Charles D. Canham · Richard K. Kobe · Erika F. Latty
Robin L. Chazdon

Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves

Abstract We examined interspecific and intraspecific variation in tree seedling survival as a function of allocation to carbohydrate reserves and structural root biomass. We predicted that allocation to carbohydrate reserves would vary as a function of the phenology of shoot growth, because of a hypothesized tradeoff between aboveground growth and carbohydrate storage. Intraspecific variation in levels of carbohydrate reserves was induced through experimental defoliation of naturally occurring, 2-year-old seedlings of four northeastern tree species – *Acer rubrum*, *A. saccharum*, *Quercus rubra*, and *Prunus serotina* – with shoot growth strategies that ranged from highly determinate to indeterminate. Allocation to root structural biomass varied among species and as a function of light, but did not respond to the defoliation treatments. Allocation to carbohydrate reserves varied among species, and the two species with the most determinate shoot growth patterns had the highest total mass of carbohydrate reserves, but not the highest concentrations. Both the total mass and concentrations of carbohydrate reserves were significantly reduced by defoliation. Seedling survival during the year following the defoliation treatments did not vary among species, but did vary dramatically in response to defoliation. In general, there was an approximately linear relationship between carbohydrate reserves and subsequent survival, but no clear relationship between allocation to root structural biomass and subsequent survival. Because of the disproportionate amounts of reserves stored in roots, we would have erroneously concluded that allocation to roots was significantly and positively related to seedling survival if we had failed to distinguish between reserves and structural biomass in roots.

Key words Carbohydrate reserves · Root allocation · Tree seedling survival

C.D. Canham (✉) · E.F. Latty[1]
Institute of Ecosystem Studies, Box AB,
Millbrook, NY 12545, USA
e-mail: ccanham@ecostudies.org, Fax: +1-914-6775974

R.K. Kobe[2] · R.L. Chazdon
Department of Ecology and Evolutionary Biology,
University of Connecticut,
Storrs, CT 06269, USA

Present addresses:
[1]Section of Ecology and Systematics,
Cornell University,
Ithaca, NY 14850, USA
[2]Department of Forestry,
Michigan State University,
East Lansing, MI 48824, USA

Introduction

Recent studies indicate that interspecific variation in juvenile tree survival, particularly under low light conditions, plays a far greater role in determining forest community dynamics than does interspecific variation in seedling or sapling growth rates (Casperson et al., in press; Kobe 1996; Kobe and Coates 1997; Kobe et al. 1995; Pacala et al. 1994, 1996; Wright et al. 1998). Numerous studies examine the ecophysiological mechanisms that underlie interspecific variation in growth of tree seedlings along gradients of light availability (e.g., Bazzaz and Miao 1993; Walters et al. 1993). By contrast, strikingly little research focuses on the physiological bases of interspecific variation in *survival* of tree seedlings (Collins 1990).

Kobe and coworkers (Kobe 1996; Kobe and Coates 1997; Kobe et al. 1995) have developed an efficient field method to predict the probability of sapling mortality as a function of growth rate. The method takes advantage of a long-recognized, positive, empirical relationship between growth and survival in mature trees (Buchman 1983; Buchman et al. 1983; Hamilton 1986, 1990; Monserud 1976). When combined with empirical functions that relate growth to ambient light levels, the method allows a quantitative assessment of rates of mortality as a function of light (e.g., Kobe et al. 1995). While this method provides a powerful tool for quanti-

tative comparison of interspecific variation in survival, it is clearly phenomenological. In particular, the method does not assume that variation in growth rate is the cause of variation in survival, but rather that both growth and survival reflect some underlying component of carbon balance (Kobe 1997; Kobe et al. 1995).

Kobe (1997) proposed that variation in allocation to carbohydrate reserves provides one mechanism for interspecific variation in survival under low light conditions. For saplings of pairs of both deciduous and evergreen species, the species with higher levels of fall root reserves had higher rates of low-light survival (Kobe 1997) and lower predicted rates of aboveground shoot growth (Pacala et al. 1994). The higher levels of stored carbon in the roots of the two "shade-tolerant" species presumably contributed to maintenance of basic metabolic functions through periods of low light, and enabled recovery from stress and damage (e.g., herbivory and winter injury).

There is substantial evidence that aboveground structural growth and storage pools represent competing sinks for carbohydrates in woody plants (Kozlowski 1992). Carbohydrate reserves generally begin to accumulate in roots and lower stems only after cessation of aboveground growth (Dickson and Nelson 1982; Kays and Canham 1991; Nelson and Dickson 1981). Many shade-tolerant species in the northeastern United States complete 90% of their shoot growth in the first month of the growing season (Kozlowski and Ward 1957a, 1957b; Marks 1975). Shade-intolerant trees, in contrast, often exhibit shoot growth throughout the growing season, either through episodic flushes of growth ("lammas" shoots), or through uninterrupted growth of individual shoots. Thus, species with the shortest duration of aboveground growth should accumulate the highest levels of fall carbohydrate reserves (after controlling for overall levels of resource availability), while species with continuous shoot growth throughout the growing season should maximize aboveground growth at the expense of allocation to carbohydrate reserves. Species with episodic growth strategies would be expected to show the greatest variation in carbohydrate reserves as a function of resource availability, with the highest allocation to reserves in low light conditions where the duration of shoot growth is truncated.

Variation in allocation to root structural biomass may represent an alternative mechanism of variation in seedling survival. Recent studies of tree seedling ecophysiology and demography in northeastern United States forests suggest that drought and associated thermal stress are a significant source of seedling mortality, particularly under high light regimes (Casperson et al., in press; Sipe and Bazzaz 1994). There is substantial inter- and intraspecific variation in root allocation among seedlings of temperate tree species, with the greatest allocation within a species generally observed under high light conditions (e.g., Canham et al. 1996). While water and nutrient demand is correspondingly high under those conditions, a high relative allocation to root structural biomass may play a significant role in the ability of tree seedlings to survive the drought and thermal stress associated with high light regimes (Gottschalk 1985; Sipe and Bazzaz 1994).

The root systems of woody plants often represent an important site for storage of carbohydrate reserves. Most previous studies of root allocation have not distinguished between root structural biomass (including fine root biomass) and nonstructural carbohydrate reserves stored in roots (e.g., Canham et al. 1996). As a result, those studies may confound the functionally distinct consequences of allocation to root structure versus carbohydrate reserves.

In the present study, we examine the generality of the relationship between survival and carbohydrate reserves documented in Kobe (1997) by looking at a wider range of deciduous species and smaller plant size, and consider two additional hypotheses: (1) variation among species in allocation to carbohydrate reserves reflects a tradeoff between the seasonal duration of aboveground growth and allocation to reserves; and (2) variation in allocation to structural root biomass also plays a critical role in patterns of seedling survival.

Methods

Study sites and species

Our research was conducted in and around Great Mountain Forest (GMF) – a northern hardwood-oak forest on Canaan Mountain in northwestern Connecticut, United States. Dominant canopy tree species include northern red oak (*Quercus rubra*), red maple (*Acer rubrum*), yellow birch (*Betula allegheniensis*), sugar maple (*A. saccharum*), beech (*Fagus grandifolia*) and hemlock (*Tsuga canadensis*), with white pine (*Pinus strobus*), white ash (*Fraxinus americana*), and black cherry (*Prunus serotina*) as subordinate species. GMF has been the site for the development and parameterization of SORTIE, a spatially explicit model of forest dynamics (i.e., Pacala et al. 1996), including the work on interspecific variation in sapling mortality patterns (Kobe et al. 1995) that motivated the research described here.

We selected four species for study: sugar maple, red oak, black cherry, and red maple. The species represent a wide range of shade tolerance and shoot growth strategies. Sugar maple is traditionally considered very shade tolerant, and is normally characterized by an early cessation of shoot growth (Marks 1975), although morphological studies indicate that it is not strictly determinate. Our previous research also suggests that shade tolerance in this species varies as a function of soil calcium supply (Kobe 1996; Kobe et al. 1995). Red oak is traditionally considered shade intolerant but the most drought tolerant of the major tree species at GMF (Casperson et al., in press; Kobe et al. 1995). Under high light conditions, this species can produce multiple flushes of shoot growth during a growing season (e.g., Kays and Canham 1991). Black cherry and red maple are both considered intermediate in shade tolerance, and show varying duration of shoot growth and leaf production within a growing season (Kays and Canham 1991). Under high light conditions, saplings of red maple, in particular, may continue to produce new leaves throughout the growing season (Kays and Canham 1991). We have deliberately minimized use of the terms "determinate" and "indeterminate" because of their inconsistent usage in the ecological and botanical literature. In studies of morphology, "determinate" describes species that form a set number of leaf primordia and internodes in one growth period. In the ecological literature, the terms determinate/indeterminate are

frequently used to describe seasonal patterns of extension growth, without regard to the morphology of dormant buds. To avoid confusion, we will describe seasonal patterns of shoot growth as "truncated" (i.e., cessation of aboveground growth early in the growing season), "episodic" (i.e., multiple flushes of growth), or "continuous" (extension growth throughout the growing season). The episodic growth strategy is considered intermediate, because of evidence that the occurrence of subsequent flushes of growth is determined by resource availability. Under this terminology, sugar maple has a truncated shoot growth pattern, while red oak is episodic, and red maple and black cherry are continuous.

Experimental design and field measurements

One of the challenges of studying mortality in perennial woody plants is that it is very difficult to recreate the diverse array of natural mortality agents under controlled conditions in a greenhouse. Accordingly, we performed a field experiment with naturally established seedlings for our research. We took advantage of large, natural cohorts of 2-year-old seedlings of each of the four study species. The cohorts presumably dated from mast seed crops of each of the four species during 1991 (with seed germination in spring; 1992), since none of the four species (with the possible exception of black cherry) is noted for seed dormancy or the presence of buried seed banks (Marquis 1975). A second challenge in assessing the relationships between biomass allocation and carbohydrate reserves and survival in small seedlings is that it is effectively impossible to estimate root biomass and/or nonstructural carbohydrate contents non-destructively. In order to experimentally induce variation in levels of carbohydrate reserves within seedlings, we applied three levels of leaf removal. The goal of the leaf removal treatments was to create populations of seedlings with different root structural biomass and masses of total nonstructural carbohydrate reserves (TNC), in which we could assess allocation to roots and reserves on a destructively harvested subsample, while monitoring subsequent survival on the remaining undisturbed seedlings. A third challenge in designing a field experiment to test our hypotheses is that both overall carbon gain and carbon allocation patterns can be expected to vary in response to variation in ambient light levels. Since light levels vary across a wide range under natural conditions, we have used light availability as a covariate in all of our analyses (see methods of estimating seasonal average light levels, below).

Thus, our basic experiment consisted of a 4×3 factorial design, with four species and three leaf removal treatments (details below), with light level as the major covariate, and with naturally occurring, 2-year-old seedlings as the experimental units. Principal response variables included (1) growth during the year treatments were applied and during the following full growing season, (2) biomass allocation patterns and TNC levels at the end of the growing season in which treatments were applied, and (3) survival, both overwinter, and during the full year following the treatments. In some analyses, plant size was also used as a covariate to control for size-dependencies in growth and carbohydrate allocation, although the range of variation among individuals was relatively small because of their uniform age.

During June of 1994, approximately 500, naturally occurring, 2-year-old seedlings of each of the four study species were located within our study sites. To assure relatively uniform sampling across the full range of ambient light levels, approximately equal numbers of seedlings were selected from each of four canopy conditions: (1) closed canopy, (2) small gap, (3) large gap, and (4) recent clearcut. Light levels were subsequently measured for each seedling using fisheye photography (methods described below), so these general categories were not used in any of the analyses. A total of 2,017 suitable seedlings were selected: 535 red maples, 511 sugar maples, 476 red oaks, and 495 black cherries.

The seedlings were randomly assigned to one of the three leaf removal treatments: *no* leaf removal (control); *partial* removal of approximately 50% of leaf area through removal of selected, entire leaves, and *complete* removal of all leaf area. We removed entire leaves rather than clipping portions of leaves to minimize variation among individuals and species in their direct response to damage. We developed simple rules to determine which leaves to remove for the partial removal treatment. For red maple and sugar maple – the two species with opposite leaf display – we removed the smaller leaf of the largest (and usually oldest) pair of leaves and the larger leaf of the smaller pair. Because leaf number and leaf size was more variable for red oak and black cherry, we developed tables of leaf area as a function of leaf length for these two species. For each seedling, the tables were used to calculate total leaf area, and then a combination of leaves that most closely totaled 50% of leaf area was selected for removal. The leaf removal treatments were applied in the 1st week of July to maximize the effect of leaf removal on accumulation of carbohydrate reserves and minimize effects on current-year, aboveground growth, since shoot growth in these four species (as in most temperate woody plants) occurs early in the growing season, and significant accumulation of root reserves occurs late in the growing season (e.g., Kays and Canham 1991). The treatments were applied only once even though new leaves were produced after the treatments in many seedlings.

Variation in seasonal average light levels was expected to affect accumulation of carbohydrate reserves through effects on both total, whole-plant, carbon gain, and through proportionate changes in allocation to reserves versus growth. Fisheye photography was used to estimate a gap light index (GLI, Canham 1988a) for each seedling. GLI estimates the percent of incident radiation (both diffuse and direct) above the canopy that penetrates through openings (of any size) in the canopy over the course of a defined growing season. Photographs were taken by placing the camera (with an Olympus 8-mm true fisheye lens) directly above each seedling (approximately 20 cm above the ground). The camera was leveled and oriented with a compass. Image orientation was accomplished through the presence of light-emitting diodes (LEDs) mounted on the east and west side of the lens. All photographs were taken with Ektachrome 400 ASA slide film, and were underexposed by 1 *f*-stop to increase contrast. Slides were scanned at approximately 800 dpi resolution with a commercial slide scanner (Microtek, Inc.) and GLI was calculated from the color, digitized images using software (GLI/C 2.0) developed by C.D. Canham. Because of effort involved in processing the slides, we only analyzed the subset of approximately 400 slides for seedlings that were randomly selected for destructive sampling of biomass allocation.

Stem basal diameter (at ground level), total seedling height, extension growth of the leader, and total number of expanded leaves were recorded at both the beginning of the experiment (late June–early July 1994) and at the end of the growing season (end of September). In order to facilitate accurate remeasurement of basal diameter, a small mark was made at the location of the measurement on the stem using a permanent marker. Extension growth and total number of leaves were also recorded at the beginning of August to document differences among species in the phenology of shoot growth and leaf display. Seedling status (live, dead, missing, and evidence of natural herbivory) was recorded at each of the three times. Overwinter survival was assessed through a census of seedling status during mid-May 1995. Stem basal diameter, total seedling height, 1995 leader extension growth, and 1994–1995 survival were measured at the end of the 1995 growing season (late September, 1995). Seedlings that could not be located during the spring and fall 1995 censuses were considered dead. These missing seedlings accounted for only 7.1% of the 1549 seedlings that remained after the fall 1994 harvest.

Laboratory methods

In mid-September 1994 (i.e., near the end of the first growing season of the experiment, but before leaf senescence) a randomly selected subsample of 40 seedlings of each of the 12 species/leaf removal treatment combinations was harvested for measurements of biomass accumulation and total nonstructural carbohydrate (TNC) reserves. Prior to harvesting, seedling height, extension growth, and stem basal diameter were measured. Seedlings were

4

harvested by carefully excavating the root system and removing excess soil in the field. The seedlings were then sealed in polyethylene bags and placed in a cooler until transported to the laboratory, where they were stored in a refrigerator for a maximum of 5 days. In the laboratory, leaves were removed and measured for total area (using a Li-Cor leaf area meter). The root systems were gently rinsed by hand, and the root and shoot systems were separated at ground level at the point of a mark recorded in the field prior to harvesting. We washed the roots by hand in an attempt to recover as much of the root system as was practical; however, our methods undoubtedly did not recover all, or necessarily even a majority, of the fine roots. Roots, stems and leaves were then dried at 100°C for 1 h, after which the temperature was reduced to 70°C until dry (2–4 weeks for woody tissues) (Smith 1973). After weighing, all samples were stored frozen until analyzed for carbohydrate concentration.

Levels of TNC in roots and shoots were analyzed separately, using an enzymatic process and colorimetric assay (DuBois et al. 1956; Smith 1969). The procedure converts sugars and starches to glucose equivalents, and then analyzes glucose content using a standard phenol-sulfuric acid colorimetric assay (DuBois et al. 1956). The entire root and shoot systems (excluding leaves) of each seedling were pulverized separately with a ball mill (Kleco, Inc.). An approximately 0.1 g subsample was then used for analysis. The subsample was sonicated in an acetate buffer for 3 min to further break up cells, since initial tests indicated that sonication increased estimated TNC levels. Samples were then placed in a shaking water bath at 80°C for 1 h with alpha-amylase added to solubilize amylopectins. Amyloglucosidase was added to each sample, and the samples were placed back in the shaking water bath at 60°C overnight. The samples were centrifuged for 10 min at 10,000° rpm, and a subsample of supernatant was removed and stored frozen until addition of phenol and sulfuric acid for colorimetric analysis with a spectrophotometer at 487 nm. Concentrations of glucose equivalents were calculated from standard curves using appropriate standards and blanks.

Statistical analyses

Most studies of root versus shoot allocation in plants lump both structural biomass and nonstructural carbohydrate reserves. Our methods allow us to distinguish between these different pools, and we believe that this is particularly valuable in studies of allocation in woody plants. Roots are an important site for storage of carbohydrate reserves in woody plants, and failure to distinguish between structural and nonstructural biomass would confound the effects of allocation to reserves versus growth. Thus, we subtracted the mass of the carbohydrate reserves in both the roots and shoots from the total biomass of those pools to estimate and analyze the total "structural" biomass of those pools.

We present results for both the total amount of TNC (mg) and TNC concentration. Since our seedlings are all the same age, the total size of the TNC pool represents the best measure of actual allocation to reserves (Chapin et al. 1990). However, the benefits of carbohydrate reserves may depend on either the total size of the pool or its concentration, depending on the specific function of the reserves (see Discussion). Similarly, we present the total structural biomass of the root system as the most appropriate measure of variation among species and treatments in allocation to root structure (again since the seedlings are all the same age). However, we also analyzed the fraction of total structural biomass allocated to roots (i.e., root structural biomass as a percentage of total seedling structural biomass), in part because of the large literature contrasting allocation to roots vs. shoots.

Seedling growth and biomass allocation (including TNC levels) were analyzed with analysis of variance or analysis of covariance (when light level was included as a covariate). The main treatment effects in the ANOVA – species and leaf removal treatment – were both considered fixed effects. Aboveground growth rates were log-transformed to improve normality before analysis. A number of our continuous response variables were expressed as percentages,

but did not require transformation to meet assumptions of either normality or homogeneity of variance. The interaction between the main effects in a model was examined to test whether response to the leaf removal treatments varied among species. Interactions between the covariate (light level) and the main effects were examined in the ANCOVAs to ensure that the homogeneity of slopes assumptions were met. Planned contrasts were used to compare the two species with truncated growth strategies (sugar maple and red oak) to the two species with continuous growth strategies (red maple and black cherry).

Seedling overwinter survival and survival for the full year following the year of the leaf removal treatments were analyzed as linear categorical models (analogous to ANOVA) with the CAT-MOD procedure in SAS, with species and leaf removal treatment as main effects. All statistical tests were done with SAS version 6.12 (SAS 1989).

Results

Shoot growth and leaf display

The leaf removal treatments were deliberately applied after the majority of shoot extension growth had been completed, in order to maximize the effects of the treatments on levels of carbohydrate reserves and root allocation. Thus, as expected, shoot extension growth at the end of the first growing season (1994) varied among species and in response to ambient light levels, but there were no significant effects of leaf removal (Table 1). Seedlings in all three treatments were combined to increase the power of our analysis of the response of shoot growth to variation in light level (giving sample sizes of 99–107 seedlings per species). All four species showed statistically significant variation in shoot growth as a function of light (GLI) ($P < 0.05$ for linear regressions – scatter plots and residuals did not justify use of non-linear models); however, the slopes of the responses to light were low, as were the proportions of variance explained by the regressions (r^2 of 6–21%).

There were strong, residual effects of the 1994 leaf removal treatments on 1995 shoot extension growth (Tables 1, 2). There was also a significant interaction between species and leaf removal treatments (Table 1).

Table 1 Results of analysis of covariance for 1994 shoot extension growth (with light as the covariate), and analysis of variance for 1995 shoot extension growth. Light levels were only assessed for the 1994 growing season. Both growth rates were $\log_{10}$ transformed prior to analysis

Variable	Factor	df	F	p
1994 Extension Growth	Species	3	3.99	0.0081
	Leaf removal	2	2.06	0.1287
	Species × Removal	6	1.33	0.2443
	Light	1	36.84	0.0001
	Species × Light	3	2.05	0.1062
	Removal × Light	2	0.99	0.3729
	Error	395		
1995 Extension Growth	Species	3	16.99	0.0001
	Leaf removal	2	33.25	0.0001
	Species × Removal	6	2.85	0.0092
	Error	1077		

Table 2 Mean and standard error of 1995 shoot extension growth (cm) by species and leaf removal treatment (*N* no leaf removal, *P* partial leaf removal, *C* complete leaf removal)

Species	Treatment	*n*	Mean	SE
Acer rubrum	N	151	1.79	0.172
	P	89	1.64	0.168
	C	72	1.05	0.118
A. saccharum	N	121	3.60	0.340
	P	105	3.02	0.338
	C	59	1.59	0.244
Prunus serotina	N	108	3.49	0.465
	P	98	2.86	0.274
	C	79	1.42	0.174
Quercus rubra	N	93	1.88	0.205
	P	94	1.48	0.172
	C	73	1.24	0.128

The two species with the greatest extension growth among control seedlings – black cherry and sugar maple – showed the greatest reduction in 1995 growth in response to complete leaf removal (Table 2).

There were no significant relationships between ambient light levels and total leaf area (measured at the end of the 1994 growing season) of the control seedlings for any of the four species. However, there was a positive relationship between light and leaf area in both of the black cherry defoliation treatments and the partial leaf removal treatment for red oak ($P < 0.05$ for linear regression of leaf area at the end of the 1994 growing season on GLI, with r^2 ranging from 10.5 to 26.6%). Thus, recovery of leaf area following defoliation was influenced by light to at least some degree in two of the four species.

Allocation to shoot and root structural biomass

Shoot and root structural biomass (total biomass minus TNC content) at the end of the 1994 growing season represents net, cumulative allocation to structural growth over the three growing seasons since germination, plus the contribution of maternal reserves from seeds. Interspecific variation in both shoot and root biomass for these 3-year-old seedlings was still roughly correlated with seed mass: red maple has the smallest seeds of the four species (Schopmeyer 1974), and had the smallest total structural biomass, while red oak has the largest seeds (Schopmeyer 1974) and had the largest root and shoot systems (Fig. 1).

While we have estimates of ambient light levels only for the 1994 growing season, seedlings were located in areas that would not have experienced significant changes in ambient light levels in the past 3 years. Patterns of variation in shoot structural biomass mirrored the patterns in shoot extension growth, varying among species and in response to light, but not in response to the leaf removal treatments (Table 3). We also examined the responses of shoot structural biomass to light for the four species individually because of a marginally signi-
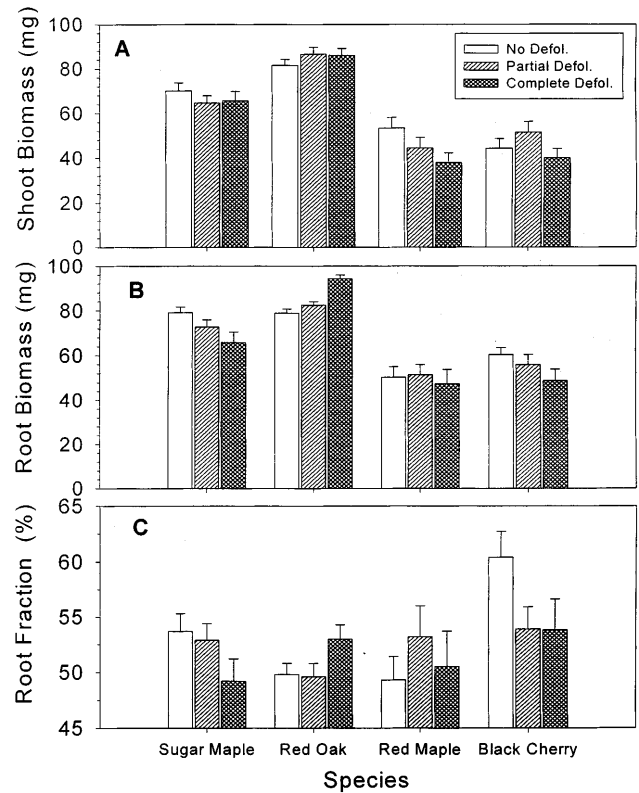

Fig. 1 Means and standard errors of **A** shoot structural biomass (mg), **B** root structural biomass (mg), and **C** root structural biomass as a percent of total structural biomass (%) for the 12 combinations of species and leaf removal treatments

Table 3 Results of analyses of covariance for structural shoot and root biomass (mg), with light as a covariate, and analysis of variance for root fraction (structural root biomass as a percentage of total structural biomass). There was no significant effect of light on root fraction, so the covariate was dropped from the analysis of that variable. There were 382 error *df* for shoot biomass, 370 for root biomass, and 348 for root fraction

Factor	df	Shoot biomass		Root biomass		Root fraction	
		F	P	F	P	F	P
Species	3	47.09	0.0001	39.99	0.0001	4.51	0.0040
Leaf removal	2	1.60	0.2027	1.71	0.1829	0.70	0.4952
Species × Removal	6	1.05	0.3930	2.74	0.0129	2.71	0.0449
Light	1	13.07	0.0003	9.36	0.0024		
Species × Light	3	2.28	0.0793	4.99	0.0021		
Removal × Light	2	0.94	0.3911	2.16	0.1168		

ficant interaction between species and light (Table 3). When analyzed individually, the two continuous-growth species (red maple and black cherry) had very weak but significant relationships between 1994 light levels and shoot structural biomass ($P < 0.05$ for simple linear regressions of shoot biomass vs. GLI, $r^2 = 12.3$ and 7.7% for red maple and black cherry, respectively). Of the remaining two species, a significant fraction of the shoot biomass of young red oak seedlings may reflect a high level of maternal investment (via large seeds), while

the relative insensitivity of young sugar maple seedlings may be a reflection of the species' high shade tolerance (Canham 1988b; Kobe et al. 1995).

Root structural biomass (both as total allocation and as a percent of total structural biomass) varied significantly among species, but there was no main effect of leaf removal on root structural biomass, in part because of differences among the species in the direction of the effect (giving a significant interaction between species and leaf removal) (Table 3, Fig. 1). The two species with truncated or episodic shoot growth (sugar maple and red oak) had significantly greater root biomass than the continuous-growth species (planned contrast, $F = 96.86$, $df = 1,370$, $P < 0.0001$) (Fig. 1). Black cherry seedlings had the highest fraction of total structural biomass in roots (56.1%, averaged across the three leaf removal treatments), while the other three species were remarkably similar in allocation to roots versus shoots (50.8–51.9% of total structural biomass) (Fig. 1). Leaf removal resulted in declines in root allocation (measured both as total pool size and as a fraction of total structural biomass) in black cherry and to a lesser extent in sugar maple seedlings, while red maple maintained relatively constant levels of root allocation and red oak seedlings increased root allocation in response to complete leaf removal (Fig. 1).

Ambient light levels had a slight effect on total root structural biomass (Table 3), but had no significant effect on the relative allocation to roots vs. shoots (i.e., as measured by the fraction of total structural biomass in roots) for any of the four species or for any of the three leaf removal treatments (Table 3). In general, root allocation was high even under the lowest light levels (i.e., GLI = 1–2% of full sun). Similar results were obtained for 1-year-old red oak seedlings in a greenhouse experiment (Canham et al. 1996); however, seedlings of the two maple species showed much greater plasticity in root allocation under greenhouse conditions, with significant reductions in root allocation under low light and/or high soil resource availability (Canham et al. 1996).

Allocation to nonstructural carbohydrate reserves

Both the total amounts and concentrations of fall carbohydrate reserves (TNC) varied significantly among the four species (Table 4), and generally conformed to our expectations based on shoot growth patterns (Fig. 2). The two species with continuous shoot growth patterns (red maple and black cherry) had the highest concentrations of carbohydrate reserves (planned contrast $F = 10.99$, $df = 1,349$, $P = 0.001$), but the two species with truncated shoot growth (sugar maple and red oak) had significantly greater total amounts of carbohydrate (planned contrast $F = 19.04$, $df = 1,349$, $P < 0.0001$) (Fig. 2).

The leaf removal treatments caused significant variation in both total amounts and concentrations of carbohydrate reserves (Table 4), largely in response to complete leaf removal (Fig. 2). There was no interaction between species and leaf removal for either total
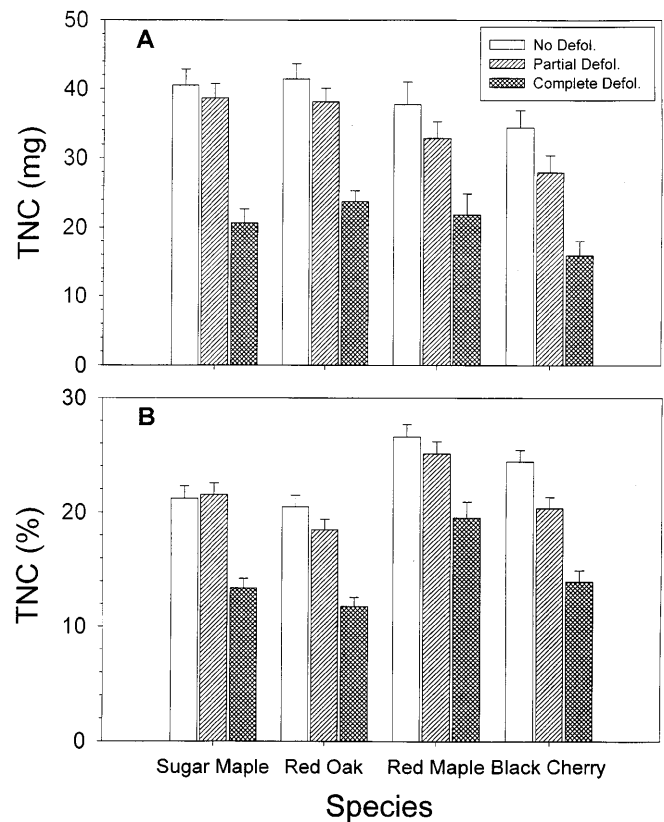

Fig. 2 Means and standard errors of **A** total seedling total nonstructural carbohydrate reserves (TNC, mg), and **B** TNC concentration (% by mass of total seedling biomass)

Table 4 Results of analyses of covariance for total nonstructural carbohydrate (TNC) reserves pool size (mg), TNC concentration (% of total plant biomass), and the fraction of TNC stored in roots (as a percentage of total TNC pool size), with light as a covariate. There were 349 error *df* for all three analyses

Factor	df	TNC (mg)		TNC concentration (%)		%TNC in roots	
		F	P	F	P	F	P
Species	3	8.79	0.0001	7.60	0.0001	11.00	0.0001
Leaf removal	2	29.79	0.0001	40.29	0.0001	3.39	0.0347
Species × Removal	6	0.91	0.4863	1.05	0.3947	2.35	0.0305
Light	1	20.26	0.0001	8.12	0.0046	1.57	0.2114
Species × Light	3	1.26	0.2880	0.47	0.7012	1.04	0.3758
Removal × Light	2	0.20	0.8148	1.23	0.2931	0.19	0.8308

amounts or concentrations of carbohydrate reserves (Table 4), indicating that carbohydrate allocation in the 4 species responded similarly to leaf removal. Allocation to carbohydrate reserves also varied positively in response to light (Table 4), although the magnitude of the response was relatively small, and with considerable scatter. Surprisingly, there was no interaction between species and light level (Table 4), indicating that the effects of light levels on carbohydrate allocation were uniform across 4 species that vary substantially in their shade tolerance (Kobe et al. 1995).

Roots were the dominant site for carbohydrate storage in all 4 species, but the relative importance of shoots vs. roots as storage sites varied both among the 4 species and in response to the leaf removal treatments (Tables 4, 5). TNC concentrations were 1–17% (by mass) higher in root tissues than in shoot tissues (Table 5). The fraction of total carbohydrate reserves stored in roots varied significantly among the four species, ranging from a high of 80.8% in the black cherry control seedlings to 51.4% in sugar maple seedlings with complete leaf removal (Tables 4, 5). The high proportion of storage in black cherry roots was a function of the combination of the second highest root tissue TNC concentration, and the largest root system relative to total seedling biomass.

Seedling survival

There was essentially no seedling mortality during the first growing season of the experiment. Only 27 of the 2029 seedlings (1.3%) were dead or missing during the fall 1994 census, and even the complete leaf removal treatment resulted in only 1.9% mortality by the end of the 1994 growing season. Thus, even complete, mid-summer defoliation failed to result in immediate mortality of these young seedlings.

Overwinter survival (fall 1994 – spring 1995) was relatively high (85.7% overall), and varied significantly in response to leaf removal – ranging from a low of 77.3% in the complete leaf removal treatment, to 89.8% and 89.1% in the partial and no (control) removal groups, respectively (categorical model $\chi^2 = 38.68$, $df = 2$, $P < 0.0001$) (Fig. 3). Overwinter survival,

however, did not vary significantly among the four species – ranging from 87.7% for red oak to 84.0% for red maple (categorical model $\chi^2 = 3.02$, $df = 3$, $P = 0.3887$). Red maple seedlings had the greatest decline in survival, ranging from 91.6% in the unclipped seedlings, to 70.2% survival in the complete leaf removal treatment.

Cumulative survival from fall 1994 to fall 1995 declined moderately to 79.3% of all seedlings, and still varied significantly among the three leaf removal treatments but not among the four species (species: $\chi^2 = 2.37$, $df = 3$, $P = 0.499$; treatment: $\chi^2 = 78.94$, $df = 2$, $P < 0.0001$) (Fig. 3). Survival of the control
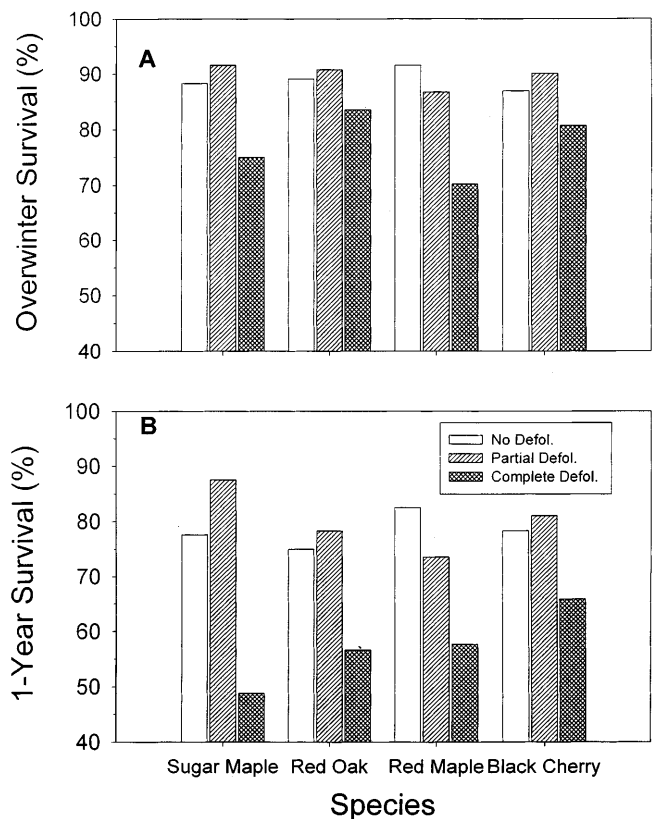

Fig. 3 A Overwinter seedling survival (%), and **B** seedling survival (%) over the 1-year period from fall 1994 to fall 1995

Table 5 Concentrations of fall carbohydrate reserves (mean %TNC by mass, with SEs below in parentheses) in roots and shoots, and the percentage of total plant TNC stored in roots, as a function of species and leaf removal treatment (*N* no removal, *P* partial leaf removal, *C* complete leaf removal)

Species Treatment	Sugar maple			Red oak			Black cherry			Red maple		
	N	P	C	N	P	C	N	P	C	N	P	C
Root TNC (%)	24.1 (1.6)	25.5 (1.2)	14.1 (1.2)	26.1 (1.6)	22.4 (1.3)	13.1 (1.1)	30.8 (1.4)	26.1 (1.2)	17.7 (1.3)	31.2 (1.7)	29.0 (1.4)	20.8 (1.5)
Shoot TNC (%)	17.8 (0.9)	16.4 (1.0)	12.7 (0.9)	15.0 (0.7)	14.1 (0.9)	10.1 (0.6)	13.7 (1.0)	12.7 (0.9)	10.0 (0.6)	20.4 (1.7)	20.1 (1.6)	16.4 (1.6)
% TNC in roots	61.6 (2.1)	65.9 (2.1)	51.4 (3.2)	63.3 (2.2)	62.5 (2.0)	58.6 (2.0)	80.8 (1.8)	74.0 (2.2)	66.3 (2.6)	60.0 (3.2)	65.6 (3.6)	61.2 (4.6)

seedlings was strikingly similar among three of the four species (with red maple as the exception, Fig. 3B). In general, survival declined sharply in response to the complete leaf removal treatment. There was a significant interaction between species and the leaf removal treatment ($\chi^2 = 14.60$, $df = 6$, $P = 0.024$), with sugar maple showing the greatest decline in survival under complete leaf removal (Fig. 3). Survival from fall 1994 to fall 1995 was independent of both 1994 growth (extension growth) and size (height) for all four species, and for 10 of the 12 separate species/treatment combinations: logistic regressions of status in fall 1995 on 1994 growth or height were non-significant, except for status as a function of height in the high leaf removal treatments for red maple and red oak.

In general, patterns of 1-year seedling survival (Fig. 3B) mirrored amounts of fall carbohydrate reserves (Fig. 2A) quite closely. Across the 12 treatment combinations, seedling survival from fall 1994 to fall 1995 was linearly related to both the total amounts ($r^2 = 0.60$) and concentrations ($r^2 = 0.54$) of carbohydrate reserves at the beginning of the period (Fig. 4A, B). In contrast, there was no clear relationship between seedling survival and either the absolute or relative allocation to root structural biomass (Fig. 5A, B), and the relationship within individual species varied from positive to negative (Fig. 5A, B). Because of the dispropor-

tionate amounts of reserves stored in roots (Table 5), we would have erroneously concluded that allocation to roots was significantly and positively related to seedling survival if we had failed to distinguish between reserves versus structural biomass in roots.

Discussion

Carbohydrate reserves and seedling survival

Our results show a clear linkage between levels of carbohydrate reserves and subsequent survival of tree seedlings, confirming previous results with both saplings (e.g., Kobe 1997) and adult trees (e.g., Webb 1981). The patterns were strongest in comparisons where we induced intraspecific variation in fall TNC levels through defoliation. Although there was statistically significant variation among species in overall levels of carbohydrate reserves, the magnitude of the variation was surprisingly small, and there was no overall variation among species in survival. In particular, the control (undefoliated) seedlings of the four species were remarkably similar in both carbohydrate reserves and 1-year survival. Leaf removal induced consistent patterns of variation in carbohydrate reserves among all four species; however, the treatments had much more divergent effects on survival
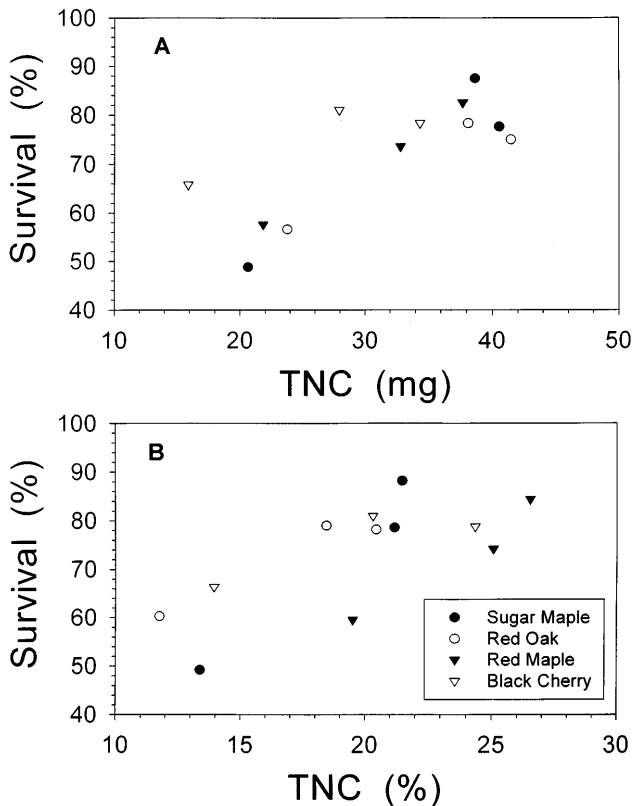

Fig. 4 Relationships between 1-year seedling survival (%) and **A** total seedling TNC (mg) and **B** seedling TNC concentration (% of total seedling biomass) for the 12 combinations of species and leaf removal treatments
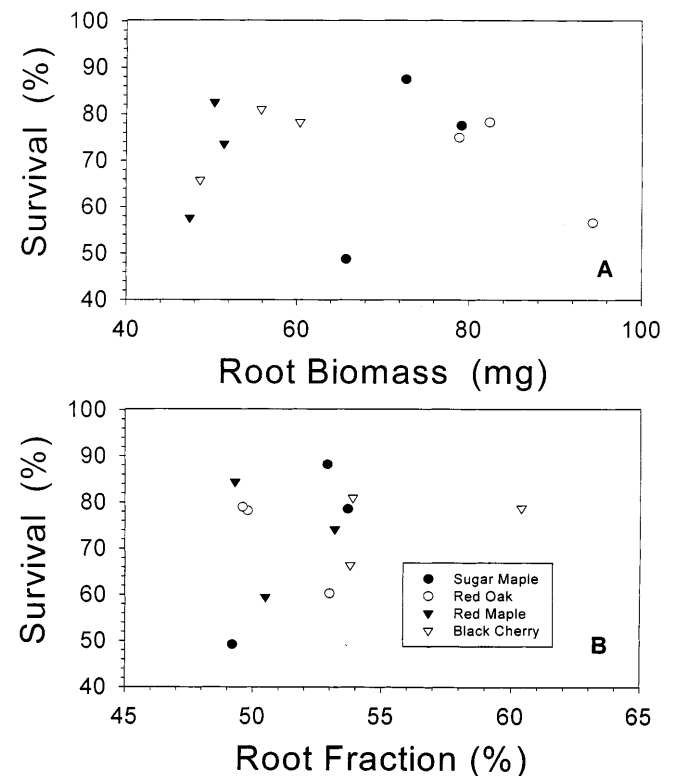

Fig. 5 Relationships between 1-year seedling survival and **A** structural root biomass (mg) and **B** structural root fraction (structural root biomass as a percent of total seedling structural biomass) for the 12 combinations of species and leaf removal treatments

(Fig. 2B). Thus, our results suggest that the quantitative relationships between levels of carbohydrate reserves and subsequent survival vary among species. For example, of the four species, black cherry had the lowest overall levels of carbohydrate reserves in each of the three treatment groups (Fig. 2A), but had relatively high survival per unit of carbohydrate reserves (Fig. 5A).

There are a number of possible mechanisms for the relationship between carbohydrate reserves and seedling survival. In the most general terms, carbohydrate reserves presumably play a crucial role in recovery of seedlings from a broad range of agents of stress and physical damage, ranging from herbivory to frost heaving. Carbohydrate reserves are also assumed to play a role in response to infection by pathogens (e.g., Carroll et al. 1983; Matson and Waring 1984; Wargo 1972, 1977; Wargo et al. 1972), and in susceptibility to attack by insects (Dunn et al. 1987, 1990). Carbohydrate reserves also play a role in winter hardening (Sakai and Larcher 1987). We suspect that the relatively minor overwinter mortality, even in the complete defoliation treatment, may have been due to an extremely mild winter in 1994–1995.

Our experiment used seedlings of uniform age and a very narrow range of sizes. Moreover, both the total amounts of carbohydrate reserves and the concentrations of those reserves responded similarly to the experimental treatments. As a result, we cannot differentiate between absolute amounts versus concentrations of carbohydrate reserves as predictors of seedling survival. The effectiveness of carbohydrates in winter hardening, for instance, should depend on carbohydrate concentration rather than the total pool of carbohydrates within the plant. In contrast, in cases where reserves buffer against mortality by being mobilized to repair or replace tissues lost to localized physical damage or consumption by herbivores, the total amount of reserves within the plant (regardless of plant size) may be a better predictor of survival. In general, survival of both seedlings and saplings of temperate trees increases with increasing size (e.g., Kobe et al. 1995). An increase in sapling size is undoubtedly accompanied by an increase in the total amount of reserves within the plant, since TNC concentrations in temperate tree saplings are roughly comparable to the levels observed in our seedlings (e.g., Kays and Canham 1991; Kobe 1997). We suspect that the increase in the total size of the reserve pool is responsible for at least some of the increase in survival with increasing juvenile tree size. However, we do not know of any critical experiments that have yet addressed the relative importance of changes in pool sizes of carbohydrate reserves for size-dependent variation in juvenile tree survival.

Carbohydrate reserves and shoot growth phenology

Our results confirmed both the general link between carbohydrate reserves and survival, and the hypothesis that interspecific variation in levels of TNC would reflect interspecific variation in the seasonal duration of shoot growth. The two species characterized by a relatively short duration of aboveground shoot growth within a growing season (sugar maple and red oak) also had the highest total quantities of carbohydrate reserves. However, in contrast to the results of the study of Kobe (1997) using larger saplings, the differences observed here in small, 2-year-old seedlings were relatively small, and we do not have any evidence of a clear tradeoff between allocation to shoot growth versus carbohydrate reserves. In general, interspecific differences in TNC levels were much smaller than intraspecific differences induced by the defoliation treatments. We were particularly surprised by the very minor effects of variation in ambient light levels (which ranged from 1 to 50% of full sun) on carbohydrate reserves. This suggests that either seedling responses to resource availability were constrained, or that allocation to storage has a very high priority that seedlings attempt to meet regardless of light level.

Root allocation and seedling survival

Our results highlight the importance of distinguishing between structural biomass and carbohydrate reserves when analyzing the functional significance of root allocation. Our results also reinforce the conclusion that there is a great deal of interspecific variation among seedlings of northeastern tree species in the responsiveness of root allocation to environmental cues (Canham et al. 1996). Root structure and function are clearly large sinks for carbon in seedlings of all four tree species. The structural biomass of roots in the control seedlings generally exceeded shoot biomass in these 3-year-old seedlings. Despite the overall magnitude of root allocation, and the obvious importance of an adequate root system for water and nutrient uptake, there was no clear relationship between variation in root structural biomass and subsequent seedling survival. Our biomass-based measures underestimate the proportion of net primary production actually allocated to root function because they do not account for the metabolic costs of fine root turnover, mycorrhizae and root exudates. Our methods also undoubtedly underestimate the total biomass of very fine roots. While fine roots represent a relatively small fraction of total root biomass, they obviously play a critical role in resource uptake. Thus, our inability to document a clear relationship between root biomass and seedling survival may simply be due to insufficient data on allocation to fine roots.

Functional ecology of seedlings versus saplings and adult trees

Our experiment was conducted on relatively small and uniformly sized seedlings. Even under the highest light

levels, almost none of the seedlings had produced lateral branches. The seedlings thus had a very simple architecture consisting of a single axis of shoot growth and a small number of leaves (generally 2–6, depending on species). Variation in ambient light levels had little measurable effect on seedling size, growth rate, biomass allocation, or survival. Other field and greenhouse experiments confirm this result: while light may have a statistically significant effect on seedling growth, the actual magnitude of the growth response in very young temperate tree seedlings is relatively small (e.g., Canham et al. 1996; Farmer 1975, 1980; Gottschalk 1985; Latham 1992; Sipe and Bazzaz 1994). We suggest that the limited response to variation in light in these seedlings is primarily due to their very limited ability to vary the architecture of leaf display (through variation in both the number of shoots and the sizes and numbers of leaves on shoots) when they are so small. This line of reasoning suggests the unorthodox conclusion that the survival of these small seedlings is relatively insensitive to variation in resource availability. However, our results suggest that seedling survival will be strongly affected by biotic or abiotic factors that cause defoliation or loss of root or shoot tissues (and the reserves stored in those tissues).

References

Bazzaz FA, Miao SL (1993) Successional status, seed size, and responses of tree seedlings to CO_2, light and nutrients. Ecology 74:104–112

Buchman RG (1983) Survival predictions for major lake states tree species (North Central Forest Experiment Station Research Paper NC-233). United States Department of Agriculture, St Paul

Buchman RG, Pederson SP, Walters NR (1983) A tree survival model with application to species of the Great Lakes region. Can J For Res 13:601–608

Canham CD (1988a) An index for understory light levels in and around canopy gaps. Ecology 69:1634–1638

Canham CD (1988b) Growth and architecture of shade-tolerant trees: response to canopy gaps. Ecology 69:786–795

Canham CD, Berkowitz AR, Kelly VR, Lovett GL, Ollinger SV, Schnurr J (1996) Biomass allocation and multiple resource limitation in tree seedlings. Can J For Res 26:1521–1530

Carroll JE, Tattar TA, Wargo PM (1983) Relationship of root starch to decline of sugar maple. Plant Disease 67:1347–1349

Casperson JP, Silander JA Jr, Canham CD, Pacala SW (in press) Modeling the competitive dynamics and distribution of tree species along moisture gradients. In: Mladenoff D, Baker W (eds) Advances in spatial modelling of forest landscape change. Cambridge University Press, Cambridge

Chapin FS III, Shulze E-D, Mooney HA (1990) The ecology and economics of storage in plants. Annu Rev Ecol Syst 21:423–447

Collins SL (1990) Habitat relationships and survivorship of tree seedlings in hemlock-hardwood forest. Can J Bot 68:790–797

Dickson RE, Nelson EA (1982) Fixation and distribution of C-14 in *Populus deltoides* during dormancy induction. Physiol Plant 54:393–401

DuBois M, Giels KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Dunn JP, Kimmerer TW, Potter DA (1987) Winter starch reserves of white oak as a predictor of attack by the twolined chestnut borer, *Agrilus bilineaus* (Weber) (Coleoptera: Buprestidae). Oecologia 74:352–355

Dunn JP, Potter DA, Kimmerer TW (1990) Carbohydrate reserves, radial growth, and mechanisms of resistance of oak trees to phloem-boring insects. Oecologia 83:458–468

Farmer RE Jr (1975) Growth and assimilation rate of juvenile northern red oak: effects of light and temperature. For Sci 21:373–381

Farmer RE Jr (1980) Comparative analysis of first-year growth in six deciduous tree species. Can J For Res 10:35–41

Gottschalk KW (1985) Effects of shading on growth and development of northern red oak, black oak, black cherry, and red maple seedlings. I. Height, diameter and root/shoot ratio. In: Dawason JO, Majerus KA (eds), Proceedings of the Fifth Central Hardwood Forest Conference. Society of American Foresters, Bethesda, pp 189–195

Hamilton DA (1986) A logistic model of mortality in thinned and unthinned mixed conifer stands of northern Idaho. For Sci 32:989–1000

Hamilton DA (1990) Extending the range of applicability of an individual tree mortality model. Can J For Res 13:601–608

Kays JS, Canham CD (1991) Effects of time and frequency of cutting on hardwood root reserves and sprout growth. For Sci 37:524–539

Kobe RK (1996) Interspecific variation in sapling mortality and growth predicts geographic variation in forest composition. Ecol Monogr 66:181–201

Kobe RK (1997) Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. Oikos 80:226–233

Kobe RK, Coates KD (1997) Models of sapling mortality as a function of growth to characterize interspecific variation in shade tolerance of eight tree species of northwestern British Columbia. Can J For Res 27:227–236

Kobe RK, Pacala SW, Silander JA Jr, Canham CD (1995) Juvenile tree survivorship as a component of shade tolerance. Ecol Appl 5:517–532

Kozlowski TT (1992) Carbohydrate sources and sinks in woody plants. Bot Rev 58:107–222

Kozlowski TT, Ward RC (1957a) Seasonal height growth of conifers. For Sci 3:61–66

Kozlowski TT, Ward RC (1957b) Seasonal height growth of deciduous trees. For Sci 3:168–174

Latham RE (1992) Co-occurring tree species change rank in seedling performance with resources varied experimentally. Ecology 73:2129–2144

Marks PL (1975) On the relation between extension growth and successional status of deciduous trees of the northeastern United States. Bull Torrey Bot Club 102:172–177

Marquis DA (1975) Seed storage and germination under northern hardwoods forests. Can J For Res 5:478–484

Matson PA, Waring RH (1984) Effects of nutrient and light limitation on mountain hemlock: susceptibility to laminated root rot. Ecology 65:1517–1524

Monserud RA (1976) Simulation of forest tree mortality. For Sci 22:438–444

Nelson EA, Dickson RE (1981) Accumulation of food reserves in cottonwood stems during dormancy induction. Can J For Res 11:145–154

Pacala SW, Canham CD, Silander JA Jr, Kobe RK (1994) Sapling growth as a function of resources in a north temperate forest. Can J For Res 24:2172–2183

Pacala SW, Canham CD, Saponara J, Silander JA Jr, Kobe RK, Ribbens E (1996) Forest models defined by field measurements:

II. Estimation, error analysis and dynamics. Ecol Monogr 66:1–43

Sakai A, Larcher W (1987) Frost survival of plants. Springer, Berlin Heidelberg New York

SAS (1989) SAS/STAT guide for personal computers, version 6. SAS Institute, Cary

Schopmeyer CS (1974) Seeds of woody plants in the United States (Agriculture Handbook 450). USDA Forest Service, Washington

Sipe TW, Bazzaz FA (1994) Gap partitioning among maples (*Acer*) in central New England: shoot architecture and photosynthesis. Ecology 75:2318–2332

Smith D (1969) Removing and analyzing total nonstructural carbohydrates from plant tissue (Research report 41). Wisconsin Agriculture Experiment Station, Madison

Smith D (1973) Influence of drying and storage conditions on nonstructural carbohydrate analysis of herbage tissue: a review. J Br Grassland Soc 28:129–134

Walters MB, Krueger EL, Reich PB (1993) Growth, biomass distribution and CO_2 exchange of northern hardwood seedlings in high and low light: relationships with successional status and shade tolerance. Oecologia 94:7–16

Wargo PM (1972) Defoliation-induced chemical changes in sugar maple roots stimulate growth of *Armillaria mellea*. Phytopathology 62:1278–1283

Wargo PM (1977) *Armillariella mellea* and *Agrilus bilineatus* and mortality of defoliated oak trees. For Sci 23:485–492

Wargo PM, Parker J, Houston DR (1972) Starch content in roots of defoliated sugar maple. For Sci 18:203–204

Webb WL (1981) Relation of starch content to conifer mortality and growth loss after defoliation by the Douglas-fir tussock moth. For Sci 27:224–232

Wright EF, Coates KD, Canham CD, Bartemucci P (1998) Species variability in growth response to light across climatic regions in northwestern British Columbia. Can J For Res 28:871–886