

CHAPTER I

INTRODUCTION

In many forest biomes, understories composed of dense evergreen shrubs have been implicated in the suppression or alteration of normal tree recruitment processes. In Chilean forests, thickets of dwarf bamboo (*Chusquea* spp.) inhibit regeneration of dominant canopy oak (*Quercus*) and beech (*Nothofagus*) species (Veblen, 1982). In Costa Rican tropical forests, Denslow et al. (1991) found that canopy tree recruitment was impeded by broad-leaf palm understories. Ericaceous, evergreen shrubs are significant examples in temperate and boreal forests: *Kalmia angustifolia* (Small) Fernald. in conifer forests of Canada, where inhibition may be due to strong allelopathy (Mallik, 1995); *Rhododendron ponticum* L. in the oak woodlands of the U.K. (Mitchell et al., 1997), where it has become a rapidly-spreading pest species; and *Rhododendron maximum* L. in mixed-oak southern Appalachian forests, where its effects on tree recruitment have been long observed (Whittaker, 1956; Swank and Crossley, 1988; Clinton et al., 1994; Baker and Van Lear, 1998; Nilsen et al., 2001).

Forest understories dominated by *Rhododendron maximum* are a particularly important example of the inhibition of tree regeneration for a number of reasons. First, *R. maximum* has dramatically increased its dominance of the forest floor in the last several decades (in the Coweeta Basin; Dobbs, 1995; Baker and Van Lear, 1998), spreading through clonal reproduction and dispersal of tiny, windborne seeds. Logging, fire suppression and major compositional shifts in the forest canopy (due to the chestnut blight) have created a disturbance regime suitable for *R. maximum*'s invasion from

riparian areas into coves and north-facing slopes. Once established, the shrub rapidly forms a thick, evergreen subcanopy layer and a dense root mat in the organic horizon (Baker and Van Lear, 1998). Prior studies have found that established shrub thickets lower resource availability, essentially have a 'priority effect' by excluding forest floor vegetation, and may neutralize the facilitative effect of canopy gaps on resources and microclimate (Boettcher and Kalisz, 1990; Clinton and Vose, 1996; Semones, 1999, Nilsen et al., 2001). Thickets of *R. maximum* have become an issue for forest managers concerned about the loss or degradation of high quality hardwood production sites and wildlife habitats, and have prompted studies on the suppression of its spread (Hooper, 1969; Romancier, 1971). Another understory shrub species of interest in Appalachian forests is the evergreen *Kalmia latifolia* L. (Mountain Laurel), which also forms dense thickets, either in combination with *R. maximum* or on more xeric soils at higher elevations. The canopy of a *K. latifolia* thicket is sparse and generally short in stature (2-5m) in comparison to thickets of *R. maximum* (Whitaker, 1956; Baker and Van Lear, 1998). The potential influence of *K. latifolia* on tree recruitment processes, as well as its ecological similarity to *R. maximum*, have not been directly investigated, and will be addressed in the following chapters.

In this chapter, I will discuss several aspects of the ecology of tree recruitment, including both pre- and post-germination processes that influence the establishment, growth and survival of tree seedlings. Previous studies demonstrating the influence of a dense shrub subcanopy on resource availability, microclimate and soil characteristics will be outlined. Further, I will describe the ecophysiology of the shade-tolerant, late-successional tree species of concern, as well as the potential impact of herbivory on

seedling performance and mortality. Within the context of inhibition by *Rhododendron maximum*, most studies indicate that seedling suppression occurs post-establishment as a result of asymmetric competition with the well-established shrubs (Clinton and Vose, 1996; Walker et al., 1999; Semones, 1999; Nilsen et al., 2001). However, a primary assumption of the resource competition hypothesis is that tree seed dispersal, seed bank entrance and germination are unaffected by a dense shrub understory. In the second chapter, I address this assumption through a factorial experiment examining seed rain and seed bank composition among shrub cover treatments. Next, the third chapter presents the results of a study examining the resource competition hypothesis along natural gradients of shrub density. The fourth and final chapter synthesizes the knowledge gained from the two experiments and briefly outlines possible directions for future research.

Factors influencing seed dispersal and seed bank characteristics

Studies of recruitment limitation of canopy trees can be very complex due to the difficulty in measuring and analyzing the fecundity, dispersal and establishment of tree propagules under a closed canopy. In fact, many studies have ignored pre-establishment processes and instead focused on resource-mediated growth limitations and factors causing mortality of young seedlings. Clark et al. (1998) outlined the primary impediments for fecundity and dispersal studies: 1) seed rain is difficult to quantify beneath a closed canopy, because seed shadows overlap, 2) wind and animal dispersed seeds are extremely difficult to track, and 3) highly variable winds, seed release heights

and microsite topography are difficult to parameterize in mechanistic models of dispersal. Yet, many studies have overcome these hardships and shed light on early life history stages where limitation can occur (see Clark et al., 1998), such as seed arrival, seed predation, germination and initial survival. Arrival of seeds in suitable microsites is dependent on the density and spatial arrangement of mature trees, their fecundity, and seed-specific dispersal distances. Seed morphology has a profound impact on dispersal and patterns of seed rain in mixed stands; for example, by increasing drag, generating lift (i.e. spinning samaras such as *Acer*) or attracting animal dispersers (Augsperger, 1986; Willson, et al., 1990; see Clark et al., 1998). Germination and establishment of seedlings is another potential stage where recruitment can be limited.

Clark et al. (1998) found that, in southern Appalachian forests, establishment of seedlings was one of the strongest limitations to recruitment. Specifically, they showed that few species manage to saturate the forest floor with seed (*Acer*, *Betula*, *Liriodendron*), while other species, despite their presence in mixed stands, are unable to do so (*Carya*, *Cornus*, *Nyssa*, *Oxydendrum*). In general, *Quercus* species had high source densities, moderate source strengths, but appeared to be strongly limited at the establishment stage (a very low seedlings / seed ratio). Similar to *Quercus*, other large seeded species (*Carya*, *Nyssa*, *Cornus*) were also limited by dispersal, which was mostly reliant on animal vectors. Finally, Clark et al. (1998) argued that all tree populations in southern Appalachian forests experience substantial recruitment limitation, at one or more stages.

Seed predation is an important component of the recruitment filter between dispersal and establishment stages. Post-dispersal predation is a critical mechanism

limiting regeneration in eastern deciduous forests and the invasion of old fields (Gill and Marks, 1991; Myster and Pickett, 1993). Prior research has shown that seed predation differs among species, decreases with distance from source trees, is often spatially heterogeneous, and may be dependent on litter type and depth (Thompson, 1982; Mittelbach and Gross, 1984; Schupp, 1988). Myster and Pickett (1993) found that seed predation was greater beneath patches of remnant woody vegetation than beneath herbaceous cover in old fields. Cornett et al. (1998) found that exclusion of predators was more beneficial for conifer seedling emergence (*Abies*, *Pinus*) than removal of inhibitory forest floor material and release from understory competition. Similar results have been observed in tropical forests (Molofsky and Fisher, 1993). Rodent predation of smaller seeds selected for larger-sized seeds, but did not influence natural recruitment patterns for wild olive trees (*Olea europaea* var. *sylvestris*; Alcantara et al., 2000). Predation by small mammals, however, often results in redistribution of some seed in caches throughout the forest (Cornett et al., 1998); and this may be an important dispersal vector for *Quercus* species during mast years, when acorn production far exceeds consumption demand.

Seed banks have been defined as those seeds that maintain dormancy for a period of time until some environmental or physical disturbance prompts germination (Grime, 1989). For many years, there has been interest in the spatial heterogeneity of seed bank density and composition, due to the important role of seed banks in post-disturbance forest regeneration (Hyatt, 1998). Since seed bank stimulation (release from dormancy followed by germination) is most commonly dependent on disturbance, the type and severity of the disturbance, as well as the changes in microclimate associated with it, are

integral to understanding what types of seedlings will emerge. For example, seeds that germinate best in high light will most likely emerge when the disturbance raises light levels on the forest floor, such as the formation of a canopy gap (Hyatt, 1998). Other disturbances that create a microenvironment more suited to germination and growth of shade-tolerant species may result in a very different composition of emergent seedlings. Many studies of temperate deciduous forests have found that the seed banks include mostly early-successional, shade-intolerant taxa (i.e. *Betula*, *Prunus*, *Rubus*, *Liriodendron*) that differ from the existing overstory composition (Hill and Stevens, 1981; Nakagoshi, 1985; Ashton et al., 1998). Some canopy tree species of *Quercus* (as well as *Carya*, *Nyssa*, *Aesculus* and *Juglans*) do not typically enter the seed bank, probably because of their large size and susceptibility to decomposition and predation by invertebrates. In forests of the northeastern U.S., topographic position had a strong impact on seed bank composition; according to Ashton et al. (1998), graminoids and trees were most abundant in cove sites, herbaceous species on midslopes, and shrubs on ridgetops; as a rule, most germinant species were not characteristic of the existing vegetation. In old growth temperate deciduous forests of Canada, germinants of woody species predominated in the seed bank substrates that were characterized by richer and moister soils, deeper litter and closed canopies, and unlike many secondary successional forests, most seedlings arising from the seed bank were late-successional, shade tolerant species (Leckie et al., 2000). Overall, most seed bank analyses have been done in secondary successional forests, with the management-oriented goal of predicting regeneration patterns post-disturbance (as reviewed by Thompson et al., 1997). Seed bank information may be crucial in the context of suppression of tree regeneration by

understory shrubs, since forest managers have considered removal of shrub thickets (Romancier, 1971; Baker and Van Lear, 1998).

Ecology of the Rhododendron understory in Appalachian forests

In southern Appalachian forests, *Rhododendron maximum* traditionally occupied a primarily riparian habitat, but in the last 25 years has increased its coverage approximately 30% by spreading into midslope areas and ridge tops (Dobbs, 1995). Many authors have suggested that the loss of the American chestnut (*Castanea dentata* L.) prompted spread of *R. maximum* and the formation of dense thickets in upland forest understories (Whittaker, 1956; Monk et al., 1985, Beckage et al., 2000); McGee and Smith (1967) concluded that virtually no canopy tree recruitment has occurred since that time. The spread of *R. maximum* has also been attributed to other concomitant disturbances, including the abandonment of agricultural land, high-grading and fire suppression (McGee and Smith, 1967; Clinton et al., 1994; Baker and Van Lear, 1998). Mallik (1995) has documented numerous examples of disturbance regimes that have contributed to the conversion of temperate forests to heaths. Baker and Van Lear (1998) found that species diversity in regeneration pools strongly decreased with increasing *R. maximum* density, and that juveniles of the shrub were very common in the regeneration layer, indicating its ability to replace itself and spread into adjacent open areas. Further, they projected that, in the absence of a severe disturbance, the future diversity of riparian forests – and upland sites as well – will be reduced as coverage by *R. maximum* continues

to increase. Certainly, *R. maximum*'s proclivity to reproduce clonally facilitates its spread in this manner (Dobbs, 1995).

The central quality of *R. maximum* that has prompted attention to its ecology is the suppression of vegetation beneath dense thickets. Inhibition of forest floor vegetation has been repeatedly observed for nearly the last 50 years (Whittaker, 1956; Romancier, 1971; Boettcher and Kalisz, 1990; Clinton et al., 1994; Clinton and Vose, 1996; Walker et al., 1999; Beckage et al., 2000; Nilsen et al., 2001). Thickets of *Rhododendron* strongly limit light availability on the forest floor (Clinton et al., 1994; Semones, 1999; Nilsen et al., 2001) and produce recalcitrant, sclerophyllous litter that decomposes slowly, often resulting in thick humus and litter layers (Boettcher and Kalisz, 1990; Baker and Van Lear, 1998, Nilsen et al., 2001). Nutrient paucity and acidic soils have been spatially correlated with shrub presence (Boettcher and Kalisz, 1990). Soil moisture and soil micronutrient cation concentrations were significantly lower beneath shrub thickets, compared to nearby open understory sites (Nilsen et al., 2001). It has been proposed that *R. maximum* produces allelopathic chemicals that inhibit growth of other species (Rice, 1979), but no evidence has been found indicating that litter leachates negatively affect seed germination and initial growth of tree seedlings (Nilsen et al., 1999). Leaf leachates from *R. maximum* litter depressed growth of ectomycorrhizal fungal hyphae, but to a lesser extent than hardwood litter (Nilsen et al., 1999). Clearly, the habitat beneath *R. maximum* thickets presents multiple stressors to which most vegetation will eventually succumb, including the strongly competitive, shade tolerant canopy tree seedlings on which future forest structure and composition is dependent.

Resource requirements for shade tolerant seedlings

Seedlings of canopy trees in climax-successional forests exhibit physiological and morphological traits that are essential for low-light survival and growth beneath a closed canopy (Canham, 1988; Pacala et al., 1996; Walters and Reich, 1996, 2000). For example, many such species have a large seed, which provides greater carbon reserves, allowing establishment of a primary root through a deep litter horizon (Molofsky and Augspurger, 1992) and increased growth, competitive ability and resilience from damage such as herbivory (Walters and Reich, 2000). However, the advantage of a large seed is only temporary, and young seedlings of shade-tolerant species have further physiological traits that promote survival. Slow growth and low tissue turnover rates are typical for these species, and these are adaptations that help maintain plant carbon balance under low resource conditions. Since most climax-successional forest habitats are relatively resource-poor (compared to early seral stages), seedling survivorship is simultaneously dependent on continued growth, storage (Kobe, 1997) and the prevention of, or resilience from, damage caused by herbivores or other sources (Coley et al., 1985; Kitajima, 1996).

Once established, seedlings must be able to persist for many seasons (under commonly low resource conditions), as longevity increases the possibility of exposure to a canopy gap (Clinton et al., 1994). Under a closed canopy, sunflecks are the primary source of direct light for seedlings (Clinton and Vose, 1996; Semones, 1999; Nilsen et al., 2001), although the high spatial and temporal variability in the location, intensity and duration of sun flecks requires that seedlings have larger leaf areas to intercept them. Walters et al. (1993) demonstrated that leaf allocation was more strongly correlated with

growth (in moderate to low light) than photosynthetic rates for several shade tolerant species. However, for a given increase in leaf area ratio, the relative increase in growth rate (and during the first two years, survivorship) afforded by larger leaf areas was much less in deep shade than moderate or high light. Growth rates in low light have been linked with survival in many studies (Walters and Reich, 1996, 2000; Kobe et al., 1995; Kobe, 1996); yet, there are costs to maximizing leaf allocation, such as: higher probability of damage to large leaves (Coley, 1988), reduced allocation to storage (Chapin et al., 1990; Kobe, 1997), and higher whole-plant respiration rates (Walters and Reich, 2000). Lastly, it is well known that responses to belowground resources are primarily dependent on light availability; many studies have suggested that seedlings minimally respond to belowground resource availability when light is less than 2% full sun (see Coomes and Grubb, 2000).

Belowground resource deficiencies in moisture and nutrients that co-limit growth in deep shade may result in reduced persistence on the forest floor (Peace and Grubb, 1982; Walters and Reich, 1997) through a number of potential mechanisms. Water stress may reduce sun fleck use efficiency in shade tolerant species by limiting stomatal conductance, and consequently limiting carbon gain during these crucial high light periods (Knapp and Smith, 1990). Xeric soils limit expansion of the root system in many *Quercus* species (Callaway, 1990), and may impede normal colonization of fine root tips with beneficial mycorrhizal fungi. Walters and Reich (1997) found that low nitrogen availability was linked to reduced photosynthetic acclimation to brief high light periods, but was not a determining factor for survivorship of shade-tolerant species grown in low light. In a later study (Walters and Reich, 2000), nitrogen amendments

improved growth and leaf allocation for large seeded, shade-tolerant species, but survivorship was most improved for small seeded, shade-intolerant species. Thus, in the short term, low nitrogen conditions may not be deleterious to shade adapted seedlings growing in low light, but the effects of nitrogen paucity may accumulate over time (Coomes and Grubb, 2000). Overall, it is likely that seedlings which encounter a suite of stressors – arising from resource paucity in combination with either increased tissue loss (i.e. herbivory) or harsh environmental conditions (i.e. drought, allelopathic or acidic soils) – have higher rates of mortality than seedlings which are primarily light-limited (Kitajima, 1996; Walters and Reich, 2000).

Impact of herbivory on tree recruitment

Herbivores exert a major impact on plant communities, and on the average, greater than ten percent of plant production is consumed annually, which is more than the typical allocation to reproductive effort (see Coley et al., 1985). By increasing mortality and / or removing biomass allocated to growth and reproduction, herbivory becomes a major selective force shaping community composition through successional stages (Hanley, 1998; Gill et al., 1996; Johnston and Naiman, 1990). Loss of tissues to herbivores generally has a much greater selective impact on late-successional species growing in resource-poor habitats (Coley et al., 1985; Hanley, 1998). Commonly, these species are less preferred by herbivores than fast-growing species growing in the same microsites, primarily due to higher concentrations of secondary metabolites such as tannins and polyphenolics in slow-growers (Coley, 1988; Molofsky and Fisher, 1993). In

the absence of herbivory, slow-growing species characteristically have low tissue turnover rates (long-lived stems and leaves), an adaptation that minimizes carbon loss in low resource conditions.

As reviewed by Coley et al. (1985), there is ample evidence that anti-herbivore defense strategies in plants are causally linked with habitat resource availability. Under a deeply shaded forest canopy, leaf tannin content may be positively correlated with light availability, for saplings of late successional species such as dogwood (Dudt and Shure, 1994). Further, it has been postulated and confirmed by simulation models that obligate defensive strategies (exhibited by slow-growing, climax successional species) are more resource efficient than facultative strategies, in which the plant responds to herbivory by either growing new tissues or increasing the concentration of defensive compounds; these compounds are energetically expensive to produce and maintain in high concentrations in plant tissues. Thus, a tenuous balance must be achieved between growth and defense, since growth is linked with survivorship (Walters, 1993) and the replacement of tissues (lost to herbivory) may be prohibitive under low resource conditions (Coley et al., 1985).

Overall, studies of the impact of herbivory at larger scales (i.e. stand, landscape) have found that the consequences of preferential browsing or insect defoliation are highly variable and dependent on numerous autogenic qualities of the forest community. Insect defoliators may only have a strong effect on community composition if the affected tree species is a major component of the forest and its fitness is severely depressed (Crossley et al., 1988; Dyer and Shugart, 1992). Preferential browsing by ungulates may have little impact in montane birch (*Betula spp.*) forests in Central Europe (Kienast et al., 1999), yet was severely damaging to xeric Scots Pine (*Pinus sylvestris* L.) forests in the

Netherlands (Jorritsma et al., 1999). Prolonged intensive browsing in many forest communities will shift normal successional trajectories, leading to assemblages of unpalatable species that may drastically alter habitat and microclimate (Bryant and Chapin, 1986; Johnston and Naiman, 1990), with possible future consequences on tree regeneration processes and primary productivity.

Research objectives and hypotheses

The central goal of this research was to investigate the influence of the evergreen understory shrubs *Rhododendron maximum* L. and *Kalmia latifolia* L. on tree recruitment processes at multiple stages: seed rain, seed bank presence, and seedling performance and mortality. While the suppression of seedling establishment beneath *R. maximum* has long been observed in the Southern Appalachians, few studies have explicitly described possible mechanisms (Walker et al., 1999; Nilsen et al., 2001). Most studies that have considered the evergreen understory in Appalachian forests have assumed that light attenuation is the primary mechanism by which seedlings are suppressed (Swank and Crossley, 1988; Clinton et al., 1994; Beckage et al., 2001; also see Semones, 1999); yet there is both observational and experimental evidence that shrub thickets influence belowground processes and availability of moisture and nutrients (Boettcher and Kalisz, 1990; Clinton and Vose, 1996; Walker et al., 1999; Nilsen et al., 2001). Further, there has been little attention to herbivory or other sources of seedling damage beneath dense understory shrubs, and how these processes affect seedling performance and survivorship (but see Thompson et al., 1993).

As I have stated previously, the consensus of most research on seedling suppression beneath *R. maximum* implies that asymmetric competition with the well-established shrubs inhibits regeneration. Clearly, this hypothesis has merit, but is ultimately dependent on the assumption that pre-establishment recruitment processes (i.e. dispersal, entrance into the seed bank, germination) are not affected by the presence of understory shrubs. If this assumption is false, then one cannot attribute suppression of seedlings to competition alone, but must consider possible mechanisms prior to establishment. On the other hand, if seeds are able to normally arrive and germinate in suitable microsites regardless of shrub presence or density, then the resource competition hypothesis has greater significance. Therefore, my first objective was to test the null model of shrub effect on seed dispersal and presence in the forest seed bank, with the following hypotheses (addressed in Chapter II):

1.1: There is no significant difference in seed rain characteristics (density and composition of seeds) between sites beneath thickets of *Rhododendron maximum* or *Kalmia latifolia* and open understories.

1.2: There is no significant difference in seed bank characteristics (density and composition of germinants) between sites beneath thickets of *Rhododendron maximum* or *Kalmia latifolia* and open understories.

If these hypotheses can be supported with regard to *R. maximum*, then it can be safely assumed that the shrub has little influence on pre-establishment recruitment

processes; Nilsen et al. (1999) found that *R. maximum* litter leachates had no adverse impact on germination of both assay species (i.e. *Lactuca sativa*) and ecologically relevant species (i.e. *Quercus spp.*). The potential allelopathic qualities of *Kalmia* litter and precipitation throughfall are currently being investigated in the Ecophysiology Lab at Virginia Tech. The results of this work may be central to understanding the role of *K. latifolia* in Appalachian forests, since the inhibitory nature of *Kalmia angustifolia* on conifer regeneration (in boreal forests of Canada) is largely due to the allelopathic qualities of its tissues (Mallik, 1995).

If the above null models are supported (and if *K. latifolia* litter is not shown to be allelopathic to seed germination), then it can be argued that seedling suppression occurs post-establishment, through resource competition and/or other mechanisms. I tested the general hypothesis of resource competition as the driving force of seedling suppression beneath *R. maximum*, and used the same approach to investigate how *K. latifolia* may influence seedling performance and survivorship. While this research is a continuation of previous studies (Semones, 1999; Walker et al., 1999; Nilsen et al., 2001), there are two important distinctions of this work from prior efforts: 1) the influence of shrubs in this work was tested using natural gradients of shrub density, instead of using a factorial approach comparing dense thickets to open understories; and 2) seedlings in this study were monitored intensively throughout the growing season, allowing detailed observations of phenology and quantification of damage rates due to herbivory, pathogens and other sources.

Using field data on resource availability, substrate (litter, humus, soil), and seedling performance parameters, I analyzed (with linear regression) three general types

of relationships: 1) shrub influence on resource availability; 2) seedling performance response to resource availability; and 3) shrub influence on seedling performance. Additionally, I utilized phenology and herbivory data to determine if any relationship existed between shrub density and the timing of foliar emergence and the rates / extent of damage to seedlings. All of this analysis was compiled to provide mechanistic evidence for what I have termed “competitive linkages” between shrubs and seedlings. For example, if a negative relationship was observed between *R. maximum* density and seedling leaf area, and light availability was an explanatory factor – shrubs attenuated light and seedling leaf area responded positively to light – then the shrub-seedling relationship would be partially explained by a light “linkage.” In other words, if shrub vs. light and light vs. seedling leaf area regressions were significant and had opposite slopes (i.e. one was negative, one was positive), then light availability was likely a mechanism for reduced seedling leaf area beneath the shrub understory.

Due to the large number of potential competitive linkages between shrubs and seedlings, I tested only three specific hypotheses, which were either based on findings from prior research (Nilsen et al., 2001) or null models addressed first by this study:

2.1: Light availability will be limited by the shrub understory, to a greater extent by *R. maximum* than *K. latifolia*, and will be positively related to seedling growth, biomass and survivorship.

2.2: Belowground resource availability (moisture, nutrients) will be negatively correlated with the density of understory shrubs *R. maximum*, and positively related to seedling growth, biomass and survivorship.

2.3: Shrub density and/or presence will have no influence on variation of seedling phenology or rates / extent of damage caused by herbivores and other agents.

In essence, I expected light to be a primary linkage explaining seedling inhibition, and certain belowground resources to be possible linkages as well. Hypothesis 2.2 was generally stated because I expected that at least one competitive linkage would involve either soil moisture and nutrients, or traits of the litter and humus horizons. The negative effect of *R. maximum* presence on the colonization of seedling root tips with ectomycorrhizal fungi found by Walker et al. (1999), and the belowground resource deficiencies associated with the shrub (Boettcher and Kalisz, 1990; Nilsen et al., 2001) supported this hypothesis. In addition, I tested null models of *K. latifolia* influence on resource availability and seedling performance because there has been little attention to this topic in the literature. For the same reason, the null model of shrub influence on seedling phenology and herbivory was tested. Since this study was largely explorative – in the sense that I sought evidence for competitive mechanisms – these hypotheses mainly acted as guidelines to help prioritize analysis of an expansive data set.

Additional objectives for this research include: 1) contributing to our knowledge on the phenomenon of seedling suppression beneath dense understory shrubs, particularly

R. maximum; 2) providing insight into possible mechanisms of inhibition that may be neutralized by management efforts; 3) developing a profile of the forest seed bank in different understory types, which should be useful management information (for example, to predict initial regeneration pools after *Rhododendron* or *Kalmia* removal); and 4) to determine if planting of greenhouse-grown seedlings leads to long-term survival of tree seedlings, beneath the rapidly expanding shrub understories in the Southern Appalachians.

CHAPTER II

The effect of a dense subcanopy shrub layer on seed rain and seed bank composition in a Southern Appalachian forest.

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ABSTRACT

The suppression of tree regeneration beneath *Rhododendron maximum* L. (Ericaceae) in southern Appalachian forests has long been observed, but few studies have uncovered specific mechanisms. Most work has focused on post-establishment resource competition as the primary mechanism, but this approach is reliant on the assumption that shrub thickets do not adversely affect dispersal and seed bank dynamics. We tested the effect of shrub presence (*R. maximum* and *Kalmia latifolia* L.) on seed rain and seed bank density and composition over a fourteen-month period at two sites in the Coweeta Basin, NC. Seed traps were located in pairs beneath either *R. maximum* or *K. latifolia* thickets and adjacent open understories, and seed bank samples were collected directly around traps. We found that total seed rain density was unaffected by *R. maximum* presence, and neither shrub cover negatively affected seed rain of dominant canopy tree species. Seed rain species richness was higher beneath *K. latifolia*, and seed bank germinant density was higher in samples collected beneath *R. maximum*, compared to open understories. The forest seed bank was overwhelmingly dominated by sweet birch (*Betula lenta* L.) and was compositionally disparate from the overstory at both sites. In conclusion, we rejected the null hypotheses of shrub influence on seed rain and seed bank dynamics, but found no strong evidence indicating that the shrubs have a negative effect on seed rain of major overstory trees, or their presence in the seed bank.

INTRODUCTION

In the Southern Appalachian forests of North America, dense understories of the thicket-forming evergreen shrub *Rhododendron maximum* L. (rosebay rhododendron, Ericaceae) have become increasingly common in the last 30 years (Dobbs, 1995). The suppression of tree recruitment beneath *R. maximum* thickets has long been observed (Whitaker, 1956; Monk et al., 1985; Swank and Crossley, 1988; Clinton et al., 1994), and many aspects of the interaction have been studied: shrub influence on gap dynamics (Clinton et al., 1994; Beckage et al., 2000), resource competition (Clinton and Vose, 1996; Semones, 1999; Nilsen et al., 2001), allelopathy (Nilsen et al., 1999), herbivory (Beier et al., *unpublished data*) and seedling ectomycorrhizae (Walker et al., 1999). Overall, these studies have indicated that inhibition occurs post-establishment, and that seedling decline is correlated with asymmetric competition with thickets of *R. maximum*, primarily for light and soil nutrients. Dense understory vegetation can negate the facilitative influence of canopy gaps on resource availability (Veblen, 1982; Clinton et al., 1994; Beckage et al., 2000), and demographic studies of recruitment have linked dense shrub understories with generally small, declining populations of tree seedlings as well as the complete absence of herbaceous species (Boettcher and Kalisz, 1990) compared to areas without *R. maximum*. Inhibition of seedling mycorrhizae and increased herbivory may also be important factors beneath *R. maximum* (Walker et al., 1999; Beier et al., *unpublished data*).

An underlying assumption of the resource competition explanation, however, is that seeds are ‘ubiquitous’ and dispersal uniform (Clark, 1993) across physical (i.e.

elevation, moisture) and biological (i.e. stand composition, fecundity, understory type) gradients within the forest community. Yet, it has been shown that seedling recruitment is limited by source tree density, distance from source trees and dispersal patterns. Furthermore, the relative importance of limiting factors at each stage of recruitment (production, dispersal, and establishment) is highly variable among species and across sites and / or stand types (Clark et al., 1998). In southern Appalachian forests, tree recruitment in canopy gaps is limited both by seed dispersal patterns and *R. maximum* understories (Beckage et al., 2000). Several possible mechanisms explaining this interaction have not been investigated, particularly the direct effect of dense *R. maximum* thickets on patterns of seed rain; which has been estimated by censuses of established seedlings (Clinton et al., 1994; Beckage et al., 2000), but not directly tested. Moreover, comparisons of seed bank compositions among microsites with and without understory shrub is yet to be determined.

The goal of this study was to test null models of shrub influence on both the dispersal of tree propagules and the composition of forest seed banks. If seed dispersal is uniform with regard to understory type (i.e. the presence of subcanopy shrubs), then seeds are able to arrive and germinate in suitable microsites beneath dense shrubs as they would beneath an open understory. In order for the post-germination resource competition hypothesis (of recruitment inhibition) to be applicable, we must first show that propagules are not impeded from reaching microsites and, for some species, entering the seed bank. We hypothesized that the shrubs *Rhododendron maximum* L. and *Kalmia latifolia* L., which both form dense evergreen subcanopies in these forests, have no significant impact on these processes. In addition, we assumed that seed germination

was not inhibited through allelopathy by *R. maximum* litter leachates (as shown by Nilsen et al., 1999). The potential allelopathic influence of *K. latifolia* leaf litter is currently being investigated through lab bioassays and field experimentation. In this paper, we present the results of a factorial experiment – testing the impact of dense shrub presence on seed rain and seed bank composition, propagule / germinant density and species richness – during a fourteen-month period in which all tree species were dispersing seed at least once, and twice for large-seeded, late-season dispersers such as oak (*Quercus* spp.) and hickory (*Carya* spp.).

METHODS

A factorial experiment was installed in the summer of 2000 at two sites in the Coweeta Hydrologic Lab, USDA Forest Service Southern Research Station. The Coweeta Basin is located in the Blue Ridge Physiographic region of the Southern Appalachians; Swank and Crossley (1988) provide a detailed description of the vegetation, climate, hydrology and ecology of Coweeta, which is largely representative of Southern Appalachian forest ecosystems. Our sites differed in elevation, aspect and stand composition: Ball Flats (BF) was a low-elevation (580m), mesic northeast-facing slope and the dominant canopy tree were oak species *Quercus rubrum* L. (red oak), *Q. alba* L. (white oak), *Q. coccinea* L. (scarlet oak), as well as hickory (*Carya glabra* L.), maple (*Acer rubrum* L.), sourwood (*Oxydendrum arboreum* L.), tulip poplar (*Liriodendron tulipifera* L.) and black tupelo (*Nyssa sylvatica* L.). Dryman Fork (DK) was a high elevation (1560m) ridge, located on a southwest-facing slope. Canopy tree

composition was similar to Ball Flats, except that *Quercus prinus* L. (chestnut oak) and *Quercus velutina* L. (black oak) replaced *Q. rubrum* and *Q. alba* as the dominant oak species. Also, at Dryman Fork, *Hamamelis virginiana* L. (witch-hazel) and *Amelanchier arborea* L. (serviceberry) were important mid-story and understory tree species. Both sites were defined on continuous slopes within a defined area of approximately 1.5-2.0 hectares that contained natural populations of *Rhododendron maximum* L. and *Kalmia latifolia* L. Most commonly, these two species occur in monoculture stands (thickets) in the understory, but can infrequently be found growing in combination (Dobbs, 1995).

The experimental design of paired traps is detailed in Figure 2.1. We located paired traps beneath open and shrub-influenced understories as closely as possible to maintain a high degree of similarity in directly influential source trees within the pair. All woody vegetation around traps was sampled (species, dbh) within a 4m radius for shrub species (*R. maximum* and *K. latifolia*) and an 8m radius for understory and canopy trees (primary stem > 10cm dbh). Trees with canopies that intersected this sampling circle around each trap were also included. We installed five pairs (ten traps) at each site to determine the effect of *R. maximum* (treatments RM+ and RM control), and the same for *K. latifolia* (treatments KL+ and KL control). Therefore, out of a total of twenty seed traps at each site, five were situated beneath RM, five beneath KL, and ten beneath an open understory. Paired *t*-tests were used to evaluate treatment effects among paired traps for total rain density, tree:non-tree seed ratio and tree species richness. The same approach was used to evaluate any treatment effects on the densities of individual tree species in the overstory. Rank-abundance relationships were also used

to determine if shrub cover treatments had any influence on seed rain and seed bank structure.

Seed traps consisted of a plastic basket 40cm in diameter (0.13m^2) lined with fine mesh fabric and covered with large mesh chicken wire to provide protection from squirrels and other seed predators. Most propagules entered traps normally, however, during field tests, we found that full-size acorns and other large seeds were possibly being deflected by the chicken wire covering. This covering was removed in August 2001 in an attempt to eliminate this error; therefore, seed predation in the traps may have potentially become a subsequent problem. Traps were installed approximately 1m above ground level, within steel tomato cages anchored at least 20cm into the forest floor. Traps were emptied monthly, and all material from traps was identified as seed, leafy-woody debris, insect frass, or leaf buds. Seeds were identified to genus, and species (if possible) using a USDA Forest Service manual. We also germinated a random sample of each seed type and categorized any unknown taxa (those which could not be identified with certainty) as either “trees” or “non-trees” for the analysis. Since we are primarily concerned with tree recruitment processes, “non-tree” seeds (including shrubs and herbaceous species) were not included in the analysis of total density or species richness.

Seed bank samples were collected in March, July and December of 2001 from four random points within a 1m radius of seed traps; from each point, we obtained mostly litter and humus material, with some upper mineral soil, using a 10cm diameter section of PVC pipe as a guideline. Any seeds residing on top of the leaf litter at the sampling point were included. All four subsamples were lumped into the single sample corresponding with the trap. We avoided re-sampling the same random points at

subsequent samplings, because prior cores were still apparent on the forest floor. Samples were stratified in a 5° C refrigerator for four weeks, and placed into greenhouse flats on a 5cm layer of potting medium, watered daily, and allowed to develop (inducing germination and growth) under high light for a period of twelve weeks. Large weeds were periodically removed as necessary from developing samples, to prevent shading and / or any possible allelopathic influence on germination of target tree species. For the analysis, we used density (total seedling density, individual species densities) and diversity indices (richness, or total number of tree species) as response parameters to treatments. As in the seed rain experiment, the same RM+, KL+ and control (OPEN) treatments corresponded to the seed bank samples, and were tested using the same *t*-testing design.

RESULTS

Seed rain

The presence of an evergreen understory had significant effects on seed rain species richness, but was not influential on total seed density or tree:non-tree seed ratios (Table 2.1). At Dryman Fork, density of tree seeds was highest beneath *K. latifolia* and lowest beneath *R. maximum*, while the ratio of tree seeds to ‘weed’ seeds (non-tree) was not significantly affected by shrub cover treatments. At Ball Flats, tree seed density was highest beneath *R. maximum* and lowest for open understories, although these differences were not significant among the paired traps. Higher species richness was observed in

traps beneath *R. maximum* thickets compared to open understories ($p < 0.05$) at Dryman Fork. Richness was also greater beneath *K. latifolia* than open understories ($p < 0.05$), an effect observed at both sites. Further, species richness was higher at the low elevation Ball Flats site than at Dryman Fork ($p < 0.05$). Seed rain density and tree:non-tree seed ratios were similar at each site.

We did not observe any effects of shrub cover treatments on individual tree species, primarily because our traps did not catch many tree seeds, with the exception of *Oxydendrum arboreum* capsules (that contain numerous tiny seeds) and *Acer rubrum* samaras. The vast majority of *Quercus* acorns in our traps were aborted, making identification to species difficult. Yet, it appears that the understory shrub layer did not impede dispersal of these large-seeded species because density was not significantly different among treatments at either site ($p > 0.05$). Furthermore, the presence of *R. maximum* and *K. latifolia* did not appear to influence the structure of seed rain at the stand scale, as indicated by similar rank abundance curves of tree seeds (Figure 2.2) at both sites. Although the difference appears large for the most common seed species (*O. arboreum*) in the rank abundance plots, there was no significant shrub cover effect on density of this species (paired t-test, $p > 0.05$), but this may have been due to high variance in our samples. It is logical that small, windborne capsules like *Oxydendrum* may be physically impeded by a subcanopy layer of foliage to a greater extent than large-seeded species (which have higher dispersal velocities).

Seed bank

Of the woody species observed, sweet birch (*Betula lenta* L.) formed the vast majority (87.4%) of emergent seedlings in all three samplings of the seed bank. Sourwood (*Oxydendrum arboreum* L.), tulip poplar (*Liriodendron tulipifera* L.), and red maple (*Acer rubrum* L.) were also observed. A summary of woody species and densities found in seed bank trials is provided in Table 2.2. We observed relatively low species richness in most samples; even when herbaceous species were included, seed banks rarely contained greater than 5 species. Sampling time had a significant effect on seedling density, with July and December samples having much greater mean density than March samples (Tukey's HSD, $p < .05$). This effect was consistent across all treatments and both sites. Mean density of woody germinants in the seed bank was not significantly affected by shrub cover treatments (paired t -tests, all $p > 0.05$). However, at the Dryman Fork site, seedling density was slightly higher for the paired OPEN treatment when compared to the corresponding shrub cover treatments. The opposite was observed at the Ball Flats site, with both shrub cover treatments (RM+, KL+) having slightly greater density than the paired OPEN treatments. Variation in mean species richness (S) of the seed bank was unrelated to sampling date, but for both sites, richness was significantly higher ($p < 0.05$) beneath *K. latifolia* understory. We observed little difference in the structure of the seed bank due to shrub cover treatments, according to rank abundance curves (Figure 2.3B). Finally, germinant density was significantly higher for Dryman Fork (high elevation) seed banks than Ball Flats (low elevation), but no site effect on species richness was observed.

The dominance of birch seedlings in our seed bank samples was not reflected in the overstory composition of either site (Table 2.2). Relative densities of birch in the seed bank were similar between sites, although the importance value of birch in the overstory was clearly disparate between sites. This may indicate that source trees providing seed bank recruits were located some distance from the low elevation Ball Flats site, where there was only one mature *Betula* individual. Many other seedlings were species that also were not dominant in the overstory of either site (i.e. *Oxydendrum arboreum*, *Liriodendron tulipifera*). As shown by Table 2.2, *Acer rubrum* was the only canopy dominant that consistently emerged from the seed bank, although in very low density. The majority of species emergent in our seed bank samples fit this early-successional profile. On the other hand, most species of *Quercus* and *Carya* do not have a seed bank, because these seeds germinate soon after contact with the ground, after the winter season, or are consumed / relocated by mammals. Thus we did not expect to find these species in the seed bank in significant numbers. *Acer rubrum* seeds are usually ubiquitous and germinate readily upon arrival on the forest floor, making the collection of intact seeds improbable.

DISCUSSION

Our results provide evidence that understory shrubs may have an impact on seed rain and seed bank dynamics in southern Appalachian forests. While Clinton et al. (1994) and Beckage et al. (2000) found that natural establishment of tree seedlings in canopy gaps was severely depressed beneath *R. maximum* thickets, the mechanisms

behind suppression were not isolated experimentally. The causes and consequences of higher species richness observed beneath shrub cover, particularly *K. latifolia*, are unclear, but indicated that the shrub understory does not inhibit the dispersal of a variety of tree seeds. Overall, this study indicates that total seed input may be different (either greater or lower) beneath *R. maximum*, compared to open understories, but the driving force of these non-significant effects was the variation in density of a single species, *Oxydendrum arboreum* (sourwood). We estimated sourwood seed density through capsule density, and did not track the fate of the numerous tiny, windborne seeds contained in the capsules. Regeneration of sourwood from the seed bank was unaffected by either shrub cover treatment. Yet, very small sample sizes for many of the large-seeded species (i.e. *Quercus*, *Carya*) prevented a robust analysis of how shrub subcanopies may influence the dispersal of these important overstory species. Dispersal of *Acer rubrum* – a species of growing importance in these forests – appeared to be unaffected by presence of *R. maximum* or *K. latifolia*.

The overwhelming dominance of birch seedlings (87.4%) in the seed bank, while not reflected in the overstory composition of either site, was consistent with observations in previous studies that have indicated the prevalence of early-successional seedlings in secondary successional forest seed banks (reviewed by Ashton et al., 1998). Birch seeds are small and wind dispersed, and probably originated from trees some distance from our sites. Sweet birch is an early-successional, disturbance-adapted tree in most areas of this forest; thus, we believe that the dominance of birch in the seed bank is due to its life-history traits (high fecundity, seed persistence, and shade-intolerance) and our experimental methodology, in which the seed bank substrate was highly disturbed and

subjected to release from low resource conditions. Further, our seed bank samples were germinated under high light, an experimental condition that has been shown to favor the emergence of shade intolerant, fast growing species (Hyatt, 1998).

Our findings indicated that seed bank germinant density may be higher in certain sites beneath *R. maximum*, and this observation may be due to a number of factors. Primarily, we believe that this effect may be a result of greater litter and humus volume beneath *Rhododendron* thickets (as shown by Boettcher and Kalisz, 1990; Nilsen et al., 2001; Beier et al., *unpublished data*), which due to our sampling methods may have provided greater substrate mass and a higher potential seed density. Furthermore, the forest floor beneath *R. maximum* is deeply shaded and typically resource-poor (lower moisture and nutrient availability), and therefore may be more conducive to maintaining dormancy of seeds in the seed bank, leading to a possible accumulation of seeds over time (Hill and Stevens, 1981). Consequently, when the seed bank is removed from *in situ* conditions and released from resource paucity, many more dormant seeds are available for germination and establishment. Substrate characteristics such as soil moisture and texture have been shown to influence regeneration from forest seed banks (Hill and Stevens, 1981; Ashton et al., 1998), although most of our knowledge about *R. maximum* substrates (i.e. lower soil water availability, nutrient availability) would indicate a suppressive effect on germination and establishment, instead of a facilitative effect.

Overall, we have found little evidence that indicates that understory shrubs act as ‘filters’ to recruitment at the dispersal and seed bank stages; conversely, we cannot completely support the null model dictating that *R. maximum* and *K. latifolia* have no

impact on these processes. Since this null hypothesis must be supported in order to apply the resource competition explanation of seedling suppression beneath *R. maximum*, we will continue this study in an effort to capture greater seed production, specifically of *Quercus* species. Further, we will investigate how shrub cover influences natural seedling establishment around our seed traps, with the goal of resolving the role of the evergreen understory during the major stages of recruitment prior to establishment.

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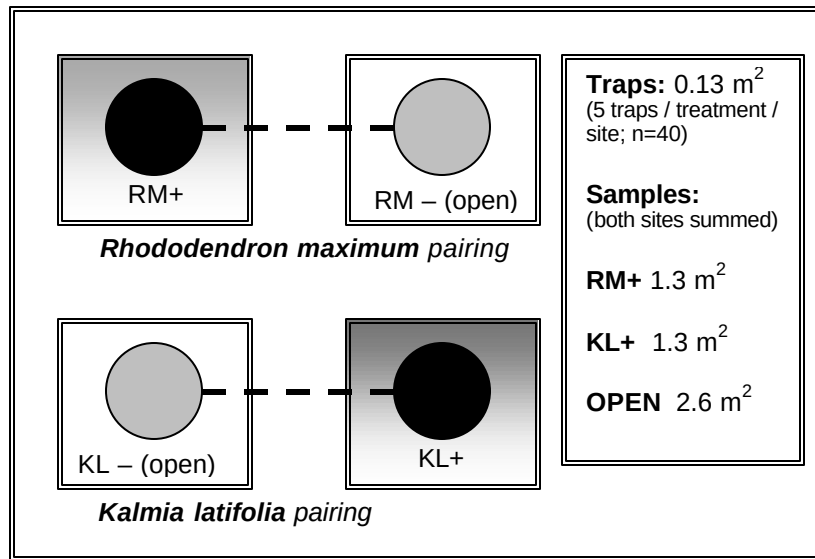


Figure 2.1. Sampling design for paired seed traps beneath *R. maximum* and *K. latifolia* shrub cover. Total trap area is shown for each treatment above; paired traps were placed as closely together as possible to minimize variation in canopy tree influence. Trees were censused within an 8m radius of each trap, shrub species (*R. maximum*, *K. latifolia*) within a 4m radius.

Table 2.1 Effects of shrub cover treatments on seed rain parameters, tested among paired traps at two sites in the Coweeta Basin. A total of five paired traps for each treatment were installed at each site; a pair consisted of one trap beneath the shrub (*R. maximum* or *K. latifolia*) closely located near another trap beneath an open understory. Means, standard errors (x (SE)) and results from paired *t*-tests are given below (* denotes a significant difference between the paired treatments, $p < 0.05$).

Dryman Fork Site

Treatment	Total density	Richness	Tree : non-tree
RM +	21.2 (3.9)	16.3 (0.9)*	1.02 (.03)
RM – (OPEN)	30.4 (12.3)	11.8 (1.6)	1.64 (.79)
KL +	68.8 (42.1)	13.4 (.51)*	1.56 (.89)
KL – (OPEN)	52.2 (33.7)	10.0 (1.3)	3.41 (1.8)

Ball Flats Site

Treatment	Total density	Richness	Tree : non-tree
RM +	62.8 (22.6)	19.8 (1.9)	1.41 (.22)
RM – (OPEN)	37.2 (4.0)	18.6 (1.5)	3.16 (1.3)
KL +	58.0 (24.6)	17.4 (.81)*	1.0 (.38)
KL – (OPEN)	56.4 (19.9)	15.0 (.32)	4.7 (2.3)

Key to abbreviations: *R. maximum* treatment (RM+), open trap paired with *R. maximum* treatment (RM – OPEN), *K. latifolia* treatment (KL+), open trap paired with *K. latifolia* treatment (KL – OPEN).

Table 2.2. Summary of woody species in seed banks sampled at two sites in the Coweeta Basin, NC. Importance values (IV=rel. dominance + rel. frequency) are given for each species based on all vegetation sampled around traps at each site (some species are understory or mid-canopy trees). For each site, the three most important tree species located around sampling areas (based on IV values) are noted.

Dryman Fork		<i>H. virginiana</i> , <i>Quercus prinus</i> , <i>Acer rubrum</i>	
Species	Density	Relative Density (%)	Canopy tree IV
<i>Betula lenta</i>	311	87.1	10.4
<i>Oxydendrum arboreum</i>	30	9.6	6.12
<i>Rubus spp.</i>	5	1.6	--
<i>Liriodendron tulipifera</i>	4	1.3	--
<i>Nyssa sylvatica</i>	3	1.0	2.21
<i>Acer rubrum</i>	1	0.3	24.4
<i>Hammamelis virginiana</i>	1	0.3	38.5
<i>Quercus rubrum</i>	1	0.3	6.98
<i>Sassafras albidum</i>	1	0.3	--
Ball Flats		<i>Quercus alba</i> , <i>Acer rubrum</i> , <i>Oxydendrum arboreum</i>	
Species	Density	Relative Density (%)	Canopy tree IV
<i>Betula lenta</i>	207	84.8	0.43
<i>Oxydendrum arboreum</i>	16	6.6	22.0
<i>Acer rubrum</i>	9	3.7	26.0
<i>Liriodendron tulipifera</i>	7	2.9	1.23
<i>Rubus spp.</i>	2	0.8	--
<i>Hammamelis virginiana</i>	1	0.4	4.49
<i>Nyssa sylvatica</i>	1	0.4	6.06
<i>Ilex opaca</i>	1	0.4	--

Table 2.3. Effects of shrub cover treatments on seed bank parameters, tested among samples collected around paired traps in the Coweeta Basin. Total seedling density (emergent seedlings from seed bank) and species richness (number of woody species in seed bank) for all paired treatments are given below. Means, standard errors (x (SE)) and results from paired *t*-tests are given below (* denotes a significant difference between the paired treatments, $p < 0.05$).

Dryman Fork Site

Treatment	Total seedling density	Woody species richness
RM +	6.53 (1.1)	1.27 (0.18)
RM – (OPEN)	7.07 (1.2)	1.80 (0.40)
KL +	4.33 (1.1)	1.13 (0.22)*
KL – (OPEN)	5.67 (0.97)	1.87 (0.27)

Ball Flats Site

Treatment	Total seedling density	Woody species richness
RM +	6.27 (1.7)	1.07 (0.23)
RM – (OPEN)	2.80 (0.69)	1.33 (0.27)
KL +	4.40 (0.72)	1.53 (0.22)*
KL – (OPEN)	2.73 (0.88)	0.87 (0.13)

Key to abbreviations: *R. maximum* treatment (RM+), open trap paired with *R. maximum* treatment (RM – OPEN), *K. latifolia* treatment (KL+), open trap paired with *K. latifolia* treatment (KL – OPEN).

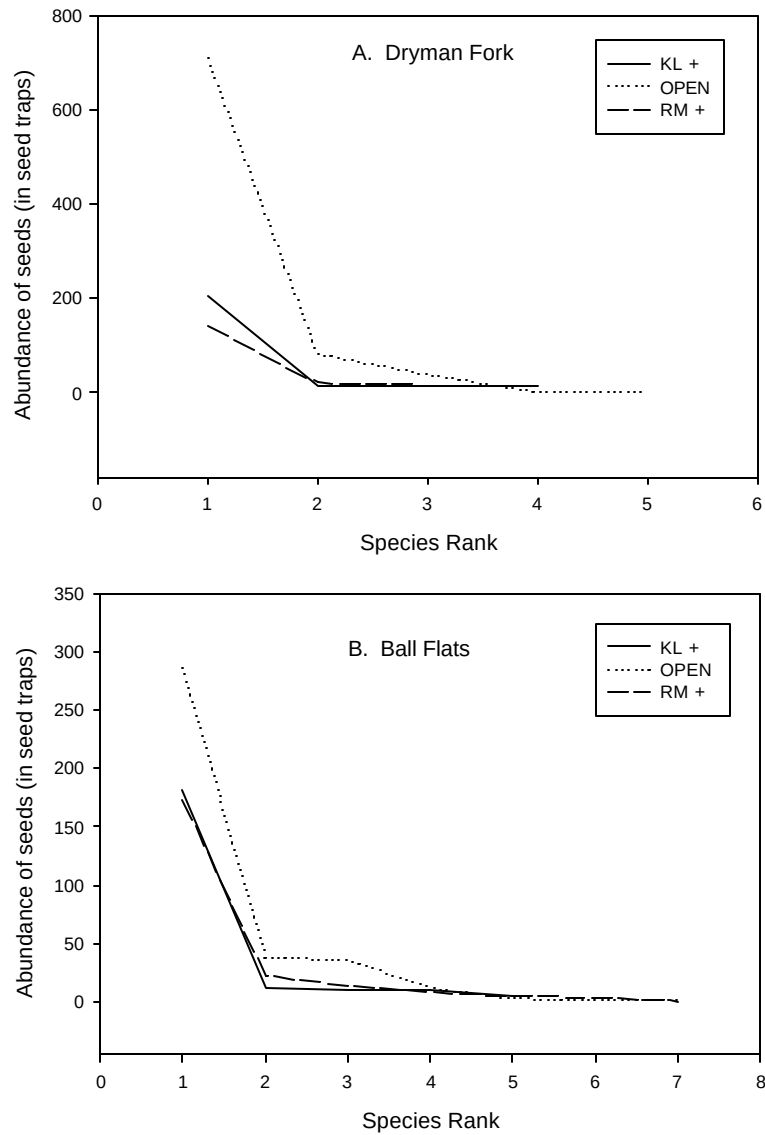


Figure 2.2. Rank abundance plots for tree seeds collected beneath *K. latifolia* (KL), *R. maximum* (RM), and open understories (OPEN) at two sites in the Coweeta Basin, NC. Seeds were collected in traps from 09/2000 to 12/2001; abundance axis is sum of all seeds collected throughout duration of the study. Each species is ranked by its specific density collected in each treatment. Trap sampling area for OPEN traps is twice that of either RM or KL treatments.

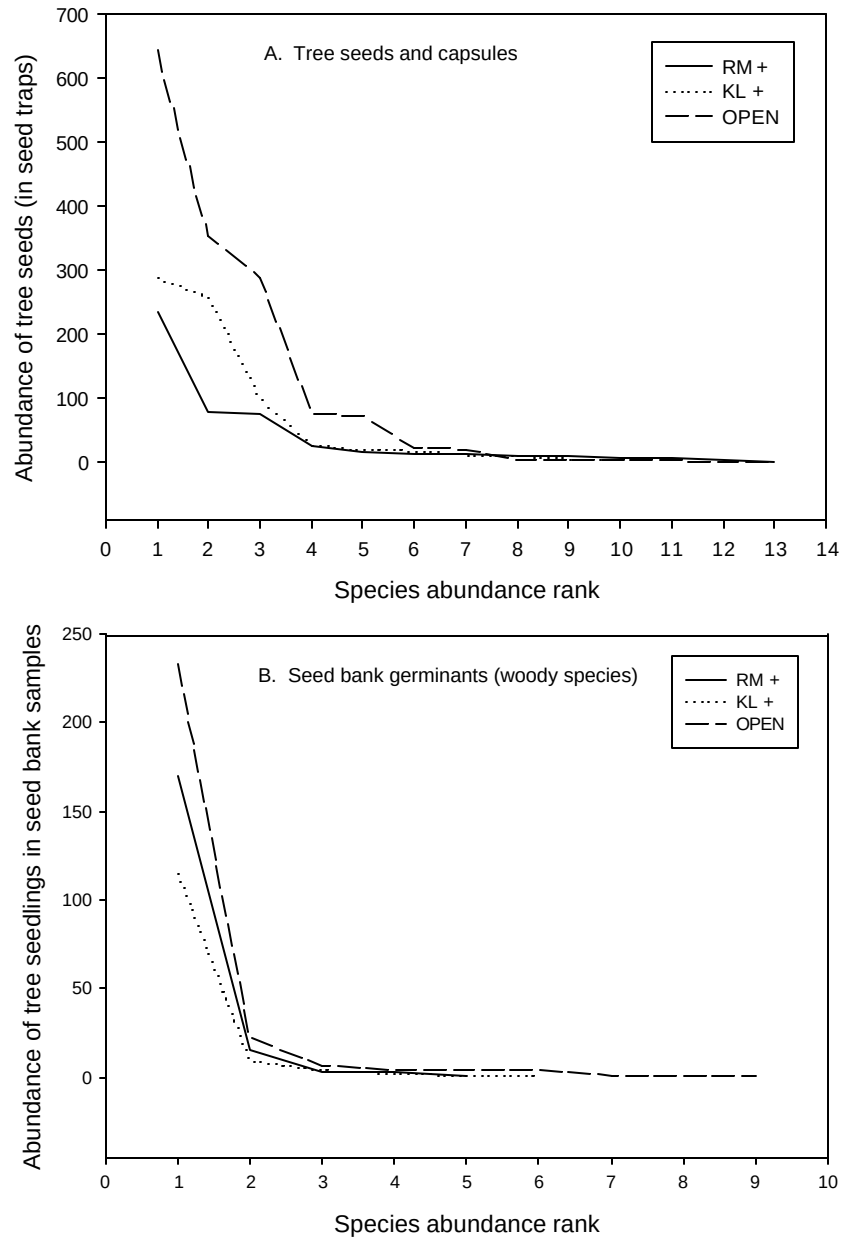


Figure 2.3. Comparison of rank abundance relationships among shrub cover treatments for tree species in seed rain and seed bank samples. Abundances axes represent summed data for the duration of the study. Species are ranked by their specific density in each treatment, OPEN sampling area is twice that of RM (*R. maximum*) and KL (*K. latifolia*) treatments.

CHAPTER III

Resource availability and tree seedling performance along natural gradients of subcanopy shrub density in an eastern deciduous forest.

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ABSTRACT

Inhibition of canopy tree recruitment beneath thickets of *Rhododendron maximum* L. (Ericaceae) has long been observed in Southern Appalachian forests, yet the mechanisms of this process remain unresolved. In this study, we examined relationships between the densities of two thicket-forming subcanopy shrubs (*R. maximum* and *Kalmia latifolia* L.), resource availability, herbivory rates and seedling performance and survivorship. We planted oak seedlings (*Quercus rubrum* L. and *Q. prinus*) in forest plots randomly located along gradients of shrub density at two contrasting sites in the Coweeta Basin, NC, and measured seedling response (growth, biomass) to resource availability. Seedling phenology, health (i.e. damage from herbivory) and survivorship were measured biweekly during the growing season. We found that numerous shrub-mediated factors interacted to influence seedling performance, particularly light attenuation, nitrogen availability and leaf herbivory. Density of *R. maximum* was negatively correlated with seedling biomass, open-canopy light availability, litter %N, and positively correlated with seedling mortality rates, soil metals (Cu, Fe, Al) and early-season herbivory rates. Seedling leaf area and growth was positively correlated with light and litter %N, while negatively correlated with soil [Al], an indicator of acidic, nutrient poor soils at both sites. *Kalmia latifolia* had relatively little influence on resources, seedling performance or herbivory rates. In contrast to previous studies that have emphasized light attenuation as the primary mechanism by which *R. maximum* inhibits recruitment, this study indicates that belowground resource deficiencies and herbivore damage are also important factors.

INTRODUCTION

In many tropical and temperate forests, tree regeneration patterns are influenced by the presence of dense understory vegetation that inhibits seedling growth and survivorship. Light availability in the forest understory is limited and strongly linked to seedling establishment and growth (Denslow et al., 1991; Pacala et al., 1994; Nilsen et al., 2001). In the temperate deciduous forests of North America, tree regeneration generally occurs in conjunction with the formation of canopy gaps (Runkle, 1982; Clinton et al., 1994; Beckage et al., 2000), which provide sufficient light intensity for many species to reach the canopy (Whitmore, 1989; Pacala et al., 1994). Dense understories can negate the facilitative influence of canopy gaps on light, moisture and nutrient availability (Clinton et al., 1994), and therefore suppress recruitment for many gap-dependent species. For example, in Chilean forests (Veblen, 1982) species of dwarf bamboo (*Chusquea* sp.) suppress recruitment of beech (*Nothofagus* sp.) and oak (*Quercus* sp.); Denslow et al. (1991) showed that broad leaf palm understories inhibit canopy tree seedling establishment in rainforests of Costa Rica. Evergreen, thicket-forming shrubs of the family Ericaceae are inhibitory to recruitment in many areas: *Rhododendron ponticum* L. in the U.K, *Kalmia angustifolia* (Small) Fernald. in the boreal forests of Canada, and *Rhododendron maximum* L. in the southern Appalachian mountains (Mallik, 1995; Clinton and Vose, 1996; Walker et al., 1999; Nilsen et al., 2001).

The influence of *Rhododendron maximum* on tree regeneration has long been observed in the southern Appalachians (Whittaker, 1956; Romancier, 1971; Monk et al., 1985; Swank and Crossley, 1988; Clinton et al., 1994; Clinton and Vose, 1996).

Although previous studies have tested possibilities, the mechanisms of inhibition beneath dense evergreen shrubs remain unresolved (Denslow et al., 1991; Walters and Reich, 1996, 2000; Nilsen et al., 2001). In particular, the effect of shrub density on herbivore damage to growth and survivorship of seedlings remain untested. It has been widely demonstrated that thickets of *R. maximum* greatly reduce light availability (approx. 2% full sun) on the forest floor, including the size and duration of sunflecks (Semones, 1999). Consequently, most of the research on this system has centered on light as the primary factor that reduces seedling recruitment in the presence of shrubs (see Semones, 1999, Beckage et al., 2000), and few studies have focused on belowground processes affecting soil moisture and nutrients. Clinton and Vose (1996) found that light attenuation beneath *R. maximum* did not fully explain inhibition of *Acer rubrum* L. seedlings, indicating the importance of belowground resources and/or soil characteristics. Lower soil moisture and soil cation concentrations were found in dense thickets of *R. maximum*, compared to areas without the shrub (Nilsen et al., 2001); and the presence of shrub thickets has been spatially correlated with soil acidification and nutrient paucity (Boettcher and Kalisz, 1990). Moreover, Walker et al. (1999) showed that mycorrhizal colonization of oak and hemlock seedlings was significantly reduced for seedlings growing beneath *R. maximum*, compared to those not directly influenced by the shrub.

Our goal in this study was to define the neighborhood interactions of *R. maximum* (RM) and *K. latifolia* (KL) with canopy tree seedlings by: 1) describing forest floor resource availability (in seedling growth space) as a function of shrub density, 2) identifying those resources most strongly associated with seedling health, growth and survival, 3) measuring growing-season phenology and damage rates to seedlings

(primarily herbivory), and 4) forming empirical ‘linkages’ among shrub, resource, herbivore damage and seedling performance parameters to provide evidence for mechanisms of inhibition. In this study, we define a ‘competitive linkage’ as an explanatory mechanism for negative relationships between shrub density and seedling performance. For example, if light availability is negatively related to shrub density, and seedling growth is positively related to light, then a ‘light linkage’ may explain an observed negative relationship between shrub density and seedling growth.

In this study, canopy tree seedlings were planted in plots along natural gradients of RM and KL density, unlike previous research on tree suppression by *R. maximum* that has focused on the effect of a dense thicket versus an open understory (such as Nilsen et al., 2001; Walker et al., 1999). Since *R. maximum* exists in both dense stands and spatially clumped populations, thicket-edge effects and the influence of smaller shrub densities should be evaluated. We used linear regression to analyze relationships between the gradient in shrub density, resource availability and seedling performance, and identified those resources that mechanistically explained inhibition (shrub-seedling regressions), while incorporating the natural heterogeneity of the forest floor environment. We also considered damage to seedlings caused by herbivores or pathogens as factors that reduced functional leaf area, which is critical for photosynthetic performance, growth and survival in low light environments (Walters and Reich, 1996). There is evidence that subcanopy ericaceous shrubs can exert an influence on insect herbivore populations (Thomson et al., 1993). Therefore, we also evaluated any associations between shrub density and the rates of extent of damage by herbivores and pathogens.

METHODS

Site Description

This study was conducted at two sites within the Coweeta Hydrologic Laboratory (35°03'N, 83°27'W), located in the Blue Ridge Physiographic Province of the Southern Appalachians. The 1626-ha Coweeta Basin ranges from 675 to 1592m in elevation, and has a mean annual precipitation of 180 cm distributed evenly throughout the year. Mean annual temperature is approximately 13 C and ranges from 24 C in July to -9 C in January. Soils are immature Inceptisols and more developed Ultisols (Swank and Crossley, 1988) in the largely oak-dominated stands used in this study. Since widespread logging in the 1920s, the chestnut (*Castanea dentata* (Marsh.) Borkh) blight was the major non-anthropogenic disturbance, which killed most canopy chestnut trees by 1940. Chestnut-oak forest was replaced by mixed-oak at mid elevations and moderate moisture regimes, between low-elevation mesic cove hardwoods and xeric oak-pine at high elevations (Whitaker, 1956; Beckage et al., 2000). Oak species at Coweeta include *Quercus rubra* L., *Q. prinus* L., *Q. alba* L., *Q. coccinea* Muenchh., and *Q. velutina* Lam.; also common are *Acer rubrum* L., *Carya glabra* L., *Liriodendron tulipifera* L., *Oxydendrum arboreum* (L.) DC, and *Nyssa sylvatica* Marsh.. Conifers include the common *Tsuga canadensis* (L.) Carr., infrequent *Pinus rigida* L. and rare *Pinus strobus* L., where it has been introduced into some of Coweeta's experimental watersheds.

Rhododendron maximum, an ericaceous, evergreen shrub, dominates much of the forest understory and has spread from 15 to 33% coverage in the Coweeta Basin since 1976 (Dobbs, 1995). *Rhododendron* has the capability to reproduce clonally and forms

dense subcanopy thickets 3-7m in height. Historically a riparian species, *R. maximum* has spread into mid-slope areas and now covers entire drainages on many sites, regardless of elevation or aspect. Stem densities in thickets can range from 5000 to 17 000 per hectare (Baker and Van Lear, 1998). *Kalmia latifolia* L. is the other major component of the ericaceous understory, and is commonly found in thickets on xeric sites, or occasionally in combination with *R. maximum*. Clonal mats of *Gaylussachia baccata* (Wang.) K. Koch., cover the forest floor in many areas lacking *R. maximum* and *K. latifolia*.

In March 2000, we installed sixty 2x2m randomly-selected experimental plots at Ball Flats (680-700m elev., northeast aspect) and Dryman Fork (approximately 1530 m elev., southwest aspect), hereafter referred to as “BF” and “DK”, respectively. Plots were placed through a mosaic of forest vegetation including open and shrub-influenced understory, creating a gradient of shrub density among plots ranging from absent to dense thicket. The distribution of shrub density among our randomly located plots (at each site) was heavily skewed towards low density, with the majority of plots having little or no shrub influence. In our effort to maintain a high degree of randomness and within-site continuity, we could not have achieved a normal distribution of shrub densities without grouping plots in open and thicket-dominated areas, or projecting transects from riparian areas outward (Baker and Van Lear, 1998).

The influence of canopy trees and shrubs on forest plots was estimated by basal area (m^2) and a ITII index, which considers both the basal area of individual trees and their distance from plot center (see Pelletier et al., 1999). The most influential canopy tree species (on plots) at BF included: oaks (*Q. alba*, *Q. falcata*, *Q. prinus*, and *Q. rubra*),

hickory (*Carya glabra*), maple (*Acer rubrum*) as well as yellow poplar (*Liriodendron tulipifera*), sourwood (*Oxydendrum arboreum*) and black gum (*Nyssa sylvatica*). At DK, we observed a similar assemblage of oaks, hickory and maple, although *Q. alba* and *Q. falcata* were rare and therefore less influential on plots than *Q. rubrum* and *Q. prinus* (the opposite of what was observed at the BF site). Common understory and mid-canopy tree species at both sites included witch-hazel (*Hammamelis virginiana* L.), American dogwood (*Cornus florida* L.), black locust (*Robinia psuedoacacia* L.), serviceberry (*Amelanchier arborea*), and flame azalea (*Rhododendron calundulaceum*), which does not form dense thickets like *R. maximum*.

Vegetation and resource availability measures

Forest vegetation was quantified in a circular area centered on each plot in a 5m radius for shrubs and an 8m radius for canopy and understory trees. Species, diameter at breast height (dbh), and distance from plot center were recorded for all canopy and understory trees; shrubs were measured identically except the stem basal diameter was recorded instead of dbh. Individual Tree Influence Index (ITII; Pelletier et al., 1999) values were calculated for canopy and understory trees. A Tree Species Influence Index (TSII, Pelletier et al., 1999, was used for the two shrub species of interest (*R. maximum* and *K. latifolia*) in order to quantify the total influence of that species on a particular plot. The shrub TSII was calculated identically to the ITII, except that all shrub stems were summed.

We measured the understory light environment using hemispherical canopy photographs taken from plot center, approximately 0.5m above the ground. Photographs were taken in each plot in March to assess shrub influence before canopy leaf-out, and in July to measure the light environment beneath a closed canopy. 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 allows 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 indirect (ISF) and direct site factor (DSF), defined as the proportion of diffuse and direct radiation respectively, received below the canopy to the amount of radiation received above the canopy (Rich, 1989). Global site factor (GSF) is a summary statistic calculated with the ISF and DSF. Site factors ranged from 0 (complete obstruction) to 1 (open sky).

Soil moisture content in each plot was measured monthly throughout 2001 using time-domain reflectometry (TDR). On each measurement date, soils were sampled twice at randomly changing points within each plot to a depth of 20cm; the TDR gives an integrated measure of volumetric soil water content throughout the 20cm of soil measured. In the analysis, we included only the soil moisture measurements bracketing the growing season, from March through August 2001 – defined by the beginning of seedling new growth in late March and seedling harvest in early September.

Soil properties were assayed in each plot on a bulked soil sample collected from the top 10 cm of mineral soil (seedling rooting zone) in four locations. Soil, humus and

litter depth in each plot were measured in late summer 2000 at the same locations where mineral soil was sampled. The soil horizons were defined as follows: litter (loose leaf material, from the forest floor surface to the humus layer), humus (decomposed leaf and organic material, most easily identified by presence of dense root mat), and mineral soil (all horizons below humus layer). Separate layers of litter and humus biomass were collected, dried and weighed. This data was used 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 an Elementar CNS Analyzer (Elementar Americas, Trenton, NJ). Soil pH and concentration of cations (Ca, Mg, P, K, Mn, Zn, Fe, Al, Cu, and B) were determined by the Soil Testing Laboratory at Virginia Tech, using inductively coupled plasma mass spectroscopy (Thermo Jarrell Ask Corp., Franklin, MA).

Plant material and seedling performance measures

Acorns of *Quercus rubra* (QR) and *Quercus prinus* (QP), and seeds of *Pinus rigida* (PR) were germinated in vermiculite and transferred to nursery cells containing a mixture of potting medium and vermiculite. Seedlings were grown in a shadehouse for 16wks, and a randomly selected sample – a “preharvest” of at least 50 seedlings per species - was analyzed for morphological and biomass parameters. Seedlings were classed into four categories based on general leaf quality and size prior to planting. We grew approximately twice as many seedlings required, and randomly selected those that were planted in the plots. The majority of planted seedlings were classed in the middle

two quality categories, with approximately equal amounts in the best and worst categories (i.e. histograms of seedling quality at planting represent normal distributions). During the spring of 2000, we planted red oak (QR) and chestnut oak (QP) seedlings at the low elevation BF site, and red oak (QR) and pitch pine (PR) seedlings at the high elevation DK site. Four seedlings of each species were planted in each plot. The seedling quality, basal diameter, primary stem length (from soil line to apical meristem) and number of leaves were recorded for QP and QR. Total height and seedling quality were recorded for PR seedlings. For oak seedlings, we estimated stem biomass of each seedling at planting using the following allometric relationship derived from the ‘pre-harvested’ seedlings:

$$\text{Eq. 1} \quad \text{stem biomass (g)} = \mathbf{m} (\mathbf{D}^2)(\mathbf{h}) + \mathbf{b} \quad [\text{for } Q. \text{ rubrum: } n = 52; r^2 = .669, \\ p < 0.0001];$$

where **D** is basal diameter, **h** is stem height (cm length from ground line to apical meristem), **m** and **b** are the parameter estimates of the regression model. The predicted initial stem biomass was used to estimate stem growth (g) in addition to the morphological parameters measured at the harvest.

Seedling aboveground growth responses were determined by harvesting two randomly selected seedlings (one on each of two harvest dates) per species from each plot. Harvests occurred in July and September 2001; the purpose of the July harvest was to determine if seedlings were able to recover from high rates of herbivore damage observed during April-June. Many seedlings that experienced extreme damage by herbivores and pathogens developed new leaves during the growing season. For each harvested seedling, total stem length (cm), green stem length (cm, new stem growth),

basal diameter (mm) and leaf area was measured (using a leaf area meter, Li-Cor Instruments, Lincoln, NE). Leaves, stems and roots were cleaned, oven-dried at 80 C, and weighed.

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). Overall health was assessed as:

Eq. 2
$$H = (L_n/L_o) (100 - \%loss)$$

L_n is the number of leaves at sampling time n , and L_o is the number of initial leaves at spring flush. The **%loss** term represents the estimated total leaf area removed or rendered abnormal by herbivory, fungal pathogens, dessication, or parasitism at sampling time n . The causes of leaf area loss were recorded, and in some cases, seedlings experienced more than one type of damage. Since this type of field measure can be fairly subjective, the same researcher performed all census sampling. Most **H** values were between 0 (mortality) and 1 (healthy), but some seedlings had additional flushes that made the (L_n/L_o) term greater than 1, raising the health value considerably. We tested the validity of the field observations of leaf loss, and found a highly significant positive correlation between H values and leaf area ($p < 0.0001$), and a negative correlation between %loss term and leaf area ($p < 0.0001$). It should be noted that 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 health value, but may have less leaf area than a larger, more damaged seedling with a subsequently lower health value. Since both the total formula (**H**) and the **%loss** component proved useful, and because the vast majority of observed damage

was due to herbivory, we have used both measures in regression analyses with shrub and seedling performance parameters.

Data analysis

Vegetation, resource availability, and seedling performance parameters were primarily analyzed using simple and multiple linear regression models (JMP Bivariate, SAS Institute Inc. 1995). We analyzed four general types of relationships: 1) shrub influence on seedling mortality, growth and biomass, 2) shrub influence on available resources, 3) seedling response to resource availability, and 4) shrub influence on seedling health and phenology (Table 3.1). Multiple linear regression analyses were used to model seedling growth responses to resource parameters (JMP Fit Model, stepwise, SAS Institute Inc., 1995) for both oak species at BF and red oak at DK. We followed a backwards stepwise procedure that sequentially eliminated parameters from the model at the $p > 0.1$ level. Two-parameter correlation analysis was also used to describe intrinsic relationships among resource parameters, and between resources and seedling performance. Due to the extremely high initial mortality of pine seedlings at the DF site, we were unable to perform sufficiently powerful statistical tests ($n=22$ at harvest, 480 seedlings initially planted) and this species has been excluded from the analysis. Mean growth responses of seedlings were compared across sites and by species using t-tests and ANOVA (JMP Bivariate, SAS Institute Inc., 1995).

In some cases, we observed a qualitative effect of RM presence/absence on seedling performance and resource variables. In plots without the shrub, the range of

parameter values was equal to the total range of the data; in plots with any degree of RM influence, there was an upper limit to parameter values. Where this qualitative effect was evident, we grouped plots by presence or absence of RM, and tested for significant differences (Tukey's HSD, JMP Bivariate) in the mean response between the two groups (see Figure 3.1). Since the experimental design was not geared towards a factorial ANOVA approach, this analysis was used only when regression lines did not adequately describe clear patterns in the data.

RESULTS

Shrub influence on seedling performance and demography

Direct relationships between shrub density and seedling performance parameters varied by site, shrub species and seedling species, and thus each grouping was addressed independently. Basal area of *Kalmia latifolia* (KL) was not related with mortality of either species of oak at either site. Chestnut oak biomass (green stem length, leaf weight, stem weight) and growth (estimated stem growth (g)) were weakly positively correlated with KL basal area (Table 3.2). KL density had no relationships with growth or biomass parameters for red oak at either site. *Rhododendron maximum* (RM) density was positively linked with mortality of oak seedlings at the BF site, while red oak seedling mortality at the DK site was not linked with the RM understory (Table 3.2). Leaf weight and leaf area of chestnut oak seedlings had a weak negative relationship with RM basal area, and while other biomass parameters showed negative trends with increasing RM density, these relationships were not significant ($p > .05$). For red oak

seedlings at BF, we found no significant relationships between shrub density and seedling performance; however, negative trends were apparent for leaf weight, green stem length and estimated stem growth (g) beneath increasing RM basal area. Lastly, at the DK site we observed negative relationships between RM density and red oak aboveground biomass, most strongly with stem growth and leaf dry weight.

Site and species differences in seedling performance included: 1) the estimated stem biomass growth (g) of QR seedlings was greater at DK than at BF ($p < 0.05$), 2) chestnut oak aboveground biomass at harvest was significantly greater ($p < 0.05$) than red oak, while our estimates of stem biomass at planting indicated no difference between species ($p > 0.05$), 3) higher growth rates for chestnut oak than red oak at the BF site, evidenced by our estimates of stem growth (total expansion in primary stem length, estimated stem biomass growth), all of which were highly significantly different ($p < 0.0001$).

Shrub influence on resource availability

Direct and diffuse light availability (site factors) prior to canopy closure in late March was negatively correlated with basal area of *R. maximum* at both sites (Table 3.3). *Kalmia latifolia* exhibited less of an influence on light availability: basal area was not correlated with March site factors at our low elevation BF site, but a significant negative correlation was observed between KL density and March site factors at the high elevation DK site. At both sites, the shading influence of RM was approximately twice as strong as KL. In most cases, plot TSII (a measure of influence on plots based on size and

proximity, see *Methods*) values for both shrubs were more strongly correlated with light availability than shrub basal area, yet both measures of shrub presence are effective in explaining the observed relationships. Under a closed canopy (mid-July), we observed a weak negative relationship between indirect site factors (ISFs) and RM and KL density at the high elevation DK site, but no relationship with direct site factors (DSFs). No correlations existed for shrub density and light availability (ISFs and DSFs) in July at the low elevation BF site (Table 3.3).

In our low elevation (BF) plots, RM basal area was positively correlated with litter dry weight, litter depth, humus depth and humus %C (Table 3.4). Overall, soil volumetric water content was not significantly correlated with RM, but a weak negative trend did exist in July ($r^2=.043$, $p>0.05$) that could be indicative of soil moisture depletion beneath RM during the growing season. This observed relationship could have been more biologically significant under less mesic conditions. It is likely that soil moisture was not limiting at the BF site during 2001, since normally high local precipitation was observed, and the site itself is located on a mesic, north-facing slope. Further, we did not observe any relationships (at BF) between RM density and soil depth, pH, or soil nutrient availability. However, higher concentrations of metallic soil cations (Fe, Al, Cu) were found with increasing RM basal area and TSII values (Table 3.4). At the high elevation DK site, litter %N decreased linearly with RM density; however, soil moisture, soil nutrients and litter/humus characteristics were not shown to be functions of shrub density. Metallic nutrient (Fe, Al, Cu) concentrations were not linked to RM-influenced soils at the DK site (all p values $>.05$), in contrast to what was observed at the low elevation BF site (all p values $<.05$). In cases where we observed a qualitative effect of

RM presence, we tested the variation in mean response (of resource parameters) between RM and non-RM influenced plots (as in Figure 3.1). At the DK site, soils beneath RM had lower mean concentrations of Ca ($p=.045$) and P ($p=.057$), lower humus %N ($p=.011$), and higher mean concentrations of Al ($p=.061$) and Cu ($p=.033$) compared to plots without RM (Tukey's HSD, JMP Bivariate). At the BF site, we did not observe any significant RM influence on the parameter distributions. *Kalmia latifolia* basal area and TSII values were generally not correlated with belowground resource parameters at BF, although soil %N and Al ppm were negatively related with KL density. We found that KL density was negatively related with soil moisture, soil pH and soil [Al] at the ridge top DK site.

Seedling response to resource availability

Multiple regression models of relationships between seedling aboveground biomass and resource availability were highly variable between sites, species and harvest dates; therefore each of the six models was analyzed independently. The models are summarized in Table 3.5. Overall, models of red oak seedlings at the high elevation DK site were the most inclusive, indicating that numerous parameters influenced seedling growth at this site. Light availability, both prior to and after canopy closure, was an important factor, as well as soil nutrient availability (particularly Ca, Mn, Al), litter %N, humus %C and %N, and volumetric soil water content (soil VWC%) during late spring. Red oak seedlings at BF did not appear to respond strongly to resource availability, but parameter components included light, soil moisture (from spring to early summer), soil

%C and %N, and litter %N. Finally, for chestnut oak seedlings at BF, there was a large discrepancy between models based on harvest date. QP seedlings harvested in mid-season responded weakly to resource availability (three parameters, $r^2=0.2$), compared to those harvested at the end of the growing season (eleven parameters, $r^2 = 0.55$). Similar to the other models, light availability, early spring soil moisture, nutrient availability (Mn, K) and litter %N were the most important components. Clearly, these models did not form a definitive picture of growth responses to resources across sites or between two functionally similar species of tree seedling. QR seedling response to resource availability was drastically different by site. Yet, models for late-season harvested seedlings were consistently more inclusive and effective in explaining the observed variation (in aboveground biomass) than models of the mid-season harvest. This indicates that factors determining aboveground biomass remained influential throughout the latter half of the growing season.

To reveal potential explanatory variables that may have been lost in multiple regression models due to redundancy, we supplemented the multiple regression approach with simple linear regression / correlation analysis of all resource and seedling performance variables. Red oak seedling leaf area and estimated stem growth (g) at DK were negatively correlated with humus yield and mean soil depth, and positively correlated with soil nutrients P, K, Mn, and B, as well as humus %N and litter %N. Chestnut oak seedling leaf area and estimated stem growth (g) at BF were negatively correlated with mean soil depth and soil %C. No further correlations were found for QR seedlings at the low elevation BF site.

Factors influencing seedling health and phenology

The majority of our planted seedlings flushed (began new growth) about 2-3 weeks prior to canopy closure, which coincided with our observations of extant (naturally occurring) oak seedlings at each site. Foliar emergence and canopy closure occurred approximately one to two weeks later at the high elevation DK site than at BF. We found no correlation between shrub density or presence and the small proportion of seedlings that flushed later in the season. There was also no observable difference in the overall timing of foliar emergence between QR and QP seedlings at the BF site.

Herbivore damage was by far the most common form of seedling leaf area loss during the growing season (Figure 3.2), with the exception of widespread (approximately 70%) dessication damage to QR new growth, observed during the first census at DK. We attributed this single event to a late frost that occurred three days prior to the census, since the new growth of affected seedlings appeared frost-burned. The majority of these seedlings developed new leaf and stem tissue by the next census. This late frost did not appear to have an effect on our seedlings at the BF site. Observed herbivore damage on planted seedlings was caused primarily by arthropods, including geometrid larvae ('leaf rollers'), other Lepidopterans, leaf miners and other unknown species. Since these seedlings were small and had relatively few leaves, mammalian herbivory was readily obvious – either stem clipping by rabbits or whole-plant browsing by deer – and did not account for a significant portion of observed leaf tissue loss. Damage that appeared to be a result of pathogens, discolorations caused by nutrient deficiencies (necrosis,

chlorosis) and leaf dessication were relatively minor causes of damage in comparison with herbivory.

Seedling damage (% leaf area loss) at DK due to the late frost event was negatively correlated with RM density, and significantly lower in the presence of the shrub (t-test, $p < .05$), regardless of density. This indicates that a late frost may be less influential on seedlings growing beneath a dense shrub understory. However, at the next census, mean plot leaf area loss due to herbivory strongly increased with RM basal area (Figure 3.3). We found no clear relationships between shrub basal area or presence and herbivore leaf damage for the rest of the census periods at DK. At the BF site, observed QR leaf area loss was positively correlated with RM basal area for the third census ($r^2 = .12$, $p < .05$), the period during which we observed the highest frequency of herbivore damage (Figure 3.3). For July harvested QR seedlings at this site, mean health (an average of H values until harvest) was negatively correlated with RM basal area ($r^2 = .16$, $p < .05$), while mean health of the corresponding QR seedlings harvested in September was not linked with RM density. QP seedling leaf area loss trended upward with increasing RM density, however, the regressions were not significant ($p > .05$). KL basal area was generally not an explanatory variable for leaf area loss, although a weak negative relationship ($r^2 = .07$, $p < .05$), indicating slightly lower herbivory beneath KL, existed for the third census of QR seedlings at BF. Seedlings of both species appeared to recover from herbivory that occurred early in the season. For both sites, July harvested seedling leaf weight was significantly lower (t-test, $p < .05$) than those harvested in September, indicating that seedlings are able to partially recover lost leaf area later in

the season. Our phenological observations verified that several heavily damaged seedlings developed new leaves late in the growing season.

DISCUSSION

Our data supports the hypothesis that suppression of canopy tree seedlings beneath dense shrub understories is mediated by a number of factors, instead of a single dominant factor such as light availability. Nitrogen content of the humus and litter layers and leaf herbivory were important ‘linkages’, in addition to light (Table 3.6). Light attenuation beneath the shrub understory was linked with seedling biomass, but the relative importance of this linkage in this study was probably less than has been estimated (Clinton and Vose, 1996; Semones, 1999; Nilsen et al., 2001) and assumed (Clinton et al., 1994; Beckage et al., 2000) in previous studies. While our observations of the ‘light linkage’ were somewhat equivocal, the role of light is unquestionably paramount in seedling establishment and growth of species with intermediate shade tolerance, such as *Quercus*. Once established, seedlings must be able to persist for many years on the forest floor, as longevity increases the possibility of exposure to the high light levels of a canopy gap (Clinton et al., 1994). Growth rates in low light have been linked with survival in many studies of shade-tolerant tree seedlings (Kobe et al., 1995; Kobe, 1996; Walters and Reich, 1996, 2000). In contrast, we found no linkage between plot light availability and mortality rates, and little evidence that growth rates of harvested seedlings were related to plot-specific mortality rates. These relationships probably become more evident in subsequent years, when seedling growth is a function of carbon

gained solely from photosynthesis (instead of seed reserves), and therefore dependent on light availability.

We found that early-season light availability was a stronger competitive linkage than light levels during the rest of the growing season. Since most of our seedlings began new growth approximately 10-20 days before full canopy closure, light availability during this period (March site factors) was a factor of equal or greater influence on seedlings than closed-canopy light levels (July site factors). March (open) canopy photographs likely provided a better representation of the light environment than the July (closed) photographs. Closure of the canopy drastically reduced light levels (70-90%) on the forest floor and (according to canopy photos) either reduced (at DK) or nullified (at BF) the shading influence of RM and KL. During this brief period when high light is available to seedlings, canopy openness is both a function of evergreen shrub density – especially for *R. maximum*, which has thicker stems and greater LAI than *K. latifolia* – and may be essential for seedling carbon gain (Walters et al., 1993; Pacala et al., 1994; Semones, 1999). Two of the three light mechanism components were open-canopy site factors (based on March canopy photos), and these components were evident at both sites and for all groups of seedlings (Table 3.6). We note that measures of light based on canopy photographs may not be as sensitive to finer variation in PAR as other methods of measuring light, such as quantum sensors; this could have masked the shading effects of dense shrubs (or other vegetation) beneath a closed canopy, which have been well established by previous research on this system (Clinton and Vose, 1996; Semones, 1999; Nilsen et al., 2001) as well as others (Veblen, 1982; Mallik, 1995; Denslow et al., 1991).

The maximization of growth rates in low light compromises allocation to storage, defense, and root system growth, and may have the effect of lowering long-term persistence in resource poor habitats (Walters and Reich, 1996, 1997). Belowground resource deficiencies can co-limit performance in deep shade, such as the reduction of lateral root development in xeric soils (Callaway, 1990) and limitation of stomatal conductance and sun fleck use efficiency (Knapp and Smith, 1990; Semones et al., 1999). In this study, seedling leaf area and stem growth were positive functions of nitrogen sequestered in the litter and humus layers, and growth was negatively correlated with soil %N, while soil %N was negatively correlated with litter and humus %N. At the high elevation site, litter %N declined strongly with *R. maximum* density. In comparison to mixed deciduous litter, the lower nitrogen content and slower decomposition rates (Dobbs, 1995; Baker and Van Lear, 1998) of RM leaves probably results in less nitrogen input (from leaf litter) beneath RM thickets (Monk et al., 1985; Nilsen et al., 2001). Given that most of our seedlings were rooted in the organic horizon, the ‘nitrogen linkage’ helps to explain the negative influence of RM on leaf area and stem growth at the high elevation DK site. Other possible belowground linkages included: 1) seedling growth and biomass was negatively correlated with soil [Al] at both sites, and soil nutrient (P, K, Mn, Ca, Mg) availability and pH was inversely autocorrelated with soil [Al]. If high [Al] is an indicator of acidic, nutrient poor soils, and is positively associated with RM density (at BF, see Table 3.4), then overall nutrient availability may be inversely correlated with shrub density. Seedling biomass and growth parameters were positively correlated with the availability of P, K, Mn, and B cations. Boettcher and Kalisz (1990) found lower pH, soil [Ca], and N mineralization beneath *R. maximum*, and

concluded that the shrub dominates soil nutrient pools and excludes herbaceous cover beneath. 2) Greater humus and litter depth (or yield) beneath RM, which would require additional root growth of a new germinant for establishment and access to moisture and nutrients in the mineral soil. Both RM and KL form a dense root mat in the organic horizon and this may spatially impede normal root development of a new seedling. However, in the process of planting seedlings, we damaged this root layer and confounded any analysis of its influence.

In forest ecosystems, herbivory is an important factor in successional dynamics (Dyer and Shugart, 1992; Jorritsma et al., 1999; Kienast et al., 1999), stand composition and regeneration (Bryant and Chapin, 1990; Nolet et al., 1994; Johnston and Naiman, 1994), and seedling establishment (Schupp, 1988; Hanley, 1988; Molofsky and Fisher, 1993). By reducing fitness, above and belowground herbivory by insects has strong selective effects on woody species (Maron, 1998). In addition to shrub-seedling linkages involving light and nitrogen availability, this study provides the first empirical evidence that leaf herbivory is influenced by an *R. maximum* understory. During the period where the highest incidence of herbivory was observed (April-June), percent loss of seedling leaf tissue was positively correlated with *R. maximum* density. Since anti-herbivore defense capability in plants has often been linked with habitat resource availability (see Coley et al., 1985), seedlings growing beneath dense shrub understories may not be able to produce adequate amounts of defensive compounds, and consequently suffer higher rates of leaf herbivory. Ericaceous understories may have an influence on insect diversity, as demonstrated by Thompson et al. (1993) in the *Rhododendron*

ponticum dominated understories of woodlands in the U.K. Further studies that specifically address seedling herbivory beneath *R. maximum* are clearly needed.

In conclusion, dense *R. maximum* understories limit resource availability, neutralize the facilitative effect of canopy gaps, and likely present a suite of stressors which result in early seedling decline and mortality. Prior studies on this system have demonstrated that these stressors include: deep shade, lower soil moisture, nutrients and soil pH, and the inhibition of mycorrhizal colonization beneath dense thickets. There is no ecologically significant evidence that inhibition occurs prior to seedling establishment (Nilsen et al., 1999). *Kalmia latifolia* appears to have little influence on seedling performance, resource availability or herbivory rates. This study verifies the resource competition hypothesis of inhibition by *Rhododendron maximum*, reaffirms the importance of light, and provides evidence that nitrogen and herbivory are additional mechanisms.

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Table 3.1. A summary of linear regression models; each explanatory variable has been regressed with all response variables listed in the category (JMP Bivariate, SAS Institute, Cary, NC).

<u>Category</u>	<u>Explanatory</u>	<u>Response</u>
Shrub x seedling	RM basal area RM TSII KL basal area KL TSII	Aboveground biomass Leaf area, leaf dry weight Total stem height, stem dry weight Estimated stem growth (g)
Vegetation x Resource	RM basal area RM TSII KL basal area KL TSII Canopy tree ITII	Light (ISF,DSF,GSF; March, July) Soil moisture (VWC%) Soil nutrients (P,K, Ca, Mg, Mn) Litter %C,N, yield (g/m ²) Humus %C,N, yield (g/m ²) Soil %C, %N Soil pH Soil cations (Zn, Fe, Cu, Al, B) Soil texture and depth
Resource x Seedling	Light (ISF,DSF,GSF) Soil moisture Soil nutrients Litter C:N, yield Humus C:N, yield Soil pH Soil cations Soil texture and depth Soil %C, %N	Aboveground biomass (dry wgt.) Leaf area Total stem height Estimated stem growth (g)
Shrub x Seedling Health	RM basal area RM TSII KL basal area KL TSII	Plot % mortality Plot mean % leaf area loss (census 1-8) Plot mean health (census 1-8) Seedling mean health (until harvest)

Table 3.2. Regression analysis of shrub influence on seedling biomass and growth parameters, for all harvested seedlings at each site. Mortality data is based on all seedlings initially planted (n=4 per species) in each plot (n=60 per site). Best fits, depending on basal area or TSII parameter measures of shrub density, are shown.

Group	Explanatory	Response	(+,-)	r ²	prob>F
Q. rubrum DK site	RM basal area	leaf weight	-	.12	<.0001
		stem weight	-	.04	.0364
		est. stem growth (g)	-	.05	.0016
	KL basal area	-- no relationships --			
Q. rubrum BF site	RM basal area	green stem length	-	.03	.0584
		plot % mortality	+	.15	.0027
	KL basal area	-- no relationships --			
Q. prinus BF site	RM basal area	leaf weight	-	.06	.0069
		plot % mortality	+	.08	.0286
	KL basal area	green stem length	+	.07	.0057
		leaf weight	+	.04	.0320
	KL TSII	stem weight	+	.06	.0100
		est. stem growth (g)	+	.06	.0104

Table 3.3. Linear regressions between basal area of *R. maximum* (RM) and *K. latifolia* (KL) and light availability in March and July, 2001. ISF is indirect site factor (diffuse light); DSF is direct site factor (direct light). All significant regression coefficients ($p < .05$) have regression lines with negative slope. Sites are Ball Flats (BF, low elevation, northeast facing) and Dryman Fork (DK, high elevation, southwest facing ridge).

Site	Date	Model	r^2	p
BF	March	RM basal x ISF	.174	.0009
		RM basal x DSF	.153	.002
	July	RM basal x ISF	.014	>.05
		RM basal x DSF	.015	>.05
BF	March	KL basal x ISF	.017	>.05
		KL basal x DSF	.005	>.05
	July	KL basal x ISF	--	--
		KL basal x DSF	--	--
DK	March	RM basal x ISF	.171	.001
		RM basal x DSF	.128	.005
	July	RM basal x ISF	.066	.048
		RM basal x DSF	.022	>.05
DK	March	KL basal x ISF	.108	.01
		KL basal x DSF	.067	.045
	July	KL basal x ISF	.072	.037
		KL basal x DSF	.029	>.05

Table 3.4. Summary of linear regressions between shrub density and belowground resources (moisture, nutrients) and substrate characteristics (soil, humus, litter). Analyses included all 2x2m plots (n=60) at each site (n=2); the (+,-) column indicates the slope of the regression line. Best fits, depending on basal area or TSII parameter measures of shrub density, are depicted below. (JMP Bivariate, SAS Institute, Cary, NC.)

Site	Explanatory	Response	(+,-)	r ²	Prob. > F
BF	RM basal area	Dry litter weight	+	.11	.0115
		Mean litter depth	+	.10	.0146
		Soil [Al] ppm	+	.12	.0070
		Soil VWC% July	-	.04	.0662
	RM TSII	Mean humus depth	+	.21	.0003
		Soil [Fe] ppm	+	.17	.0013
		Soil [Cu] ppm	+	.23	.0001
		Humus %C	+	.12	.0060
	KL basal area	-- no relationships --			
	KL TSII	Soil [Al] ppm	-	.09	.0200
		Soil %N	-	.10	.0157
DK	RM basal area	Litter %N	-	.20	.0004
	RM TSII	-- no relationships --			
	KL basal area	Soil VWC% June	-	.12	.0067
		Soil VWC% August	-	.07	.0432
		Litter %C	+	.09	.0184
		Soil [Al] ppm	-	.06	.0526
	KL TSII	Soil VWC% March	-	.08	.0268
		Soil pH	-	.09	.0172
Key to abbreviations: Ball Flats site (BF), Dryman Fork site (DK), <i>R. maximum</i> (RM), <i>K. latifolia</i> (KL), Tree Species Influence Index (TSII, <i>see Methods</i>), Volumetric Water Content (VWC%, soil moisture using TDR).					

Table 3.5. Summary of multiple regression models of oak seedling response (aboveground biomass) to measures of resource availability in forest plots. Seedlings are grouped by site (1=DK, 2=BF), species (Q. rubrum-QR, Q. prinus-QP) and harvest date (July, September).

Group	Model r^2 (r^2 adj.)	Parameter component	prob>F
1, QR, July	.51 (.40)	ISF July	.0018
		DSF July	.0012
		GSF July	.0011
		Soil VWC% March	.0626
		Soil VWC% April	.0001
		Ca ppm	.0952
		K ppm	.0170
		Soil %N	.0052
		Humus %C	.0417
		Litter %N	.0019
1, QR, Sept	.69 (.61)	ISF March	.0035
		DSF March	.0021
		GSF March	.0022
		Mean soil depth	.0001
		Soil pH	.0628
		Ca ppm	.0766
		Mn ppm	.0000
		Al ppm	.0000
		Cu ppm	.0007
		B ppm	.0677
		Humus %N	.0836
2, QR, July	.29 (.23)	ISF March	.0200
		Soil VWC% April	.0555
		Al ppm	.0288
		Litter %N	.0482
2, QR, Sept	.38 (.32)	DSF July	.0010
		Soil VWC% March	.0005
		Soil VWC% June	.0010
		Soil %C	.0206
		Soil %N	.0189
2, QP, July	.20 (.15)	Soil VWC% March	.0178
		Soil pH	.0127
		Litter %N	.0699
2, QP, Sept	.55 (.43)	ISF March	.0426
		DSF March	.0295
		GSF March	.0314
		DSF July	.0497
		GSF July	.0628
		Humus yield	.0423
		Mean soil depth	.0245
		K ppm	.0816
		Mn ppm	.0423
		Zn ppm	.0449
		Litter %N	.0122

Table 3.6. Summary of major competitive linkages between *R. maximum* (RM) and seedlings of *Q. rubrum* (QR) and *Q. prinus* (QP) in forest plots at two sites (1=DK, 2=BF) in the Coweeta Basin. RM influence on resource/interaction is for 60 plots at each site; seedling response statistics (r^2 , p) are excluded for brevity. RM x seedling column shows direct relationships between shrub density and seedling biomass/growth parameters, sorted by grouping. Seedling groups are designated by superscripts (a=QR, BF; b=QP, BF; c=QR, DK).

Linkage	Relationship with RM	Seedling response to linkage	RM x seedling
1. Light	ISF March (-) ^{1,2} $r^2 = .174$ ($p < .001$) ² $r^2 = .171$ ($p < .001$) ¹	stem growth (+) ^a leaf weight (+) ^{ac} stem weight (+) ^a	green stem (-) ^a $r^2 = .03$ ($p = .0584$)
	DSF March (-) ^{1,2} $r^2 = .153$ ($p < .005$) ² $r^2 = .128$ ($p < .005$) ¹	leaf weight (+) ^b	leaf weight (-) ^{bc} $r^2 = .12$ ($p < .0001$) ^c $r^2 = .06$ ($p < .01$) ^b
	ISF July (-) ¹ $r^2 = .066$ ($p < .05$)	leaf weight (+) ^c	
2. Nitrogen	Litter %N (-) ¹ $r^2 = .20$ ($p < .001$)	stem growth (+) ^c leaf weight (+) ^c stem weight (+) ^c	stem growth (-) ^c $r^2 = .05$ ($p < .005$) leaf weight (-) ^c $r^2 = .12$ ($p < .0001$) stem weight (-) ^c $r^2 = .04$ ($p < .05$)
3. Herbivory	C-3 Health (-) ^{ab} $r^2 = .04$ ($p < .05$) ^{ab}	leaf weight (+) ^{ab}	leaf weight (-) ^{bc} $r^2 = .06$ ($p < .01$) ^b $r^2 = .12$ ($p < .0001$) ^c
	C-2 Health (-) ^c $r^2 = .14$ ($p < .005$) ^c	leaf weight (+) ^c	
	Mean C-2 % loss (+) ^c $r^2 = .34$ ($p < .0001$) ^c	leaf weight (-) ^c	

Influence of *R. maximum* on *Q. rubrum* leaf biomass at Dryman Fork

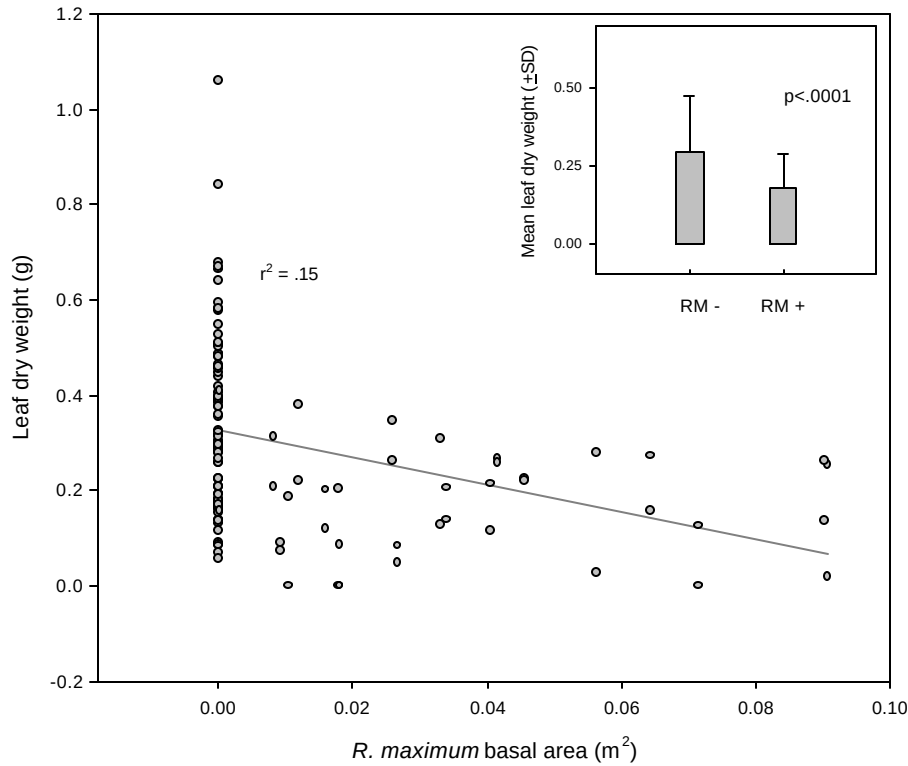
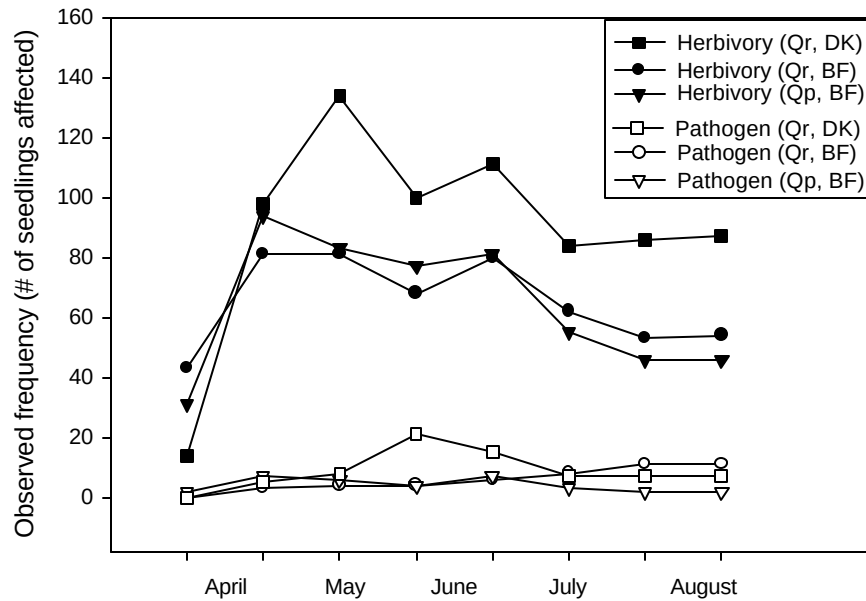


Figure 3.1. An example of a qualitative effect of *R. maximum* (RM) on a seedling response parameter (i.e. leaf biomass). Variance of the parameter beneath RM is approximately half of the total variance of the parameter when RM density equals zero. This difference between means of RM present versus RM absent (see inset) is significant according to a student's t-test ($p < 0.0001$).

Frequency of seedling damage during growing season



QR = *Quercus rubrum*

QP = *Quercus prinus*

BF = Ball Flats site

DK = Dryman Fork site

Figure 3.2. Frequency of observed seedling damage by herbivores and pathogens during the 2001 growing season. A total of 480 seedlings of each group were censused at each period. Seedlings were censused biweekly from mid-April to early September (prior to the second harvest). The July harvest occurred after the fifth census, and reduced the number of seedlings (per species) per plot to three; however, most damage occurred prior to this date. Leaf herbivory and pathogens were the two most important causes of damage observed, and are thus shown.

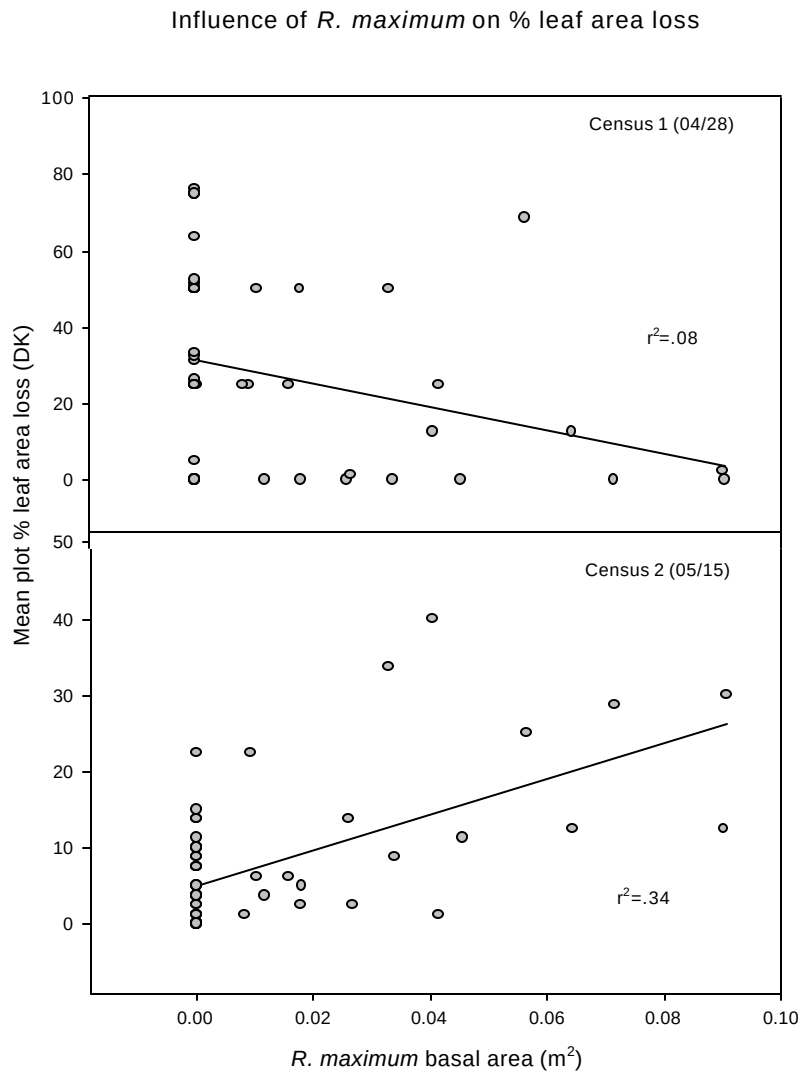


Figure 3.3. Relationships between *R. maximum* density and mean leaf area loss observed for all seedlings in each plot (n=4) for the first two census periods at Dryman Fork (DK). Most of the damage observed during the first census was caused by a late frost, and for the second census, herbivory was the predominant cause. Most seedlings affected by the late frost developed new leaves by the second census.

CHAPTER IV

The primary goal of this research was to reveal the potential mechanisms by which evergreen understory shrubs suppress the regeneration of canopy trees, and to determine at which stage(s) of recruitment these mechanisms are most important. Clearly, the resource competition hypothesis has the most support concerning *Rhododendron maximum* in Appalachian forests (Monk et al., 1985; Clinton et al., 1994; Clinton and Vose, 1996; Walker et al., 1999; Nilsen et al., 2001) of any explanation proposed thus far. Most research has focused on deep shade beneath *R. maximum* thickets as the primary cause of seedling decline. Lower moisture and nutrient availability, as well as acidic soils and lower mycorrhizal colonization of tree seedling root tips, have been found to occur in conjunction with light attenuation beneath *R. maximum*. Studies have repeatedly shown that shade tolerant seedlings cannot tolerate other resource deficiencies when growing in low light (Walters, 1993; Walters and Reich, 1996, 1997) and thus should suffer higher mortality. Virtually all of the evidence provided by prior observations and experiments has supported this resource competition explanation, but few studies have addressed two important aspects: 1) the specific mechanisms of inhibition and their relative importance to seedling performance, and 2) the assumption that shrub thickets do not negatively affect recruitment prior to seedling establishment.

Proving the latter assumption to be true is essential if one is to attribute seedling suppression exclusively to resource competition. Thus, I addressed the potential influence of understory shrubs on two aspects of tree recruitment prior to germination, seed rain and seed bank dynamics:

1.1: There is no significant difference in seed rain characteristics (density and composition of seeds) between shrub cover treatments (*Rhododendron maximum*, *Kalmia latifolia*) and open understories.

1.2: There is no significant difference in seed bank characteristics (density and composition of germinants) between shrub cover treatments (*Rhododendron maximum*, *Kalmia latifolia*) and open understories.

My findings indicated that while overall seed rain density appeared to be lower beneath *R. maximum* than an open understory, most of this effect was driven by a single species, *Oxydendrum arboreum*. This tree forms numerous capsules that contain many tiny seeds, and the fate of those individual seeds is nearly impossible to track. The lack of a shrub presence effect on the regeneration of *O. arboreum* in seed bank samples was an indication that dispersal of its seeds was not inhibited by shrub cover. Moreover, I could not find any overstory dominant species whose seed rain densities were significantly affected by presence of either *R. maximum* or *K. latifolia*. Species richness of the seed rain was highest beneath *R. maximum* and lowest beneath open understories, and the proportion of tree seeds (of the total rain) was unaffected by either shrub treatment.

The analysis of seed bank composition was very limited because *Betula lenta* seedlings overwhelmingly dominated in all treatments at both sites. Species richness of all seed bank samples was low and unrelated to shrub cover treatments. Density of woody seedlings was highest for seed bank material collected beneath *R. maximum*, and

similarly lower for seed banks beneath *K. latifolia* and open understories. This finding was probably a result of one or more shrub-mediated factors. First, greater litter and humus volume beneath *R. maximum* may have led to a proportionally larger substrate samples, and the potential for more seeds to be present. Secondly, the ability of dense shrub thickets to reduce resources and neutralize the effect of canopy gaps may act to maintain seed dormancy for longer periods, allowing the accumulation of seeds over time. In either case, it does not appear that regeneration from the seed bank is inhibited by presence of *R. maximum*, at least for the early-successional species that I observed. In fact, many of the important canopy trees in late-successional Appalachian forests do have seeds capable of dormancy (i.e. *Quercus*, *Carya*) and generally do not form a seed bank. Further, there is no ecologically significant evidence that leachates from *R. maximum* litter have an allelopathic effect on the germination of tree seeds (Nilsen et al., 1999).

Therefore, I concluded that both null hypotheses (1.1 and 1.2) could not be completely supported based on my findings. There were effects of the shrub thickets that denied the null model. However, the purpose of testing these hypotheses was to prove that seedling suppression does not occur prior to establishment, and I found little evidence indicating that shrub thickets negatively impact dispersal or regeneration of important overstory tree species. Based on those findings, I accept the assumption that the suppressive influence of *R. maximum* occurs post-establishment; this makes a mechanistic analysis of competition between shrubs and seedlings more valuable and ecologically relevant.

To uncover potential mechanisms explaining the decline of tree seedlings beneath *R. maximum*, I examined how shrubs influenced resource availability, how seedlings responded to *in situ* resource conditions and how shrubs influenced the rates of seedling damage (i.e. herbivory). In the second manuscript, I defined these mechanisms as ‘competitive linkages’ in which a negative shrub-seedling relationship (i.e. declining seedling leaf area with increasing shrub density) could be described by shrub-resource and resource-seedling regressions with opposite slopes (Table 3.6). The ‘linkage’ is termed for the resource or interaction that the shrub-seedling relationship may potentially be reliant on. Based on prior work, I tested the following hypotheses:

- 2.1: Light availability will be limited by the shrub understory, to a greater extent by *R. maximum* than *K. latifolia*, and will be positively related to seedling growth, biomass and survivorship.
- 2.2: Belowground resource availability (moisture, nutrients) will be negatively correlated with the density of understory shrubs *R. maximum*, if any relationship exists, and positively related to seedling growth, biomass and survivorship.
- 2.3: Shrub density and/or presence will have no influence on variation of seedling phenology or rates / extent of damage caused by herbivores and other agents.

I found that light was an important linkage, and that early season light availability was more significant to seedling performance than light levels after canopy closure. During the brief high light periods when seedling leaves have emerged but the canopy was still

partially open, *Rhododendron maximum* density had a strong negative relationship with diffuse and direct light availability (site factors). Once the canopy had closed, the relationship between *R. maximum* density and light availability was severely muted. As expected, seedling leaf area and absolute growth was positively correlated with light. The importance of light limitation as a mechanism has been verified by numerous studies in several different forest communities (Veblen, 1982; Denslow et al., 1991; Clinton and Vose, 1996; Nilsen et al., 2001).

Nitrogen content in the litter and humus layers was another linkage between shrubs and seedlings. Litter %N was negatively related with *R. maximum* density, and seedling growth was a positive function of litter nitrogen content. The litter of *R. maximum* is dense and slow to decompose, and thus may provide lesser nitrogen input (into the organic horizon) than mixed hardwood litter. Further experiments using nitrogen amendments *in situ* may be useful in testing this potential mechanism.

Perhaps the most interesting linkage found between shrubs and seedling was herbivory. I found that the rates of early-season herbivore damage were strongly positively related to *R. maximum* density. At the high elevation site, a late frost affected many of the seedlings, but damage due to this event was negatively correlated with *R. maximum* density. Cover provided by the dense shrub thicket may have afforded some protection from this frost, but the mechanisms are unclear. Within the following two weeks, seedlings recovered leaf area (lost to the frost), but appeared to be more heavily eaten by herbivores with increasing shrub cover (Figure 3.2). This is a particularly important observation, because it is generally thought that shade tolerant seedlings, which have inherently low tissue turnover rates and high defense levels, may be unable to

recover from severe herbivory (Coley et al., 1985). By reducing leaf area, herbivory also reduces the ability of seedlings to capture sun flecks, an essential source of light on the forest floor. Replacing tissues lost to herbivory while growing under low resource conditions may shift the plant carbon balance into the negative, thus limiting further growth and storage, and potentially limiting the ability to persist for many growing seasons. Clearly, my observations of herbivory beneath *R. maximum* are not adequate to show conclusively that seedlings suffer greater damage beneath the shrub, but do provide an important new direction for research into the suppression phenomenon. Therefore, I accepted the first two hypotheses (2.1 and 2.2) and rejected the third hypothesis (2.3) concerning seedling phenology and health.

It is important to mention that only one of the aforementioned ‘competitive linkages’ may be the primary driving force of the negative shrub-seedling interaction, and any other mechanisms may be ‘ghost’ mechanisms that arise from autocorrelation and / or redundancy. For example, suppose that herbivore reduction of leaf area is the true mechanism reducing seedling leaf area beneath *R. maximum*; and both light and litter nitrogen availability are negatively related with shrub density, positively related with seedling performance, and thus may appear to be important mechanisms. Therefore, if low light and nitrogen availability are correlated with high herbivory rates beneath *R. maximum*, then light and nitrogen may be secondary to the herbivory linkage. Overall, I believe that all three linkages may be important; yet, I did not attempt to isolate a single linkage as the most important. In order to do so, a more specifically tailored experimental design would be required that could include the use of herbivore exclosures and nitrogen amendments.

In conclusion, it is my opinion that seedling suppression beneath *R. maximum* occurs post-establishment, primarily through asymmetric competition and increased herbivory on seedlings. There appears to be little influence of either shrub species on overstory tree seed dispersal or presence in the seed bank. Although it occupies a similar growth habit, *Kalmia latifolia* appears to have little influence on the performance of tree seedlings. Allelopathy, however, may be an important aspect of the habitat beneath *Kalmia* species (Mallik, 1995). Both species are endemic to the southern Appalachian flora, but forest managers have considered removal of *R. maximum*, which has spread extensively in the last 30 years due to numerous disturbances (Dobbs, 1995). There is much concern over the future structure of the forest if canopy trees are unable to regenerate normally in canopy gaps occupied by *Rhododendron*. This research has shown that management of forests with *R. maximum* understories may require removal, because several different factors associated with the shrub may contribute to seedling decline. However, removal of *R. maximum* will be surely be very difficult, due to the species' sprawling growth habit and its inherent capacity to reproduce clonally. Severe disturbance of the forest substrate would be concomitant with shrub removal, and would probably create early-successional regeneration patches that may be re-invaded by shrub thickets. From this research, it is unclear whether greenhouse grown and planted *Quercus* seedlings perform better than naturally recruits. Likewise, the value of mycorrhizal inoculation treatment (as indicated by Walker et al., 1999) is still being evaluated. Over the long term, continuation of this research should provide a better picture of how normal regeneration processes can be restored in these highly productive and diverse ecosystems.

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VITA

Born in 1977 in Newport News, VA, Colin Beier attended Ferguson High School and the Governors Magnet School for the Sciences until his graduation in 1994. He then attended Boston University and completed a year of coursework in biomedical engineering. After deciding that 'engineering nature' was not reconcilable with his philosophical beliefs, Colin transferred to Virginia Commonwealth University in Richmond, VA, and completed a Bachelor's degree in Biology, with a focus on plant ecology. Following graduation from VCU in 1999, Colin worked as a field technician for Dean Urban, Ph.D. (Duke University) at the USDA Forest Service Coweeta Hydrologic Lab in Otto, NC. At Coweeta, Colin was introduced to the research program of Erik Nilsen, Ph.D. (et al.) at Virginia Tech, studying seedling suppression beneath *Rhododendron* thickets. In the fall of 2000, he began work on a Master of Science degree with Dr. Nilsen at Virginia Tech, continuing the research on *Rhododendron* in southern Appalachian forests. After graduation from Virginia Tech in the summer of 2002, Colin will pursue doctoral studies at University of Alaska Fairbanks, with Terry Chapin, Ph.D. At UAF, Colin will participate in the NSF-IGERT Regional Resilience and Adaptation program, working on multidisciplinary approaches to regional environmental issues and systems modeling of human-fire-ecosystem-climate interactions. In his spare time, he enjoys guitar and percussion, backcountry hiking and camping, fresh and saltwater angling, and the culinary arts (often moonlighting as a part-time chef).