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Carbon limitation leads to suppression of first year oak seedlings beneath evergreen understory shrubs in Southern Appalachian hardwood forests

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Abstract

Inhibition of canopy tree recruitment beneath thickets of the evergreen shrubs *Rhododendron maximum* L. and *Kalmia latifolia* L. has long been observed in Southern Appalachian forests, yet the mechanisms of this process remain unresolved. We present a first-year account of suppression of oak seedlings in relation to *Rhododendron* and *Kalmia* basal area, light and resource availability, seedling performance and the rates of seedling damage (i.e., herbivory). We found no evidence of first-year seedling suppression or significant resource deficiencies beneath thickets of *K. latifolia* in mature mixed hardwood stands. Suppression beneath *R. maximum* was apparent during the first growing season. We found that seedling biomass, light availability prior to canopy closure, and seedling tissue C:N ratios were negatively correlated with *R. maximum* basal area. Basal area of *R. maximum* was positively correlated with seedling mortality rates, soil [Al], and early-growing season leaf herbivory rates. Seedling growth was positively correlated with light and tissue C:N, while negatively correlated with soil [Al]. Overall, our results support the inhibition model of shade-mediated carbon limitation beneath dense understory shrubs and indicate the potential importance of herbivory and aluminum toxicity as components of a suppression mechanism beneath *R. maximum* thickets. We present a causal model of first year inhibition beneath *R. maximum* in the context of our findings and the results of prior studies.

Introduction

In many tropical and temperate forests, tree regeneration patterns are influenced by the presence of dense understory vegetation that inhibits seedling establishment, growth and longevity. Through suppression of advance regeneration and herbaceous cover, shrub understories can have both short and long-term effects on forest structure, diversity and function. In Chilean forests, thickets of dwarf bamboo (*Chusquea* spp.) suppress recruitment of beech (*Nothofagus* spp.) and oak (*Quercus* spp) (Veblen 1982). Similarly, broad leaf palm understories inhibit canopy tree seedling establishment in rainforests of Costa Rica (Denslow et al. 1991). In temperate forests, ericaceous shrubs are often the culprits, forming dense evergreen thickets that suppress regeneration on a variety of sites, forest types, and in many regions: *Rhododendron ponticum* L. in the United Kingdom, *Kalmia angustifolia* (Small) Fernald. in the boreal forests of Canada, and *Rhododendron maximum* L. in the Southern Appalachian range of the U.S. (Monk et al.

1985; Mallik 1995; Clinton and Vose 1996; Nilsen et al. 2001).

Evergreen *Rhododendron maximum* (RM) dominates much of the forest understory and has rapidly increased its coverage in Southern Appalachian watersheds in recent decades (from 15 to 33% coverage during 1976–1995; Dobbs 1995). *Rhododendron maximum* can reproduce clonally and forms dense subcanopy thickets 3–7m in height. Historically a riparian species, RM has spread into mid-slope areas and now covers entire drainages on many sites, regardless of elevation or aspect (Dobbs 1995). Expansion in RM coverage has likely been due to past parallel disturbances of chestnut blight, fire suppression and high-grade logging (Monk et al. 1985; Clinton et al. 1994). Stem densities can range from 5000 to 17,000 per hectare (Baker and Van Lear 1998), resulting in deeply shaded and densely littered forest floor conditions beneath thickets. *Kalmia latifolia* (KL) is the other major component of the evergreen ericaceous understory in Southern Appalachian forests. The shrub occurs in dense thickets on dry to mesic slopes, is abundant in the understory of high-elevation conifer stands, and can be found in mixture with RM on more mesic sites (Horsley 1998). Rapid growth of dense KL thickets has been implicated in regeneration failures after logging in Appalachian forests (Marquis et al. 1984).

Most research on this system has centered on deep shade (< 2% full sun) as the primary factor that reduces seedling performance in the presence of RM (Clinton and Vose 1996; Semones 1999; Beckage et al. 2000; Nilsen et al. 2001). However, Clinton and Vose (1996) found that shading beneath RM did not fully explain inhibition of *Acer rubrum* L. seedlings, suggesting the importance of other mechanisms. In addition to lower soil moisture and nutrients found beneath RM (Nilsen et al. 2001), the presence of shrub thickets has been spatially correlated with soil acidification and nutrient paucity (Boettcher and Kalisz 1990). Moreover, Walker et al. (1999) found suppression of ectomycorrhizae on *Quercus* seedlings grown beneath dense RM. Herbivory and other interactions of potential importance have not been investigated.

In this article, we describe shrub-mediated inhibition of planted oak seedlings during the first full growing season after establishment. While suppression occurs over a period of several years after establishment beneath RM (Monk et al. 1985; Nilsen et al. 2001), we sought to better understand the initial factors that drive a seedling's trajectory towards mortality or vitality. Using correlation and linear regression analyses, we describe 1) seedling performance and mortality as related to shrub basal area; 2) shrub influences on light and resource availability; 3) seedling response to resources, e.g., the relationships among light, tissue C:N and seedling growth; and 4) observations of seedling damage along shrub basal area gradients. From the first year observations, we present a hypothetical causal model of early inhibition beneath RM based on a fully randomized explanting study at two sites differing in aspect, elevation and stand composition. The likelihood of seedling inhibition beneath KL thickets and the impacts of leaf herbivory on plant carbon balance are also discussed in the context of these results.

Methods

Experimental design

This study was conducted at the Coweeta Hydrologic Laboratory (35°03′N, 83°27′W), located in the Blue Ridge Physiographic Province of the Southern Appalachians, USA (for a complete description of regional characteristics, see Swank and Crossley 1988). The region is characterized as humid and subtropical, with an annual average precipitation of 180cm distributed evenly throughout the year. Soils are well-drained, immature Inceptisols and more developed Ultisols in the second-growth mixed oak stands used in this study.

Study sites were selected to represent the different conditions of mesic cove and xeric high elevation stands: the mesic Ball Flats site (680–720m, northwest-facing slope) and the dry ridge-top Dryman Fork site (approximately 1530 m, northeast-facing ridge), hereafter referred to as 'BF' and 'DK', respectively. Both second-growth mixed-oak stands contained dense understory RM and KL thickets, as well as smaller clumps of each species, and 'open' areas of understory without evergreen vegetation. At DK, clonal mats of deciduous *Gaylussacia baccata* [(Wangenh.) K. Koch] covered much of the forest floor where RM thickets were absent. At both sites, while some extant juvenile tree seedlings were observed beneath RM, there was an almost complete absence of herbaceous species or tree saplings beneath mature RM thickets.

In March 2000, we installed sixty $2m^2$ seedling plots which were randomly located along four transects running along the contour at each site. Plot transects passed through a mosaic of forest vegetation including open and shrub-influenced understories, creating a gradient of shrub basal area among plots ranging from absent to dense thicket. We quantified shrub basal area in 5m diameter circular plots centered on the seedling plots. Basal area metrics have been reliably used in allometric estimates of RM and KL aboveground biomass (McGinty 1972). Measurements of the light and soil environment were taken within the $2m^2$ plot in which seedlings were planted. We measured the understory light environment using hemispherical canopy photographs taken from plot center, approximately 0.5m above the ground. Canopy images were taken prior to leaf-out (March) and after overstory canopy closure (July). Photographs were taken on Kodak Tri-X film using a fish-eye 8mm lens fitted to a Nikon FM2 camera (with a red filter to maximize sky-foliage contrast) mounted on a leveled tripod. Magnetic north was marked at the top of the image, which allowed the solar track to be superimposed onto the photograph. Negatives were scanned, digitized and analyzed using HemiView canopy analysis software (Delta-T Devices, Cambridge, U.K.). HemiView calculates the proportion of diffuse (indirect site factor, ISF) and direct (direct site factor, DSF) radiation received below the canopy to the amount of radiation received above the canopy (Rich 1989). The global site factor (GSF) is a summary statistic calculated from ISF and DSF. Site factors can range from 0 (complete obstruction) to 1 (open sky), and are strongly related to PPFD measures in these forests, based on regression analysis (Horton *unpublished data*).

Volumetric soil water content (θ_v) in each plot was measured monthly throughout 2001 using time-domain reflectometry, a method based on the differential conductivity of water and soil particles (Campbell Scientific, Logan, UT). Soil properties were assayed from a bulked sample of the top 10 cm of mineral soil from four locations within each plot. Soil, humus and litter depth in each plot were measured in late summer 2000 at the same locations where mineral soil was sampled. Soil horizons were defined as: litter (loose leaf material), humus (decomposed leaf and organic material), and mineral soil (all horizons below humus layer). Separate layers of litter and humus biomass were collected, dried and weighed to estimate litter and humus yield ($g\ m^{-2}$) per plot. Humus, litter and mineral soil samples from each plot were analyzed for carbon and nitrogen content using a CNS analyzer (Elementar Americas, Trenton, NJ). Soil pH and cation analysis was performed by the Virginia Tech Soil Testing Laboratory using inductively coupled plasma mass spectroscopy.

Plant material and seedling performance measures

We germinated acorns of *Quercus rubra* L. and *Quercus prinus* L. and seeds of *Pinus rigida* L. in coarse vermiculite and grew seedlings in nursery cells with vermiculite. Seedlings were grown in the greenhouse for at total of 16 weeks, and seedlings were fertilized weekly with 25% Hoagland's solution after 8 weeks. Prior to planting, fifty randomly selected seedlings from each species were analyzed for biomass parameters used in allometric equations. During early summer 2000 we planted *Q. rubra* and *Q. prinus* seedlings at the low elevation BF site, and *Q. rubra* and *P. rigida* seedlings at the high elevation DK site. *Pinus rigida* seedlings suffered very high post-transplant mortality and were excluded from the experiment. Four seedlings of each species were planted with equal spacing in each plot, and we recorded seedling height, general quality, basal diameter and number of leaves. We estimated stem biomass of each seedling at planting with an allometric equation based on harvested greenhouse seedlings:

$$\text{stem biomass} = m\,(D^2)(h) + b \quad (1)$$

(e.g., for *Q. rubra*: $n = 52$; $r^2 = 0.669$, $p < 0.0001$)

Where D is basal diameter, h is stem length (from ground line to apical meristem); m and b are regression model parameters. Separate equations were developed for the analysis of each species. Predicted initial stem biomass (based on the allometric model) was used to estimate changes in stem biomass during the growing season.

Seedling performance was determined by analysis of growth, morphology and biomass parameters of randomly selected seedlings harvested from each plot. We harvested seedlings during the middle (July) and end (September) of the growing season; a total of two seedlings per species per plot. We harvested in July to determine if seedlings recovered leaf tissue from high rates of herbivore damage observed during

April-June. In addition to morphological measurements, leaves, stems and roots were cleaned, oven-dried at 80 °C, weighed and prepared for carbon-nitrogen (CN) analysis.

In addition to harvest measurements, we censused seedlings every two weeks during the growing season to record survival, phenology and the extent and causes of seedling damage (health). Health was assessed as a function of damage to tissues:

$$H = (L_n/L_o)\,(100 - \%\text{loss}) \qquad (2)$$

Where L_n is the number of leaves at sampling time n, and L_o is the initial number of leaves at spring flush. The %loss term represents a cumulative estimate of removed leaf tissue for each seedling. Due to the subjectivity of %loss estimate, the same researcher did all census sampling and recorded the apparent causes of damage (herbivory, pathogens, desiccation). The index does not predict actual leaf area, but estimates a percentage loss based on the overall size of the leaves for each seedling. Thus a seedling that has intact, small leaves will have a high H, but may have less leaf area than a larger, more damaged seedling with a subsequently lower H value.

Data analysis

Because plots were selected randomly along natural gradients of shrub basal area, we used multivariate correlation and linear regression approaches to identify relationships indicative of seedling inhibition. We first identified any seedling performance trends over the gradient of shrub basal area, using Pearson's *r* pair-wise correlations. Next, correlations between shrub basal area and belowground resource / substrate variables were described for each site, using the seedling plot as the experimental unit (n = 60 per site). Linear regression was used to model shrub influence on light availability (as a function of canopy openness above each plot), due to the strong shading effects of RM shown in previous studies (Monk et al. 1985; Clinton et al. 1994; Semones 1999). Using stepwise multivariate regression models, we identified the resource variables that influenced seedling growth and biomass during the first growing season, and (with these variables) developed 'streamlined' correlation matrices describing seedling response to resource availability. Correlation matrices were a preferable approach because co-linearity in the 'explanatory' resource variables could be explained in the correct biological context. Lastly, we used correlation analysis for shrub influence on seedling damage (i.e., herbivory) during the growing season. Seedling performance data was initially stratified by site, species and harvest date for all analyses. In the following section, we present results comparing harvests only when we observed qualitatively different relationships based on harvest date. For brevity, analyses where both harvests were similar are presented as a single sample based on site and species.

Results

Shrub influence on seedling performance and survivorship

We found that seedling growth, biomass and survivorship were negatively correlated with basal area of RM. At BF, mortality of *Q. rubra* (10.0%) and *Q. prinus* (17.9%) was positively correlated with RM density, and higher than *Q. rubra* mortality at DK (4.6%). The strongest negative correlations between RM basal area and seedling performance measures were observed at DK. However, there was no evidence that RM was related with mortality of *Q. rubra* seedlings at DK (Table 1). Basal area of *K. latifolia* (KL) was unrelated with survivorship, and was positively correlated with measures of *Q. prinus* performance (total biomass and leaf area; Table 1). In some cases, we observed a categorical effect of RM presence in scatter-plot diagrams. Seedling parameters had reduced variability in plots with RM than in plots without RM. For example, leaf dry weight of *Q. rubra* at DK was much lower and less variable in the presence of RM than in plots where RM was absent (Figure 1). We observed similar trends beneath RM for several *Q. rubra* parameters at DK, including stem growth and aboveground biomass.

Shrub influence on light and soil resources

Mean shrub densities and the relative dominance of RM and KL around plots were variable by site. For BF plots, mean KL density ($0.021 m^2$) was higher than RM ($0.01 m^2$). Conversely, mean plot density of RM ($0.012 m^2$) was greater than KL ($0.004 m^2$) at the DK site. Maximum basal area observed around plots was consistently higher for RM than KL at both sites,

Table 1. The influence of understory shrub density on first-year *Quercus* seedlings. Correlations among basal area of Rhododendron maximum (RM) and Kalmia latifolia (KL), measures of seedling performance (biomass, growth , tissue nutrition), and first-year mortality in forest plots representing a gradient of shrub density. Pearson's *r* coefficients are significant at the $p < 0.05$ level when denoted by (*).

	RM basal	KL basal	dry biomass	root C:N	leaf C:N
Dryman Fork (DK) ***Quercus rubra***					
dry biomass	−0.303*	0.0142	– – –		
root C:N	−0.3499*	0.0646	0.5973*	– – –	
leaf C:N	−0.4535*	0.0217	0.4178*	0.6564*	– – –
stem growth	−0.2106*	−0.0119	0.6568*	0.384*	0.3395*
leaf area	−0.2938*	0.1622	0.7731*	0.5174*	0.3486*
plot % mortality	−0.0306	−0.1128			
Ball Flats (BF) ***Quercus prinus***					
dry biomass	−0.1072	0.2041*	– – –		
root C:N	−0.0557	0.0122	0.3338*	– – –	
leaf C:N	−0.2369*	−0.0029	0.3321*	0.4889*	– – –
stem growth	−0.028	0.1473	0.8207*	0.1835*	0.2232*
leaf area	−0.1563*	0.2186*	0.921*	0.2606*	0.3908*
plot % mortality	0.2827*	−0.0603			
Ball Flats (BF) ***Quercus rubra***					
dry biomass	−0.1019	0.095	– – –		
root C:N	−0.0437	0.0576	0.3271*	– – –	
leaf C:N	−0.1668*	0.0013	0.1416	0.3823*	– – –
stem growth	−0.1205	0.0455	0.6515*	0.193*	0.148
leaf area	−0.1742*	−0.0499	0.7709*	0.2573*	0.0652
plot % mortality	0.3802*	−0.0623			

probably a result of morphological differences. RM tends to have thicker stems and greater spacing between individuals in a mature thicket.

Open-canopy light availability was a weak negative function of RM basal area at both sites (Open ISF, DSF- Table 2). The shading influence of RM was stronger than KL (with one exception, at DK in July). Canopy closure reduced the influence of RM and KL on site factors, but to a lesser degree at the DK site, where several large canopy gaps had recently been formed. After canopy closure, our measures of light were inadequate to isolate the shrub influence based on site factors. Prior research has found deep shade (< 2% full sun) beneath RM in forest understories very similar to those sampled in this study (Monk et al. 1985; Clinton and Vose 1996).

We found no correlations indicating major nutrient deficiencies beneath RM at either site, yet some factors detrimental to plant nutrition were observed. At both sites, mineral soils were acidic with mean pH below 5 (DK = 4.67, BF = 4.91) where soil [Fe] was strongly correlated with low pH. Soil [Al] and humus C:N ratios were positively correlated with RM at both sites (Table 3, Table 4), but no significant RM-soil pH relationships were observed. Litter and humus yield ($g\ m^{-2}$) was a positive function of RM density at BF, but not at DK. Basal area of KL was negatively correlated with soil [Al] at both sites, and was related with lower pH and soil moisture at the dry DK site. Mineral soil N content declined with KL density at BF.

Seedling response to resource availability

Multivariate regression modeling of seedling response to resource availability allowed us to identify a group of variables most commonly related with seedling performance. The most important variables in these models were light (open and closed canopy site factors), litter and humus C:N ratios, soil moisture, soil [Al] and other nutrient cations. Overall, seedling correlations with light were positive, variable with soil moisture, negative with soil [Al] regardless of RM cover, and weakly positive with soil [P], [K], [Mg] and [Ca] (Table 5, Table 6).

Seedling growth and tissue C:N responses to light availability before and after canopy closure were variable, but generally positive; and differences between harvest groups were qualitatively apparent (Table 5). Open-canopy site factor (GSF) was correlated with

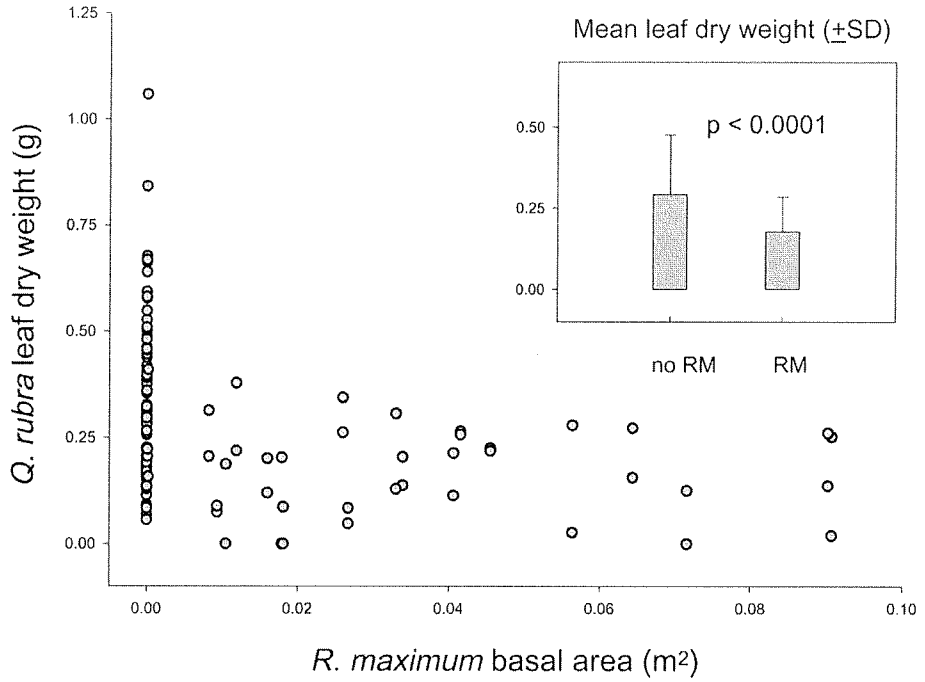

Figure 1. An example of a non-continuous relationship between shrub basal area and a seedling performance variable. Variance in leaf dry weight beneath *R. maximum* (RM) is approximately half of the total variance when RM basal area equals zero. Analysis of variance based on RM presence/absence (student's *t*-test) indicates a highly significant difference between group means (see inset).

leaf C:N and total dry biomass for July-harvested *Q. rubra* seedlings at DK, although September-harvested seedlings from the same plots showed no significant response to light (Table 5a). *Quercus prinus* seedlings at BF (Table 5b) responded positively to light for both harvests, with the strongest correlations between open-canopy GSF and leaf C:N, as well as root C:N, total biomass and leaf area. However, in the same plots at BF, *Q. rubra* growth, tissue C:N and biomass were mostly unrelated with our measures of light availability (Table 5c).

Seedling parameters were also correlated with soil [Al], organic horizon N content and mid-season soil moisture (Table 6). Growth, biomass and tissue C:N were negatively correlated with soil [Al] at the high elevation DK site. Soil [Al] appeared to have no negative influence on either oak species at BF. Total biomass and leaf area of *Q. rubra* were positively related with the leaf litter N in DK plots, while root C:N was co-linear with humus N. At BF, *Q. rubra* leaf area and *Q. prinus* stem growth were positively correlated with litter N content. Leaf C:N and growth increased with soil moisture at the mesic BF site, in contrast to the dry ridge DK site, where seedling vigor was unrelated with soil moisture.

Phenology and seedling damage beneath understory shrub thickets

Most planted seedlings began new growth about 2–3 weeks prior to canopy closure, and we found no correlation between shrub basal area and the small proportion (6%) of late-flush seedlings. During the 2001 growing season, the two most important sources of observable seedling damage were arthropod herbivores and a late frost event at the high-elevation DK site. Cumulative frequencies of observed herbivore and pathogen damage over the growing season are shown in Figure 2. For the late frost, mean seedling damage per plot was 28% of the spring flush tissue, affecting over 70% of our planted *Q. rubra* at DK by the first census period (April 28). Frost damage was negatively related with RM basal area (Figure 3) and significantly lower in the presence of the shrub (student's *t*-test; $p < 0.05$). Within two weeks of the frost-related damage, most seedlings had produced a

Table 2. Shading influence of understory shrubs: linear regressions between basal area of *R. maximum* (RM) and *K. latifolia* (KL) and light availability as interpreted by hemispherical canopy photograph analysis in open (before leaf out) and closed (after closure) canopies. ISF is indirect site factor (diffuse light); DSF is direct site factor (direct light). A significant regression coefficient ($p < 0.05$) indicates the light parameter was a negative function of RM or KL basal area.

Canopy	Model	r-square	p
Ball Flats (BF) – 685m, NW-facing slope			
Open	RM basal x ISF	0.179	0.0009
	RM basal x DSF	0.153	0.002
Closed	RM basal x ISF	0.014	> 0.05
	RM basal x DSF	0.015	> 0.05
Open	KL basal x ISF	0.017	> 0.05
	KL basal x DSF	0.005	> 0.05
Closed	KL basal x ISF	< 0.001	> 0.05
	KL basal x DSF	< 0.001	> 0.05
Dryman Fork (DK) – 1590m, NE-facing ridge			
Open	RM basal x ISF	0.171	0.001
	RM basal x DSF	0.128	0.005
Closed	RM basal x ISF	0.066	0.048
	RM basal x DSF	0.022	> 0.05
Open	KL basal x ISF	0.108	0.01
	KL basal x DSF	0.067	0.045
Closed	KL basal x ISF	0.072	0.037
	KL basal x DSF	0.029	> 0.05

second flush of leaves. Our subsequent census observations indicated that mean plot leaf area loss due to herbivory increased with RM basal area (Figure 3); although actual damage was low, averaging 8% leaf area per plot at DK.

There were no relationships between KL basal area and our measures of seedling health through the growing season ($p > 0.05$ on all sample dates). With the exception of the first census, *Q. rubra* seedling health at BF was negatively correlated with RM basal area throughout the growing season. Similar trends were observed for *Q. prinus* seedlings in the same BF plots, although the correlations were mostly non-significant. For all seedlings at both sites, we observed a strong 'pulse' of herbivory prior to the second census period (May 15; Figure 2) that increased in severity as RM basal area increased (Pearson's r, all $p < 0.05$). Actual damage was low at BF, averaging less than 10% leaf area removed per seedling. Seedlings of both species recovered leaf area from early-season herbivore damage. For both sites and species, July-harvested seedling leaf weight was significantly lower (student's t-test, $p < 0.05$) than those harvested in September. Census observations verified that several heavily damaged seedlings developed new leaves late in the season.

Discussion

Carbon limitation of *Quercus* seedlings was evident in the first growing season after establishment beneath RM thickets. Shade beneath RM was linked with lower leaf carbon content for *Q. prinus* at the mesic BF site and *Q. rubra* at the dry DK site. Mortality of *Q. prinus* was linked with RM density at BF, but not for *Q. rubra* at DK where increased herbivory and aluminum toxicity beneath RM were secondary stressors that may have exacerbated the carbon-limited condition (Walters and Reich 1996; Hanley 1998). Yet overall, our observations of mechanism (i.e., C-limitation, Al toxicity) were unrelated with increased mortality beneath RM because the best examples of these interactions occurred at different sites. The only obvious exception to this was for *Q. prinus* at BF, where carbon limitation and increased mortality were both linked with RM. Overall for *Q. rubra*, we observed lower-stress, higher-mortality at BF and higher-stress, lower-mortality at DK. These paradoxes are probably reflective of both the heterogeneity of this system and inherent limitations of this study: mortality due to transplanting shock, the single-year duration, and the role of carbon stores of nursery-grown seedlings in buffering against carbon imbalance.

The multiple factors contributing to suppression of *Q. rubra* seedlings beneath *R. maximum* at the high-elevation DK site are presented in Figure 4. As RM basal area increased and light availability decreased, seedling tissue C:N ratios declined, indicating that limited carbon gain may have caused a decrease in the relative proportion of carbon in tissues. On average, the C:N ratio of *Q. rubra* seedlings was approximately 15-20% lower when grown beneath RM, compared to those beneath an open understory. While the biological significance of this reduction in tissue C may be uncertain, the trend is indicative of a physiological trajectory towards slow growth, reduced leaf area, and early mortality (Semones 1999). Carbon limitation has been proposed as a primary mechanism of shade-tolerant seedling decline in deeply shaded understories where light availability is strongly linked to seedling establishment and growth

Table 3. Influence of Rhododendron maximum density (RM basal) on soil resources and forest floor traits, as well as co-linearities among soil cations and pH, at the high elevation Dryman Fork (DK) site. Mean mineral soil concentrations of cations of Cu^{2+} Al^{3+} and Fe^{2+} are provided. Pearson's *r* coefficients are significant at the $p < 0.05$ level when denoted by an asterisk (*), SD is standard deviation.

	RM basal	pH	Ca	Mg	P	K	Fe	Al
pH	0.0391	– – –						
Ca	– 0.1814*	– 0.0699	– – –					
Mg	– 0.0208	– 0.0427	0.7703*	– – –				
P	– 0.1349	– 0.2071*	0.6185*	0.7267*	– – –			
K	– 0.0664	– 0.3249*	0.5947*	0.6068*	0.5685*	– – –		
Fe	0.0782	– 0.5709*	0.0832	0.2582*	0.4082*	0.3608*	– – –	
Al	0.1812*	0.1643	– 0.1968*	– 0.3449*	– 0.308*	– 0.046	– 0.1284	– – –
Cu	0.198*	0.3014*	0.02	0.2817*	0.2218*	0.0288	– 0.0174	– 0.2844*
Litter yield	0.107							
Soil depth	0.1357		Soil ppm	Cu	Fe	Al		
Humus yield	0.1389							
Soil C:N	– 0.0412		Mean	0.42	27.5	696.3		
Humus C:N	0.2198*		Max	1.27	49.4	1150.8		
Litter C:N	0.4987*		SD	0.22	10	150		

Table 4. Influence of Rhododendron maximum density (RM basal) on soil resources and forest floor traits, as well as co-linearities among soil cations and pH, at the low elevation Ball Flats (BF) site. Mean mineral soil concentrations of cations of Cu^{2+} Al^{3+} and Fe^{2+} are provided. Pearson's *r* coefficients are significant at the $p < 0.05$ level when denoted by an asterisk (*), SD is standard deviation.

	RM basal	pH	Ca	Mg	P	K	Fe	Al
pH	– 0.0925	– – –						
Ca	– 0.1239	0.4133*	– – –					
Mg	0.0628	0.601*	0.3493*	– – –				
P	– 0.0821	– 0.2326*	0.039	– 0.0673	– – –			
K	0.0963	– 0.1179	– 0.0262	0.2079*	– 0.0517	– – –		
Fe	0.2845*	– 0.4567*	– 0.1983*	0.0394	0.2137*	0.3685*	– – –	
Al	0.3446*	– 0.1551	– 0.1873*	– 0.1484	0.0476	0.3449*	0.1852	– – –
Cu	0.3814*	– 0.0142	– 0.4715*	– 0.0545	– 0.201*	0.2509*	0.2418*	0.3808*
Litter yield	0.3242*							
Soil depth	0.142		Soil ppm	Cu	Fe	Al		
Humus yield	0.2177*							
Soil C:N	0.0954		Mean	1.05	16.1	382.1		
Humus C:N	0.3994*		Max	1.8	30.1	545.6		
Litter C:N	– 0.0569		SD	0.4	4.3	60.9		

(Denslow et al. 1991; Pacala et al. 1994; Mallik 1995; Semones 1999). In temperate forests, tree regeneration generally occurs in conjunction with the formation of canopy gaps (Runkle 1982; Canham 1988, Clinton et al. 1994), which if large enough provide sufficient light intensity for many species to reach the canopy (Whitmore 1989). Dense understories can negate the facilitative influence of canopy gaps on light, moisture and nutrient availability (Clinton et al. 1994, Beckage et al. 2000). As a result, even the most shade-tolerant seedlings experience slow or insignificant growth (Walters et al. 1993), limited allocation to storage (Walters and Reich 1996), decreased survivorship (Kobe et al. 1995) and may commit less energy to defensive compounds (Coley et al. 1985; Dudt and Shure 1994). Chronic carbon limitation represents a positive feedback loop that may lead to hastened mortality, particularly when herbivory exacerbates the depletion of carbon stores via tissue removal (Hanley 1998) or additional resource deficiencies exist in conjunction with deep shade (Walters and Reich 1996).

During the first year of growth, *Quercus* seedling damage was correlated with RM density: less damage from a late frost (at DK), and greater leaf area loss due to herbivores (at both sites). Although the absolute amount of tissue loss observed was too low to directly cause mortality, increased damage beneath

Table 5. First-year *Quercus* seedling responses to light availability in forest plots, based on correlation analysis. Seedling groupings are based on site, species and harvest date. Global Site Factor (GSF) is a measure of indirect and direct light based on canopy photographs. Photos were taken prior to deciduous leaf-out (March) and after canopy closure (July). Pearson's *r* coefficients are significant at the $p < 0.05$ level when denoted by an asterisk (*).

July Harvest			September Harvest		
	GSF March	GSF July		GSF March	GSF July
a) Dryman Fork – *Quercus rubra*					
root C:N	0.1503	0.3111*	root C:N	−0.065	−0.0955
leaf C:N	0.3205*	0.1502	leaf C:N	−0.0285	0.0554
total biomass	0.3152*	0.2714*	total biomass	0.0341	−0.0351
leaf area	0.2303	0.205	leaf area	−0.0047	−0.1103
stem growth	0.1398	−0.0151	stem growth	−0.0788	0.1275
b) Ball Flats – *Quercus prinus*					
root C:N	0.1845	−0.0295	root C:N	0.3045*	−0.0336
leaf C:N	0.5108*	0.0824	leaf C:N	0.0411	−0.0796
total biomass	−0.0103	0.0601	total biomass	0.3403*	−0.0501
leaf area	−0.0115	0.1316	leaf area	0.3319*	−0.0995
stem growth	−0.1073	0.1194	stem growth	0.1478	−0.0846
c) Ball Flats – *Quercus rubra*					
root C:N	0.1244	−0.1366	root C:N	−0.129	−0.0425
leaf C:N	0.1409	−0.2238	leaf C:N	−0.085	0.0053
total biomass	0.1308	0.0865	total biomass	0.0443	−0.2741*
leaf area	0.1352	0.0882	leaf area	0.0797	−0.1372
stem growth	−0.0209	0.0751	stem growth	0.1924	−0.1703

Table 6. Influence of soil [Al], litter and humus N content, and June volumetric soil water content (θ_v) on first-year *Quercus* seedling biomass, growth and tissue nutrition. Correlation matrices are abridged for brevity, showing only Pearson's *r* when significant at the $p < 0.05$ level (i.e., empty spaces indicate no statistical relationship was found).

	Soil [Al]	Litter N	Humus N	June θ_v
Dryman Fork (DK) *Q. rubra*				
dry biomass	−0.2524	0.3843	–	–
root C:N	−0.2154	–	0.3221	–
stem C:N	−0.1886	–	–	–
leaf C:N	−0.1879	–	–	–
stem growth	−0.2144	–	–	–
leaf area	−0.152	0.2439	–	–
Ball Flats (BF) *Q. prinus*				
dry biomass	–	–	–	0.1944
leaf C:N	–	–	–	0.3543
stem growth	–	0.2933	–	–
leaf area	–	–	–	0.2721
Ball Flats (BF) *Q. rubra*				
leaf C:N	–	–	–	0.2721
leaf area	–	0.1959	–	–

RM could worsen carbon-limitation and lead to lower survivorship. Loss of tissues to herbivores generally has a much greater selective impact on late successional species (such as *Quercus*) growing in resource-poor habitats (Coley et al. 1985; Bryant and Chapin 1986). Further, our *Quercus* seedlings grew new leaves in response to herbivory; these replacement costs are more detrimental to plants competing for resources in forest understories (Hanley 1998). In Figure 4, we propose a linkage between tissue C:N and the positive correlation between herbivory damage and RM (see dotted line). Declining leaf C:N ratios with increasing RM basal area could indicate lower concentrations of defensive compounds (which require large C investment) and higher palatability of tissues to herbivores (due to a higher relative proportion of tissue N). *Quercus* species rely on chemical defenses to deter herbivory, and shade-mediated limitations to defense beneath RM may have contributed to the increased rates of damage observed beneath shrub thickets. For example, light-limited seedlings of late-successional dogwood (*Cornus florida* L.) produced below-normal concentrations of defensive compounds (Dudt and Shure 1994). Further experimental study of herbivory beneath *R. maximum* is clearly needed.

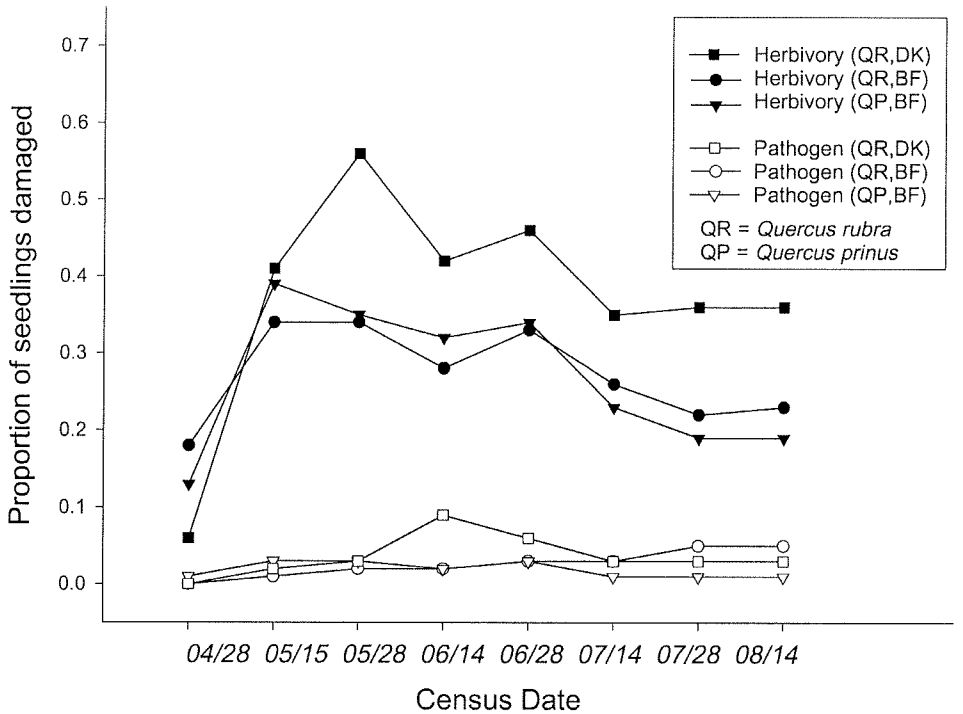

Figure 2. Frequency of observed seedling damage by herbivores and pathogens during the 2001-growing season. Seedlings were censused biweekly from mid-April to early September (prior to the second harvest). Leaf herbivory and pathogens were the two most important causes of damage observed, with exception to a late frost event at the DK site (not depicted).

Aluminum toxicity may have been another shrub-mediated factor that negatively influenced seedling growth and survivorship (Table 6). Soil [Al] in forest plots was positively related with the basal area of RM surrounding those plots. Aluminum toxicity interferes with the uptake, transport and use of essential elements such as Ca, Mg, P, K, and Fe (Foy 1983). We observed weak but negative correlations between soil [Al] and various measures of seedling performance. In addition, lower N content of litter and humus material beneath RM was linked with seedling biomass and tissue nutrition. Therefore, aluminum toxicity in concert with slight nutrient cation deficiencies and lower organic N availability may have generated further stress to carbon-limited seedlings beneath RM thickets.

Kalmia latifolia, while often implicated in post-harvest regeneration failures in Appalachian hardwood stands (Marquis et al. 1984), did not appear to have an inhibitory influence on our first-year *Quercus* seedlings. None of our measures of seedling performance were negatively correlated with basal area of KL. There were no indications that seedlings were carbon-limited beneath KL shrubs or that KL influenced survivorship.

The suppression of canopy tree recruitment observed beneath *R. maximum* has engendered concern by managers and ecologists over the future viability of these productive and diverse forests. Neither empirical nor anecdotal accounts have indicated that canopy trees have recruited successfully after thicket formation in the understory (McGinty 1972; Baker and Van Lear 1998). Coverage of RM thickets has expanded dramatically in recent decades (Dobbs 1995), occupying a range of forest and soil types outside of its typical riparian niche (Baker and Van Lear 1998). Our high elevation DK site is typical of the stands where RM has become well established in recent decades (Dobbs 1995). At this site, *Q. rubra* seedlings established beneath *R. maximum* thickets encountered a number of simultaneous stressors. We have shown that these stressors initiate a carbon-limited condition in the first growing season, and propose that if persistent, this condition will lead to early de-

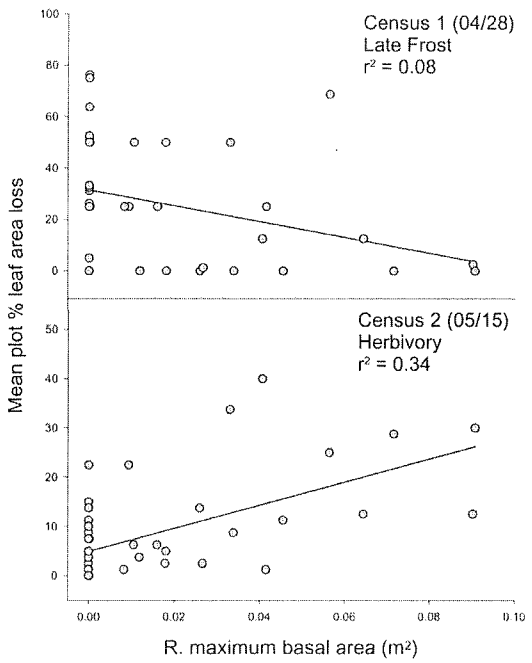

Figure 3. Regression analysis of *R. maximum* influence on seedling leaf damage for two consecutive census periods during the early growing season of 2001. Late frost affected about 70% of seedlings and mean tissue loss was approximately 28%. Most seedlings developed new leaves by the second census. Actual leaf area loss to herbivory averaged less than 10% per seedling for this census period.

cline and mortality of *Quercus* regeneration beneath a *R. maximum* understory.

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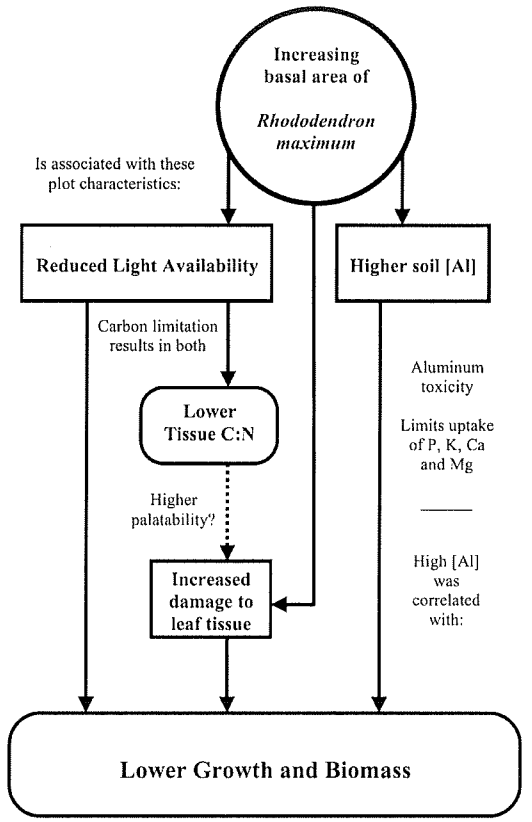

Figure 4. Hypothetical causal model of first-year seedling inhibition beneath understory thickets of *R. maximum*, based on observations of *Quercus rubra* seedlings grown at the xeric, high elevation DK site. Solid lines reflect statistical relationships observed in this study; the mechanism indicated by the dotted line is theoretical and is addressed further in the discussion. Square boxes contain resource or interaction variables influenced by shrub basal area, and rounded rectangles contain seedling response variables.

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