (g / g)	59.4 (3.0)	66.9 (2.8)	61.9 (1.6)	71.2 (1.1)	35.5 (1.9)	49.7 (3.7)
Root Mass Ratio (g / g)	28.9 (2.6)	20.4 (1.8)	26.3 (2.1)	17.7 (1.2)	53.2 (1.3)	41.7 (3.4)

Table 2.3. Sources of variation p-values from ANOVA for growth parameters of red spruce seedlings grown at 17°C and 21°C at weeks 8, 12 and 16. As *t*-tests and ANOVAs are 2-tailed tests, *p*-values were considered significant at 0.10, to obtain 1-tailed α of 0.05.

Week 8	Leaf		Stem	Root		Plant	SLA	SRL	LMR	RMR
	Area	Mass	Mass	Length	Mass	Mass				
Temperature	0.009	0.001	0.002	0.022	0.419	0.004	0.155	0.094	0.001	0.001
Provenance	0.234	0.755	0.842	0.242	0.567	0.943	0.314	0.350	0.773	0.721
Elevation	0.400	0.326	0.631	0.379	0.778	0.368	0.966	0.890	0.239	0.218
T x P	0.701	0.259	0.959	0.877	0.017	0.095	0.473	0.025	0.274	0.148
T x E	0.675	0.814	0.679	0.016	0.861	0.852	0.482	0.457	0.235	0.420
P x E	0.429	0.801	0.177	0.025	0.142	0.391	0.183	0.004	0.079	0.236
T x P x E	0.837	0.712	0.554	0.083	0.603	0.703	0.839	0.105	0.650	0.695
Block	0.112	0.000	0.016	0.843	0.038	0.000	0.000	0.001	0.000	0.000
Week 12	Leaf		Stem	Root		Plant	SLA	SRL	LMR	RMR
	Area	Mass	Mass	Length	Mass	Mass				
Temperature	0.000	0.003	0.016	0.280	0.162	0.007	0.001	0.435	0.001	0.006
Provenance	0.032	0.539	0.487	0.184	0.387	0.481	0.000	0.718	0.705	0.623
Elevation	0.274	0.289	0.156	0.675	0.447	0.274	0.739	0.283	0.986	0.765
T x P	0.278	0.599	0.349	0.940	0.999	0.619	0.572	0.615	0.917	0.610
T x E	0.234	0.314	0.100	0.884	0.757	0.318	0.305	0.619	0.929	0.552
P x E	0.898	0.788	0.787	0.996	0.891	0.899	0.561	0.836	0.771	0.716
T x P x E	0.374	0.612	0.442	0.982	0.740	0.744	0.108	0.579	0.180	0.996
Week 16	Leaf		Stem	Root		Plant	SLA	SRL	LMR	RMR
	Area	Mass	Mass	Length	Mass	Mass				
Temperature	0.000	0.000	0.000	0.003	0.002	0.000	0.942	0.082	0.000	0.000
Provenance	0.001	0.014	0.004	0.786	0.358	0.014	0.381	0.847	0.140	0.049
Elevation	0.157	0.282	0.942	0.941	0.781	0.481	0.831	0.565	0.726	0.748
T x P	0.001	0.013	0.006	0.365	0.069	0.006	0.027	0.156	0.717	0.656
T x E	0.104	0.130	0.093	0.644	0.808	0.163	0.764	0.912	0.739	0.381
P x E	0.004	0.005	0.034	0.334	0.080	0.004	0.255	0.182	0.156	0.229
T x P x E	0.006	0.022	0.022	0.889	0.089	0.011	0.146	0.130	0.539	0.808
Block	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000

elevation interactions were evident (table 2.3). After further review, it was found that genotypes from RB at 1650m were the cause of each interaction. This genotype displayed the greatest increase in plant mass, leaf area, leaf mass, stem mass and root mass when exposed to elevated growth temperatures (figure 2.7).

Non-structural carbohydrate concentrations

Among the four carbohydrate parameters tested at week 16, concentrations of root starch and needle starch were significantly different between temperature treatments (Table 2.4). In seedlings in the 17°C treatment, the concentration of needle starch was 268% greater than needles of seedlings grown at 21°C (figure 2.8). Overall, whole-plant mean total carbohydrate concentrations declined from 214.06 mg/g dw to 165.55 mg/g dw ($p < 0.001$) with the increase in temperature from 17 to 21°C. A provenance effect was observed with RB genotypes accumulating more root and needle starch than CD genotypes. We also noticed a temperature by provenance interaction for root starch, needle sugar and needle starch. Seedlings from RB were more sensitive to a 4°C increase in growth temperature, illustrated by a sharp decline in root and shoot starch. CD genotypes showed greater needle sugar sensitivity to elevated growth temperatures. A growth temperature by provenance by elevation interaction was also observed with genotypes from RB at 1650m accumulating the greatest difference in needle starch and sugar with an increase in temperature (figure 2.7).

Discussion

Temperature response of carbon assimilation

For the first 12 weeks of temperature treatment, red spruce seedlings grown at 17°C exhibited higher rates of P than seedlings grown at an elevated (+4°C) temperature, irrespective of measurement temperature. A similar response was reported in a study of loblolly pine (*Pinus taeda* L.) seedlings grown for two months at constant temperatures of 25, 30 and 35°C (Teskey and Will 1999). Seedlings grown at 21°C adjusted with time to the stress of higher than natural temperatures, to produce P rates near equal to seedlings grown at 17°C. By week 16, no difference in P was exhibited,

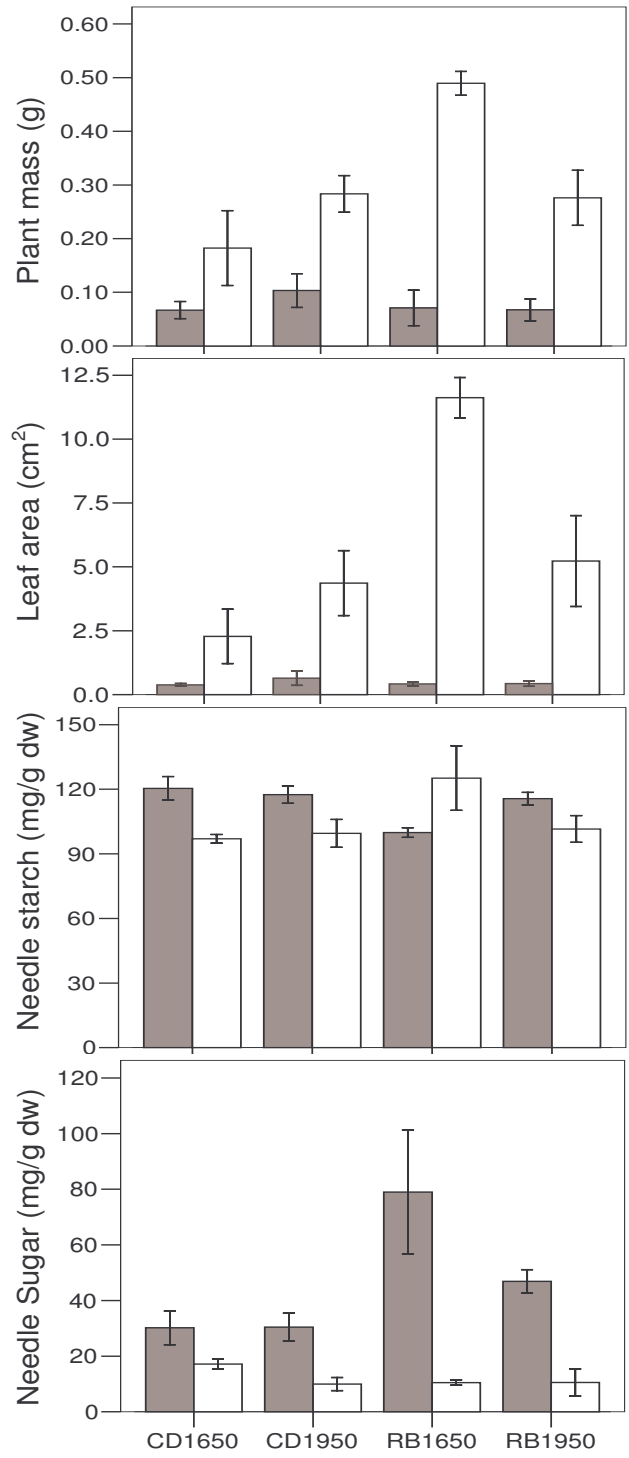


Figure 2.7. Red spruce genotype variation in response to elevated growth temperatures, for plant mass, leaf area, needle starch and needle sugar. Gray bars represent growth temperatures of 17°C and open bars represent growth temperatures of 21°C. Values represent means \pm SE of n = 12 -24 seedlings for all parameters. CD = Clingman's Dome provenance, RB = Richland Balsam provenance.

Table 2.4. Analysis of variance probabilities ($P > F$) of main effects and interactions on non-structural carbohydrate concentrations of red spruce seedlings grown at 17 and 21°C for 16 weeks. Bold values are significant at $p < 0.10$.

	Root		Needle		Total Carbohydrates
	Sugar	Starch	Sugar	Starch	
Temperature	0.121	0.015	0.178	0.000	0.000
Provenance	0.789	0.025	0.775	0.005	0.037
Elevation	0.452	0.503	0.705	0.050	0.139
T x P	0.123	0.023	0.026	0.001	0.764
T x E	0.203	0.174	0.132	0.199	0.384
P x E	0.190	0.693	0.729	0.193	0.212
T x P x E	0.985	0.344	0.053	0.047	0.793
Block	0.341	0.185	0.242	0.273	0.179

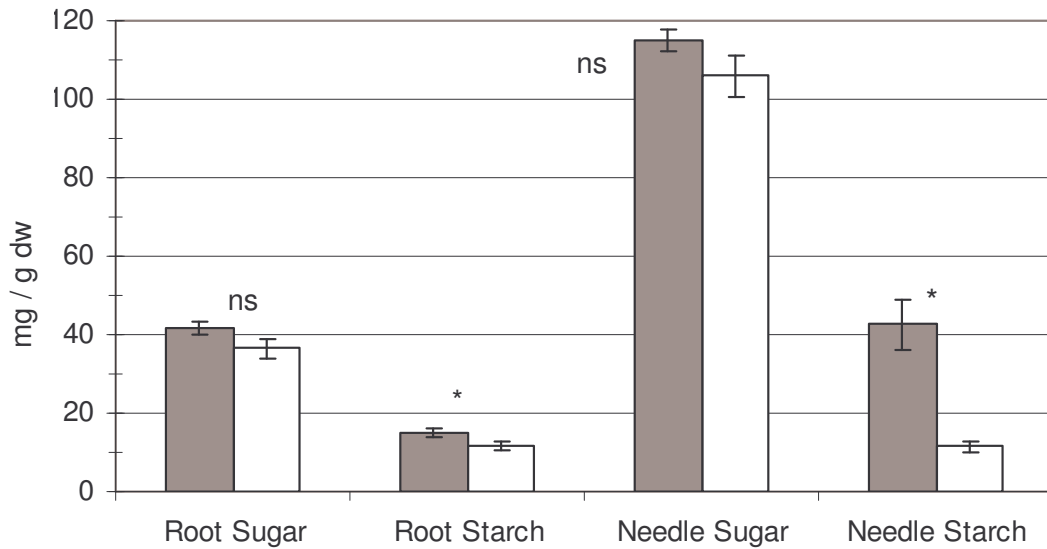


Figure 2.8. Total non-structural carbohydrate concentrations (mg / g dw) of red spruce seedlings grown at 17 (gray bars) and 21°C (open bars) mean temperatures. Values represent means \pm 1 SE of $n = 12$ to 20 seedlings. Asterisks represents significant difference between temperature treatments at $p < 0.05$, ns = not significant.

suggesting an acclimation response to growth temperature. As gas exchange rates of seedlings grown at 17°C did not change over time, this adjustment of seedlings at the higher temperature was probably not an age effect. This finding indicates exposure to supraoptimal growth temperature (in our case 21°C) has detrimental effects on photosynthetic capacity of red spruce that can be compensated for by a long-term acclimation response.

Rates of R were not affected by growth temperature, aiding in the equality of CUE between the seedlings from the two growth temperatures. CUE previously has been found to be relatively constant among species and under different environmental conditions (van Iersel 2003). Here it is shown that CUE adjusted with time, likely as a result of adjustments of P with time. Plants grown at 17°C were much more efficient with carbon utility at week 8, but by week 12 any differences in such efficiency were nullified. Thus, despite the differences in P with temperature, by week 12 CUE was equal for both sets of seedlings suggesting evidence of acclimation over the period of weeks. The hypothesis that higher growth temperatures will result in comparatively suboptimal carbon assimilation was not supported.

The negative response of P to increasing VPD in red spruce has been described previously (Day 2000), resulting in high sensitivity to high VPD (> 2.00 kPa). Current conditions of high-elevation southern Appalachian sites are generally cool and moist with VPD less than 2.00 kPa (NCDC 2005). A +4°C increase in air temperature will likely increase VPD, regardless if RH remains constant (Prenger and Ling 2001), which has also been forecast to change with global change. As there was an effect of growth temperature on VPD (data not shown), our findings suggest a reduced sensitivity of P to high VPD for seedlings grown at higher temperatures.

The observed decline of P performance with an increase in measurement temperature meant we were unable to define an optimal temperature for red spruce in either growth chamber. This consistent decline in P with increasing temperature, suggests that the optimum temperature for P may be below measurement temperatures applied. Similarly we can not detect any shifts in temperature optimum, however both set of seedlings displayed a significant drop in P at +5°C over ambient. That the rate of P does not continue to increase with measurement temperature has been attributed, in part, to reduced CO₂ uptake, lower gas solubility at higher temperatures, and increased CO₂ evolution caused by photorespiratory activities (Ku and Edwards 1977, Salisbury

and Ross 1978). As elevated temperatures decrease the Rubisco activation of carboxylation for P within a plant cell, it increases the activation of oxygenation for R , which is precisely what we observed with our temperature response curves.

The short-term temperature coefficient for respiration, Q_{10} , was not significantly different between the two sets of seedlings at week 16, and averaged 2.1 for both groups of seedlings. This value is consistent with those commonly reported for woody plant species, averaging about 2.3 (see review in Atkin et al. 2005), and suggests short-term acclimation over the period of hours. The long-term temperature coefficient for R was 1.33 when averaged across all genotypes, suggesting a partial acclimation of R to growth thermal environment over the period of weeks. A complete acclimation would result in identical rates of R when measured at the growth temperature, creating a value of 1.0 (Larigauderie and Körner 1995). No acclimation has occurred if the acclimation ratio and Q_{10} values are equal, since the short-term temperature response would result in a similar long-term response. As seedlings grown at 21°C exhibited partial respiratory acclimation to temperature, we can not accept the hypothesis that seedlings in higher growth environments will show a greater sensitivity to pulse measurement temperatures when compared to seedlings grown at 17°C.

We found no consistent variation among provenance or elevation for gas exchange measurements, thus we can not accept the hypothesis that genotypes from higher elevations will show a greater response to high growth temperatures. This suggests that the two provenances tested are able to partially acclimate to changes in their growth temperature. Collection sites were selected along a small altitudinal gradient of 300m, which has been estimated to represent a local Appalachian lapse rate difference of 1.89°C (Leffler 1981). Other studies have reported altitudinal variants for optimal P temperature as a response to microclimate (e.g. Fryer and Ledig 1971), thereby suggesting that reduced P of trees at high elevation sites are at least in part related to differences in carbon utilization and allocation at each site. Similar to the present findings, McLaughlin and colleagues (1990) observed a uniform P of red spruce between their high elevation (1935m) and low elevation sites (1720m) from area-based field measurements. These results provide evidence for a balance between genetic and environmental control on carbon synthesis.

Carbon partitioning and growth

Over sixteen weeks, seedlings in elevated (+4°C) temperatures had greater mean RGR and produced 214% more biomass than trees in ambient temperatures. The growth rate declined with time and age yet, due to the initial advanced growth of seedlings grown at 21°C, the difference in plant weight increased over time. These findings can not support the research hypothesis that simulated warming will result in limited photosynthate supply. This observation is in agreement with the temperature effects found on other boreal conifers jack pine (*Pinus banksiana* Lamb.), tamarack (*Larix laricina* K.Koch), and black spruce (*Picea mariana* Mill. B.S.P.) (Tjoelker et al. 1998). The authors noted a growth increase in response to elevated temperatures, in concert with CO₂ enrichment, but only up to 21°C, when the response was stable until a decline at 27°C.

Elevated temperatures influenced above ground growth and biomass accumulation more so than below ground area or mass, as displayed by a three-fold increase in stem weight and nearly six-fold increase in foliage weight for seedlings grown at 21°C as compared to seedlings grown at 17°C (table 2.3). LMR was consistently higher in seedlings grown at 21°C, than in seedlings grown at 17°C. Relatively small increases in dry matter towards photosynthesizing tissue can increase growth rate profoundly. The increased partitioning to leaf rather than root partially explains the elevated productivity of our warm treated seedlings. This is, in part, because increased dry matter to leaves decreases the proportion of carbon used in respiration by the root mass, and increases leaf area (light interception) which increases available carbon for growth, compared to seedlings with disproportionately higher below ground allocation. Seedlings grown at 21°C exhibited comparative rates of area-based *P*, but leaf area was consistently twice as large as seedlings grown at 17°C, so whole-plant *P* is assumed to be considerably greater.

Concentration of mobile carbon pools, or non-structural carbohydrates (NSC), is the measurable result of the balance between supply by photosynthesis and demand by growth and metabolism (Körner 2003). The accumulation of NSC at week 16 for plants grown at 17°C may reflect changes in the production and use of newly fixed carbon, and the biosynthesis intermediates. Low NSC displayed by red spruce seedlings at 21°C probably reflects carbon use associated with the construction and maintenance of new tissue, rather than for cryoprotective purposes. The physiologic responses that help

provide protection against frost damage simultaneously reduce growth rate. Previous studies have observed a negative correlation between RGR and frost hardiness (Greer et al. 2000). Though not plotted, our seedlings with greater RGR had lower NSC reserves. The accumulation of carbohydrates clearly is energy intensive and therefore inhibitory to growth. One growing season however, represents a fraction of the lifespan of any age class of needles, and exposure to temperature treatments during such a limited time may not impair physiologic function to the point of inducing visible alterations in carbon allocation or growth.

Ecological considerations

Separate studies have identified the presence of a strong genetic trade-off between cold tolerance (Loehle 1998), heat tolerance (Bigras 2000) and annual growth at the interspecific and intraspecific levels for several tree species. As red spruce seedlings in elevated temperatures increased their growth rate in response to warming, the timing and degree of cold tolerance of these individuals may come in to question during early freezes. Although the precise effects of winter injury on red spruce carbon budgets have not been fully quantified, it is logical that repeated, severe events may be associated with tree decline and mortality (Lazarus et al. 2004).

Schaberg et al. (2000) specifically relate the function of red spruce foliage as a storage reservoir for carbohydrates to the decline of the species. As our seedlings grown in elevated temperatures had significantly reduced levels of total stored carbohydrates, the degree and timing of cold tolerance may be jeopardized as these high-elevation systems are prone to unseasonable frost conditions (Nicholas 1992). When plotting our one-time data points of needle sugar concentrations on published curves over time (e.g. Alscher et al. 1989, Schaberg et al. 2000), we can extrapolate a delay of roughly two weeks worth of accumulation for seedlings grown at 21°C. As needles and buds are important tissues in the whole-plant carbon balance of trees, a severe winter storm leading to losses of these tissues will severely disrupt the carbon economies of red spruce trees and could lead to further spruce decline and mortality.

Common garden experiments reveal that growth rate, biomass partitioning and carbohydrate dynamics are a genetic expression (Oleskyn et al. 1998, Oleskyn et al. 2000), but significant plasticity occurs within and between individual plants of the same

species in response to growth environment. High-elevation red spruce have been found to display lower absolute leaf area and mass along with lower SLA (Nicholas 1992, Richardson et al. 2001), when compared to low elevations, possibly as a result of thicker cell walls within the needles (Körner 2003). The current study did not reveal any significant or consistent altitude or provenance effect on any biomass parameter. One collection site however, did display greater sensitivity to elevated growth temperatures over any other site. Genotypes from 1650m at RB showed the greatest increase in whole-plant mass, leaf area, leaf mass, root mass and needle starch levels with an increase in growth temperature. These same individuals exhibited the greatest decline in needle sugars with an increase in temperature. Enhanced metabolic activity in these individuals in response to elevated temperatures is most likely a genetically controlled acclimation feature stimulated by growth environment.

In this paper, we show that the assumption that red spruce seedling growth necessarily declines at higher temperatures is invalid, and that stable southern range boundaries result from processes other than temperature limitations alone. Extreme and sudden temperature shifts may be more important to this system than mean annual or seasonal temperatures. As displayed in the present study, higher growth temperatures do not interfere with an individual's temperature response curve, thereby not necessarily resulting in a major decline of growth rate. Highly competitive invading species concurrently moving along temperature response curves in response to warmer temperatures will not gain much from their competitive advantage in the face of existing individuals of boreal species. Thus any displacement of the ecotone will be delayed until existing overstory individuals die, which may be delayed for hundreds of years. As alpine environments are often dynamic, high-stress ecosystems, evidence is needed to address the potential expansion of deciduous species under current and future alpine climates.

Although field verification is needed, we postulate that, because of temperature acclimation, increases in growth temperature (and subsequent changes in air moisture conditions) may not be as detrimental to juvenile red spruce physiology as currently predicted by some models. An increase in summer temperatures of +4°C did not result in changes in carbon balance, thereby not supporting our hypotheses or many of the published models. As a result of current information about foliar responses, physiologically-based models should incorporate temperature acclimation of metabolic

pathways, although the addition of genetic and geographic determinants of various patterns remains a challenge.

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Part 3.

Summary and Conclusions

Summary and Conclusions

This thesis has focused on the whole-plant response to growth environment of the conifer species red spruce. Forest gap models yield realistic forecasts on the status of this species as a result of climatic change on montane forests. While a general decline is projected due to the impacts of climate change, no process-based study has been conducted to verify this projected result. The main question addressed in this paper then is, which is affected more by elevated growth temperature, synthesis or investment of carbon assimilates? To address this question, research was conducted on red spruce from high-elevation southern Appalachian provenances. As red spruce is a cool-adapted boreal species, the specific hypothesis addressed was that a higher growth temperature will negatively affect carbon assimilation, leading to a decline in investment when measured at growth temperature.

In Part 1, an overview was provided of the biogeography, ecology and biology of the species, which dominates the high-elevation forests of the eastern United States. Red spruce clearly has a restricted range – both geographical and ecological – although can grow right up to the high-elevation treeline. Red spruce is extremely shade tolerant, slow-growing and long-lived, thereby contributing to the complex stand dynamics over time. This section also introduced the photosynthetic carbon reduction (Calvin) cycle as the primary pathway for fixation of atmospheric carbon dioxide. This cycle plays a central role in plant metabolism, providing intermediates not only for starch and sucrose biosynthesis, but also for constructive cellulose.

In Part 2, we showed that the rate of carbon fixation of red spruce seedlings is insensitive to growth temperature, reflecting the steady state behavior of the metabolic and physiological processes involved. Such acclimation of enzymatically mediated reactions strongly suggests that such processes are likely regulated by substrate availability. Dewar et al. (1999) suggest short-term increase in respiration with temperature is driven by the availability of labile carbon, but longer-term respiration is limited by the supply of substrate from photosynthesis. Thus, in the long term, respiration acclimates to substrate supply, not temperature. In the present study, we observed no difference in respiration between temperature treatments, although carbon use efficiency was equivalent by week 16. This observation suggests a photosynthetic acclimation to temperature, rather than respiration. We conclude therefore that carbon assimilation in

southern Appalachian red spruce has a reduced sensitivity to elevated growth temperature.

Of the forecasted aspects of climate change, the increase in atmospheric CO₂ concentration is perhaps the most certain (IPCC 2001). Growth CO₂ concentration does not however appear to have a predictable, systematic effect on gas exchange of leaves, stems and roots of trees, even though the species studied share the same C₃ photosynthetic pathway (see review in Atkin et al. 2005). Several authors now consider that growth response to elevated temperatures is likely to be considerably more important than responses that may occur solely due to elevated CO₂ (Prentice et al. 1991, Sykes and Prentice 1995, Olszyk et al. 1998). For additional analyses, an evaluation of elevated CO₂ concentrations in concert with elevated growth temperatures on the rates of gas exchange, growth and carbohydrate sinks of red spruce may be prudent. As the present examination provided evidence of physiologic acclimation to temperature, a similar response to CO₂ would be predicted, irrespective of ontogeny.

Part 2 also showcased that morphology, growth rate and biomass allocation characteristics for red spruce were phenotypically plastic. Red spruce responded to high growth temperatures by developing nearly twice the whole-plant mass as plants grown at ambient temperatures. Enhanced growth at high temperatures has been found elsewhere (e.g. Teskey and Will 1999, Tjoelker et al. 1999) and may be caused by the combined effects of high temperature on metabolism, cell division, meristematic growth, leaf development and soil nutrient uptake. Plant growth is determined both by rates of carbon uptake and allocation of fixed carbon to autotrophic tissue or heterotrophic tissue (Carey et al. 1998). Although climate change may affect leaf-level assimilation rates and short-term growth, long-term growth may be influenced more by changes in carbon allocation than by changes in photosynthetic rate. Greater biomass allocation to above-ground structures may help maintain an above-ground competitor advantage at lower altitude where plant canopies are dominated more by deciduous broadleaved trees.

Previous studies of mature trees at or near treeline have reported decreases in photosynthetic carbon gain with increasing altitude and latitude, suggesting a 'carbon limitation hypothesis' of range limits (Körner 2003, Johnson et al. 2004) where carbon uptake is insufficient to support maintenance and growth of trees. However, an increase in carbon storage in older trees, along with higher leaf nitrogen and chlorophyll content, has also been found with increasing altitude, and has been interpreted as demonstrating

phenotypic limitations to carbon processing, rather than carbon gain (Oleskyn et al. 1998, Hoch and Körner 2003, Smith et al. 2003). This suggests a 'growth limitation hypothesis' of range limits, where biosynthetic processes may not match the minimum rates required for growth and substrate renewal, irrespective of the supply of raw materials.

These limitation hypotheses of environmental effects on plant distribution may be applied to the present study of the effects of elevated temperatures. The contrasting temperature treatments did not produce disequilibrium of carbon balance of uptake and loss, thereby eliminating the carbon limitation hypothesis. Red spruce seedlings grown at 17°C accumulated greater concentrations of non-structural carbohydrates compared to seedlings grown at 21°C, which used newly assimilated carbon for construction, rather than for storage. This finding, in part, fails to support the growth limitation hypothesis of red spruce range limits. Alternatively, this increase in stored carbon may represent a genotypic adaptation for survival in a dynamic, high-stress environment by serving as a reserve for physiologic processes to ensure survival in particularly harsh years. Additional examinations on levels of cold tolerance of seedlings could satisfy this remaining question. Based on the present findings, it does not appear that temperature alone is limiting the distribution of upland red spruce.

As alpine environments are often high-stress ecosystems, evidence is needed to address the potential expansion of deciduous species under current and future alpine climates. However, because climate change can affect the distributional area of each species independently, classical community-level questions need to be approached as community-level field studies. Competition is an important factor driving plant development, and the results from the present study may not automatically be transferred to plants grown under interspecific competition as typically found in nature. Few studies have examined species acclimation potential to elevated temperatures (or any other forecasted climate deviation) outside of monoculture and this is certainly an approach that must be attempted.

The global pattern of change is far more important than any individual study, but process-based field studies of any degree will only help our understanding of natural processes. Assessing the effects of climate change on extant forests must include the potential for species to adapt, and individuals to acclimate, to changing environmental conditions. From the current thesis, we can hypothesize that niche breadth does not

appear to be correlated with genetic diversity, with which red spruce is limited, but rather by individual plasticity. By utilizing two provenances and two elevations from within each provenance, the results suggest that plasticity is the balanced response of phenotype and genotype. Neither carbon assimilation nor investment was jeopardized as a response to elevated temperatures alone, failing to support the original research hypothesis. Therefore we conclude that, protected from disturbance, juvenile red spruce of the southern Appalachians will show persistence to the potential impacts of climate change.

The observed decline in red spruce must be attributed to other factors, of which acidic deposition, elevated ozone levels and indirect effects of the balsam wooly adelgid are the primary suspects. These disturbances, whether natural or human induced, can be regarded as a catalyst that facilitates synergistic vegetational adjustment to a changing environment. These disturbances are providing *opportunities* both for the entry of new species and the removal of ageing and non-regenerating populations. These disturbed spruce-fir populations will react to climate change with a faster response time than undisturbed forests. Therefore, the complexity of responses to elevated temperature presented here may not be automatically transferred to natural (disturbed) forest systems. This present laboratory examination provides a foundation or a stimulus for which additional large-scale mixed-culture field studies can build upon.

Associations between elevation and forest composition often lead to the assumption that the natural distribution of tree species is controlled by climate. The elevation – temperature relationship may be modified by topographic variables, such as slope angle and parent material, and indeed these factors have been shown to play a secondary role in explaining vegetation pattern in the mountains of the northeastern United States (Richardson et al. 2004, Lee et al. 2005). These results in concert with the results from the current study provide evidence that elevational patterns of species abundance are a consequence of *both* edaphic and climatic factors. The relative insensitivity to temperature exhibited by red spruce coupled with substrate restrictions of many hardwoods, e.g. rooting depth, may be of greater importance to ecotone placement than one factor alone. As such, climatic warming might not simply result in a simple upward shift of present species limits and abundance patterns.

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Vita

Jonathan W. Hagen was raised and developed in Charlotte, North Carolina. He attended primary and secondary schools in the Charlotte public school system, where he graduated in May 1994. Being constantly surrounded by piedmont flora, Jonathan headed west to the mountains of North Carolina where he matriculated at Western Carolina University for undergraduate work. It is there that he developed an unhealthy interest in boreal spruce-fir systems of the Appalachians. He completed his Natural Resource Management degree, specializing in Forest Resources, in May of 1998.

At this point, Jonathan Hagen went off to see the northern Appalachians of New England. Whilst residing in New Hampshire, he held a position as a plant physiologist specializing in IPM strategies. After living a transient life, he returned to the hot and humid southeast and entered the Graduate program of Forestry at The University of Tennessee in January 2004. Maintaining an interest in plant physiology and spruce-fir systems, he developed the thesis project enclosed.

Jonathan Hagen is married to the former Shiley K. Brisini, who was his travel partner for the period between schooling. He and his wife enjoy time with their daughter Elizabeth. The future holds....