

Inhibition of Canopy Tree Seedlings by Thickets of *Rhododendron  
maximum* L. (Ericaceae) in an Eastern Deciduous Forest

by

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(ABSTRACT)

*Rhododendron maximum* L. (Ericaceae) is an evergreen shrub that grows in dense thickets and currently covers large areas of the understory in the deciduous forests of the southeastern United States. Thickets of *R. maximum* are inhibitory to recruitment and regeneration of many understory plants including canopy tree seedlings. By effectively lowering the survivorship of woody species trying to establish within thickets, *R. maximum* could influence stand level regeneration patterns and ultimately the community structure of these deciduous forests. This dissertation outlines research conducted to determine if: 1) below and above ground resources are lower within thickets of *R. maximum* when compared to forest sites where *R. maximum* is absent; 2) *Quercus rubra* and *Prunus serotina* seedlings growing in thickets have lower mid-day photosynthetic rates; 3) *Quercus rubra* and *Prunus serotina* seedlings growing within thickets are low light acclimated when compared to seedlings growing in forest without *R. maximum*; 4) the presence of *R. maximum* constrains CO<sub>2</sub> assimilation of *Quercus rubra* seedlings exposed to light flecks of different durations and intensities; 5) the presence of *R.*

*maximum* constrains the light fleck responses of *Quercus rubra* seedlings exposed to eight light flecks in rapid succession; and 6) canopy openness regulates the capacity of *Quercus rubra* seedlings to assimilate carbon when exposed to eight consecutive light flecks.

*Rhododendron maximum* thickets altered resource availability for seedlings when compared to areas of forest without *R. maximum*. Diffused photosynthetically active radiation (PAR) averaged less than  $5 \mu\text{mol m}^2 \text{s}^{-1}$  throughout the growing season in sites with *R. maximum* in comparison to  $10\text{-}30 \mu\text{mol m}^2 \text{s}^{-1}$  in sites without *R. maximum*. Soil moisture content, measured using Time Domain Reflectometry was approximately 6% lower in forest sites with *R. maximum* compared to sites without *R. maximum* throughout the growing season. Most nutrient concentrations (e.g., C, N and most cations) and nitrogen mineralization rates were significantly lower in sites with *R. maximum*. Temperature and atmospheric relative humidity are slightly lower under thickets of *R. maximum*. In general, sites with *R. maximum* are associated with lower resource availability above and below ground in comparison with sites without *R. maximum*.

Attenuation of below canopy PAR by thickets of *R. maximum* negatively influences the photosynthetic capacity of *Quercus rubra* and *Prunus serotina* seedlings as indicated by measurements of mid-day photosynthesis. In 1996, the seasonal mean mid-day photosynthetic rate of first year *Q. rubra* seedlings growing in *R. maximum* thickets ( $1.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was 62% lower than the seasonal mean mid-day photosynthetic rate ( $2.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) of seedlings growing in forest sites without *R. maximum*. For second year seedlings in 1997, seasonal mean mid-day photosynthesis was 183% higher for

plants growing outside of thickets ( $1.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) compared to the mean rate ( $0.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for plants located within thicket sites. The mean mid-day PAR available to seedlings located in forest sites without *R. maximum* during measurements of photosynthesis was 354% higher in 1996 and 257% higher in 1997. First year *Prunus serotina* seedlings growing in forest without *R. maximum* also had greater seasonal mean mid-day photosynthesis ( $0.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) when compared to the mean rate ( $-0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for plants growing within thickets. *Prunus serotina* seedlings located in the presence of *R. maximum* received on average 67% less PAR.

Photosynthetic acclimation to low light was assessed for *Q. rubra* and *P. serotina* seedlings growing under both forest conditions by measuring photosynthetic responses to light *in situ* using even aged one-year old seedlings. *Quercus rubra* seedlings growing in forest sites without *R. maximum* had significantly higher light saturated rates of photosynthesis. For both species, photosynthetic responses to light were otherwise similar irrespective of the presence or absence of *R. maximum*.

The impact of the *R. maximum* subcanopy on understory PAR and subsequent influence on canopy tree seedling photosynthetic capacity implies that sunflecks are critical for seedling net carbon gain in these forest understory environments. To determine the effect of *R. maximum* on the photosynthetic response to sunflecks of oak seedlings, light flecks were simulated on 288 randomly chosen, even aged, two-year old seedlings *in situ*. Half of the seedlings were located within *R. maximum* thickets. Seedlings were randomly assigned one of four light fleck durations (30, 60, 120, and 300s) and one of three intensities (100, 500, 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Half of all seedlings

were dark pre-acclimated prior to light fleck simulations by covering with aluminum foil for at least 12 hours, while the remaining seedlings were pre-acclimated under ambient conditions.

Analysis of covariance showed that a significant, positive, linear relationship exists between the length of a light fleck and total carbon gain during a light fleck for seedlings in forest sites with and without *R. maximum* regardless of pre-acclimation status, or light fleck intensity. Furthermore, there was a significant effect of *R. maximum* on the slope of the relationship such that following ambient pre-acclimation, seedlings located within thickets assimilated significantly less carbon with increasing light fleck length than seedlings located in forest sites without *R. maximum*. When seedlings were dark pre-acclimated there was no difference in carbon gain with increasing fleck length between seedlings in forest with and without *R. maximum* except for flecks of  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The data lead to the conclusion that under natural conditions the presence of *R. maximum* likely prohibits *Q. rubra* seedlings from utilizing sunflecks as effectively as seedlings growing in forest sites where *R. maximum* is absent.

Because sunflecks often occur clustered together during a short period of time during the day, another field study was conducted to further characterize the effect of *R. maximum* on the photosynthetic response of oak seedlings to eight consecutive light flecks. Within 10 paired sites, (i.e., with and without *R. maximum*) 3 even aged three-year old *Q. rubra* seedlings were selected. Over each seedling, a hemispherical canopy photograph was taken and analyzed for percent canopy openness. Each seedling was dark pre-acclimated for 12 hours and then exposed to eight light flecks in rapid

succession during which time photosynthesis was logged every two seconds. Each light fleck was  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  in intensity and lasted for 120s. Following each light fleck, leaves were exposed to  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR for 60s before the next light fleck.

Mean carbon gain and maximum photosynthesis achieved during each light fleck was significantly lower for seedlings located in the presence of *R. maximum* for all flecks in an eight-fleck simulation. In addition, seedlings located within thickets generally had significantly lower pre-illumination photosynthesis following the first of eight light flecks. The mean photosynthetic light use efficiency of seedlings located in forest with *R. maximum* was significantly lower for the first six of eight light flecks in succession. Using regression analysis and analysis of covariance, percent canopy openness was used to explain the variation in carbon gained from all eight light flecks in succession for seedlings under both forest conditions. However, significant relationships failed to exist between under either forest condition and precluded using analysis of covariance.

The results from these studies lead to the conclusion that light limitation is a major mechanism responsible for the extirpation of canopy tree seedlings from within thickets of *R. maximum*. Tree seedlings growing in forest sites with *R. maximum* receive less solar irradiance, have lower mid-day photosynthesis, fail to acclimate to the lower light conditions within thickets, and utilize sunflecks less effectively as well as less efficiently when compared to plants growing in forest sites without *R. maximum*.

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## **Introduction**

### **Invasive subcanopy evergreen plants**

Deciduous forests cover approximately one third of all land area in the continental United States. Some of these forests are narrow bands of riparian vegetation that extend west along river courses into non-forested regions. However, the majority of the deciduous forests lie east of the 95°W longitude and south of the 45°N latitude (i.e., the eastern deciduous forests) (Barbour et al. 1987). The eastern deciduous forests are certainly not spatially or temporally homogenous in species composition (Braun 1950; Monk et al. 1985). Braun (1950) used dominant and co-dominant overstory tree species to separate the region into nine separate forest associations. Since then, these associations have been revised so that now much of the eastern deciduous forests can be classified into essentially five forest types or associations: mixed mesophytic, oak-hickory, southern mixed hardwood, beech-maple, and hemlock northern hardwood (Barbour et al. 1987).

The factors that control or regulate the development and persistence of these distinct forest types are still under study. In fact, this topic has been fervently addressed by plant ecologists for a majority of the 20<sup>th</sup> century. One factor gaining recognition, which may even be a form of disturbance, is the presence of invasive plants. Subcanopy evergreen shrubs and grasses inhibit canopy tree recruitment in many temperate and tropical forests globally. For example, coniferous forests of the northwestern United States have a subcanopy of *Galtheria shaloni* which can interfere with seedling survival

(Klinka et al. 1989; Messier 1993). In Chile and Costa Rica (Veblen 1982; Widmer 1998), species of dwarf bamboo (*Chusquea sp.*) inhibit regeneration of southern beech (*Nothofagus sp.*) and oak (*Quercus sp.*). Other species of dwarf bamboo (*Sasa sp.*) in the temperate deciduous forest of SW China and Japan inhibit regeneration of *Abies sp.* and *Betula sp.* and most other canopy tree seedlings (Nakashizuka and Numata 1982; Taylor and Qin 1992). Broad leaf palms inhibit canopy tree seedling recruitment in Costa Rican rain forest (Denslow et al. 1991). Moreover, the invasive species *Rhododendron ponticum*, inhibits regeneration of canopy trees in the United Kingdom (Fuller and Boorman 1977; Cross 1981; Mitchell et al. 1997). The best examples of this phenomenon in eastern North America are *Rhododendron maximum* in the southern Appalachian mountains and *Kalmia angustifolia* in northeastern Canada (Clinton et al. 1994; Mallik 1995). Despite all of the evidence for adverse effects of evergreen subcanopy species on other understory plants, the exact mechanisms responsible for inhibition in many of these systems remain unclear. These factors need further elaboration because differential influence of subcanopy shrubs on tree species recruitment and regeneration could modify forest structure.

A model system for the study of invasive subcanopy plants is present in the southern Appalachian mountains of the United States. Approximately, 2.5 million hectares of these forests are covered by thickets of the evergreen, subcanopy species, *Rhododendron maximum* L. (Ericaceae), and *Kalmia latifolia* L. (Ericaceae), (Kurmes 1967; Monk and Day 1985). The abundance of these two species within deciduous forest ecosystems varies with position on the mountain slope and edaphic characteristics (Day

and Monk 1974; Graves and Monk 1985). For example, *K. latifolia* is often found in greater abundance on south facing slopes and *R. maximum* on north facing slopes.

*Rhododendron maximum*, rosebay or great laurel, is an upright shrub or small tree (3-6m tall) that grows in dense thickets or slicks. Thickets may be as large as 20 hectares and apparently exclude most understory vegetation. *Rhododendron maximum* thickets are normally located in riparian zones along streams, in coves, or on north facing slopes. These locations are critical for ecosystem processes (such as nutrient cycling) because they constitute the interface between terrestrial and aquatic ecosystems (Gregory et al. 1991; Yeakley et al. 1994). The shrubs are shade tolerant, can occupy the space under residual trees and are able to persist. After disturbance, broken stems and root crowns can re-sprout to rapidly fill canopy openings. The high relative abundance of *R. maximum* in southern Appalachian forests, its distribution in critical habitats for ecosystem processes, and its apparent suppressive affect on forest productivity, makes the interaction between *R. maximum* and canopy trees the most important shrub-tree interaction in southern Appalachian forests. This dissertation will focus on the influence of *R. maximum* on tree regeneration.

### **Influence of *R. maximum* on subcanopy tree seedlings**

Prior research has conclusively shown that thickets of *R. maximum* L. have an inhibitory effect on the establishment and growth of canopy tree seedlings (Minkler 1941; Wahlenberg 1950; Phillips and Murdy 1985; Plocher and Carvell 1987; Baker and Van Lear 1998). In fact, the importance of *R. maximum* to forest productivity has been

recognized by foresters for more than 4 decades and has led to many studies on how *R. maximum* could be eradicated or suppressed on high quality hardwood production sites (Wahlenberg and Doolittle 1950; Yawney 1962; Hooper 1969; Romancier 1971). Mature shrubs create dense shade, a deep dense root mat, and produce heavy litter that decomposes slowly in terrestrial systems. Some still undetermined aspect of thicket formation inhibits regeneration of woody species.

There is increasing evidence that the percentage cover of *Rhododendron* thickets in eastern deciduous forests has increased over the last two decades (Monk et al. 1985; Baker and Van Lear 1998). Consequently, the inhibitory impact of *R. maximum* on canopy-tree regeneration, and productivity could become spatially more apparent and influential. As a result, thicket formation and expansion has important long-term implications for ecosystem processes and forest management because regeneration of many temperate forest trees is highly dependent upon the establishment and persistence of their seedlings in understory conditions (Canham 1985; Canham 1989; Canham 1990). Therefore, in order to understand the long-term dynamics of this ecosystem and to appropriately manage the southern Appalachian forest it is critical to understand the basic mechanisms by which the evergreen shrubs are inhibiting recruitment of seedlings and growth of canopy trees.

Despite accruing evidence concerning the influence of *R. maximum* in deciduous forests, the exact mechanisms responsible for the inhibitory interaction between *R. maximum* and canopy tree seedlings remain unknown. Several mechanisms have been explored however and include: 1) the production of allelochemicals that leach into the

thicket soil; 2) reduced soil resources within thickets; 3) inhibition of canopy tree mycorrhizae within thicket perimeters; 4) increased herbivory within thickets; and 5) reduced seed bank within *R. maximum* thickets. The conclusions from these studies are that none of these mechanisms appear to be entirely responsible for the suppressive nature of *R. maximum* on understory plants and seedling regeneration.

Perhaps the most dramatic and least studied impact of *R. maximum* on the forest understory environment is the strong attenuation of photosynthetically active radiation (PAR). The dense evergreen foliage attenuates subcanopy PAR creating shadier conditions. Hence, impaired seedling regeneration in these forest sites with *R. maximum* could be the result of aboveground resource limitation resulting in rapid carbon limitation for seedlings when compared to plants growing in forest sites without *R. maximum*. This dissertation presents a series of studies that were conducted in an effort to elaborate the underlying mechanisms of thicket inhibition focussing on the influence of light limitation for tree seedlings in these forest systems. The question of light attenuation by *R. maximum* and subsequent effect on seedling physiology and ultimately successful regeneration has not been adequately addressed within deciduous forests of the southeastern United States.

### **Plant growth in the understory**

Light is often the most limiting resource for plant carbon gain in forest understory environments (Chazdon et al. 1988; Pearcy 1990) because the overstory canopy attenuates the majority of direct solar radiation incident upon forests. The wavelengths of

photosynthetically active radiation (PAR), 400-700 nm, most strongly absorbed by the overstory canopy are the same wavelengths that are most useful to the plants in the understory. Short-term survival of plants in the forest understory is dependent upon the capacity to utilize the very low levels of diffuse irradiance present the majority of the time. Consequently, plants from low light environments usually invest more energy into light harvesting capabilities (e.g., larger, thinner, leaves, a thinner cuticle, solar tracking, etc.) in order to maximize photon penetration into the leaf from an environment of low PAR (Nilsen and Orcutt 1996). The acclimation of these light-harvesting processes must be achieved concomitant with minimized losses of energy and carbon through respiration (Chazdon 1988; Björkman 1973). However, the long-term success of plants existing in the forest understory is ultimately dependent upon their ability to capture and efficiently utilize high energy sunflecks that vary spatially and temporally in quantity and quality (Chazdon 1988). This negative trade-off poses a dilemma for plants that are acclimated to low light because periods of direct solar irradiance can be damaging, often leading to photoinhibition, and can subsequently reduce the capacity to take advantage of successive periods of illumination.

### **The sub canopy light environment**

#### *Diffuse Radiation*

The majority of light in the forest understory is in the form of diffuse solar radiation, occasionally punctuated by periodic pulses of direct solar radiation called

sunflecks. In most forests, less than 2% of the PAR incident above the canopy actually reaches the forest floor (Chazdon 1988). Photosynthetically active radiation on the floor of tropical rainforests can be less than 1% of that incident above the canopy (Björkman and Ludlow 1972). For deciduous forests, such as the one at Coweeta Hydrological Forest Laboratory, between 1-5% of the incident PAR above the canopy reaches the forest floor (Hicks and Chabot 1985). As a result, plants that exist in the understory receive less solar irradiance than plants growing in open environments. Often in the forest understory, light levels exist well below saturating values for net photosynthesis of most canopy tree seedlings (Percy 1983; Semones et al. manuscript submitted). More importantly though, understory light levels may exist below the light compensation point (i.e., that point where respiratory loss is balanced by photosynthetic gain) for many of these plants. The lower quantity of PAR available to plants in these forests reduces potential net carbon gain and ultimately retards regeneration of woody species. As a result, effective use of direct PAR in the form of sunflecks is critical for seedling survival in the forest understory.

### *Light Quality*

Light quality as well as light quantity is strongly influenced by the forest canopy. Alteration in light quality will affect plants because they use environmental cues to gather information about prevalent growth conditions. Light signals perceived by specific photoreceptors play a fundamental role in the detection of light opportunities. In addition to the two photosystems contained within the chloroplasts of plants, higher plants have at



least three additional families of photoreceptors: phytochromes, (B)/UV-A pigments and a less characterized UV-B receptor. These photoreceptors are molecular devices that translate electronic excitations of light into specific cellular signals (Smith 1992), which in turn lead to altered cellular metabolism and ultimately influence plant growth, development and reproduction.

Phytochrome is the most characterized of all plant photoreceptors. Phytochromes absorb radiation over a wide range from the U-V to the far red with maxima around 660nm for the Pr form, and around 730nm for the Pfr form. Phytochrome exists in these relatively stable forms with absorption maxima in the R, (red) and Fr, (far red) regions of the electromagnetic spectrum respectively. Each form of the protein is converted into the other upon absorption of light in photoconversion reactions. White light contains both red and far red light so both forms of the pigment are exposed simultaneously to photons that are efficient in promoting photoconversion. After a brief period of illumination, a photoequilibrium is established in which the rates of photoconversion between pigment forms are equal, and the proportions of each type of Phytochrome, the Pfr:Pr ratio, are constant. Under natural polychromatic light the Pfr:Pr ratio is highly dependent upon the ratio of R to FR photon fluxes in incident radiation. Values for the Pfr:Pr ratio fluctuate between ca. 0.6 for full sunlight, to approximately 0.15 for light filtered through a dense leaf canopy (Ballaré 1994).

Many physiological responses in plants are well correlated with the photoequilibrium of phytochrome forms, (e.g., seed germination, the detection of shading by other plants, flowering, pigment formation and plastid changes). The Pfr form

of the photoreceptor is biologically active (i.e., triggers a response such as seed germination), whereas the Pr form is inactive. The pigment molecule can thus act as a biological switch turning responses on or off depending upon the light environment. The specific mechanisms of phytochrome function have yet to be established. It is clear however, that a number of phytochrome regulated morphogeneses result from changes in gene transcription. Plants in the forest understory not only have to develop mechanisms to deal with rapid changes in light quantity but also light quality relative to plants located in environments characterized by more homogenous light.

#### *Direct Light*

One of the most important sources of PAR for plants in the forest understory comes in the form of sunflecks (Chazdon 1988; Pearcy 1990). Sunflecks are pulses of direct PAR that penetrate through openings or gaps in the forest canopy thereby increasing the PAR incident on the plant up to 20 fold in as short a time as one second (Pearcy and Sims 1994). The characteristics of sunflecks depend upon the canopy structure (i.e., height, leaf area distribution, flexibility etc.) as well as the prevailing climatic conditions (Pearcy and Sims 1994). For that reason, a typical sunfleck is difficult to characterize. In fact, the characterization of transient light is quite subjective and usually defined by the observer. Generally, in ecophysiological studies the sunfleck has been defined as some excursion of PAR above some threshold limit greater than the shade light present in the understory (Pearcy and Sims 1994).

Short duration sunflecks generated through canopy shading usually range from <1 sec to ca. 1 min. (Chazdon 1988), and primarily influence CO<sub>2</sub> uptake (Chazdon and Pearcy 1986a). In environments characterized by these shorter duration sunfleck periods, the number of sunflecks that occur through time is inversely proportional to the mean length of sunflecks (Chazdon 1988; Pearcy 1990). Flecks of direct solar irradiance may only occur during 10% of the day, but they may contribute 20-90% of all the photosynthetically active light in the understory environment (Chazdon 1988; Pfitsch and Pearcy 1989; Pearcy 1990).

After penetration of the forest canopy, smaller more ephemeral pulses of light experience a great deal of scattering, or penumbra, thus creating a shaded border around the center of the incident sunfleck (Smith et al. 1989). Direct sunlight, on leaves of forest understory, can result in higher than normal leaf temperatures, transpiration, water stress, and wilting. If the period of direct light persists, the photosynthetic capacity of these plants may be reduced. For that reason, it is believed that penumbral effects may be very important for plants in these extremely low light environments, because the penumbra can pre-adapt the leaves for the direct light that follows. (Anderson and Miller 1974; Myneni and Impens 1985; Smith et al. 1989).

Sunpatches are generally longer in duration and somewhat larger than sunflecks (i.e., several meters in diameter) (Smith et al. 1989). In more open forest types, sunpatches are more representative of the heterogeneous light environment than are sunflecks (Chazdon and Pearcy 1986a; Chazdon 1988; Smith et al. 1989). Variations in PAR caused by intermittent clouds or diurnal solar movement across larger canopy

openings typically result in sunpatches that last from several minutes to hours (Young and Smith 1979; Knapp and Smith 1988; Smith et al. 1989). During sunpatch periods, peak PAR on the forest floor will often reach full sun irradiance. Also, levels of diffuse irradiance between consecutive sunpatch events tend to be higher than between more transient sunflecks (Smith et al. 1989). It is during these longer periods of direct light and short intermittent shade that stomatal conductance and xylem pressure potential may play an important and interactive role with responses in net photosynthesis (Knapp 1992).

### **Photosynthesis and carbon balance in the sub-canopy**

#### *Induction: the factor that regulates light use*

The capacity of understory plants to use transient light efficiently depends on the photosynthetic induction state, (essentially a state of “readiness” for the plant), maximum photosynthesis, and the capacity for post illumination CO<sub>2</sub> fixation (Chazdon 1988; Pearcy 1990). Pools of ribulose 1,5-bisphosphate, Rubisco activation and stomatal response physiologically regulate this capacity. When leaves are first exposed to high light levels after an extended period of low light or darkness, 10-40 minutes of induction are required before the plant is considered to be fully induced and at steady state or maximum photosynthetic capacity (Chazdon and Pearcy 1986a). During this induction period, the photosynthetic rate increases slowly relative to a plant that has been exposed to light previously. The induction status at 60 seconds (IS<sub>60</sub>), is defined as the ratio of photosynthesis measured 60 seconds following an increase in light compared to

maximum photosynthesis at that light intensity, and is often used as a measure of leaf induction state (Chazdon and Pearcy 1986a; Pons et al. 1992). Thus, the longer the sunfleck, the greater the induction state attained during the sunfleck. Carbon gain in understory plants may be limited by a low induction state when shorter duration sunflecks are preceded by extended periods of darkness or low light. Moreover, a higher induction status might also be achieved and maintained following a series of frequent, but short sunflecks. As a result, short duration sunflecks that are clustered in time are in some cases utilized more efficiently than longer sunflecks that are spaced farther apart (Chazdon 1988).

#### *Post Illumination CO<sub>2</sub> Fixation*

Another important photosynthetic characteristic of understory plants is post illumination CO<sub>2</sub> assimilation. Following a sunfleck, many plants will continue to assimilate CO<sub>2</sub> as the rate of photosynthesis slowly decreases back to a pre-illumination state. Post illumination CO<sub>2</sub> fixation occurs because of a capacity for rapid reduction of 3-phosphoglyceric acid (PGA) to triose phosphate (TP) and other reduced high energy metabolites during the sunfleck, which can then be utilized for continued CO<sub>2</sub> fixation following the sunfleck (Percy and Sims 1994). This post illumination CO<sub>2</sub> fixation can contribute significantly to the total carbon gain during highly transient sunflecks. Post illumination CO<sub>2</sub> fixation is inversely proportional to sunfleck length. The longer the sunfleck, the quicker the rate of CO<sub>2</sub> assimilation declines following a decrease in light

due to exhausted pools of reduced high energy metabolites such as PGA, thereby reducing the potential for extended post illumination CO<sub>2</sub> fixation.

The efficiency with which a plant utilizes transient light is also inversely proportional to the length of the sunfleck. Net plant carbon gain attributed to transient light, increases with sunfleck length. However, plant light use efficiency (i.e., the ratio of integrated carbon gain due to light / expected maximum carbon gain, assuming an instant response to a light increase = LUE), has been shown to decrease with sunfleck length (Pearcy et al. 1987). Many plants that successfully inhabit environments that are characterized by frequent brief sunflecks, such as a tropical forest understory, will have LUE's greater than 100%, reflecting a significant capacity for post illumination CO<sub>2</sub> fixation. In environments where larger, longer duration sunpatches occur, or strong penumbral effects characterize the transient light, rapid induction and post illumination CO<sub>2</sub> fixation may not be as important for carbon gain as in those environments where shorter sunflecks or reduced penumbral effects are more common.

### **Sunfleck Research and Significance**

The importance of sun flecks for plant carbon gain in the forest understory has been realized for many years (Evans 1939). Still, due to technological constraints, there has been relatively little experimental work designed to examine the utilization of sunflecks by plants, and their actual importance for plant carbon gain. In addition, despite the dynamic nature of the forest understory environment, many gas exchange studies have been conducted under steady state conditions (Langenheim et al. 1984;

Weber et al. 1985). This method of measuring gas exchange and net carbon gain likely overestimates the actual net carbon gain of the plant; because the efficiency with which a plant utilizes sunflecks depends not only on the irradiance level but also the frequency and duration of transient light (Pearcy et al. 1991). Furthermore, under natural conditions, the photosynthetic utilization of longer duration sunflecks may be inhibited by factors not conducive for maximum steady state photosynthesis. These factors include loss of induction during extended low light periods, restricted stomatal opening to conserve water loss, wilting, high leaf temperatures, and photoinhibition (Chazdon 1988; Pearcy 1990).

Only recently have the physiological responses of understory plants to fluctuating or non steady state light environments been characterized in detail (Young and Smith 1979; Knapp and Smith 1989, 1990a & b; Pearcy 1990; Chazdon and Pearcy 1991). By studying transient light as a limiting resource, the importance to specific physiological and ecological processes can be considered along with the interactive effects of other critical resources, such as water and nutrients. Studies on plant photosynthetic response to rapid fluctuations in light have been carried out on native species from the Northern Australian rainforest (Chazdon and Pearcy 1986a & b; Kirschbaum and Pearcy 1988a-c;), on North American understory herbs (Young and Smith 1979; Pfitsch and Pearcy 1989a & b), on Hawaiian tree species (Pearcy 1983) and on Mid-Western canopy trees (Ellsworth and Reich 1992). However, very little gas exchange work has been done on woody shrubs or trees in transient light environments from eastern deciduous forests.

## Dissertation objective

There is evidence now that evergreen subcanopy plants inhibit successful recruitment and regeneration understory plants, including canopy tree seedlings (Fuller and Boorman 1977; Cross 1981; Nakashizuka and Numata 1982; Veblen 1982; Klinka et al. 1989; Denslow et al. 1991; Taylor and Qin 1992; Messier 1993; Clinton et al. 1994; Mitchell et al. 1997; Widmer 1998; Baker and Van Lear 1998). Furthermore, the inhibition of canopy tree seedling recruitment is critical to forest dynamics and may ultimately impact stand level regeneration patterns of woody species. As a result, the spread and invasive nature of subcanopy evergreen shrubs need to be recognized as potentially affecting forest community structure. *Rhododendron maximum* provides an excellent model for the effects of subcanopy evergreen species on forest processes. Although *R. maximum* is known to inhibit canopy tree seedling recruitment the mechanisms that mediate inhibition are still undefined. This dissertation presents several studies conducted to evaluate inhibition of tree seedling recruitment due to light limitation in a mature, uniform forest containing a mosaic of *R. maximum* thickets.

All of the research was conducted at Coweeta Hydrological Forest Laboratory, a long term ecological research site, where it has been reported that incident PAR under *R. maximum* stands is 14-34% of that found in the surrounding forest understory (Clinton and Vose 1996). Furthermore, it is not uncommon for light levels to drop as low as 0.2% full sunlight under thickets of *R. maximum*. In these forests, there is no doubt that light is a major limiting resource for tree seedling carbon gain, especially within the densest thickets of *R. maximum*. Consequently, efficient sunfleck utilization by understory tree



seedlings is likely critical for establishment, successful growth and long term survival (especially within the thicket environment). Yet, the influence of light limitation on canopy tree seedlings within thickets of *R. maximum*, or their capacity to utilize available sunflecks, has not been fully elucidated.

The purpose of this study is to determine if: 1) light is limiting for *Quercus rubra* L. (Fagaceae) and *Prunus serotina* Ehrl. (Rosaceae) seedlings in *R. maximum* thickets; 2) *Quercus rubra* and *P. serotina* seedlings exhibit greater low light acclimation within the thicket environment than in forest sites without *R. maximum*; 3) *Quercus rubra* seedlings within *R. maximum* thickets exhibit constrained photosynthetic response to sunflecks compared to seedlings in forest sites without *R. maximum*; and 4) differences in carbon gain during sunflecks by *Q. rubra* seedling can be explained using an indirect measure of light availability derived from canopy photographs taken above individual seedlings.

### **Specific chapter objectives of the dissertation**

The objective of the research presented in chapter one is to evaluate resource availability above ground and below ground in forest sites with and without thickets of *R. maximum*. We hypothesized that the presence of *R. maximum* would result in a reduction in light availability, a decrease in air temperature, and an increase in humidity at the height of seedlings compared to forest without *R. maximum*. In addition, we hypothesized that the presence of *R. maximum* will be associated with a decrease in soil water availability, a decrease in soil pH, a decrease in nitrogen mineralization rate, and an overall decrease in soil nutrition compared with forest lacking *R. maximum*.

The purpose of chapter two is to address three questions concerning the effect of *R. maximum* on the photosynthetic capacity of *Q. rubra* and *P. serotina* seedlings. First, are above ground climatic conditions the same between forest sites with and without *R. maximum*. Second, are light levels within both mature forest and thickets of *R. maximum* sufficient for *Q. rubra* and *P. serotina* seedlings to maintain a positive daily photosynthesis? If so, do *Q. rubra* and *P. serotina* seedlings growing within thickets of *R. maximum* exhibit lower mid-day photosynthetic rates relative to those growing in forest without *R. maximum*? Third, do *Q. rubra* and *P. serotina* seedlings growing within thickets of *R. maximum* exhibit a greater degree of low light adaptation relative to the seedlings growing in the forest without *R. maximum*?

Chapter three determines whether *Q. rubra* seedlings growing in forest with *R. maximum* experience constrained photosynthetic response to light flecks of variable lengths and intensities when compared to seedlings growing in forest without *R. maximum*. Regression analysis is used to relate carbon gained by *Q. rubra* seedlings during light flecks to fleck duration for three different fleck intensities.

The main objective of chapter four is to determine if canopy openness, an indirect measure of light availability can be used to describe the variation in carbon gained from sunflecks by *Q. rubra* seedlings in forest with and without *R. maximum*. The rationale is that if carbon gained from light flecks can be explained using canopy openness as the independent variable, then curves from different sites in the forest should not differ with respect to slope or intercept, unless something other than light is regulating light fleck response. In addition, chapter four explores the effect of an evergreen subcanopy on

additional parameters derived from photosynthetic responses of *Q. rubra* seedlings to repeated light flecks. This chapter attempts to further substantiate that light limitation within thickets of *R. maximum* is a major if not the controlling mechanism of canopy tree seedling inhibition in these forests.

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## **Chapter 1**

**Resource availability for canopy tree seedlings in the understory of Southern Appalachian forests with and without a *Rhododendron maximum* L. (Ericaceae) subcanopy.**

## Abstract

Evergreen subcanopy species in forest around the world inhibit the recruitment of canopy trees to different extents. One subcanopy evergreen shrub that strongly inhibits seedling recruitment, *Rhododendron maximum* L. (Ericaceae), occurs in the southern Appalachian Mountains of the United States. However, the mechanisms by which species such as *R. maximum* suppresses seedling survival of canopy species are largely unknown, but may include competition for resources. The purpose of this study was to determine if the presence of a *R. maximum* thicket in the subcanopy of an otherwise uniform community is associated with low resource availability above and below ground. *Rhododendron maximum* thickets altered light availability patterns for seedlings compared to the areas of the forest without *R. maximum*. Diffused PAR averaged less than  $5 \mu\text{mol m}^2 \text{s}^{-1}$  throughout the growing season in sites with *R. maximum* in comparison to  $10\text{-}30 \mu\text{mol m}^2 \text{s}^{-1}$  in sites without *R. maximum*. Soil moisture content was 6% lower in forest sites with *R. maximum* compared with sites without *R. maximum* throughout the growing season. Most nutrient concentrations (particularly cations) and nitrogen mineralization rates were significantly lower in sites with *R. maximum*. In general, sites with *R. maximum* are associated with lower resource availability above and below ground in comparison with sites without *R. maximum*. The low resource availability coupled with the inhibition of seedlings under *R. maximum* thickets suggests that resource limitation (light) and (nutrients) may be important mechanisms by which *R. maximum* inhibits seedling recruitment.

Key Words: Resource availability, light availability, subcanopy evergreen shrubs,  
*Rhododendron maximum*, microclimate, soil, nutrients, Appalachian Mountains

## Introduction

*Rhododendron maximum* L. is one of the most important evergreen shrub species in the southern Appalachians because of its dominance in the understory. It has been estimated that 0.5 million hectares of the southern Appalachian Mountains are covered by dense thickets of *R. maximum* (Wahlenberg 1950). Most *Rhododendron maximum* thickets are located in riparian zones along streams, in coves, or on north facing slopes. These locations are critical for ecosystem processes (such as nutrient cycling) because they constitute the interface between terrestrial and aquatic ecosystems (Gregory et al. 1991; Yeakley et al. 1994). The high relative abundance of *R. maximum* in southern Appalachian forests and its distribution in critical habitats for ecosystem processes, makes the potential interaction between *R. maximum* and canopy trees the most important shrub-tree interaction in southern Appalachian forests.

It has long been known that populations of *R. maximum* substantially influence regeneration and growth of canopy tree species (Minkler 1941). Wahlenberg (1950) states that "the hardwoods are hopelessly inhibited within the *R. maximum* slicks in comparison to outside the slick." When studying hemlock regeneration, Barden (1979) purposely avoided areas occupied by *R. maximum* because of the confounding influences of this species on canopy species composition. *Rhododendron maximum* may reduce hardwood production by sequestering large quantities of the available nutrient pool in its evergreen leaves creating a slow nutrient turnover rate (Monk et al. 1985). Hardwood regeneration by seedlings in or out of canopy gaps is slowed in the presence of *R. maximum* stands (Phillips and Murdy 1985; Clinton et al. 1994; Neiring and Egler 1956;



Cross 1975; Fuller and Boorman 1977; Clinton et al. 1993; Clinton and Vose 1995).

There are also many reports that identify *R. maximum* as a significant weed for forest management in the southern Appalachians (Smith 1963; Romancier 1970, 1971; Martinez 1975). There is little doubt that thickets of *R. maximum* inhibit canopy tree regeneration and productivity in Appalachian forests.

The importance of *R. maximum* to forest productivity has been recognized by forestry industries for more than four decades, which has led to many studies by forest biologists on how *R. maximum* could be eradicated or suppressed on high quality hardwood production sites (Wahlenberg and Doolittle 1950; Yawney 1962; Hooper 1969; Romancier 1971). Thus, the suppression of forest canopy tree seedlings by *R. maximum* thickets has important implications for both natural ecosystem processes and forest management. The ecological mechanisms causing this suppression need to be determined in order to predict impacts of *R. maximum* on ecosystem processes and to develop appropriate management protocols.

There are many possible mechanisms by which *R. maximum* could inhibit tree seedling establishment. Some of these mechanisms include the influence of *R. maximum* on: 1) resource availability for developing canopy tree seedlings; 2) the abundance of allelopathic compounds in the forest floor substrate; 3) the abundance of mycorrhizae and canopy tree-mycorrhizae synthesis; 4) herbivore abundance and activity; and 5) canopy tree seed dispersal. In this study, we concentrate on mechanism one. If resource availability is lower in the seedling environment under *R. maximum* thickets compared to

forest understory without *R. maximum*, then competition for resources may be an important factor of canopy tree seedling inhibition by *R. maximum*.

The objective of the research presented here was to evaluate resource availability above ground and below ground in the seedling environment with and without the presence of *R. maximum*. Above ground micro-climatic conditions were also monitored. We hypothesized that the presence of *R. maximum* would result in a reduction in light availability, a decrease in air temperature, and an increase in humidity at the height of seedlings compared to forest without *R. maximum*. In addition, we hypothesized that the presence of *R. maximum* will be associated with a decrease in soil water availability, a decrease in soil pH, a decrease in nitrogen mineralization rate, and an overall decrease in soil nutrition compared with forest lacking *R. maximum*. The information presented here is part of a larger research program evaluating several mechanisms by which *R. maximum* may inhibit seedling establishment in the southern Appalachian region.

## **Methods and Materials**

### *Site description and sampling design*

This study was conducted in a mature mixed-hardwood montane forest at Coweeta Hydrologic Laboratory in the Nantahala mountains of western North Carolina (35° 02' 29" N, 83° 27' 16" W). The research site was as uniform as possible in topography and soil type. The site was a north-facing slope (60%) at an elevation of

1000 m dominated by northern red oak forest with thickets of *R. maximum* in the subcanopy of half the total site area. The *R. maximum* thickets had leaf area distributed at 3-4 m above the ground surface. The soil type at the experimental site was a deep, well drained coarse-loam of the Edneyville series (Thomas 1996). The regional climate is classified as maritime, humid with cool summers, mild winters, and adequate rainfall during all seasons (Swank and Crossley 1988). Within our study site, 90 2X2 m plots were subjectively located in an effort to adequately represent the forest conditions as best possible. Forty-five of the plots were within the boundaries of *R. maximum* thickets and 45 were within forest where *R. maximum* was absent. For this study, these plots were treated as both the experimental and observational units.

#### *Measurement of resource availability*

In each plot, soil samples were taken at a depth of 10 centimeters in May for nutrient analysis. Soil was removed from the tips of an isosceles triangle (50 cm sides) at the center of each plot. The three samples were pooled prior to nutrient analysis. Soil pH and cations were determined by the Soil Testing Laboratory at Virginia Polytechnic Institute and State University using inductively coupled plasma mass spectroscopy. Carbon, N, and H content of the soils were determined with a CHN analyzer (Perkin-Elmer model 2400 CHN elemental analyzer, Norwalk, Connecticut ).

Nitrogen mineralization was determined in each plot using a 28-day *in situ* incubation (Adams and Attiwill 1986) in July 1996. Two cores (PVC pipe 4.3 cm in diameter and 15 cm long) were driven into each plot to a depth of 10 cm. One core was

immediately collected ( $t = 0$ ) from each plot and one was left *in situ*. The soil was kept cool, returned to the laboratory, and stored at 4°C until processed within 48 h. Samples were sieved to exclude particles >6 mm. Five grams of sieved soil were shaken for 1 h in 2 M KCL at 1:4 soil/extractant ratio and then centrifuged at 3715 x g (6000 rpm) for 15 min. Concentrations of NO<sub>3</sub> and NH<sub>4</sub> in the extractions were determined on an autoanalyzer (Technicon Instruments Corp., Tarrytown, NY) using a cadmium column (U.S. EPA, 1985a) and alkaline phenol (U.S. EPA, 1985b) methods, respectively. After 28 d ( $t=1$ ), the *in situ* cores were collected and processed using the methods described above. Net N-mineralization was determined by taking the difference in NO<sub>3</sub>-N and NH<sub>4</sub>-N between  $t=0$  and  $t=1$ .

Soil water content was measured monthly in 1996 at a depth of 0-15 cm in the center of every plot by Time Domain Reflectometry (Tektronix model 1502C TDR cable tester, Heerenveen, The Netherlands).

Microclimatic conditions were evaluated continuously in two of the 90 plots (one of the forest sites without *R. maximum* and one with a *R. maximum* thicket) using permanently installed instrumentation. Each installation contained: 1) a Li-Cor model 190s quantum sensor to determine the amount of photosynthetically active radiation (PAR) (Li-Cor Inc., Lincoln, Nebraska), 2) copper constantan thermocouples to determine air temperature at 20 cm height, and 3) a shielded relative humidity sensor (Campbell Scientific, Inc., model 217, Logan, Utah). Data from all sensors were recorded every minute, and the minimum, maximum, and average for every 10-min

interval were computed and stored using a model 21x micro-data logger (Campbell Scientific Inc., Logan, Utah).

In order to determine if our single installation reflected the light conditions of a wider area, 8 gallium arsenide photo-diodes equally spaced within a 7.2 m diameter circle were each placed 50 cm above the ground surface in forest with *R. maximum* and one forest site without *R. maximum*. The photo-diodes were calibrated against a LI-190s quantum sensor and the signal corrected for each individual diode. Light intensity was recorded over 24hr periods. The results were compared with the quantum sensors positioned at the permanent forest and *Rhododendron* locations. In a similar manner, the spatial heterogeneity of air temperature was determined by placing 8 shaded thermocouples in a similar configuration as that for the photo-diodes. Air temperatures were also recorded simultaneously in the two forest types (with and without *R. maximum*) over 24hr periods for 10 days, and compared with the air temperature recording at the permanent locations.

### *Statistical Analysis*

Significant differences in measures of spatial resource availability (soil nutrient, nitrogen mineralization, and pH) between forest types were analyzed using a two sample t-test. Significant effects of forest type on temporal variation in soil moisture did not conform to conditions required for repeated measure ANOVA (Huynh-Feldt value<1); therefore, repeated measures analysis using MANOVA was used as suggested by Potvin et al. 1990. Statistical tests were performed using SAS PROC GLM (SAS 1988).

## Results

### *Above ground resources*

The amount of photosynthetically active radiation (PAR), as indicated by the quantum sensors was greater throughout the day in the absence of *R. maximum*. During summer, the mean midday (1100-1400 hr EST) PAR in forest plots without *R. maximum* was  $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ , while the mean midday PAR was  $2 \mu\text{mol m}^{-2} \text{s}^{-1}$  under the *R. maximum* thicket (Figure 1.1). The difference due to weather condition can also be clearly seen (Figure 1.1, August). Late in the growing season (October), mean midday PAR increased to  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  in forest plots but remained the same ( $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in *R. maximum* plots.

Forest understory light intensity, as indicated by the photo-diodes, clearly showed spatial heterogeneity within and between forest canopy types (Table 1.1, Figure 1.2). Both the total accumulated PAR and maximum PAR during a 24hr period in July was five fold greater in the forest understory compared with that under the *Rhododendron* canopy. In the forest understory, the mean length of time when PAR was greater than  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  was 3 hr while that under the *R. maximum* canopy was only 8 min. The photo-diode results show that the quantum sensors positioned at the permanent sites were within the range of spatial variation within each forest canopy type and can represent the temporal pattern of light over the season.

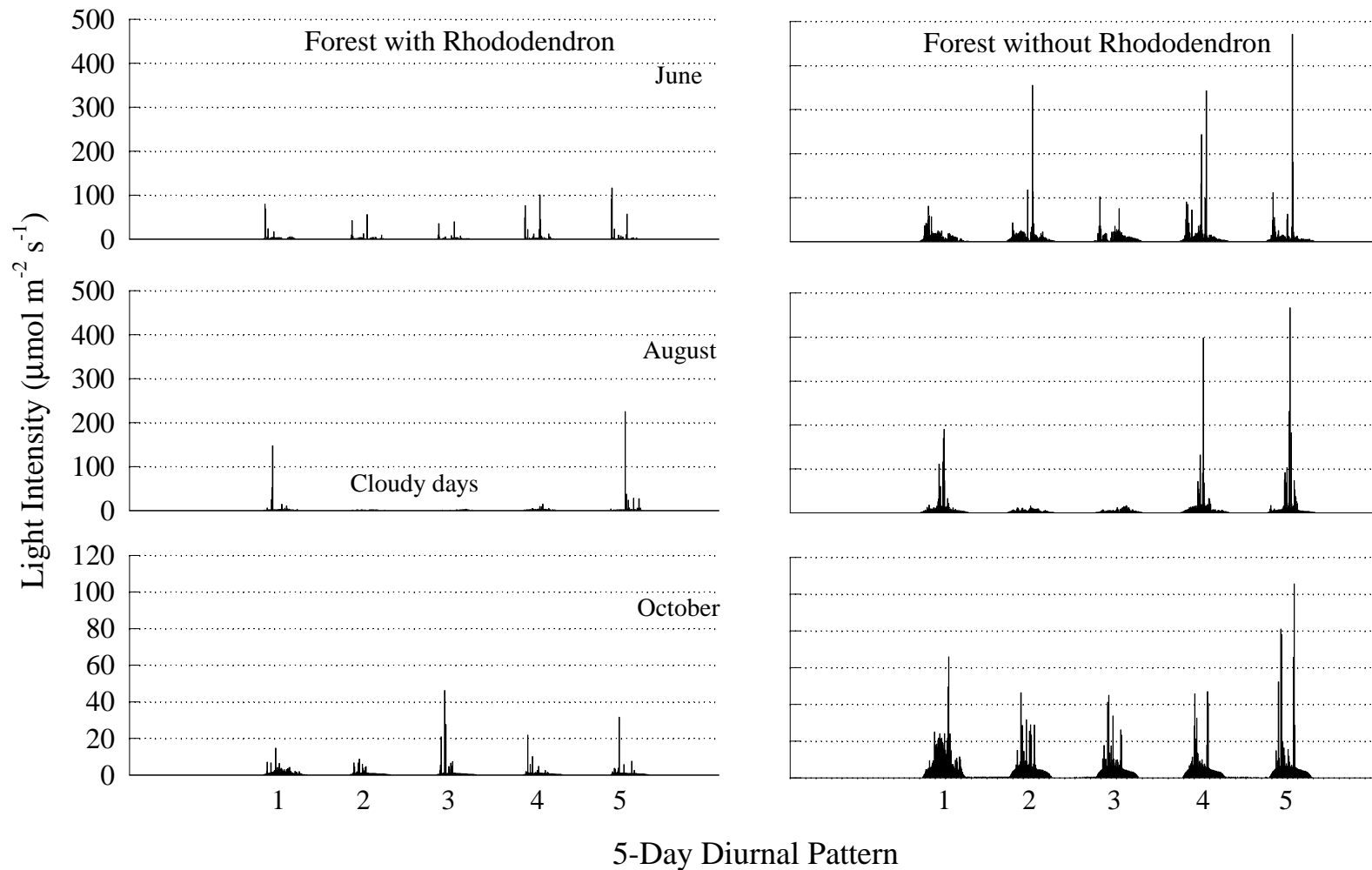


Figure 1.1: Representative variation in diurnal cycles of photosynthetically active radiation in the subcanopy of forest sites with *R. maximum* or without a thicket of *R. maximum* at Coweeta. The 3 horizontal pairs of panels represent a 5-day period in June, August and October 1996 when light intensity was measured simultaneously at the two sites. Notice different scales for light intensity among dates.

Table 1.1: Quantifiable characteristics for diurnal cycles of light intensity recorded by photo-diodes at 50 cm above the forest floor at Coweeta Hydrological Laboratory in the southern Appalachians. Photo-diodes are placed in a forest with (*Rhododendron* Plots) or without (Forest Plots) a subcanopy stand of *R. maximum*.

Characteristic	Photo-Diode number								Mean	± SE	Quantum Sensor
	1	2	3	4	5	6	7	8			
Forest Plots											
Total PAR*	689	1034	632	647	7210	337	974	310	729	114	794
MAX PAR	40.3	103	62	48	92.9	48.4	202	36.9	79.2	19.5	65.8
# of events <10 μmol	65	49	72	73	43	83	73	84	67.8	5.25	59
# of events > 10 μmol	21	37	14	13	43	3	13	2	18.3	5.25	27
Rhododendron Plots											
Total PPFD	121	160	167	115	88	182	183	112	141	12.9	112
MAX PPFD	3.42	4.06	39.1	5.68	2.01	13.2	41.9	21.5	16.4	5.74	6.23
# of events <10 μmol	86	86	85	86	86	84	84	85	85.3	0.31	86
# of events > 10 μmol	0	0	1	0	0	2	2	1	0.75	0.31	0
* PAR = photosynthetically active radiation (μmol m <sup>-2</sup> d <sup>-1</sup> )											



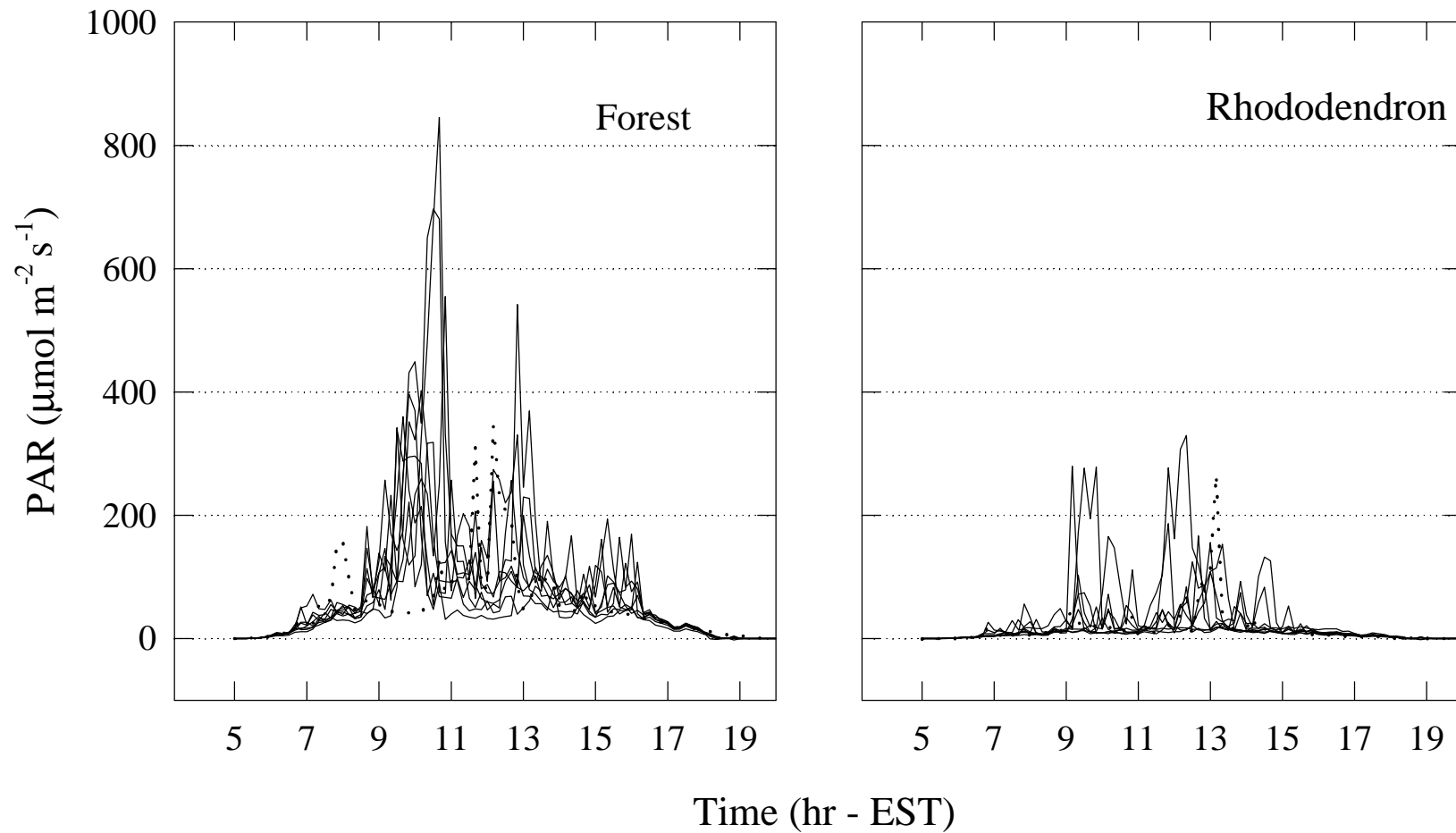


Figure 1.2: Diurnal cycles of photosynthetically active radiation in the subcanopy of southern Appalachian forest sites without (Forest) or with (Rhododendron) a thicket of *Rhododendron maximum*. Each solid line refers to light intensity recorded at 50 cm above the forest floor by gallium arsenide photo-diodes, and the stipled line refers to light intensity recorded with a quantum sensor.

Air temperature at seedling height in forest plots was normally within 1°C of that in *R. maximum* plots throughout the day on all measured days (insert of Figure 1.3). Mean monthly midday air temperature during the growing season was 21°C with a range of 18°C to 22°C, and the two sites had similar maximum, minimum and mean monthly air temperature during all months sampled (Figure 1.5). Although substantial diurnal temperature change occurred in both forest sites with and without *R. maximum*, spatial variation in each site was small (< 1°C, Figure 1.3).

Relative humidity (RH) commonly started near 100% in the morning hours (until approximately 1030 hr EST) then decreased to 70% late in the day (Figure 1.4). Late in the growing season relative humidity decreased in general but followed a similar diurnal pattern as that during the growing season. Between July and October, the average maximum RH was always 100% while the average mean and minimum values increased to a high in August then declined towards October, indicating a drier and more variable level of RH in late summer (Figure 1.5). In a similar manner to that of air temperature, relative humidity did not differ between the two forest types throughout the season (Figure 1.5). Because the air temperature and relative humidity remained the same between the two forest conditions there was no difference in the vapor pressure deficit between sites with and without *R. maximum*.

#### *Below ground resources*

Soil moisture decreased from its highest value at the beginning of the growing season in April to its lowest value in July (Figure 1.6). Following the low point in July, soil moisture increased through the rest of the growing season. There was a large amount

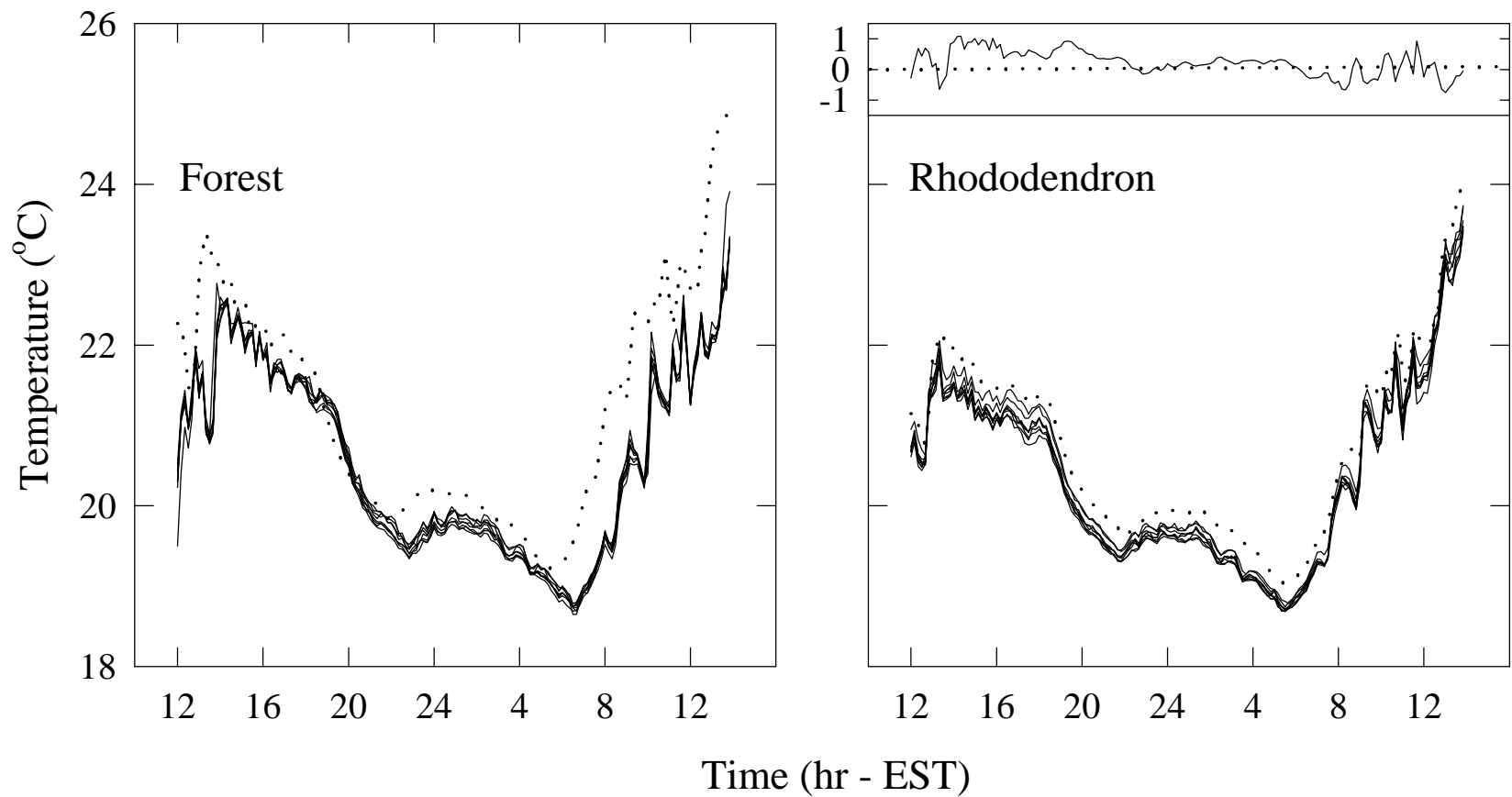


Figure 1.3: Diurnal cycles of air temperature in the subcanopy of southern Appalachian forest sites without (Forest) or with (Rhododendron) a thicket of *Rhododendron maximum*. Each solid line refers to air temperature recorded by a shaded 36 gauge thermocouple. Thermocouples were equally spaced over a 7.2 m radius at 50 cm above the ground surface. The dashed line refers to air temperature recorded with the thermister component of a relative humidity sensor. The insert in the Rhododendron panel indicates the mean air temperature of the 8 thermocouples from the forest without *R. maximum* minus that from the forest with *R. maximum*.

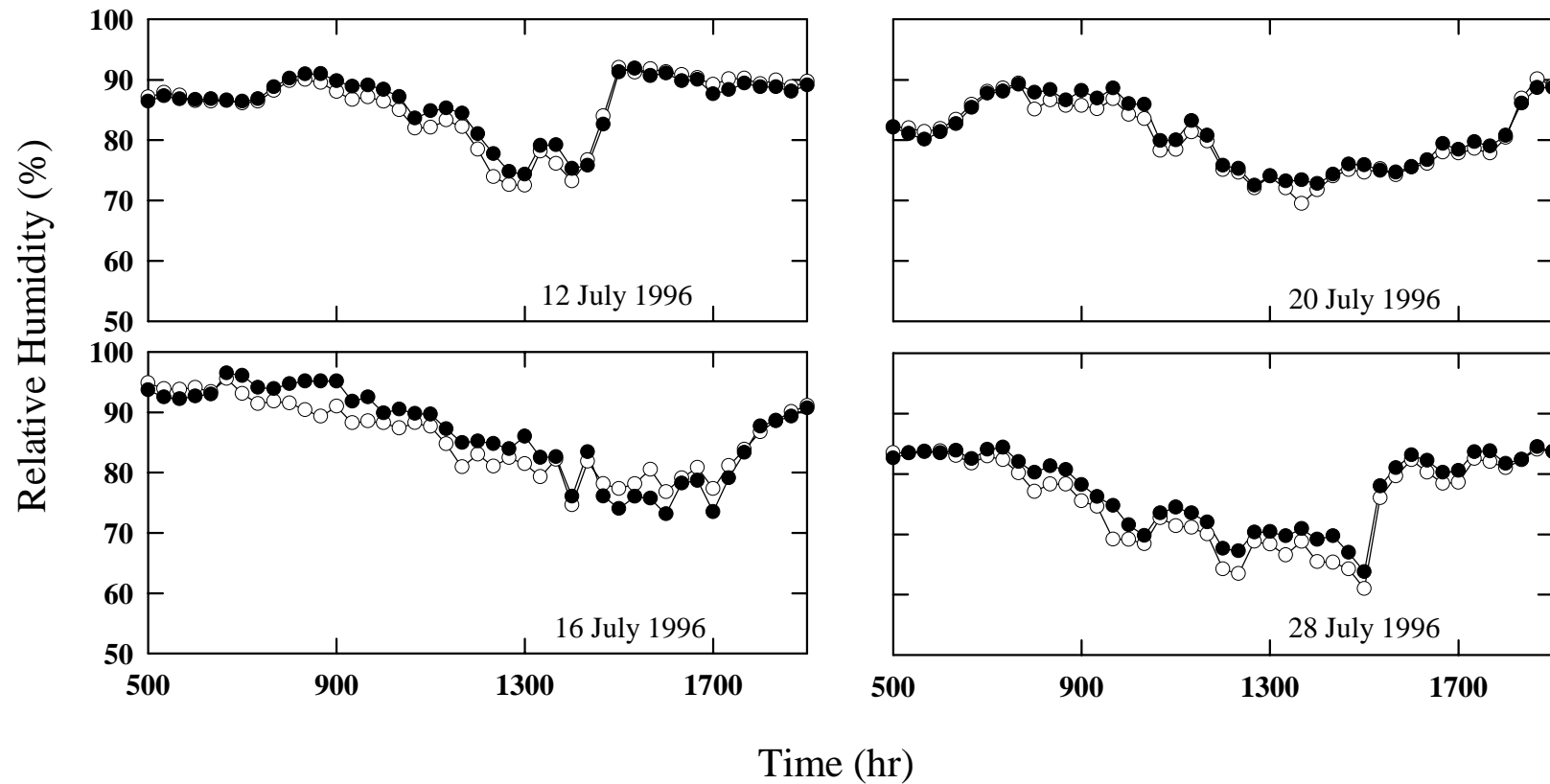


Figure 1.4: Daily variation in atmospheric relative humidity on four dates in 1996. Data were recorded with a shielded relative humidity sensor at seedling height. Open symbols are data recorded in forest without *R. maximum* while closed symbols show RH recorded in forest with *R. maximum*.

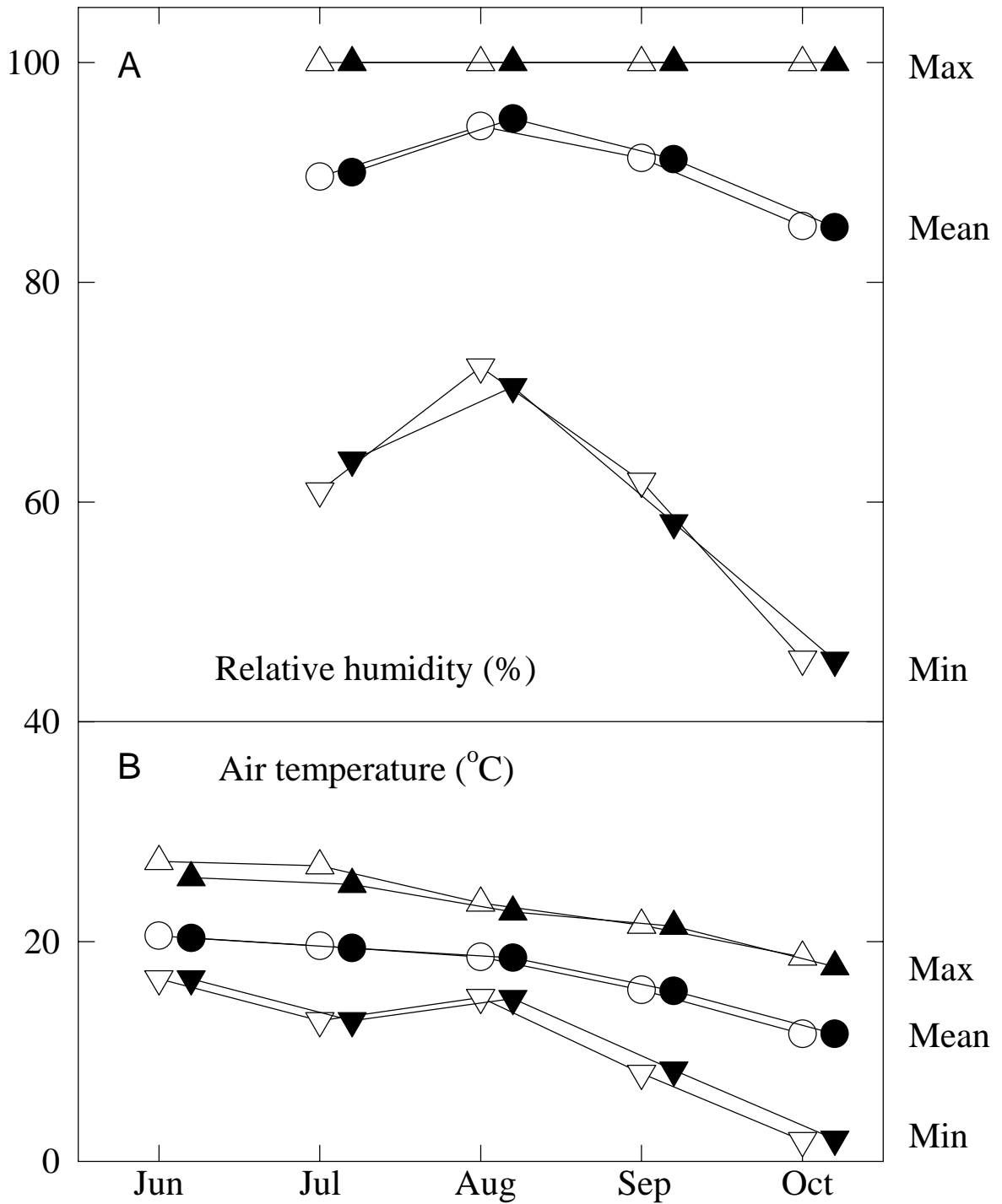


Figure 1.5: Daily midday maximum, minimum, and mean relative humidity (A) and air temperature (B) averaged by month in southern Appalachian forest sites with (*R. maximum* plot: closed symbols) or without (Forest plot: open symbols) a thicket of *R. maximum*.

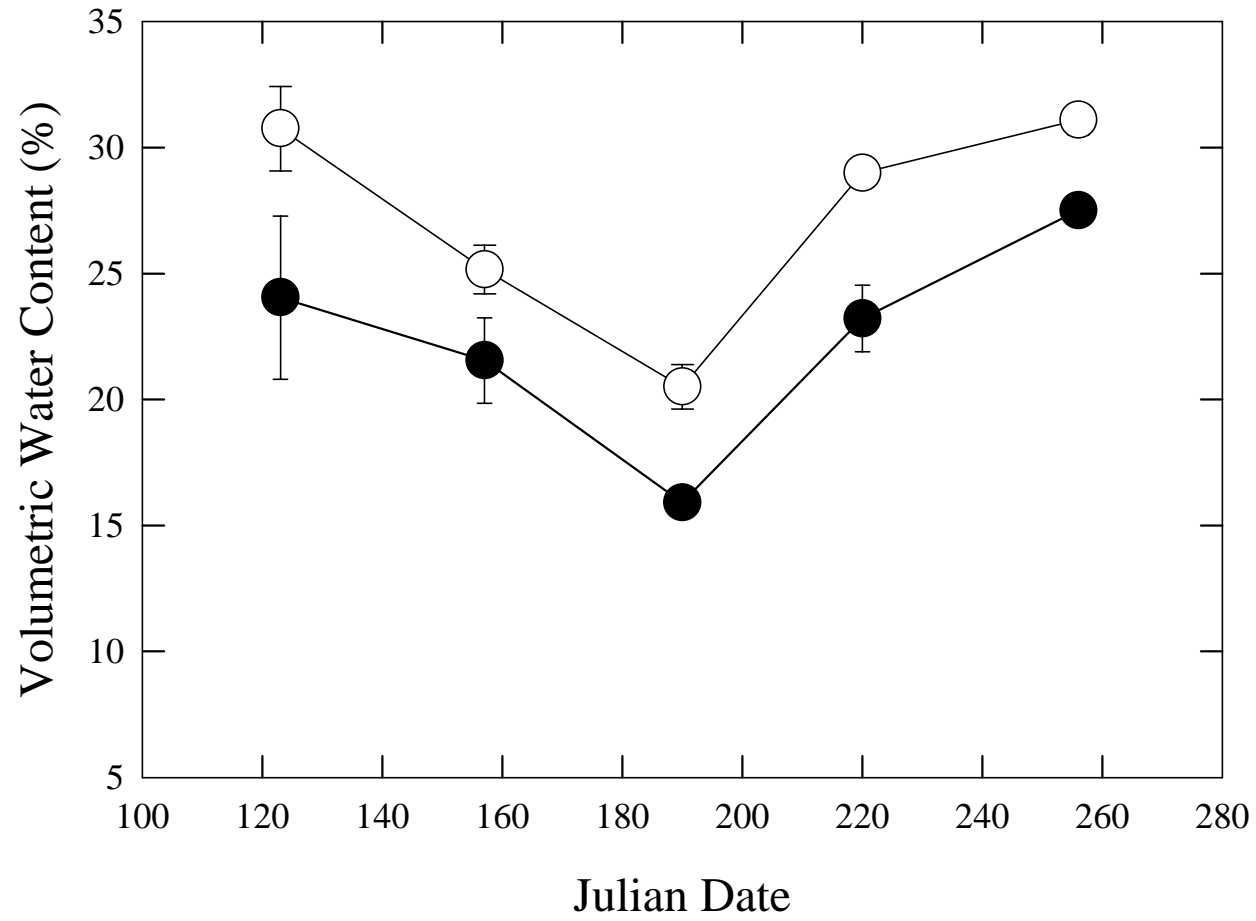


Figure 1.6: Soil moisture content at a depth of 0-15cm in Coweeta forest sites with (*R. maximum* plots: closed symbols) or without (forest plots: open symbols) a thicket of *R. maximum*. Each point refers to a mean of 45 samples. Error bars refer to one standard deviation on each side of the mean.

of variation in soil moisture among plots in each forest condition, but soil moisture was consistently lower by about 6% in *R. maximum* plots compared with forest plots (Figure 1.6) throughout the growing season. The repeated measure MANOVA indicated that there was a significant temporal variation (Pillai's trace=0.94,  $P < 0.001$ ), and the temporal variation between forest sites with and without *R. maximum* was also significant (Pillai's Trace=0.15,  $P = 0.01$ ), which indicated a significantly lower soil moisture in the forest when a *R. maximum* thicket was present.

The carbon content in the mineral soil of this forest ranged between 2.0 and 7.5 %. The mean carbon and hydrogen content of mineral soil in *R. maximum* plots was significantly lower than that of mineral soil in forest plots (Table 1.2). Soil nitrogen concentrations ranged from 0.1 to 0.34 %, and the mean N content of soil was lower in *R. maximum* plots than soil in forest plots. The mean carbon/nitrogen ratio (22.1) was consistent among samples (21 - 22.5), and there was no significant difference between forest soil types. Extractable nitrate was undetectable in all soil samples while extractable ammonia ranged from 0.5 to 2.0 ppm. There was no significant difference between extractable ammonium pool between forest types (Table 1.2). Nitrogen mineralization rates ranged between 3 and 10 mg N kg<sup>-1</sup> soil, and the average N mineralization rate was lower (but not significant,  $\alpha = 0.05$ ) in soil from *R. maximum* plots compared with that with forest plots (Table 1.2).

All soil samples were acidic with a pH ranging between 4.5 and 5.2. There was a significant difference in pH between forest soil types but that difference was small (Table 1.2). As with other acidic soils, these soils were characterized by high extractable

Table 1.2: Soil pH and nutrient concentrations for mineral soil at a depth of 10-15 cm in forest with a thicket of *R. maximum* present (Rhododendron) and without a *R. maximum* thicket (Forest). P-values represent significance level as evaluated by a two tailed, paired student's T test (n=45). P-values below 0.05 are considered to be significant.

Element	Unit	Rhododendron	Forest	p value
pH		4.75	4.85	0.006
Carbon (C)	% of soil mass	3.55	4.41	<0.001
Hydrogen (H)	% of soil mass	0.93	1.03	0.014
Nitrogen (N)	% of soil mass	0.16	0.20	<0.001
C/N	ratio	22.2	22.0	0.825
Nitrate (NO <sub>3</sub> <sup>-</sup> )	ppm in soil	0.001	0.001	---
Ammonium (NH <sub>4</sub> <sup>+</sup> )	ppm in soil	1.43	1.86	0.122
N-mineralization	mg N kg <sup>-1</sup> soil	5.96	8.61	0.086
Phosphorus (P)	ppm in soil	0.01	0.02	0.530
Potassium (K)	ppm in soil	31.99	42.44	<0.001
Calcium (Ca)	ppm in soil	27.04	46.12	<0.001
Magnesium (Mg)	ppm in soil	12.72	16.88	<0.001
Manganese (Mn)	ppm in soil	11.68	22.64	<0.001
Zinc (Zn)	ppm in soil	1.00	1.48	<0.001
Iron (Fe)	ppm in soil	40.92	38.24	0.212
Aluminum (Al)	ppm in soil	623.0	640.6	0.546
Copper	ppm in soil	0.67	0.56	<0.001
Boron (B)	ppm in soil	0.12	0.13	0.036



aluminum and iron averaging 630 and 40 ppm, respectively. Among heavy metal moieties only Cu, Zn and Mn had significantly different concentrations in the two soil types (Table 1.2). Copper content of the soil was higher in *R. maximum* plots, while Mn and Zn concentrations were lower in soil from *R. maximum* plots compared with soil from forest plots.

Extractable soil cation concentrations (K, Ca, Mg) were all significantly lower in soils from *R. maximum* plots than soil from forest plots (Table 1.2). Differences in cation concentrations (including Mn) between soil types were larger and more significant (lower P value) than that of any other soil elemental analysis.

Extractable phosphorus (P) concentrations ranged from undetectable to 1.6 ppm in the soil. The mean concentration of P in the soil was close to zero in both soil types and not significantly different. The concentration of P was the lowest among all extractable macro- and micro-elements analyzed in these soils.

## **Discussion**

### *Above ground resources*

The results of this study support our hypothesis on light intensity but did not support our hypothesis on air temperature and relative humidity. The most significant impact that *R. maximum* thickets have on the above ground environment of seedlings is the reduction of light availability. These results agree with those obtained by ceptometers (Clinton 1995). During the growing season, light intensity is normally below  $5 \mu\text{mol m}^{-2}$

$\text{s}^{-1}$  during daylight hours under a *R. maximum* thicket. These intensities are characteristic of extremely low light availability. The light intensity under the *R. maximum* thicket is below the light compensation point of photosynthesis for many woody seedlings of the eastern deciduous forest (Larcher 1995). There are infrequent periods of high irradiance (sunflecks) below *R. maximum* thickets that attain values close to  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  for a short time (from a few seconds to minutes). Given the extremely low diffused light, these periods of high light intensity should be carefully studied because they are likely to be critical for seedling survival in the understory of a *R. maximum* thicket.

#### *Below ground resources*

Our hypothesis on below ground resources is supported by the results for both soil moisture and nutrient availability. The 6% difference in soil moisture constitutes a 20% reduction in water availability for seedlings growing under *R. maximum* thickets compared to those growing in forest without *R. maximum*. This difference in soil moisture is probably due to increased interception, hence greater loss by evaporation, as well as, increased water use due to the greater total leaf area in sites occupied by *R. maximum*. The data presented in this study do not indicate that the observed difference in soil moisture has a significant impact on seedlings. However, low soil moisture availability may influence the ability of subcanopy seedlings to keep stomata fully open during sunflecks (Knapp and Smith 1990; Tinoco-Ojanguren and Pearcy 1993), thereby reducing the ability of seedlings to maximize carbon gain during sunflecks. Consequently, the lower soil moisture content under *R. maximum* thickets may

exacerbate the effect of light limitation by reducing seedling efficiency at utilizing sunflecks. Additionally, Clinton and Vose (1996) implicated lower soil moisture conditions under *R. maximum* thickets in reduced seed germination rates. They concluded that higher porosity of the organic mat below *R. maximum* thickets (compared with other forest sites) resulted in faster dry-down periods following rain events, resulting in seed radical desiccation following emergence. As a result, seedling establishment below *R. maximum* thickets could be inhibited by water stress during germination and during early seedling growth.

The soils at this site are extremely low in N and P availability. Because these nutrient resources are so low, it is possible that competition for these resources occurs. In the case of P, resource levels are approximately equal between forest types which suggests that the potential for competition in each forest type is approximately equal. However, N resources are lower under the *R. maximum* thickets suggesting that competition for N may be more intense under *R. maximum* thickets than in forest without *R. maximum*. Potential competition for resources under *R. maximum* thickets is also increased relative to the forest alone because of the large mass of *R. maximum* roots in upper soil layers (Semones personal observation).

Cation concentrations in soils under *R. maximum* thickets are particularly depleted compared to forest plots without *R. maximum* present. These results agree with data on nutrient accumulation by *R. maximum* (Monk et al. 1985). The living leaf mass of *R. maximum* stores a large quantity of cations such as Ca and Mg but does not retain a large quantity of N and P (Monk et al. 1985). Therefore, it is likely that the presence of a *R.*

*maximum* thicket depletes the availability of cations in the mineral soil because those nutrients are retained in the shrub biomass.

### *Conclusion*

The above and below ground microenvironment of establishing seedlings is strongly impacted by the presence of a *R. maximum* thicket in the southern Appalachian Mountains. Below ground resources could act synergistically with above ground processes by decreasing the ability of seedlings to maximize carbon gain during transient periods of higher light availability. These resource limitations may result in competition for resources between *R. maximum* and seedlings or adults of other species. Competition, particularly for light (as influenced by below ground resources), may be an important mechanism preventing seedlings of canopy tree species from surviving under *R. maximum* thickets. Resource manipulation experiments (fertilization, watering, etc.) are required to determine the potential significance of competition to seedling survival under *R. maximum* thickets.

### **Acknowledgments**

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## **Chapter 2**

**Low light inhibition of canopy tree seedlings in a temperate deciduous forest: the adverse influence of an expanding subcanopy evergreen plant.**

## Abstract

Thickets of *Rhododendron maximum* L. (Ericaceae) are spreading in eastern deciduous forests. This subcanopy evergreen species inhibits canopy tree seedling establishment, growth and survival. The purpose of this study was to determine if: 1) *R. maximum* influences above ground microclimate and light availability relative to forest sites without *R. maximum*; 2) mid-day photosynthesis of *Quercus rubra* L. (Fagaceae) and *Prunus serotina* Ehrl. (Rosaceae) seedlings is constrained within thickets relative to forest sites where *R. maximum*; and 3) tree seedlings within *R. maximum* thickets are low light acclimated relative to seedlings in forest without *R. maximum*.

Daily measurements of temperature and atmospheric relative humidity showed little difference in the presence and absence of *R. maximum*. Quantum sensors indicated that light intensity below *R. maximum*, was often 10 times lower during mid-day when compared to forest sites where *R. maximum* is absent. Attenuation of below canopy PAR by thickets of *R. maximum* negatively influenced the mid-day photosynthetic capacity of *Quercus rubra* and *Prunus serotina* seedlings. In 1996, the seasonal mean mid-day photosynthetic rate of first year *Q. rubra* seedlings growing in *R. maximum* thickets ( $1.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was 62% lower than the seasonal mean mid-day photosynthetic rate ( $2.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) of seedlings growing in forest sites without *R. maximum*. For second year seedlings in 1997, seasonal mean mid-day photosynthesis was 183% higher for plants growing outside of thickets ( $1.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) compared to the mean rate ( $0.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for plants within thicket sites. The mean mid-day PAR available to seedlings located

in forest sites without *R. maximum* during measurements of photosynthesis was 354% higher in 1996 and 257% higher in 1997. First year *Prunus serotina* seedlings growing in forest without *R. maximum* also had greater seasonal mean mid-day photosynthesis ( $0.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) when compared to the mean rate ( $-0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for plants growing within thickets. *Prunus serotina* seedlings located in the presence of *R. maximum* received on average 67% less PAR.

Photosynthetic acclimation to low light was assessed for *Q. rubra* and *P. serotina* seedlings growing under both forest conditions by measuring photosynthetic responses to light *in situ* using even aged one-year old seedlings. *Quercus rubra* seedlings growing in forest sites without *R. maximum* had significantly higher light saturated rates of photosynthesis. For both species, other derived parameters from photosynthetic responses to light were similar irrespective of the presence or absence of *R. maximum*. Forest community composition and succession will be influenced by the spread of *R. maximum* because extremely low light availability will preclude many tree species from establishing within shrub thickets.

**Key Words:** *Rhododendron maximum*, PAR, Photosynthesis, Light Response Curves, and Thicket.

## Introduction

Ecologists have long sought to determine the factors that regulate or influence forest community structure, production and dynamics. One such factor, expanding or invading evergreen subcanopy plant species, is slowly affecting temperate, tropical and boreal forests globally. In many of these systems, evergreen subcanopy plants are inhibitory to canopy tree recruitment and production, thus, affecting forest species composition and ultimately ecosystem functions over the long term. For example, in the western US, coniferous forests of the Cascade Mountains often contain a subcanopy of *Galtheria shaloni* that can interfere with seedling survival (Klinka et al. 1989; Messier 1993). Also, broad leaf palms in the understory of Costa Rican rain forests inhibit recruitment of canopy tree seedlings (Denslow et al. 1991). Furthermore, in Chile (Veblen 1982), two species of *Chusquea* (dwarf bamboo) inhibit regeneration of southern beech (*Nothofagus*), and in Costa Rica, *Chusquea* inhibits regeneration of oak species in high elevation forests (Widmer 1998). The invasive species, *Rhododendron ponticum* inhibits regeneration of canopy trees in the United Kingdom (Fuller and Boorman 1977; Cross 1981; Mitchell et al. 1997). Species of dwarf bamboo in the temperate deciduous forest of SW China inhibit regeneration of *Abies* and *Betula* (Taylor and Qin 1992), while another species of dwarf bamboo (*Sasa*) can completely exclude all other vegetation on the forest floor (Nakashizuka and Numata 1982). Despite mounting evidence of the inhibitory influence of many subcanopy evergreen species, the mechanisms of inhibition have yet to be determined.

In deciduous forests of the eastern United States, a model system exists for the study of evergreen subcanopy influence on forest communities. The evergreen shrub *Rhododendron maximum* L. (Ericaceae) covers approximately 7.4 million hectares of the understory in these Appalachian forests, often growing in dense thickets up to 20 hectares in area (Monk and Day 1984; Monk et al. 1985). Shrub thickets are frequently located in riparian zones, coves, or along mesic, north facing slopes. These sites are usually rich in hardwood tree species and are believed to be critical for nutrient exchange between terrestrial and aquatic ecosystems (Gregory et al. 1991; Nilsen et al. manuscript submitted). Following disturbance, the shrubs can quickly re-sprout vegetatively and rapidly layer to fill canopy openings. Thickets modify the above and below ground microenvironment by creating dense shade, producing heavy litter, and forming very thick, dense root layers.

Various studies have demonstrated an inhibitory influence of *R. maximum* on the establishment, growth, and survival of canopy tree seedlings (Minkler 1941; Wahlenberg 1950; Niering and Egler 1956; Cross 1975; Fuller and Boorman 1977; Phillips and Murdy 1985; Monk et al. 1985; Plocher and Carvell 1987; Clinton et al. 1993; Clinton and Vose 1996; Nilsen et al. manuscript submitted). There is also evidence that mature thickets might limit the growth and production of mature canopy trees located within a thicket perimeter (Monk et al. 1985; Elliot and Vose 1995), possibly by sequestering nutrients in the long-lived evergreen leaves and slowing the nutrient turnover rate. However, as with many evergreen subcanopy species, the exact mechanisms responsible for the inhibitory interaction between *R. maximum* and canopy tree species still remain

unknown. Because the percentage cover of *R. maximum* in eastern deciduous forests is increasing (Monk et al. 1985; Dobbs 1995; Baker and Van Lear 1998), the effects of thicket establishment on forest communities will become more pronounced.

Consequently, mechanisms of thicket inhibition need further elaboration.

Several plausible mechanisms are currently being explored. Initial research indicates that below ground resource availability is lower within thickets of *R. maximum* than in forest lacking *R. maximum*. Thicket soils have lower soil water content, cation availability, slower nitrogen mineralization rates, (See Chapter 1) and depressed mycorrhizal colonization potential (Walker et al. 1999). Below ground resource limitations within thicket perimeters could therefore place understory plants at a competitive disadvantage relative to plants located outside of thicket influence.

Perhaps the most dramatic impact of *R. maximum* thickets on understory plants is the result of above ground resource limitation. The multi-layered evergreen subcanopy that develops, further attenuates canopy photosynthetically active radiation (PAR) in comparison with forest without *R. maximum* (Clinton and Vose 1996; See Chapter 1). In fact, average ambient PAR within thickets of *R. maximum* is thought to be lower throughout the day when compared to forest without *R. maximum* (See Chapter 1). Such a reduction in diffuse radiation within *R. maximum* thickets could be detrimental for regenerating tree seedlings because light is often the limiting resource for plant carbon gain in the forest understory (Chazdon 1988; Pearcy 1983; Kobe et al. 1995; Walters and Reich 1996). Tree seedlings that germinate within *R. maximum* could ultimately suffer

from carbon limitation, and as a result, successful regeneration and survival of tree seedlings, regardless of shade tolerance, would be inhibited (Kobe et al. 1995).

Although the suppressive nature of *R. maximum* thickets has been recognized for some time, an understanding of tree seedling physiology within these forests is virtually non-existent. To better understand the ecological mechanisms of thicket inhibition, the physiological responses of forest tree seedlings in these changing systems need to be determined. The purpose of this study was to answer three questions concerning the effect of *R. maximum* on the photosynthetic capacity of forest tree seedlings. First, are above ground climatic conditions the same between forest sites with and without *R. maximum*. Second, are light levels in forest sites with and without thickets of *R. maximum* sufficient for seedlings to maintain a positive daily photosynthesis? If so, do seedlings growing within thickets of *R. maximum* exhibit lower mid-day photosynthetic rates relative to those growing within the forest without *R. maximum*? Third, is the photosynthetic response to light of seedlings in forest with *R. maximum* the same as that for seedlings growing in forest without *R. maximum*? In other words, do seedlings growing within thickets of *R. maximum* have the capacity to acclimate to the lower quantities of PAR present within most thickets, such that daily net carbon gain is not compromised? The answers to these questions will improve our current model detailing the mechanisms by which evergreen subcanopy species inhibit canopy tree seedlings in the southern Appalachian Mountains and globally.

## Methods

### *Site description*

This study was conducted at Coweeta Hydrologic Forest Laboratory. A 2,185 ha US Forest Service experimental station, Coweeta is a member site of the Long Term Ecological Research (LTER) networks. The basin is located in the Nantahala Mountains of the Blue Ridge Mountain physiographic province near Otto, North Carolina (35°02' N, 83°24' W;). Vegetation includes northern hardwood, cove hardwood, oak, and oak-pine communities (Day et al. 1988). Elevations range from 675 m to 1592 m. Yearly average precipitation is 180 cm at the base climate station, and increases with elevation to over 220 cm (Swank and Crossley 1988). High moisture levels and mild temperatures, characteristic of the basin, place Coweeta in the marine, humid, temperate category of Koppen's climatic classification scheme (Swift et al. 1988). The study site selected was a mature, mixed-hardwood forest, located on north facing, 60% slope, at an elevation of 1000 m. A mosaic of *R. maximum* thickets covers at least half of the total area. Dominant overstory tree species include *Quercus rubra* L. (Fagaceae) and *Prunus serotina* Ehrl. (Rosaceae).

Within the study area six 0.25 ha plots (3 sites with *R. maximum* and 3 sites without *R. maximum*) were subjectively located, and in each large 0.25 ha plot fifteen 2X2 m subplots were selected. *Quercus rubra* acorns were collected from parent trees in the vicinity of the study site in the fall 1995 and subsequently cold stratified for 90 days at 5°C. *Prunus serotina* seeds, collected from the same physiographic region, were purchased from a local nursery and cold stratified for 90 days at 5°C. *Quercus rubra* is a



moderately shade tolerant species, and *P. serotina* is a shade intolerant species. Approximately fifteen randomly chosen *Q. rubra* acorns and *P. serotina* seeds were germinated directly in each 2X2 m subplot located within three sites with *R. maximum* and 3 sites without *R. maximum* sites (i.e., 15 subplots per site). Emerged seedlings of both species were tagged and utilized for gas exchange measurements in 1996 and 1997. Sufficient *P. serotina* seeds did not germinate until the spring of 1997; therefore, gas exchange data for *P. serotina* seedlings were only collected during the spring and summer of 1997.

#### *Microclimatic data*

Air temperature, atmospheric relative humidity (RH), and PAR were monitored daily in two forest locations (one with and one without *R. maximum*) 20 cm above the soil surface, to assess above ground, site specific differences in prevailing microclimatic conditions at seedling height. Air temperature was determined using copper constantan thermocouples. Atmospheric relative humidity was monitored using shielded relative humidity sensors (Campbell Scientific, Inc., model 217, Logan, Utah). PAR intensity was measured using level Li-Cor model 190-s quantum sensors (Li-Cor Inc., Lincoln, Nebraska). Data were collected every minute, and the ten-minute mean, minimum, and maximum were recorded using a model 21x micro-data logger (Campbell Scientific, Inc., Logan, Utah).

### *Diurnal photosynthesis*

All photosynthesis measurements were made using a Li-Cor model 6400 portable infrared gas analyzer, (IRGA) (Li-Cor, Inc., Lincoln, Nebraska). PAR intensity was recorded simultaneously with photosynthesis using an external 190-s quantum sensor (Li-Cor, Inc., Lincoln, Nebraska). CO<sub>2</sub> concentration within the leaf chamber was maintained constant at 390  $\mu\text{mol m}^{-2} \text{s}^{-1}$  using the 6400-01 external CO<sub>2</sub> source assembly. RH of the leaf chamber and the vapor pressure deficit (VPD) of the leaf were maintained within 1% by manually adjusting the system flow rate through the desiccant. To determine the timing of maximum photosynthesis and potential photosynthetic depressions, diurnal curves of photosynthesis were periodically measured on six, randomly chosen extant *Q. rubra* seedlings from both forest conditions (i.e., with and without *R. maximum*). During each diurnal cycle, photosynthesis was measured on the same 12 leaves in their natural orientation, approximately every 90 minutes, under ambient conditions. Each leaf was subsampled ten times at each measurement time.

### *Mid-day photosynthesis*

Mid-day maximum photosynthetic rates were collected on a randomly chosen subset of all seedlings in a randomly chosen subset of all subplots ( $n = 90$ ) on any given day. Mid-day photosynthesis was recorded for *Q. rubra* on four dates in 1996 and five dates in 1997, while data for *P. serotina* were collected on five separate dates in 1997. Sampling dates for both species ranged from the third week in May (following canopy

closure) to the second week in August. Photosynthetic rates for both species were measured on sunny days between 0900-1400 EST as determined by diurnal curves of photosynthesis.

### *Light response curves*

In 1997, light response curves of photosynthesis were measured in the field on seventeen, randomly selected, two-year-old *Q. rubra* seedlings (10 within *R. maximum* thickets) and thirteen *P. serotina* seedlings (7 within thickets of *R. maximum*). Photosynthetic responses to light were measured using the Li-Cor 6400 portable IRGA fitted with the 6400-02 LED light source. Light response curves were generated by inducing seedlings at saturating light ( $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 15 minutes then incrementally reducing the light intensity. Photosynthesis was logged when the photosynthetic rate at each light level stabilized and the total coefficient of variance of the IRGA's fell below 1%, 2 % at light intensities below  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Between 4 and 20 measurements were recorded at each light level for each leaf. At lower PAR intensities (i.e.,  $<20 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), photosynthesis was less stable and warranted recording more data points to capture the total variation.

Photosynthetic responses to light were fitted using a convergence algorithm that determined the best fitting quadratic function of the form:  $f(y) = (bx+c)/2/a - ((bx+c)^2 - 4abcx)^{0.5}/2/a$  (Sigma Plot v. 4.0, Sigma Plot). The light saturation point of photosynthesis ( $I_s$ ) was chosen as the point at which the rate of photosynthesis did not increase more than

5 % with any additional increase in light. Light saturated photosynthesis ( $A_{\max}$ ) was determined as the maximum photosynthesis at 1000-1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . To delineate the light compensation point ( $I_c$ ) and apparent quantum yield ( $\Phi_i$ ), photosynthetic rates between 0 and 40-60  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR were fitted using linear regression of the form  $y = mx + b_0$  where  $y$  = photosynthesis,  $m$  is the  $\Phi_i$ ,  $b_0$  is the apparent dark respiration, and  $I_c$  is PAR intensity  $x$  when  $y=0$ . A two sample t-test was used to detect significant differences in derived parameters within a species growing in forest sites with and without *R. maximum* (Sigma Stat v. 2.0, Jandel Corp.).

## Results

### *Microclimate*

Examples of diurnal cycles of air temperature, RH, and ambient PAR from two separate sunny days in July 1996 are shown (Figure 2.1). Daily RH was uniformly high within and outside of *R. maximum* thickets, and ranged between 78-90% for both forest conditions (Figure 2.1A & B). Relative humidity was often near 80-90% in the morning and gradually declined with increasing air temperature reaching a minimum in the afternoon. Frequent afternoon thunderstorms, common to the Coweeta basin, or late afternoon cooling as the sun set often caused RH to increase after 1300 EST (Figure 2.1A & B). Mean RH throughout the day did not differ significantly among forest sites but was often 1-5 % higher within the *R. maximum* thicket (Figure 2.1A, & B).

Mean air temperature was within 2° C between forest sites with and without *R. maximum* throughout the day (Figure 2.1C & D). Average daily temperatures ranged between 19-22°C for both forest conditions. From the two days shown the maximum temperature recorded was 24.8°C on 12 Jul 96 in the forest site without *R. maximum*, while the minimum temperature, 16.5°C, was recorded on 28 Jul 96 in the forest site with *R. maximum*. Moderately higher air temperatures occurred during mid-day hours in forest without *R. maximum* likely due to higher intensities of PAR in the absence of a *R. maximum* subcanopy. Temperature response curves demonstrate no change in photosynthesis over the range of temperatures and temperature differences measured throughout the study (unpublished data).

Ambient PAR was consistently higher throughout the day in forest without *R. maximum* (Figure. 2.1E & F) compared to forest with *R. maximum*. Daily mean PAR, between 0500-1900 EST was between 7-15  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the forest site without *R. maximum* and 1-4  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in forest with *R. maximum*. During the growing season mean mid-day PAR (1100-1400 EST) within thickets of *R. maximum* was less than 5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  while in the forest mean mid-day PAR was 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (See Chapter 1)). Daily mean PAR throughout the growing season was 81% higher in the forest plot without *R. maximum* than in forest with *R. maximum* (Data not shown). Although there were considerable spatial and temporal variations in light quantity (Figure 2.1E & F), light availability within forest without *R. maximum* was almost always higher when compared to forest with *R. maximum*.

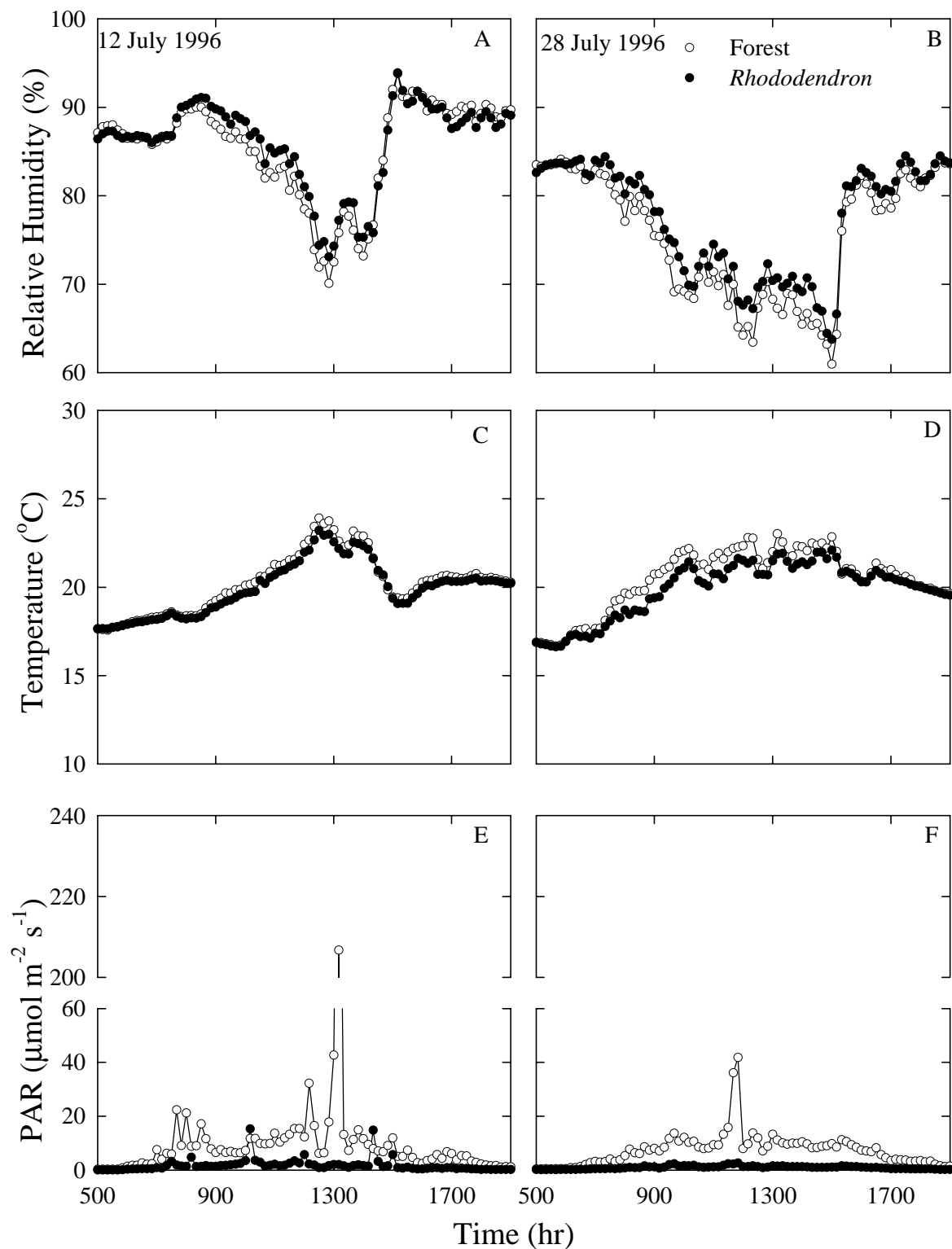


Figure 2.1: Diurnal curves of atmospheric relative humidity (A and B) air temperature (C and D) and PAR (E and F) recorded on two separate dates in July 1996. Individual points represent the mean PAR recorded every ten minutes.

### *Gas exchange northern red oak*

Diurnal patterns of photosynthesis for *Q. rubra* seedlings were determined on sunny days using randomly chosen seedlings within both forest conditions to determine the period of maximum photosynthesis during the day and if a photosynthetic depression independent of light occurs after mid-day (Figure 2.2). Seedling photosynthesis fluctuated consistently with light intensity during the day. The highest photosynthetic rates occurred between 0900 and 1300 EST. Mid-day photosynthetic depressions were not apparent except during occasional afternoon cloud cover. Other diurnal curves measured at the same site have demonstrated similar results (Data not shown) with maximum rates of photosynthesis occurring between 0900-1500 hr depending upon available PAR intensity. Notice in the early afternoon that seedlings within forest with *R. maximum* had higher photosynthesis than seedlings in forest without *R. maximum* corresponding to ephemerally higher PAR at that time.

In 1996, the seasonal mean mid-day photosynthetic rate for all seedlings measured in forest plots without *R. maximum* ( $2.1 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.1 \text{ S.E.}$ , n=120) was 62% higher than that measured for seedlings in plots with *R. maximum* ( $1.3 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.1 \text{ S.E.}$ , n=111; Figure 2.3). Likewise, mean PAR intensity incident upon seedlings located in forest sites without *R. maximum* ( $32.3 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 8.1$ , n=120) was 355% higher than the incident PAR experienced by seedlings found within thickets of *R. maximum* ( $7.1 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 1.7$ , n=110), (Figure 2.3). Although spatial and temporal variation in PAR and mid-day photosynthesis is evident for seedlings in both forest

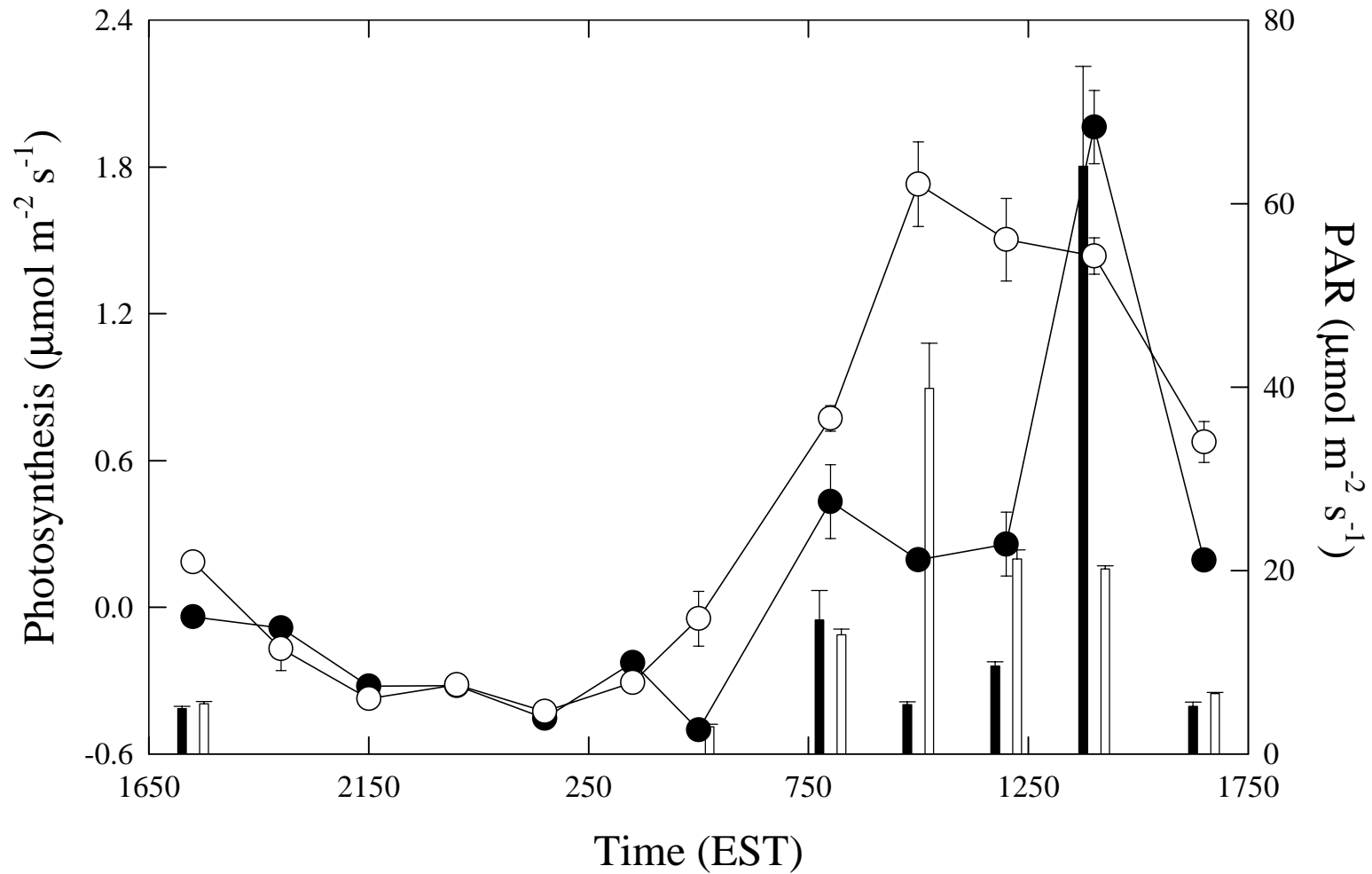


Figure 2.2: Representative diurnal curve of photosynthesis for *Q. rubra* seedlings located in forest without *R. maximum* (open symbols) and with *R. maximum* (closed symbols). Bars represent PAR incident upon the leaf when photosynthetic measurements were recorded. Each symbol and bar represent the mean  $\pm$  1 standard error ( $n = 60$ ; 6 plants subsampled 10 times).



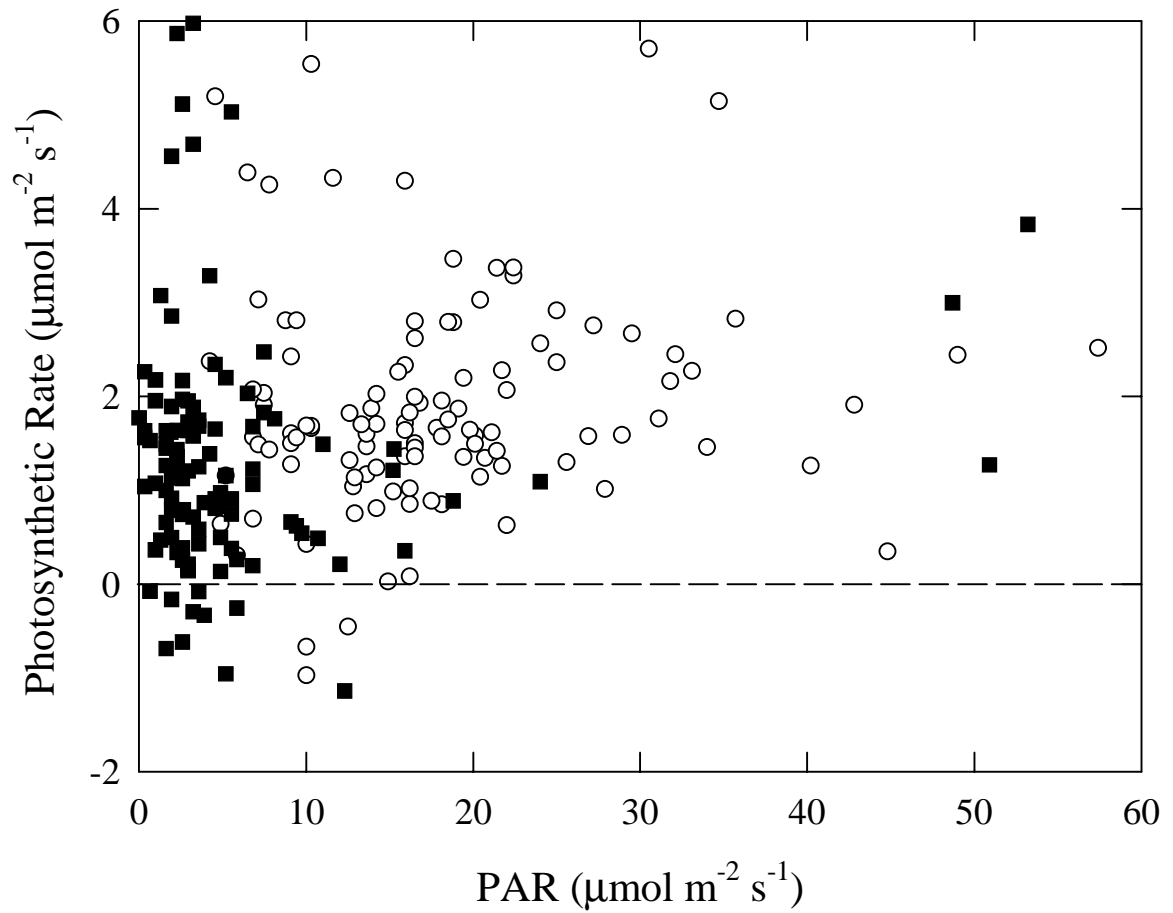


Figure 2.3: A summary of 1996 mid-day photosynthetic rates measured on first year *Q. rubra* seedlings. Mid-day photosynthetic rates for seedlings located in forest plots with *R. maximum* (closed symbols) and forest plots without *R. maximum* (open symbols) are graphed against incident PAR at the time photosynthesis was measured for the entire growing season. Symbols represent individual photosynthetic measurements collected on randomly chosen seedlings.

conditions, mid-day photosynthesis was generally higher for seedlings located in forest sites without *R. maximum* in 1996 (Figure 2.3). These data indicate that seedlings located within forest without *R. maximum* have a greater chance of experiencing higher PAR intensities during the growing season and as a result have higher mid-day photosynthesis when compared to seedlings in forest with *R. maximum*.

After pooling all 1997 data, the seasonal mean photosynthetic rate for oak seedlings located in forest sites, ( $1.7 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.2$ , n=80) was 183% higher than the seasonal mean photosynthetic rate, ( $0.6 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.1$ , n=121) of seedlings growing within a thicket (Figure 2.4A). Similar to patterns in 1996, the seasonal mean PAR experienced by oaks located within forest without *R. maximum* during photosynthetic measurements, ( $100 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 16.9$ , n=80) was 257% higher than the PAR incident upon seedlings located in thickets of *R. maximum* ( $28 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 5.1$ , n=121).

In 1997, the seasonal mean photosynthetic rate for *Q. rubra* seedlings located in forest without *R. maximum* was 19% lower than in 1996 despite seasonal mean PAR that was 213% higher. For seedlings located within *R. maximum* thickets, seasonal mean mid-day photosynthesis was 52% lower in 1997 than in 1996 despite a 291% increase in mean ambient PAR intensity. Average levels of PAR appear to be greater in 1997 because measurements of seedling photosynthesis were initiated two weeks earlier in May; consequently, the overstory canopy had not completely closed. However, an overall decline in mid-day photosynthesis is evident in two-year-old *Q. rubra* seedlings when compared to first year seedlings irrelevant of PAR intensity.

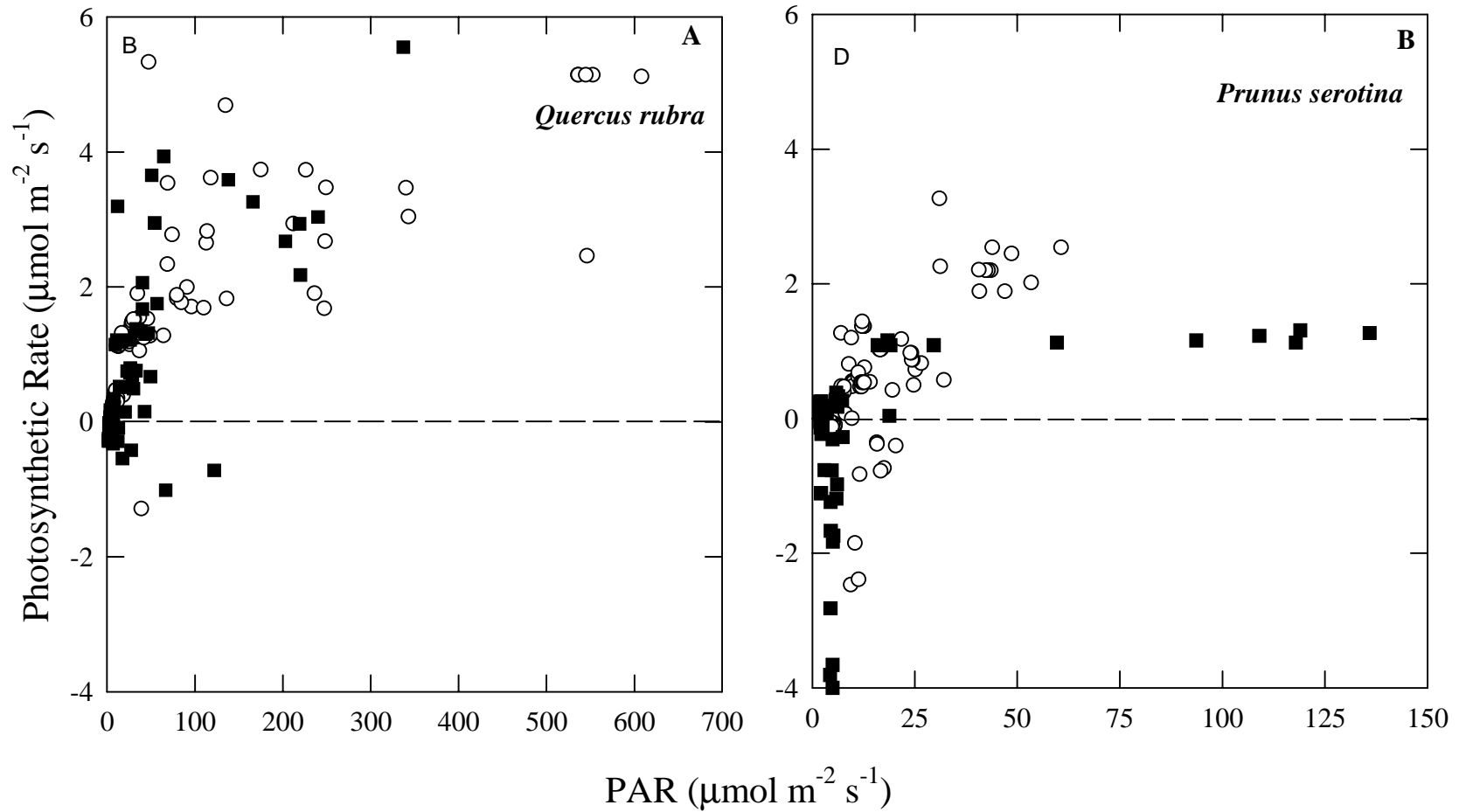


Figure 2.4: A summary of 1997 mid-day photosynthetic rates collected on both second year *Q. rubra* (A) and first year *P. serotina* (B) seedlings. Mid-day photosynthetic rates are graphed against incident PAR (at the time of photosynthesis measurement) for seedlings located in forest plots with *R. maximum* (closed symbols) and forest plots without *R. maximum* (open symbols) over the entire growing season. Each symbol represents an individual photosynthetic measurement taken on a randomly chosen seedling.

### *Gas exchange black cherry*

Mean mid-day photosynthesis in 1997 was greater for *P. serotina* seedlings located in forest without *R. maximum* ( $0.7 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.1$ , n=74) than for plants located within *R. maximum* thickets which exhibited a mean negative photosynthetic rate ( $-0.1 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.1$ , n=108; Figure 2.4B). Mean PAR intensity experienced by cherry seedlings within the open forest, ( $18 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 1.5$  n=74), was 67% greater, than the mean PAR intensity experienced by seedlings located under the *R. maximum* subcanopy ( $10.8 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 2.4$ , n=108).

### *Photosynthetic response to light*

The  $I_c$  point,  $\Phi_I$ ,  $I_s$  point, and  $A_{\text{max}}$  were derived for seedlings of both species from light response curves (Figures 2.5 & 2.6). Photosynthetic response to light was similar regardless of forest location. There were no differences in the  $I_c$  point,  $\Phi_I$ , or  $I_s$  point of *Q. rubra* seedlings in 1997 (Table 2.1). However,  $A_{\text{max}}$  was significantly higher ( $P < 0.05$ ) for *Q. rubra* seedlings located in forest without *R. maximum* (Table 2.1). Site location (i.e., with or without *R. maximum*) had no significant effect on derived light response parameters for *P. serotina* seedlings (Table 2.1). Light compensation points were extremely low for *Q. rubra* and *P. serotina* seedlings growing in both forest sites (Figure 2.6) corresponding to low respiration rates characteristic of shade grown plants. Apparent quantum yields of *Q. rubra* and *P. serotina* seedlings were similar (0.04-0.07  $\mu\text{mol CO}_2$  assimilated  $\mu\text{mol photons absorbed}^{-1}$ ) in both forest conditions.

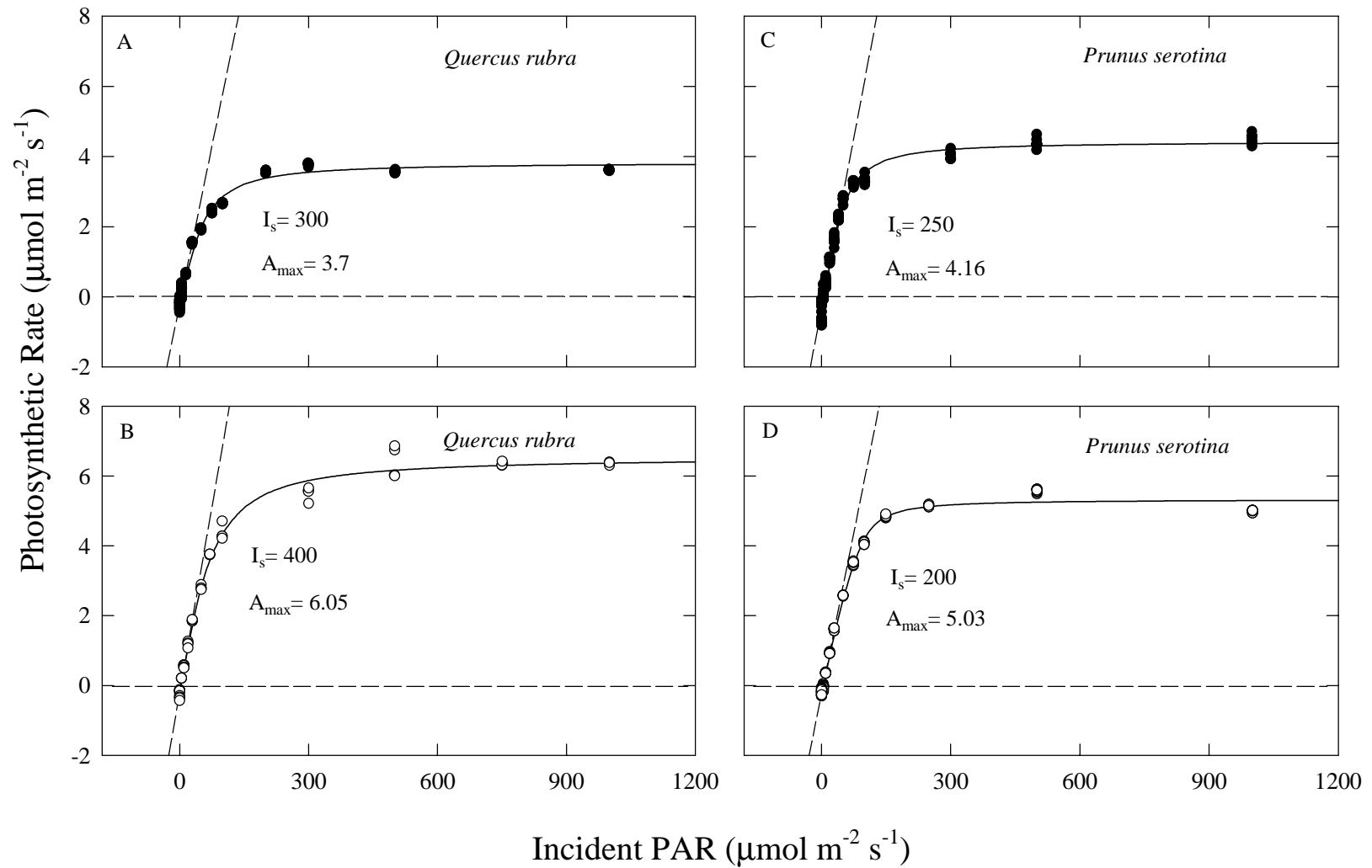


Figure 2.5: Representative light response curves measured during 1997 on individual *Q. rubra* and *P. serotina* seedlings located in forest with (closed symbols) and without (open symbols) *R. maximum*.

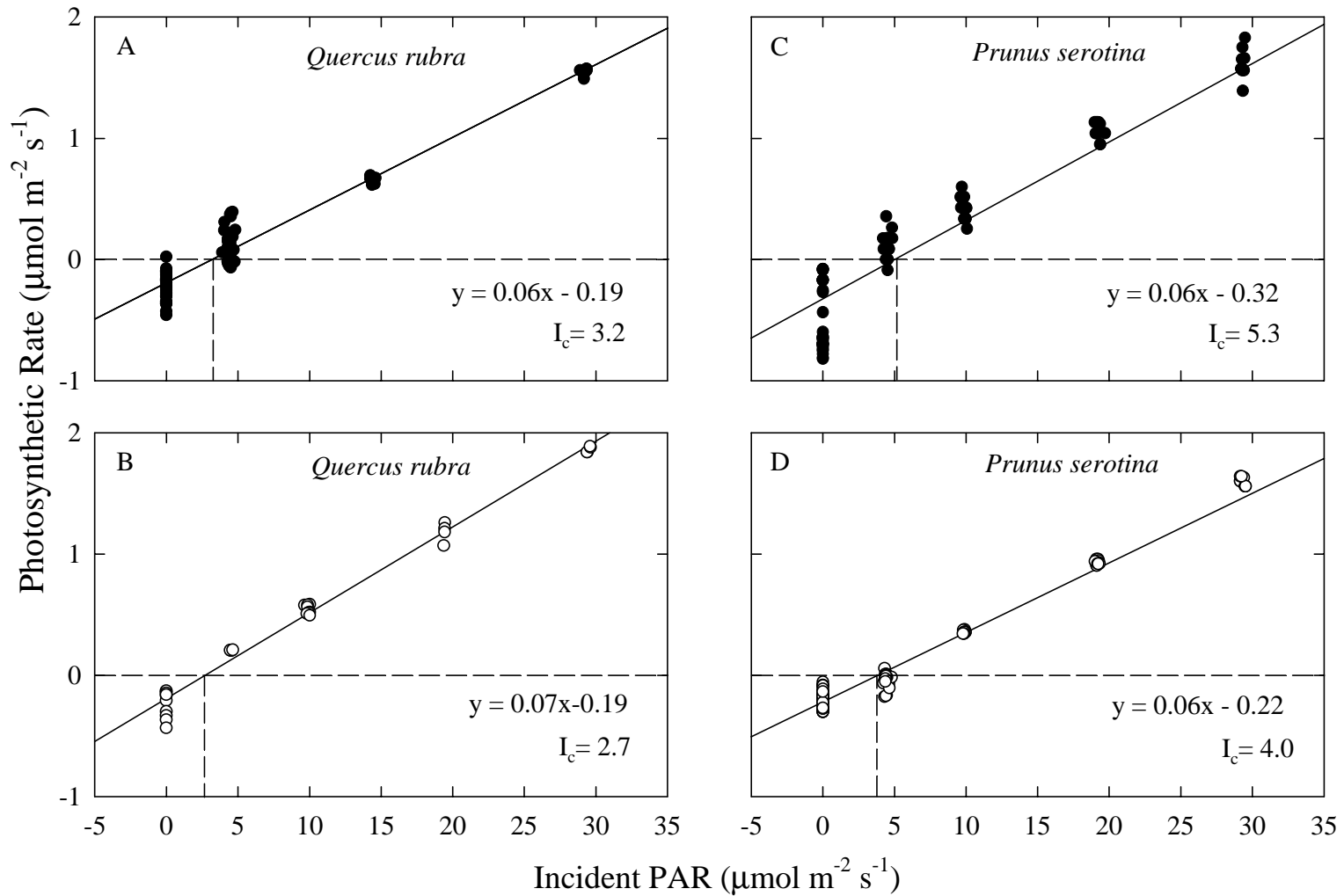


Figure 2.6: Estimates of quantum yield (slopes of the representative light response curves) and light compensation points for seedlings growing in forest with, (open symbols) and without, (closed symbols) *R. maximum*.  $I_c$  = light compensation point.

Table 2.1: Derived parameters from all light response curves generated during 1997 on both *Q. rubra* and *P. serotina* seedlings. Significant differences in means between forest and *R. maximum* sites were determined using a two sample t-test. Significantly differences are denoted by asterisks,  $\alpha = 0.05$ . Units:  $\Phi_1 = \mu\text{mol CO}_2$  assimilated/ $\mu\text{mol photons absorbed}$ ;  $I_c = \mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $I_s = \mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $A_{\text{max}} = \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$

Species	Site	Parameter	n	mean	std. error
<i>Q. rubra</i>	forest	Quantum yield ( $\Phi_1$ )	7	0.06	0.006
	<i>R. maximum</i>		10	0.05	0.006
	forest	Light compensation point ( $I_c$ )	7	2.8	0.3
	<i>R. maximum</i>		10	5.2	1.3
	forest	Light saturation point ( $I_s$ )	7	400	68.1
	<i>R. maximum</i>		10	360	53.6
	forest	Light saturated Pn ( $A_{\text{max}}$ )	7	5.2*	0.35
	<i>R. maximum</i>		10	3.4*	0.25
<i>P. serotina</i>	forest	Quantum yield	6	0.05	0.004
	<i>R. maximum</i>		7	0.06	0.004
	forest	Light compensation point	6	5.2	1.2
	<i>R. maximum</i>		7	4.6	0.85
	forest	Light saturation point	6	211	23.9
	<i>R. maximum</i>		7	192	24.3
	forest	Light saturated Pn	6	3.70	0.54
	<i>R. maximum</i>		7	3.0	0.38

## Discussion

There is increasing evidence that invasive thickets of *R. maximum* have a negative impact on successful canopy tree seedling regeneration within eastern deciduous forests (Minkler 1941; Wahlenberg 1950; Monk et al. 1985; Phillips and Murdy 1985; Plocher and Carvell 1987). Various mechanisms potentially responsible for this negative interaction have been proposed and include: 1) reduced above and belowground resource availability for regenerating seedlings in *R. maximum* thickets; 2) decreased seed rain due to the dense thicket subcanopy; 3) inhibition of canopy tree mycorrhizae in *R. maximum* soils; 4) higher concentrations of allelochemicals in thicket substrates; and 5) increased herbivory within shrub perimeters. Separately or together these mechanisms could inhibit forest tree seedling establishment.

The influence of low light intensity on photosynthetic capacity has been investigated on plants from ecosystems such as northern hardwood (Ellsworth and Reich 1992a), tropical (Chazdon and Field 1987), Virginia piedmont (Groninger et al. 1996), European deciduous (Johnson et al. 1997), and coastal plain forests (Jones and Mcleod, 1990) as well as old fields (Burton and Bazzaz 1995). All of these studies conclude that among all environmental factors measured photosynthetic capacity of understory plants is most highly dependent upon PAR. However, few studies have investigated the effects of PAR as low as that in mature forests at Coweeta on the photosynthetic capacity of understory plants.

Diffuse levels of PAR within mature forests at Coweeta are extremely low and variable (Figure 2.1E & F). Mid-day PAR during the growing season is approximately



1% full sun (i.e.,  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in those forests not covered by *R. maximum*, and less than 0.5% following thicket attenuation. These light levels are lower than any reported in the literature. Also, on days with maximum insolation in July 1997 (Figure 2.1E & F), ambient PAR did not reach photosynthetic saturation levels for either *Q. rubra* or *P. serotina* seedlings (Figure 2.1E & F and Table 2.1). However, ambient PAR was sufficient for *Q. rubra* seedlings to maintain positive  $\text{CO}_2$  assimilation rates in 1996 during 97% of all mid-day photosynthetic measurements in forest without *R. maximum* and 92% of the measurements under *R. maximum* (Figure 2.3). A similar trend was evident again in 1997 when 99% of the measured  $\text{CO}_2$  assimilation rates collected from *Q. rubra* seedlings were positive in sites where *R. maximum* was absent, while 69% of the photosynthesis measurements taken on seedlings under thickets of *R. maximum* were positive (Figure 2.4A). Of all photosynthesis measurements taken on *P. serotina* seedlings growing under *R. maximum* in 1997 62% were positive, while 77% of all measurements taken on seedlings in forest without *R. maximum* were positive (Figure 2.4B). Therefore, *Q. rubra* and *P. serotina* seedlings have a higher chance of having a positive net photosynthesis in forest without *R. maximum* than when growing in forest with *R. maximum*. However, seedlings growing under a thicket have a greater than 60% chance of having positive net photosynthesis.

The low ambient PAR in both forest types limits the photosynthetic capacity of *Q. rubra* and *P. serotina*. However, seedlings that germinate under the *R. maximum* subcanopy have a much greater chance of experiencing PAR that is near or below the light compensation point for photosynthesis. In fact, shade intolerant *P. serotina*

seedlings growing under *R. maximum* had a negative seasonal (bulking all measurements) mean mid-day photosynthetic rate while seedlings in forest without *R. maximum* had a positive seasonal mean mid-day photosynthetic rate. Consequently, *Q. rubra* and *P. serotina* seedlings that happen to germinate within thickets have lower daily and seasonal photosynthetic rates when compared to seedlings growing in forest where *R. maximum* is not present. These data provide unambiguous evidence that *R. maximum* shrub thickets inhibit the photosynthetic capacity of forest tree seedlings due to light limitation relative to plants in forest where shrub thickets have not spread.

Low light acclimation by seedlings growing in thickets is possible because ambient PAR in shrub thickets is strongly attenuated relative to forest without *R. maximum*. Typically, shade acclimated plants have lower  $A_{\max}$ , dark respiration rates and  $I_c$  points and higher carboxylation efficiency under lower PAR (Nilsen and Orcutt 1996); as a result, such plants are capable of withstanding longer periods of low-light suppression in the forest understory (Canham 1989). Thus, increased shade acclimation by *Q. rubra* and *P. serotina* seedlings could prolong survival under *R. maximum*, by improving seedling chances of experiencing and capitalizing on a gap in the overstory.

Both *Q. rubra* and *P. serotina* seedlings growing in forest with and without *R. maximum* exhibited little or no change in photosynthetic acclimation (Figures 2.5 & 2.6) despite dramatically different PAR intensity across forest sites. Seedlings of both species exhibited similar  $\Phi_P$ , extremely low  $I_c$  points and dark respiration rates, as well as low  $A_{\max}$  (Table 2.1), characteristic of shade acclimated plants (Figures. 2.5 and 2.6)(Pfitch and Pearcy 1989b; Larcher 1995; Nilsen and Orcutt 1996). Other studies have also failed

to demonstrate an effect of increased shade on photosynthetic acclimation (Midgely et al. 1992; Mitchell and Arnott 1995; Groninger et al. 1996), although none of these experiments were conducted in light environments as low as those present in the mature forests at Coweeta. In contrast, *Q. rubra* seedlings in forest without *R. maximum* did have significantly higher  $A_{\max}$  when compared to seedlings under shrub thickets. This result alone was consistent with other studies that have demonstrated an effect of increased PAR on maximum photosynthesis (Loach 1967; Teskey and Shrestha 1985; Ellsworth and Reich 1992; Holmes and Cowling 1993). Therefore data collected in this study do not support the hypothesis that *Q. rubra* or *P. serotina* seedlings within thickets of *R. maximum* exhibit a greater tolerance to lower PAR intensities and subsequently persist longer in these environments.

Analysis of light response curves also indicated that light saturation of photosynthesis occurred at approximately 10% full sun for *P. serotina* seedlings and 20% full sun for *Q. rubra* seedlings which is interesting in comparison to their relative low light tolerance. These results are important because rarely do light levels within either forest condition at Coweeta reach these intensities. Only in the presence of some sunflecks is the light saturation point succumbed by the light intensity. Therefore, efficient utilization of incident PAR during sunflecks is undoubtedly critical for seedling persistence not only in thickets of *R. maximum*, but also mature forest where PAR is still extremely low.

In general, the importance of light as a limiting resource for plant carbon gain in forest understory environments has been well documented (Pearcy 1983; Chazdon 1988;

Pfitch and Pearcy 1989a; Kobe et al. 1995; Walters and Reich 1996). Differential low light tolerance among plant species is likely the most important factor influencing forest succession and community composition (Glitzenstein et al.1986; Kobe et al. 1995; Walters and Reich 1996). In mature deciduous forests, such as those at Coweeta, ambient PAR intensity is not only far below saturating levels for photosynthesis but often can fall below the light compensation point (especially within thickets of *R. maximum*) for regenerating tree seedlings (Larcher 1995).

If greater light availability confers the advantage of increased carbon gain to seedlings in the forest understory, (Bazzaz 1979; Canham 1985; Canham 1989; Canham 1990), then plants growing in *R. maximum* thickets would likely experience a lower net yield of carbon over time. Reduced growth of saplings and seedlings under low light in other systems has been linked to increased plant mortality (Kobe et al. 1995; Walters and Reich 1996). Therefore, should light levels within thickets of *R. maximum* limit the photosynthetic capacity of forest tree seedlings, then the establishment, growth and longer-term survival of these seedlings will most certainly be inhibited. Except for those species that are either extremely low-light tolerant, or most efficient at utilizing PAR during sunflecks,

Of course, the relationship between light, photosynthesis, and ultimately carbon gain may not be perfectly correlated. Climatic conditions, above and belowground resource limitations, herbivory, allelopathy, as well as carbon allocation can affect seedling photosynthesis and growth irrespective of light availability. Various studies have shown that low light levels in conjunction with other stresses (e.g., reduced soil

moisture, nitrogen availability, or flooding) can limit maximum CO<sub>2</sub> assimilation as well as the growth and survival of tree seedlings and saplings (Daniels et al. 1979; Hicks and Chabot 1985; Jones et al. 1989; Reich et al. 1989; Ellsworth and Reich 1992). In addition, large site specific differences in air temperature and RH such as those resulting from changes in canopy cover can alter microsite VPD influencing stomatal conductance, and CO<sub>2</sub> assimilation of forest tree seedlings (Farquar and Sharkey 1982; Turner et al. 1984; Turner 1991). Minor differences in air temperature and RH evident between open forest sites and those covered by *R. maximum* (Figure 2.1) were not considered substantial enough to affect seedling photosynthesis under ambient light. Likewise, although seasonal soil moisture content is lower and nitrogen mineralization rates are slower in thicket soils (See Chapter 1), it is difficult to determine from these results if low-light inhibition of CO<sub>2</sub> assimilation experienced by understory seedlings is exacerbated by other stresses.

### *Conclusion*

Our research has demonstrated that light levels within both forest conditions at Coweeta are extremely dim, and ambient PAR in thickets of *R. maximum* is significantly attenuated when compared to forest where *R. maximum* is absent. Light levels are often sufficient for positive photosynthesis during mid-day, but it is apparent that light is an important limitation to CO<sub>2</sub> assimilation of tree seedlings and this limitation is accentuated under *R. maximum* thickets. These results together imply that sunflecks are critical for seedling CO<sub>2</sub> assimilation in these forests especially in those forests where *R. maximum* has spread. Finally, despite contrasting light conditions in forest environments

at Coweeta, seedlings under *R. maximum* thickets were not more shade tolerant than seedlings located in forest sites without *R. maximum*. The continued spread of *R. maximum* in eastern deciduous forests will alter community composition by precluding recruitment of most forest tree seedlings except for those that are extremely low light tolerant from establishing within these environments.

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## **Chapter 3**

**Do Subcanopy Evergreen Shrubs Inhibit Photosynthetic Response to Sunflecks?**

**Thicket Influence on the Carbon Gain of Oak Seedlings during Light Flecks.**

## Abstract

Thickets of *Rhododendron maximum* L. (Ericaceae) are increasing in total area in deciduous forest of the southeastern United States. This increase has important implications for forest dynamics because *R. maximum* thickets have been shown to inhibit the regeneration of woody species. Diffuse radiation is significantly lower within thickets of *R. maximum* when compared to forest without *R. maximum*. Because light is often the most limiting resource for understory plant carbon gain, maximizing carbon gain during sunflecks is critical for survival in the forest understory. The purpose of this study was to determine if the carbon gain of *Quercus rubra* L. (Fagaceae) seedlings during sunflecks is reduced within thickets of *R. maximum* when compared to seedlings in forest without *R. maximum*.

To determine the effect of *R. maximum* on the photosynthetic response to sunflecks of oak seedlings, light flecks were simulated on 288 randomly chosen, even aged, two-year old seedlings *in situ*. Half of the seedlings were located within *R. maximum* thickets. Seedlings were randomly assigned one of four light fleck durations (30, 60, 120, and 300s) and one of three intensities (100, 500, 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Half of all seedlings were dark pre-acclimated prior to light fleck simulations by covering with aluminum foil for at least 12 hours, while the remaining seedlings were pre-acclimated under ambient conditions.

Analysis of covariance showed that a significant, positive, linear relationship exists between the length of a light fleck and total carbon gain during a light fleck for seedlings in forest sites with and without *R. maximum* regardless of pre-acclimation



status, or light fleck intensity. Furthermore, there was a significant effect of *R. maximum* on the slope of the relationship such that following ambient pre-acclimation, seedlings located within thickets assimilated significantly less carbon with increasing light fleck length than seedlings located in forest sites without *R. maximum*. When seedlings were dark pre-acclimated there was no difference in carbon gain with increasing fleck length between seedlings in forest with and without *R. maximum* except for flecks of  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The data lead to the conclusion that under natural conditions the presence of *R. maximum* likely prohibits *Q. rubra* seedlings from utilizing sunflecks as effectively as seedlings growing in forest sites where *R. maximum* is absent.

**Key Words:** *Rhododendron maximum*, PAR, photosynthesis, sunfleck, and carbon gain.

## Introduction

Plant ecologists have long sought to determine the factors that regulate or influence forest community structure, production and dynamics. One such factor, expanding or invading evergreen subcanopy plant species affects forests on a global scale. In many forests, evergreen subcanopy plants are inhibitory to canopy tree recruitment, thus, potentially affecting forest community composition. For example, in the western US, coniferous forests of the Cascade Mountains often contain a subcanopy of *Galtheria shaloni* that can interfere with tree seedling survival (Klinka et al. 1989; Messier 1993). Also, broad leaf palms in the understory of Costa Rican rain forests inhibit recruitment of canopy tree seedlings (Denslow et al. 1991) while *Chusquea* (dwarf bamboo) inhibits regeneration of oak species in high elevation forests (Widmer 1998). Furthermore, in Chile two species of *Chusquea* inhibit regeneration of southern beech (*Nothofagus*) (Veblen 1982). Moreover, species of dwarf bamboo in the temperate deciduous forest of SW China inhibit regeneration of *Abies* and *Betula* (Taylor and Qin 1992), while another species of dwarf bamboo (*Sasa*) can completely exclude all other vegetation on the forest floor (Nakashizuka and Numata 1982). *Rhododendron ponticum*, an invasive species, inhibits regeneration of canopy trees in the United Kingdom (Fuller and Boorman 1977; Cross 1981; Mitchell et al. 1997). Despite mounting evidence indicating an inhibitory influence of many subcanopy evergreen species, the mechanisms of inhibition have yet to be determined.

In deciduous forests of the southeastern United States, an excellent system exists for the study of evergreen subcanopy influence on forest communities. The evergreen

shrub *Rhododendron maximum* L. (Ericaceae) covers approximately 7.4 million hectares of the understory in these Appalachian forests, often growing in dense thickets up to 20 hectares in area (Monk et al. 1985; Monk and Day 1984). Shrub thickets are frequently located in riparian zones, distributed through coves, or along mesic, north facing slopes. The interface sites that *R. maximum* inhabits are usually rich in hardwood tree species and are believed to be critical for ecosystem processes such as nutrient cycling (Gregory et al. 1991; Nilsen et al., manuscript submitted). Following disturbance, *R. maximum* can regenerate vegetatively or by seed and can rapidly fill canopy openings. Thickets modify the above and below ground microenvironment by creating dense shade, producing heavy litter, and forming dense root layers.

Various studies have demonstrated an inhibitory influence of *R. maximum* on the establishment, growth, and survival, of canopy tree seedlings (Minkler 1941; Wahlenberg 1950; Niering and Egler 1956; Fuller and Boorman 1977; Phillips and Murdy 1985; Monk et al. 1985; Plocher and Carvell 1987; Clinton et al. 1993; Clinton and Vose 1996; Baker and Van Lear 1998). Furthermore, there is also evidence that mature thickets might limit the growth and production of mature canopy trees located within a thicket perimeter (Monk et al. 1985; Elliot and Vose 1995; Baker and Van Lear 1998), possibly by sequestering nutrients in the long-lived, evergreen leaves and slowing nutrient turnover rates. As with many evergreen subcanopy species, the exact mechanisms responsible for the inhibitory interaction between the shrub and woody species remain unclear. Certainly, the effects of thicket establishment on forest communities will become more pronounced because the percentage cover of *R. maximum* in eastern deciduous

forests is increasing (Monk et al. 1985; Dobbs 1995; Baker and Van Lear 1998).

Consequently, the exact mechanisms of thicket inhibition need further elaboration.

Several plausible mechanisms of *R. maximum* thicket inhibition have been explored. Recent research indicates that below ground resource availability is lower within thickets of *R. maximum* than in forest lacking *R. maximum* (See Chapter 1). For instance, thicket soils have lower soil water content, cation availability, slower nitrogen mineralization rates, and depressed mycorrhizal colonization potential (Nilsen et. al manuscript submitted; Walker et al. 1999). Hence, soil resource limitations within thicket perimeters could place understory plants at a competitive disadvantage relative to plants growing in forest without *R. maximum*.

Perhaps the most dramatic impact of *R. maximum* thickets on understory plants results from above ground resource limitation. The multi-layered evergreen subcanopy that develops further attenuates photosynthetically active radiation (PAR) below the canopy (Clinton and Vose 1996; Nilsen et al. manuscript submitted). In fact, average ambient PAR within many thickets of *R. maximum* is significantly lower, often 10 times as low, throughout the day when compared to forest without *R. maximum* (See Chapter 1), plus the frequency and duration of sunflecks is also reduced (Lei et al., unpublished data). Such a reduction in diffuse and direct radiation within *R. maximum* thickets could be detrimental for regenerating tree seedlings because light is often the most limiting resource for plant carbon gain in the forest understory (Percy 1983; Percy and Calkin 1983; Chazdon 1988; Kobe et al. 1995; Walters and Reich 1996).

It is generally perceived that in the forest understory diffuse radiation is often well below saturating values for net photosynthesis of most canopy trees (Pearcy, 1983). More importantly, understory light levels may be below the light compensation point (i.e., that point where respiratory loss is balanced by photosynthetic gain) for many of these plants. Prior research has shown that this might be a common occurrence for plants located in forest with *R. maximum* present (Semones et al. manuscript submitted). Consequently, tree seedlings that germinate in forest with *R. maximum* could ultimately suffer more rapid carbon limitation than those that germinate in forest without a *R. maximum* subcanopy, and as a result, successful regeneration and survival of these tree seedlings, regardless of shade tolerance, would be inhibited (Kobe et al. 1995).

Although the suppressive nature of *R. maximum* thickets has been recognized for some time, an understanding of the photosynthetic response of tree seedlings to variable light within these forests is non-existent. If light is the limiting resource in these forest environments, then it is necessary to better understand seedling response to sunflecks. It has been shown that flecks of direct solar irradiance may only occur during 10% of the day, but they may contribute 20-90% of all the photosynthetically active light in the understory environment (Chazdon 1988; Pfitsch and Pearcy 1989). The purpose of this study is to determine whether seedlings growing in forest with *R. maximum* thickets experience constrained carbon gain during sun flecks of variable lengths and intensities when compared to seedling growing in forest without *R. maximum*.

## Methods

### Site description

This study was conducted at Coweeta Hydrologic Laboratory. A 2,185 ha US Forest Service experimental station, Coweeta is a member site of the Long Term Ecological Research (LTER) network. The basin is located in the Nantahala Mountains of the Blue Ridge Mountain physiographic province near Otto, North Carolina (35°02' N, 83°24' W). Vegetation has been described as northern hardwood, cove hardwood, oak, and oak-pine communities (Day et al. 1988). Elevations range from 675 m to 1592 m. Yearly average precipitation is 180 cm at the base climate station, and increases with elevation to over 220 cm (Swank and Crossley 1988). High moisture levels and mild temperatures, characteristic of the basin, place Coweeta in the marine, humid, temperate category of Koppen's climatic classification scheme (Swift et al. 1988). The study site selected was a mature, mixed-hardwood forest, located on north facing, 60% slope, at an elevation of 1000 m. A mosaic of *R. maximum* thickets covers at least half of the total area. Dominant overstory tree species include *Quercus rubra* L. (Fagaceae).

### *Light fleck simulations*

To assess seedling response to sunflecks in relation to forest type (i.e., with and without *R. maximum*) light flecks of various lengths and intensities were simulated in the field on seedlings *in situ*. The study was conducted over a six-week period in the summer of 1998 following full canopy closure using naturally occurring seedlings.

Within the study site, 144 even aged (two-year old) *Q. rubra* (red oak) seedlings were randomly selected from forest sites with *R. maximum* and 144 from sites without *R. maximum*.

All seedlings in both forest conditions with or without *R. maximum* were assigned one of two pre-acclimation light conditions, (i.e., dark prior or ambient prior). Dark pre-acclimated seedlings were covered with foil at least 12 hours prior to light fleck simulation to cause complete loss of photosynthetic induction. The purpose of dark pre-acclimation was to address seedling light fleck response with plants starting at an equivalent PAR exposure history. Ambient acclimated seedlings were exposed to light fleck simulations regardless of prevailing light conditions in the forest at that time, thus they were at some intermediate induction state between non induced and full induction. It was generally believed that under ambient pre-acclimation, seedlings growing in forest sites without *R. maximum* would be starting light fleck simulations at a higher pre-induction state than those in forest with *R. maximum* because of the higher ambient light levels in forest without *R. maximum*. Also, in each forest condition (i.e., with and without *R. maximum*) every northern red oak seedling was randomly assigned one of three light fleck intensities (i.e., 100, 500, or 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and one of four light fleck durations (30, 60, 120, 300s). Fleck durations were approximate to desired lengths because equipment limitations made it difficult to simulate the same length light fleck twice. Therefore, each light fleck duration, fleck intensity, and prior light combination (i.e., ambient or dark) was replicated six times for a total of 288 simulations.

Light flecks were simulated, and photosynthetic responses of seedling leaves were measured using the Li-Cor 6400 portable IRGA fitted with the 6400-02 LED light source (Li-Cor, Inc., Lincoln, Nebraska). An autoprogram was written and used to generate light fleck response curves. CO<sub>2</sub> concentration within the leaf chamber was maintained constant at 390  $\mu\text{mol m}^{-2} \text{s}^{-1}$  using the Li-Cor model 6400-01 external CO<sub>2</sub> source assembly (Li-Cor Inc., Lincoln, Nebraska). Relative humidity of the leaf chamber and the vapor pressure deficit (VPD) of the leaf were maintained within 1% by manually adjusting the system flow rate through the desiccant. Seedlings were pre-exposed to 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light for 10 minutes prior to light fleck simulation regardless of pre-acclimation treatment. Each light fleck response curve consisted of: 1) a 2 minute period at 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; 2) a high light fleck of desired intensity and duration; and 3) five minutes again at 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Photosynthesis was logged every 2-3 seconds during the entire light fleck response curve (Figure 3.1).

Four parameters were derived from each curve: 1) average photosynthesis prior to fleck at 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; 2) total carbon gain from fleck + 120s post fleck illumination at low light; 3) maximum photosynthesis reached during light fleck; 4) average photosynthesis at 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  following the light fleck. The effect of the presence or absence of *R. maximum* on the average induction of seedlings prior to light flecks was assessed using a two-sample t-test. Analysis of covariance was used to determine the relationship between fleck length and carbon gain or fleck length and maximum photosynthesis for seedlings from both forest with and without *R. maximum*, and to assess significant effects of the *R. maximum* subcanopy on total carbon gain, maximum photosynthesis, and the



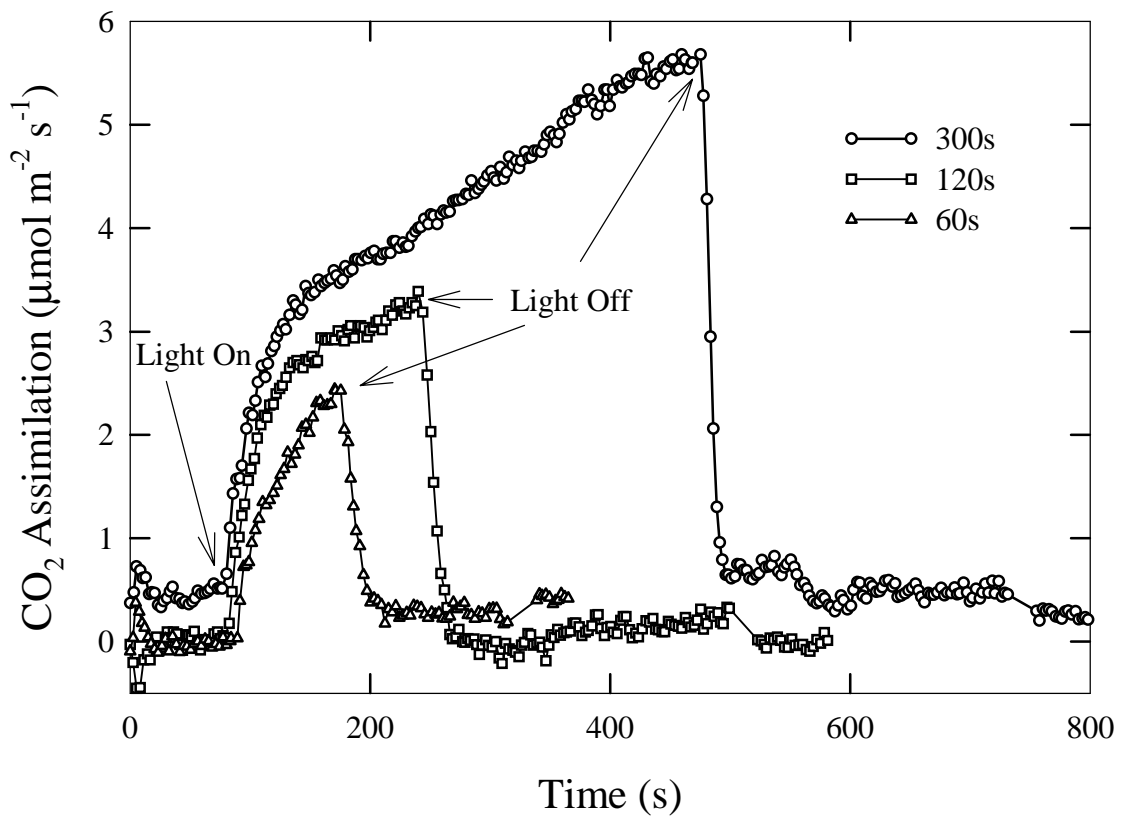


Figure 3.1: Photosynthetic response of northern red oak seedlings, located in forest without *R. maximum*, to three different duration light flecks at  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Post and pre fleck PAR was  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Seedlings were pre-acclimated under ambient PAR.

average induction after the fleck at  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Data were analyzed using SAS Proc GLM (SAS, SAS Institute Inc., Cary, N.C.).

## Results

### *Average ambient induction prior*

There was a significant effect of canopy type on the mean photosynthesis of *Q. rubra* seedlings at  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  before light fleck simulations regardless of whether seedlings were previously dark or ambient acclimated (Table 3.1). Under both acclimation conditions, seedlings in forest with *R. maximum* had significantly higher photosynthesis at low light than seedlings in forest without *R. maximum*.

### *Carbon gain from fleck + 120s post fleck illumination*

To determine if the presence of an evergreen subcanopy (i.e., canopy effect, with *R. maximum*) constrains total carbon gain during light flecks for oak seedlings, linear regression was used to relate total carbon gain from the light fleck, plus 120s post illumination  $\text{CO}_2$  assimilation, to fleck length. This was done separately for all three light intensities and for both prior acclimation conditions (Figures 3.2 & 3.3). Under both pre-acclimation conditions, and for all fleck intensities, fleck length described a significant amount of the variation in carbon gain for seedlings growing in both forest types, with and without *R. maximum*. Linear regressions demonstrate that as fleck length

Table 3.1: Average photosynthesis of *Q. rubra* seedlings at  $10 \mu\text{mol m}^{-2} \text{s}^{-2}$ . Data compiled for all curves for three light fleck intensities (i.e. 100, 500, and  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), four light fleck durations (i.e. 30, 60, 120, and 300s) under two pre-acclimation conditions (i.e. dark and ambient). Different letters denote significant differences between mean values. Notice comparisons are made between seedlings in forest sites with *R. maximum* and seedlings in forest sites without *R. maximum* not dark versus ambient pre-acclimation.

Pre-acclimation	Seedling photosynthesis in forest without <i>R. maximum</i>	Seedling photosynthesis in forest with <i>R. maximum</i>
Dark	$0.271 \pm 0.023^a$ n = 72	$0.348 \pm 0.025^b$ n = 72
Ambient	$0.257 \pm 0.023^a$ n = 72	$0.358 \pm 0.025^b$ n = 72

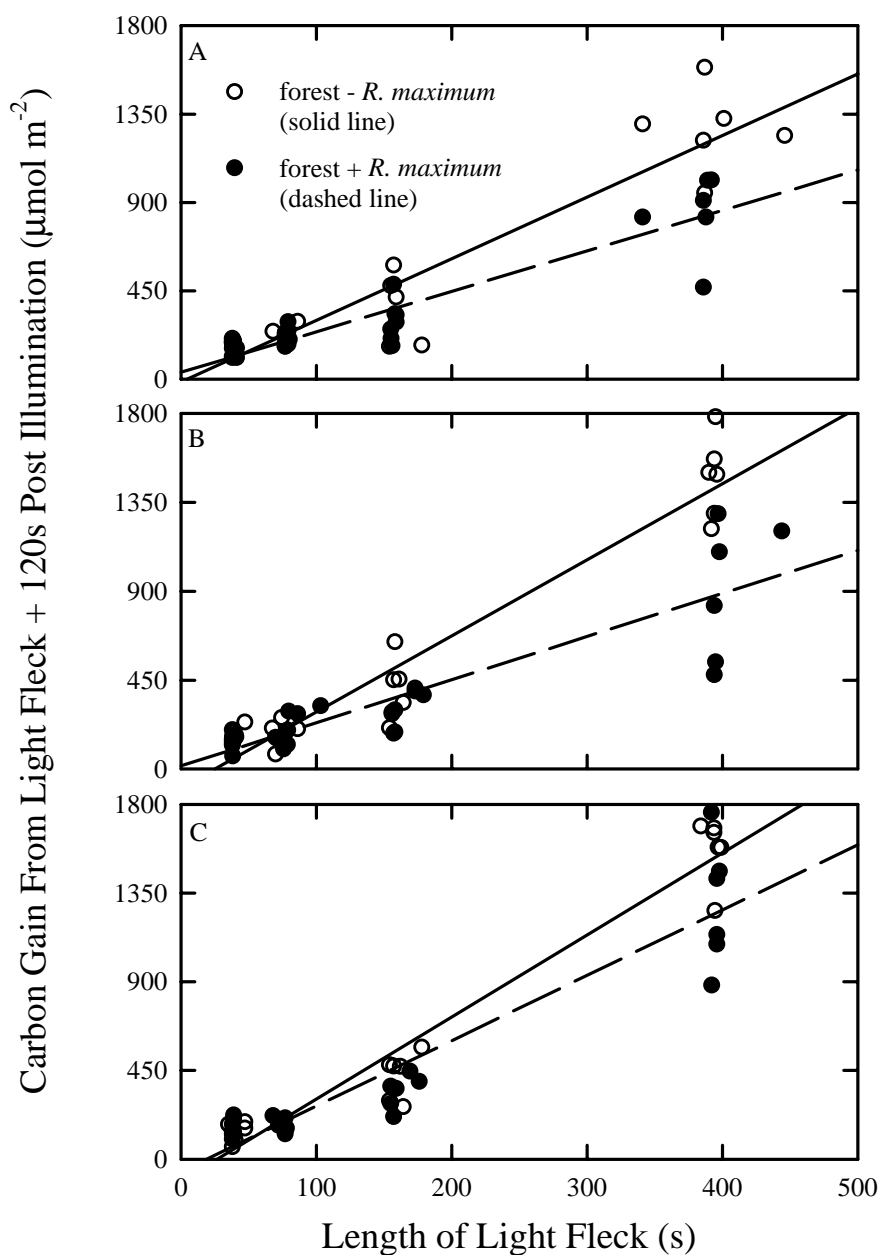


Figure 3.2: Significant positive regressions of seedling carbon gain from light fleck + 120s post fleck  $\text{CO}_2$  fixation at low light ( $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) versus light fleck length. Seedlings were pre-acclimated under ambient PAR. Each symbol represents light fleck response from one seedling. Lines are best-fit linear regressions. (A).  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 2.06x + 36.84$ ;  $\text{Rsqr} = 0.85$ ; Solid line:  $y = 3.14x - 14.55$ ;  $\text{Rsqr} = 0.88$ . (B).  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 2.18x + 15.74$ ;  $\text{Rsqr} = 0.89$ ; Solid line:  $y = 3.85x - 97.03$ ;  $\text{Rsqr} = 0.92$ . (C).  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 3.31x - 62.43$ ;  $\text{Rsqr} = 0.88$ ; Solid line:  $y = 4.16x - 110.16$ ;  $\text{Rsqr} = 0.95$ .

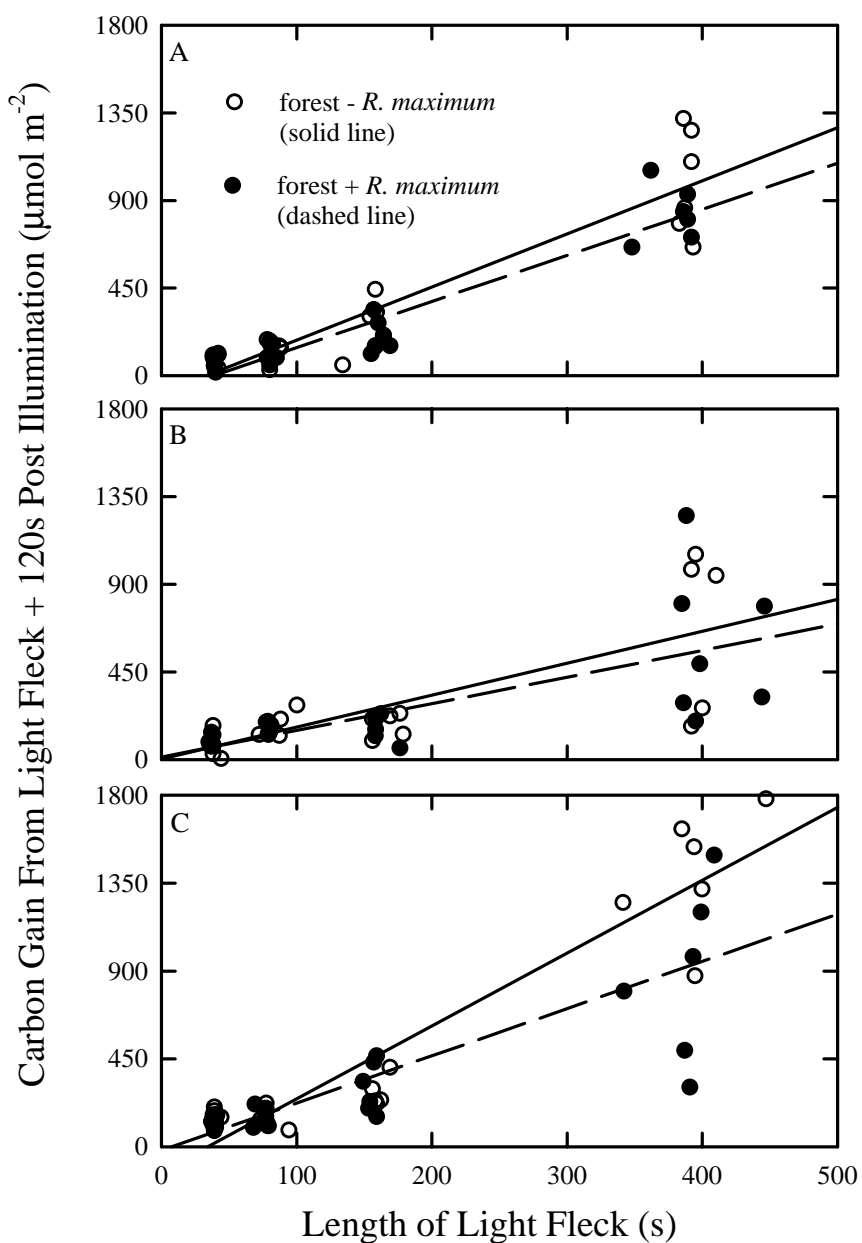


Figure 3.3: Significant positive regressions of seedling carbon gain from light fleck + 120s post fleck  $\text{CO}_2$  fixation at low light ( $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) versus light fleck length. Seedlings were pre-acclimated in the dark. Each symbol represents light fleck response from one seedling. Lines are best-fit linear regressions. (A).  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 2.37x - 91.67$ ;  $\text{Rsqr} = 0.90$ ; Solid line:  $y = 2.73x - 90.00$ ;  $\text{Rsqr} = 0.86$ . (B).  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 1.07x + 35.14$ ;  $\text{Rsqr} = 0.60$ ; Solid line:  $y = 1.77x - 6.74$ ;  $\text{Rsqr} = 0.62$ . (C).  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 2.42x - 17.83$ ;  $\text{Rsqr} = 0.77$ ; Solid line:  $y = 3.73x - 126.89$ ;  $\text{Rsqr} = 0.90$ .

increases, the carbon gained by seedlings growing in forest sites with *R. maximum* increased more slowly than seedlings growing in forest sites without *R. maximum* (Figures 3.2 & 3.3).

In all simulations, fleck length had a significant positive effect on carbon gain (Table 3.2). No significant differences in intercepts were noted (i.e., no significant canopy effects in table 3.2). However, under ambient pre-acclimation, a significant canopy\*fleck length interaction was found for all light intensities, which indicates that the effect of the *R. maximum* subcanopy was significant. Following dark acclimation, only at  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  was there a significant interaction (Table 3.2).

#### *Effect of fleck length on maximum photosynthesis during light fleck simulation*

For seedlings growing in both forest sites (i.e., with and without *R. maximum*) fleck length could be used to describe a significant amount of the variation in maximum photosynthesis for all light fleck intensities and under both prior acclimation conditions. The relationships, as indicated by R-square values, were not as strong as those were for carbon gain versus fleck length (Figures 3.2-3.5).

Analysis of covariance showed there were no significant effects of canopy type alone on the maximum photosynthesis of seedlings, irrespective of fleck intensity or prior acclimation status (Table 3.3). Regardless of prior pre-acclimation status of seedlings, for all fleck intensities there was a significant positive effect of fleck length on maximum photosynthesis achieved during light flecks (Table 3.3). The slopes of the curves from forest sites with *R. maximum* and without *R. maximum* under dark pre-acclimation at  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  were significantly different indicating a significant canopy effect at

Table 3.2: Analysis of covariance summarizing the effect of canopy, (i.e. with or without *R. maximum*), on seedling carbon gain from light fleck simulations + 120s post illumination at  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  using fleck length as a covariate. F values and P values obtained from the Type III SS for the effect of canopy, fleck length and the canopy by fleck length interaction on seedling carbon gain under three fleck intensities and both pre-acclimation conditions. Also shown is the coefficient of determination from the analysis of variance.

Prior Acclimation	Fleck Intensity	R-Square	F Values and Pr > F		
			Canopy	Fleck Length	Canopy*Fleck Length
Ambient	100	0.87	$F_{1,42} = 0.53$	<b><math>F_{1,42} = 259.23</math></b>	<b><math>F_{1,42} = 11.19</math></b>
			$P = 0.469$	<b><math>P = 0.0001</math></b>	<b><math>P = 0.0017</math></b>
	500	0.88	$F_{1,46} = 2.33$	<b><math>F_{1,46} = 313.29</math></b>	<b><math>F_{1,46} = 23.91</math></b>
			$P = 0.1338$	<b><math>P = 0.0001</math></b>	<b><math>P = 0.0001</math></b>
	1000	0.92	$F_{1,39} = 0.34$	<b><math>F_{1,39} = 435</math></b>	<b><math>F_{1,39} = 5.57</math></b>
			$P = 0.5658$	<b><math>P = 0.0001</math></b>	<b><math>P = 0.0233</math></b>
Dark	100	0.88	$F_{1,42} = 0.006$	<b><math>F_{1,42} = 291</math></b>	$F_{1,42} = 1.46$
			$P = 0.9795$	<b><math>P = 0.0001</math></b>	$P = 0.2342$
	500	0.58	$F_{1,42} = 0.05$	<b><math>F_{1,42} = 55.44</math></b>	$F_{1,42} = 0.9$
			$P = 0.8330$	<b><math>P = 0.0001</math></b>	$P = 0.3493$
	1000	0.85	$F_{1,44} = 1.41$	<b><math>F_{1,44} = 233</math></b>	<b><math>F_{1,44} = 10.63</math></b>
			$P = 0.2410$	<b><math>P = 0.0001</math></b>	<b><math>P = 0.0022</math></b>

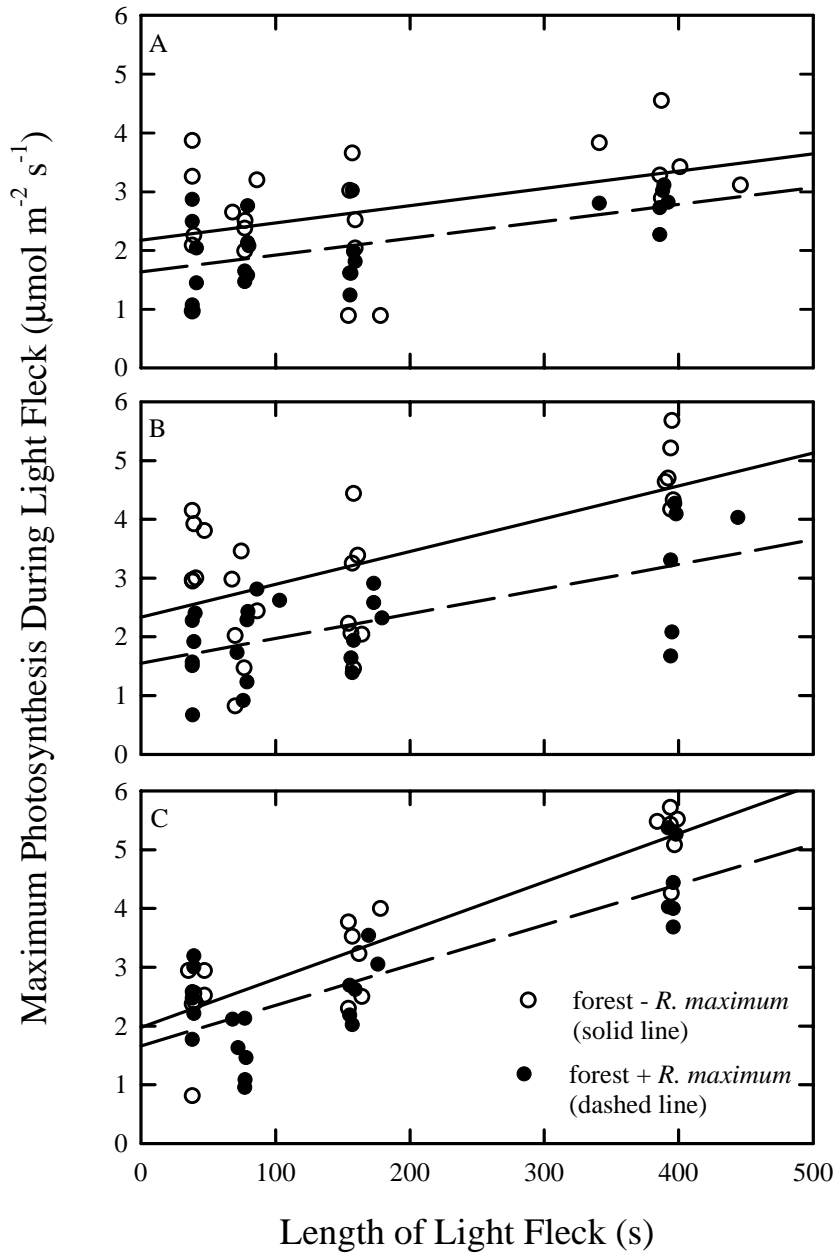


Figure 3.4: Positive regressions of maximum photosynthesis reached during a light fleck versus light fleck length. Seedlings were pre-acclimated in ambient PAR. Each symbol represents maximum photosynthesis from one. Lines are best-fit regressions through. (A).  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 0.003x + 1.64$ ;  $\text{Rsqr} = 0.34$ ; Solid line:  $y = 0.003x - 2.12$ ;  $\text{Rsqr} = 0.19$ . (B).  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 0.004x + 1.55$ ;  $\text{Rsqr} = 0.41$ ; Solid line:  $y = 0.006x + 2.34$ ;  $\text{Rsqr} = 0.38$ . (C).  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 0.007x + 1.66$ ;  $\text{Rsqr} = 0.66$ ; Solid line:  $y = 0.008x + 1.97$ ;  $\text{Rsqr} = 0.81$ .



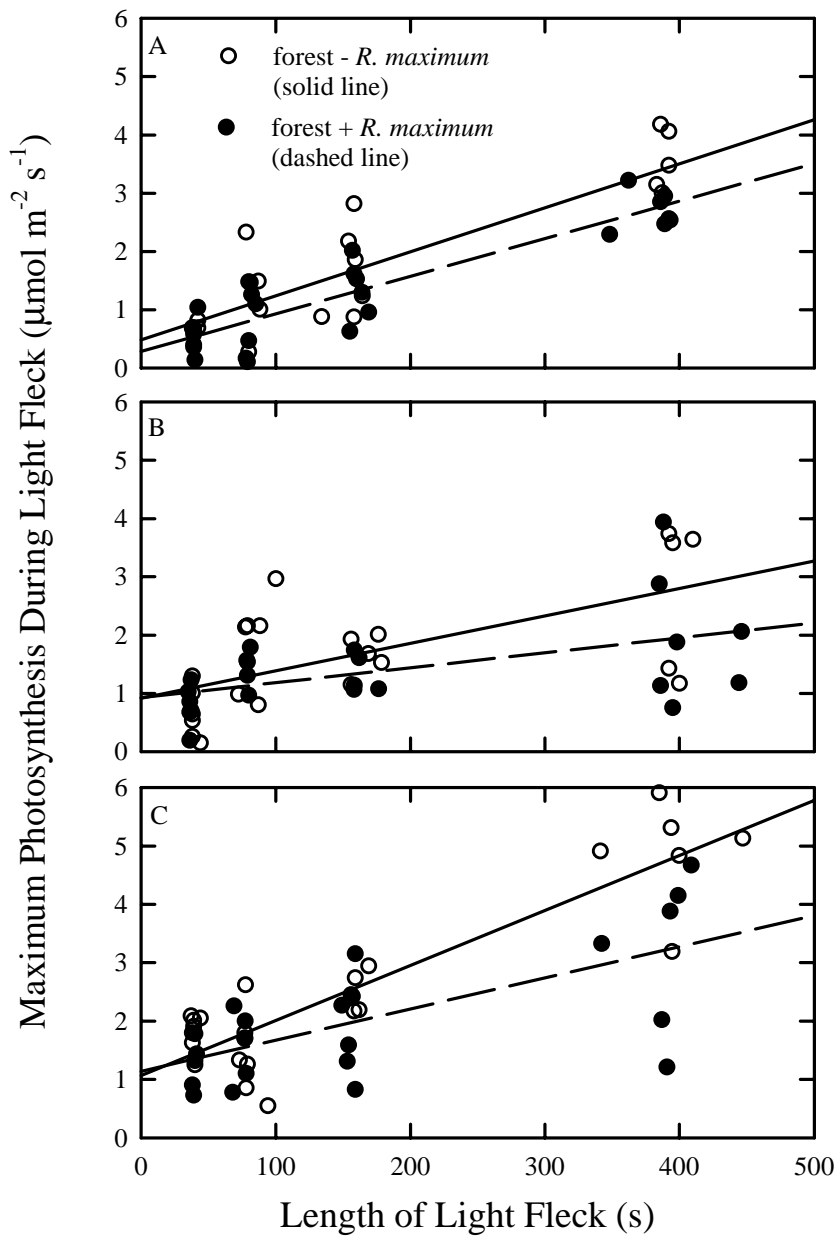


Figure 3.5: Positive regressions of maximum photosynthesis reached during a light fleck versus light fleck length. Seedlings were pre-acclimated in the dark. Each symbol represents maximum photosynthesis from one seedling. Lines are best-fit regressions. (A).  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 0.007x + 0.287$ ;  $\text{Rsqr} = 0.81$ ; Solid line:  $y = 0.008x + 0.486$ ;  $\text{Rsqr} = 0.76$ . (B).  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 0.003x + 0.93$ ;  $\text{Rsqr} = 0.26$ ; Solid line:  $y = 0.005x + 0.917$ ;  $\text{Rsqr} = 0.39$ . (C).  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  light fleck; Dashed line:  $y = 0.005x + 1.14$ ;  $\text{Rsqr} = 0.54$ ; Solid line:  $y = 0.010x + 1.07$ ;  $\text{Rsqr} = 0.80$ .

Table 3.3: Analysis of covariance summarizing the effect of canopy, (i.e. forest with or without *R. maximum*), on seedling maximum photosynthesis achieved during light fleck simulations using fleck length as the covariate. F values and P values obtained from the Type III SS show the effect of canopy, fleck length and the canopy by fleck length interaction on seedling maximum photosynthesis under three fleck intensities and both pre-acclimation conditions. Also shown is the coefficient of determination from the analysis of variance.

Prior Acclimation	Fleck Intensity	R-Square	F Values and Pr > F		
			Canopy	Fleck Length	Canopy*Fleck Length
Ambient	100	0.32	$F_{1,42} = 2.35$	$F_{1,42} = \mathbf{13.05}$	$F_{1,42} = 0.002$
			$P = 0.1329$	$P = \mathbf{0.0008}$	$P = 0.9641$
	500	0.50	$F_{1,46} = 3.97$	$F_{1,46} = \mathbf{29.19}$	$F_{1,46} = 0.58$
			$P = 0.0522$	$P = \mathbf{0.0001}$	$P = 0.4512$
	1000	0.76	$F_{1,39} = 0.89$	$F_{1,39} = \mathbf{109}$	$F_{1,39} = 0.91$
			$P = 0.3517$	$P = \mathbf{0.0001}$	$P = 0.3452$
Dark	100	0.79	$F_{1,42} = 0.63$	$F_{1,42} = \mathbf{145}$	$F_{1,42} = 0.90$
			$P = 0.4326$	$P = \mathbf{0.0001}$	$P = 0.3489$
	500	0.36	$F_{1,42} = 0.001$	$F_{1,42} = \mathbf{20.59}$	$F_{1,42} = 1.8$
			$P = 0.9709$	$P = \mathbf{0.0001}$	$P = 0.1867$
	1000	0.71	$F_{1,44} = 0.04$	$F_{1,44} = \mathbf{98.82}$	$F_{1,44} = \mathbf{7.61}$
			$P = 0.8331$	$P = \mathbf{0.0001}$	$P = \mathbf{0.0084}$

that light intensity alone. There were no significant differences in the slopes of the regressions at all other fleck intensities for either pre-acclimation status (Table 3.3).

#### *Average photosynthesis at $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ following light fleck simulation*

There was no relationship between fleck length and average photosynthesis at low light following light fleck simulations for all light intensities and either pre-acclimation condition (Data not shown).

## **Discussion**

There is increasing evidence that the percent cover of subcanopy evergreen shrubs (e.g., *R. maximum* and *Kalmia latifolia*) is increasing in deciduous forests of the southeastern United States (Dobbs 1995; Clinton and Vose 1996; Baker and Van Lear 1998). This trend is alarming because these Ericaceous shrubs have been identified as “weeds” that interfere with the regeneration and growth of important hardwood timber species in the southern Appalachian Mountains (Smith 1963; Romancier 1971; Martinez 1975; Elliot and Vose 1995). A major mechanism of seedling inhibition by shrubs is the reduction of above ground PAR below the light compensation point for many of the hardwood tree species such as *Q. rubra* (Semones et al. manuscript submitted). As a result, it is possible that for large periods of the day (e.g., early in the morning and late in the afternoon) when diffuse PAR drops below  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ , canopy tree seedlings

under thickets of *R. maximum* have reduced net photosynthesis. This will result in reduced net daily carbon gain relative to seedlings in forest without *R. maximum*.

Although they might only occur for a small part of the day, sunflecks can account for 20-90% of the total daily PAR in a variety of forests (Chazdon 1988; Pearcy 1990). A similar percentage of daily CO<sub>2</sub> assimilation by leaves in the understory can be attributed to sunfleck utilization. (Pearcy and Calkin 1983; Pearcy 1987; Pfitsch and Pearcy 1989; Pearcy 1990). Because light limitation is thought to play a role in inhibiting seedling regeneration within thickets of *R. maximum*, effective utilization of incident PAR during sunflecks should be critical for seedling, daily net carbon gain and ultimately seedling survival. Do seedlings within thickets of *R. maximum* use sunfleck PAR as efficiently as seedlings in forest without *R. maximum*? Recent studies on sunfleck utilization have suggested that shade leaves might be more capable of utilizing sunflecks more efficiently than sun leaves (Chazdon and Pearcy 1986; Küppers and Schneider 1993; Tang et al. 1993). This does not necessarily correspond to increased carbon gain though and may ultimately be unimportant for seedling survival when growing within a thicket.

The research reported here was designed instead to determine if seedlings in forest with *R. maximum* experience reduced total carbon assimilation relative to seedlings outside of the thicket influence when exposed to sunflecks of similar intensity and duration. For example, are there locations in forest sites with shrub thickets where seedlings can utilize sunflecks as effectively (indicated by total carbon gain) as those plants in forest without thickets? To answer this question seedlings growing *in situ* in

forest sites with or without a thicket of *R. maximum* were exposed to artificial light flecks in the field, and the response curves were analyzed for carbon gain characteristics.

At low light (i.e.,  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) under both pre-acclimation conditions, seedlings in forest with *R. maximum* had significantly greater average photosynthetic rates than seedlings in forest without *R. maximum*. This result indicates that seedlings located within forest sites without *R. maximum* have optimized light harvesting capabilities to maximize utilization of lower quantities of diffuse PAR in the forest understory. Prior work on the photosynthetic response to light of northern red oak seedlings showed that seedlings in thickets had significantly lower light saturated photosynthetic rates (Semones et al. manuscript submitted). Consequently, seedlings within forest sites with *R. maximum* could be at a higher induction state, under ambient conditions, than seedlings in forest without *R. maximum*.

Following ambient pre-acclimation, there is a significant effect of the evergreen subcanopy on the light fleck response of *Q. rubra* seedlings such that seedlings located within thickets suffer constrained carbon assimilation relative to seedlings not within thickets. This trend is especially manifest during the longer duration light flecks (i.e., >120s) at all light intensities. For short duration light flecks, there appears to be little effect of subcanopy presence on the carbon assimilation of oak seedlings, and in fact it is possible that seedlings in forest with *R. maximum* might utilize short duration light flecks better than seedlings in forest without *R. maximum*. There is evidence for shade plants using sunflecks as efficiently as sun plants (Chazdon and Pearcy 1986; Küppers and Schneider 1993; Tang et al. 1993). It is important to note that the effect of *R. maximum*

thickets on seedling carbon gain affects the slope of the line with increasing fleck length and not the intercepts of the lines. As a result, the lines become more divergent over time such that longer duration sunflecks are certainly more beneficial for seedlings in forest without *R. maximum* when compared to seedlings growing within a thicket. This is a direct result of seedlings in forest without *R. maximum* having higher maximum CO<sub>2</sub> assimilation rates at any given light intensity than those plants in forest with *R. maximum*.

Seedlings pre-acclimated in the dark, showed little difference in the carbon gain between plants in either forest type. Only under the brightest light fleck intensity was a significant canopy effect noticed, and again this appeared to only be important for the longest duration flecks. These data (in comparison to the results from ambient pre-acclimated seedlings) imply that sunflecks are more critical for survival of those plants in forest without *R. maximum* than for plants in forest with *R. maximum*, because under ambient acclimation seedlings in sites with *R. maximum* have an apparently higher induction status than plants in sites without *R. maximum*. It is also possible that plants in the forest areas with *R. maximum* have a lower respiration rate than seedlings in sites without *R. maximum* causing higher net photosynthesis at low light levels.

There are three important components for effective sunfleck response. First is the rate of photosynthetic response. Plants that respond more quickly to a change in light should use the fleck energy more effectively than more slowly responding plants. Second is the maximum photosynthesis achieved during the sunfleck. Plants that have a higher photosynthetic rate during a fleck have the potential to assimilate more CO<sub>2</sub>. Third is post illumination CO<sub>2</sub> fixation. Plants that continue assimilating CO<sub>2</sub> at higher rates once

light levels have returned to ambient levels should again utilize light flecks more effectively. There are three physiological components that control these responses including ribulose 1,5-bisphosphate regeneration capacity, the light modulation of Rubisco activity and stomatal conductance.

. Although there were apparent differences, our data failed to demonstrate a significant difference in the maximum photosynthetic rate obtained by seedlings during a light fleck regardless of the presence or absence of *R. maximum* and the duration of the light fleck. Previous research on photosynthetic response to light demonstrated that at steady state and saturating PAR, seedlings located in forest without *R. maximum* had significantly greater maximum photosynthesis relative to seedlings in forest with *R. maximum*. However, other parameters from light response curves, such as quantum yield, light saturation level and light compensation point, were not different between seedlings growing in sites with and without *R. maximum*. Hence, it is difficult to predict what characteristic of the thicket environment constrains light fleck response. It is possible that some resource other than light availability and light acclimation affects the capacity of seedlings to utilize light flecks effectively.

Light fleck duration turned out to be a poor predictor of post fleck photosynthetic rate for all fleck intensities as indicated by regression analyses (results not presented). Furthermore, for each fleck intensity when the data for all fleck lengths were lumped together and analyzed there was no difference in the photosynthetic rate of seedlings located in forest with *R. maximum* and those in forest sites without *R. maximum*. As a result, at lower PAR following a sunfleck, it is difficult to make any conclusions

concerning the state of readiness of seedlings in both forest with and without *R. maximum*. This aspect of sunfleck response needs further elaboration because one way a plant can achieve a higher induction status, other than exposure to a long duration, high intensity sunfleck is to be exposed to several, short duration flecks in rapid succession (Percy 1990). In fact, most understory plants receive the majority of their sunflecks in relatively rapid succession at some point during the day. This study fails to address the issue of repeating light flecks.

### *Conclusion*

The results of this study imply that seedlings located within forest with *R. maximum* accumulate significantly less carbon than do seedlings in forest without *R. maximum* when seedlings are exposed to sunflecks of similar length and intensity. These data support the notion that regenerating tree seedlings within thickets of *R. maximum* experience carbon limitation relative to seedlings in forest without *R. maximum*. If these plants are carbon limited because of reduced carbon gained during sunflecks, then survivorship of plants in thickets of *R. maximum* would likely be reduced when compared to seedlings in forest sites without *R. maximum*. This study provides further evidence that thickets of *R. maximum* have a negative impact on understory plants. Furthermore, the results imply that light limitation could be the major mechanism inhibiting the survival of understory plants within thicket sites. Consequently, by differentially affecting survivorship of forest understory plants, such as canopy tree seedlings,



expanding thickets of *R. maximum* will potentially affect the composition and structure of eastern deciduous forests where the shrubs are found in the understory.

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## **Chapter 4**

**Why do subcanopy evergreen shrubs reduce tree seedling survival? Is light limitation the only mechanism?**



## Abstract

Thickets of the subcanopy evergreen shrub *Rhododendron maximum* L. (Ericaceae) are expanding in eastern deciduous forests. These shrubs inhibit the survivorship of understory species including canopy tree seedlings. A field study was conducted to characterize the effect of *R. maximum* on the photosynthetic response of oak seedlings to eight consecutive light flecks. Within 10 paired sites, (i.e., with and without *R. maximum*) 3 even aged three-year old *Q. rubra* seedlings were selected. Over each seedling, a hemispherical canopy photograph was taken and analyzed for percent canopy openness. Each seedling was dark pre-acclimated for 12 hours and then exposed to eight light flecks in rapid succession during which time photosynthesis was logged every two seconds. Each light fleck was  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  in intensity and lasted for 120s. Following each light fleck, leaves were exposed to  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR for 60s before the next light fleck.

Canopy openness, an indirect measure of PAR availability was significantly lower within *R. maximum* thickets when compared to forest sites without *R. maximum*. Mean carbon gain and maximum photosynthesis achieved during each light fleck was significantly lower for seedlings located in the presence of *R. maximum* for all flecks in an eight-fleck simulation. In addition, seedlings located within thickets generally had significantly lower pre-illumination photosynthesis following the first of eight light flecks. The mean photosynthetic light use efficiency of seedlings located in forest with *R. maximum* was significantly lower for the first six of eight light flecks in succession. Percent canopy openness was used to explain the variation in carbon gained from all

eight light flecks in succession for seedlings under both forest conditions. However, a significant linear relationship was not found between percent canopy openness and carbon gain in either forest condition (i.e., with and without *R. maximum*) and precluded using analysis of covariance to test for a significant subcanopy effect on seedling carbon gain. Light limitation is could be a major mechanism responsible for inhibition of tree seedlings from within thickets of *R. maximum*.

**Key Words:** Thicket, PAR, photosynthesis, light use efficiency, and induction status.

## **Introduction**

Within forests, canopy architecture as well as leaf height and distribution influence the spatial and temporal availability of understory photosynthetically active radiation (PAR) (Bradshaw and Spies 1992; Brown and Parker 1994; Baldocchi and Collineau 1994; Nicotra et al. 1999). Light is often the most limiting resource for growth and ultimately survival of many forest understory species, including canopy tree seedlings (Percy 1983; Percy and Calkin 1983; Chazdon 1988; Kobe et al. 1995; Chazdon et al. 1996; Whitmore 1996; Walters and Reich 1996). Consequently, spatial patterns of light availability within forests are likely to influence seedling survivorship and eventually stand level regeneration patterns of woody species (Clark and Clark 1992; Clark et al. 1996). However, very few studies have examined the variation in spatial patterns of light distribution in forests (Clark et al. 1996; Walter and Himmler 1996; Nicotra et al. 1999), or linked microsite variation in light availability to community and landscape-level patterns of woody regeneration (MacDougall and Kellman 1992; Clark et al. 1996). In order to characterize the influence of light variation on forest structure, it is not only important to understand spatial differences in PAR between and within stands but also to understand the physiological responses of canopy tree seedlings regenerating in these heterogeneous light conditions.

Various factors can affect forest structure and ultimately influence spatial and temporal distribution of understory PAR. One such factor, expanding or invading evergreen subcanopy plant species is affecting forests globally. Evergreen subcanopies can inhibit recruitment and regeneration of canopy tree seedlings and thus, influence

forest community composition. For example, in the western US, coniferous forests of the Cascade Mountains often contain a subcanopy of *Galtheria shalon* that can interfere with tree seedling survival (Klinka et al. 1989; Messier 1993). Also, broad leaf palms in the understory of Costa Rican rain forests inhibit recruitment of canopy tree seedlings (Denslow et al. 1991) while *Chusquea* (dwarf bamboo) inhibits regeneration of oak species in high elevation forests (Widmer 1998). Furthermore, in Chile two species of *Chusquea* inhibit regeneration of southern beech (*Nothofagus*) (Veblen 1982). Moreover, species of dwarf bamboo in the temperate deciduous forest of SW China inhibit regeneration of *Abies* and *Betula* (Taylor and Qin 1992), while another species of dwarf bamboo (*Sasa*) can completely exclude all other vegetation on the forest floor (Nakashizuka and Numata 1982). *Rhododendron ponticum*, an invasive species, inhibits regeneration of canopy trees in the United Kingdom (Fuller and Boorman 1977; Cross 1981; Mitchell et al. 1997). Despite the mounting evidence that indicates many subcanopy evergreen species exclude understory plants, very few studies have investigated the influence of subcanopy evergreen plants on the spatial or temporal availability of PAR, (Clinton and Vose 1996; Lei et al. unpublished data), or more importantly the subsequent impact on understory plant photosynthesis. In order to assess the influence of subcanopy evergreen species on seedling carbon gain and survival, we first have to understand their influence on leaf photosynthesis of seedlings.

In deciduous forests of the southeastern United States, a model system exists for the study of evergreen subcanopy expansion on forest dynamics. The evergreen shrub *Rhododendron maximum* L. (Ericaceae) covers approximately 7.4 million hectares of the

forest understory, often growing in dense thickets up to 20 hectares in area (Monk et al. 1985; Monk and Day 1984). Shrub thickets are frequently located in riparian zones, distributed through species rich coves, or along mesic, north facing slopes. The sites that *R. maximum* inhabits can be especially rich in hardwood tree species and these sites are believed to be critical for ecosystem processes such as nutrient cycling (Gregory et al. 1991; Nilsen et al. manuscript submitted). Following disturbance, *R. maximum* can regenerate vegetatively or by seed and rapidly fill canopy openings. Thickets modify the above and below ground microenvironment by creating dense shade, producing heavy litter, and forming dense root layers.

Numerous studies have demonstrated an inhibitory influence of *R. maximum* on the establishment, growth, and survival, of canopy tree seedlings (Minkler 1941; Wahlenberg 1950; Niering and Egler 1956; Fuller and Boorman 1977; Phillips and Murdy 1985; Monk et al. 1985; Plocher and Carvell 1987; Clinton et al. 1993; Clinton and Vose 1996; Baker and Van Lear 1998). Furthermore, there is also some evidence that mature thickets might limit the growth and production of mature canopy trees located within a thicket perimeter (Monk et al. 1985; Elliot and Vose 1995), possibly by sequestering nutrients in the long-lived, evergreen leaves and slowing nutrient turnover rates. Yet, as with many evergreen subcanopy plants, the exact mechanisms responsible for the inhibition of understory plants within thickets of *R. maximum* remain unclear. Certainly, the effects of thicket establishment on forest community structure will become more pronounced because the percentage cover of *R. maximum* in eastern deciduous forests is increasing (Monk et al. 1985; Dobbs 1995; Baker and Van Lear 1998). As a

result, the exact mechanisms that make thickets inhibitory to understory plant survival need further examination.

Several plausible mechanisms that could help explain the inhibitory nature of *R. maximum* thickets have been explored. For example, recent research indicates that below ground resource availability is lower within thickets of *R. maximum* than in forest lacking *R. maximum*. Thicket soils have lower soil water content, cation availability, slower nitrogen mineralization rates (Nilsen et al. manuscript submitted), and depressed mycorrhization potential (Walker et al. 1999). Hence, abiotic and biotic soil resource limitations within thicket perimeters could place understory plants at a competitive disadvantage relative to plants located outside the influence of thickets.

Perhaps the most dramatic impact of *R. maximum* thickets on understory plants is the result of above ground resource limitation. The multi-layered evergreen subcanopy that develops attenuates below canopy photosynthetically active radiation (PAR) (Clinton and Vose 1996; Nilsen et al. manuscript submitted). In fact, average ambient PAR within many thickets of *R. maximum* is significantly lower, often 10 times as low, throughout the day when compared to forest without *R. maximum* (Nilsen et al. manuscript submitted); plus, the frequency and duration of sunflecks is also reduced (Lei et al., unpublished data). Reductions in both diffuse and direct PAR in forest with *R. maximum*, could be detrimental for regenerating tree seedlings if it directly results in significantly lower carbon gain relative to plants growing in forest sites without *R. maximum*, or if some other resource limitation indirectly constrains leaf carbon gain during sunflecks.

It is generally perceived that in the forest understory light levels exist well below saturating values for net photosynthesis of most canopy trees (Pearcy 1983). More importantly, understory light levels may exist below the light compensation point (i.e., that point where respiratory loss is balanced by photosynthetic gain) for many of these plants. Prior research has shown that this might be a common occurrence for seedlings located in forest with *R. maximum* (Semones et al. manuscript submitted). Consequently, tree seedlings that germinate in forest with *R. maximum* could ultimately suffer more rapid carbon limitation than those in forest without *R. maximum*, and as a result, successful regeneration and survival of these tree seedlings, regardless of shade tolerance, would be inhibited.

Although the suppressive nature of *R. maximum* thickets has been recognized for some time, an understanding of the photosynthetic response of tree seedlings to variable light within these forests is virtually non-existent. If light is the limiting resource in these forest environments, then it is necessary to better understand seedling response to sunflecks. It has been shown that flecks of direct solar irradiance may only occur during 10% of the day, but they may contribute 20-90% of all the photosynthetically active light in the understory environment (Chazdon 1988; Pfitsch and Pearcy 1989; Pearcy 1990). Semones et al. (See Chapter 3), found that seedlings growing under thickets of *R. maximum* often assimilate significantly less carbon than seedlings in forest without *R. maximum* when exposed to light flecks of varying duration and intensity. The question remains though, are differences in light fleck response a result of the PAR available during leaf development, or are other factors associated with thicket presence

constraining photosynthetic response of tree seedlings to light flecks. For example, does the presence of *R. maximum* inhibit seedling carbon gain or photosynthetic efficiency for those seedlings growing in sites with similar canopy openness.

The purpose of this study is to determine whether seedlings growing in forest with *R. maximum* experience constrained photosynthetic response to light flecks and if the response of these plants is simply a direct result of reduced PAR availability within thickets. To address these objectives we exposed northern red oak seedlings growing *in situ* to a series of light fleck simulations in a temperate deciduous forest and recorded their photosynthetic responses. In addition, we indirectly assessed PAR availability to these seedlings under full canopy closure using hemispherical canopy photographs in order to develop a relationship between light environment and carbon gain attributable to light flecks.

## **Methods**

### *Site selection*

This study was conducted in a montane, cool, temperate forest at Coweeta Hydrologic Laboratory, a 2,185 ha US Forest Service experimental station. Coweeta is a member site of the Long Term Ecological Research (LTER) network. The basin is located in the Nantahala Mountains, near Otto, North Carolina (35°02' N, 83°24' W), and is part of the Blue Ridge Mountain physiographic province. Vegetation has been described as northern hardwood, cove hardwood, oak, and oak-pine communities (Day et



al. 1988). Elevations range from 675 m to 1592 m. Yearly average precipitation is 180 cm at the base climate station, and increases with elevation to over 220 cm (Swank and Crossley, 1988). High moisture levels and mild temperatures, characteristic of the basin, place Coweeta in the marine, humid, temperate category of Koppen's climatic classification scheme (Swift et al. 1988). The study sites selected for this project were within a mature, mixed-hardwood forest, located on north facing, 60% slope, at an elevation of approximately 1000m. A mosaic of *R. maximum* thickets covers at least half of the total area of the forest. Dominant overstory tree species include *Quercus rubra* L. (Fagaceae).

Throughout the selected forest 10 paired locations (approximately 0.25 ha/location) were chosen such that one site from each pair was covered by *R. maximum* shrubs. Paired locations were selected so they were at least 20m apart and possessed naturally regenerating *Q. rubra* seedlings. Within each site location, five even aged, (three-year old) northern red oak seedlings were randomly chosen and tagged for later identification. Individual seedlings were at least ten meters apart. Of the five seedlings, three were eventually selected for light fleck simulations, thus giving a sample size of 60 seedlings (i.e., 30 in forest with *R. maximum* and 30 in forest without *R. maximum*).

#### *Light availability*

The availability of PAR above each seedling was assessed using hemispherical canopy photographs. Photographs were taken at 0.5m height using a leveled Nikon 8 mm fish-eye lens (180° field of view) mounted on a Nikon F3HP body. The lens was fitted

with a compass for indicating magnetic north. A high contrast black and white film (ASA 400, Tri X, Eastman Kodak, Incorporated, Rochester, New York) and red, built in filter were used for taking pictures with enhanced contrast. Black and white negatives were digitized using a slide scanner (Polaroid SprintScan 35 ES) and converted to bitmap files for later analysis. Canopy photos were analyzed using FEW4.0 (PC Windows95) developed by Dr. Ishizuka of FFPRI (Tsukuba, Japan) to obtain weighted canopy openness (WCO). This indirect measure of light availability weights openings directly overhead greater than openings around the perimeter of the photograph.

#### *Light fleck simulations and photosynthesis measurements*

In order to control prior PAR exposure history, and also cause complete loss of photosynthetic induction, seedlings were covered to exclude all light for at least 12 hours prior to light fleck simulation. Light flecks were simulated, and photosynthetic responses of seedling leaves were measured using the Li-Cor 6400 portable IRGA fitted with the 6400-02 LED light source (Li-Cor, Inc., Lincoln, Nebraska). An autoprogram was written and used to generate light fleck response curves. CO<sub>2</sub> concentration within the leaf chamber was maintained constant at 390  $\mu\text{mol m}^{-2} \text{s}^{-1}$  using the Li-Cor model 6400-01 external CO<sub>2</sub> source assembly (Li-Cor Inc., Lincoln, Nebraska). The relative humidity of the leaf chamber and the vapor pressure deficit (VPD) of the leaf were maintained within 1% by manually adjusting the system flow rate through the desiccant. Seedlings were pre-exposed to 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light for 10 minutes prior to light fleck simulation.

Each light fleck simulation consisted of: 1) a 60s period at low light ( $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ); 2) 90s at high light ( $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ); and 3) 60s at low light again. This was repeated 8 times for each leaf resulting in a response curve (Figure 4.1). After fleck simulations, plants were allowed to reach maximum steady state photosynthesis at  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Photosynthesis was logged every 2-3s over the entire eight light fleck simulation.

From each individual simulation (8 flecks/leaf), seven parameters were derived, (average pre-illumination photosynthesis, maximum photosynthesis, carbon gain during the fleck + 30s post illumination  $\text{CO}_2$  assimilation, induction status at 0, 60 and 90s and light use efficiency). In addition, total carbon gain and maximum photosynthesis attained for the entire light fleck simulation (i.e., eight flecks + intermediate low light periods) were determined.

Leaf induction state at the three time periods was calculated using the equation:

$$\text{Induction state (\%)} = (P_{\text{LF}} - P_{\text{L}})/(P_{\text{H}} - P_{\text{L}}) \times 100$$

where  $P_{\text{LF}}$  is the  $\text{CO}_2$  assimilation rate at 0, 60 or 90 seconds, and  $P_{\text{L}}$  and  $P_{\text{H}}$  are the steady state  $\text{CO}_2$  assimilation rates at low and high light respectively (Chazdon and Pearcy 1986a). Light fleck PAR was above light saturation as determined from steady state light response curves generated for the species, but did not induce photoinhibition. Also, low light PAR was just above the light compensation point and similar to diffuse radiation at these sites.

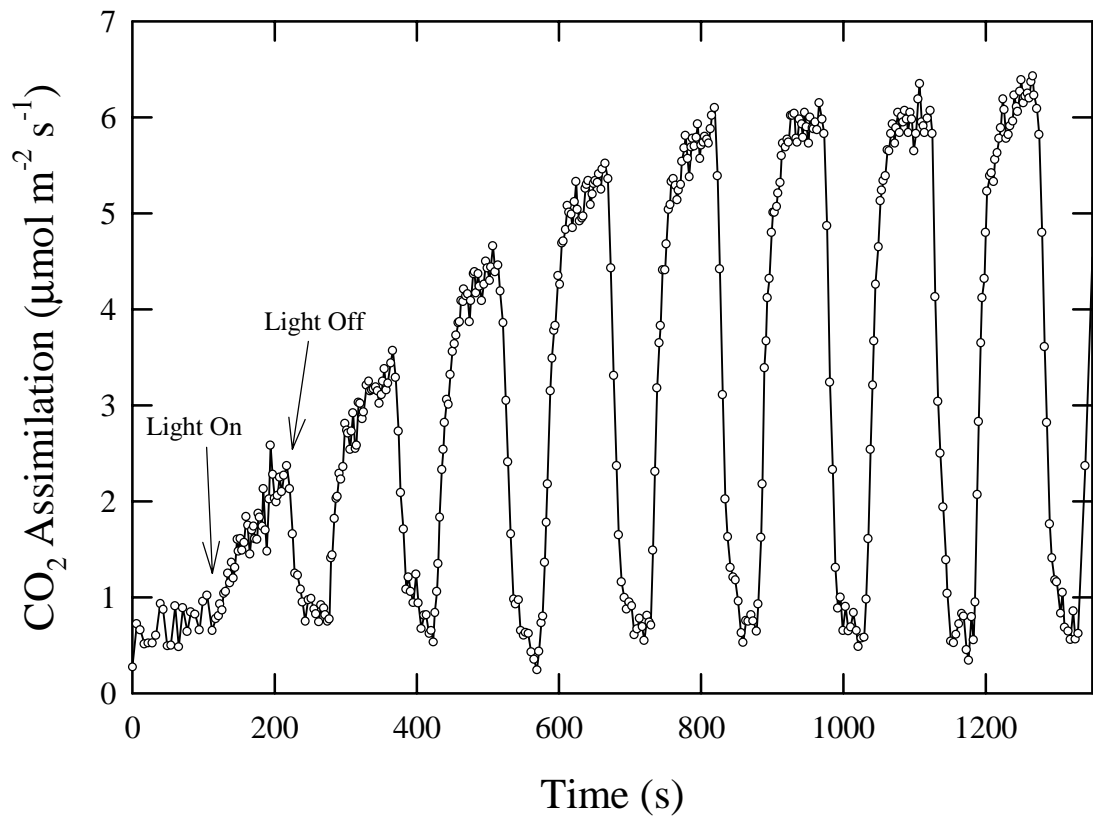


Figure 4.1: Representative light fleck simulation illustrating eight periods of high light at  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  and intermediate periods of low light at  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Response from a seedling located in forest without *R. maximum*.

The photosynthetic light use efficiency (LUE) during light flecks (relative to steady state photosynthesis) was calculated by comparing the measured carbon gain during the light fleck plus 30s post illumination CO<sub>2</sub> assimilation at low light with the predicted carbon gain calculated from steady state rates measured at low and high light levels (Chazdon and Pearcy 1986b).

$$\text{LUE} = (\text{Measured carbon gain} + 30\text{s post illumination Pn} / \text{predicted carbon gain}) \times 100$$

Linear regression was used to relate total carbon gain (attributed to the eight light fleck simulation sequence) to canopy openness. All other parameters were analyzed for each individual fleck, not across flecks, using a generalized randomized block design ANOVA with the presence or absence of *R. maximum* as the treatment factor. Variation attributed to site differences was removed by blocking. All statistical analyses were completed using SAS PROC GLM, (SAS, SAS Institute Inc., Cary, N.C.).

## **Results**

### *Canopy openness and total carbon gain*

Mean percent canopy openness (WCO) was significantly greater in forest without *R. maximum* than in forest with *R. maximum* (Tables 4.1 & 4.2). However the effect of evergreen subcanopy cover did behave differently depending upon the site location as indicated by the strong interaction effect (Table 4.2). Maximum canopy openness recorded in this study for forest without *R. maximum* was 17.2% while the minimum was

Table 4.1: Summary statistics for three parameters derived from each eight light fleck simulation on seedlings in either forest with *R. maximum* or without *R. maximum*. Data represent mean values. Numbers in parentheses are standard errors.

	Canopy openness	Maximum CO <sub>2</sub> assimilation	Total carbon gain
	(%)	( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	( $\mu\text{mol m}^{-2}$ )
Forest	8.17	5.49	2907
	(0.49)	(0.20)	(156)
	n = 30	n = 30	n = 30
<i>R. maximum</i>	6.72	4.11	1955
	(0.46)	(0.18)	(136)
	n = 30	n = 30	n = 30

Table 4.2: Results from GRBD ANOVA showing F statistics and associated P values for canopy openness, total carbon gain attributed to all eight flecks and maximum photosynthesis for all eight flecks. There were ten sites, and the treatment is the presence or absence of *R. maximum*.

Variable	df	SS	MS	F	P
<b>Canopy Openness</b>					
Site	9	131.7	14.6	3.99	0.0011
Treatment	1	36.8	36.8	10.04	0.0029
Site*Treatment	9	120.8	13.4	3.66	0.002
Error	40	146.7	3.67		
<b>Total Carbon Gain</b>					
Site	9	12708254.7	1412028.3	2.95	0.0088
Treatment	1	13606938.8	13606938.8	28.43	0.0001
Site*Treatment	9	5397791.4	599754.6	1.25	0.2920
Error	40	19145689.3	478642		
<b>Max Pn for all Flecks</b>					
Site	9	26.6	2.95	4.5	0.0004
Treatment	1	28.6	28.6	43.6	0.0001
Site*Treatment	9	8.2	0.911	1.39	.2252
Error	40	26.2	.656		

4.7%. Maximum canopy openness recorded in forest with *R. maximum* was 12.3% in a site with a predominant gap. The lowest value obtained under a thicket was 3.1% in a dense slick.

Maximum photosynthesis for each eight fleck series was determined for seedlings in forest with *R. maximum* and without *R. maximum* across all ten sites. The mean maximum CO<sub>2</sub> assimilation rate attained by seedlings during eight flecks in succession was significantly higher for plants located in forest without *R. maximum* (Tables 4.1 & 4.2). The maximum photosynthesis recorded for northern red oak seedlings in forest without *R. maximum* was 7.84 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, while the maximum rate logged in forest with *R. maximum* was 6.38 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. These data were consistent with light response curve analysis showing significantly higher rates of assimilation for plants located in forest sites without *R. maximum* which presumably receive higher levels of daily PAR (Semones et al. manuscript submitted). Contrary to the canopy openness data there was not a significant interaction effect on maximum CO<sub>2</sub> assimilation as indicated by the site by treatment interaction.

In conjunction with higher maximum rates of photosynthesis, seedlings in forest without *R. maximum* assimilated significantly greater amounts of carbon during the eight fleck simulation than plants located in forest with *R. maximum* (Tables 4.1 & 4.2). Mean carbon gain during an eight-fleck simulation was significantly higher for seedlings located in forest without *R. maximum*. Maximum carbon gain for seedlings in forest without *R. maximum* was 4459 μmol CO<sub>2</sub> m<sup>-2</sup> and the minimum carbon gain attributed to an eight fleck simulation was 552 μmol CO<sub>2</sub> m<sup>-2</sup>. For all seedlings located in forest with



*R. maximum*, the maximum amount of carbon gained was 3832  $\mu\text{mol CO}_2 \text{ m}^{-2}$  while the minimum was 274  $\mu\text{mol CO}_2 \text{ m}^{-2}$ . Analysis of variance indicated that the effect of *R. maximum* in the subcanopy on carbon gain was the same across all ten sites because of the non-significant interaction between site and treatment.

A main goal of this research was to determine if reduced light availability in forest with *R. maximum* sites solely regulates the capacity to assimilate carbon during sunflecks. Consequently, linear regression and analysis of covariance were used to evaluate the relationship between canopy openness above each seedling and total carbon gain each seedling accumulated from an eight fleck simulation for seedlings in both forest conditions (with or without *R. maximum*)(Figure 4.2). The rationale behind the analysis was to determine if a significant relationship exists between the canopy openness and total carbon gained and if so are the slopes or intercepts of the curves significantly different. If significant relationships between canopy openness and carbon gained during light flecks existed, but the curves differed significantly, then this would be an indicator of some factor other than light influencing light fleck use. The analyses indicated that a significant positive trend definitely exists for seedlings located in both forest conditions. However, the independent variable, canopy openness, failed to explain a significant amount of the variation present in the dependent variable, carbon gain (Figure 4.2). Because the regressions were not significant there was no reason to test for the effect of subcanopy on the slopes or intercepts of the curves using analysis of covariance.

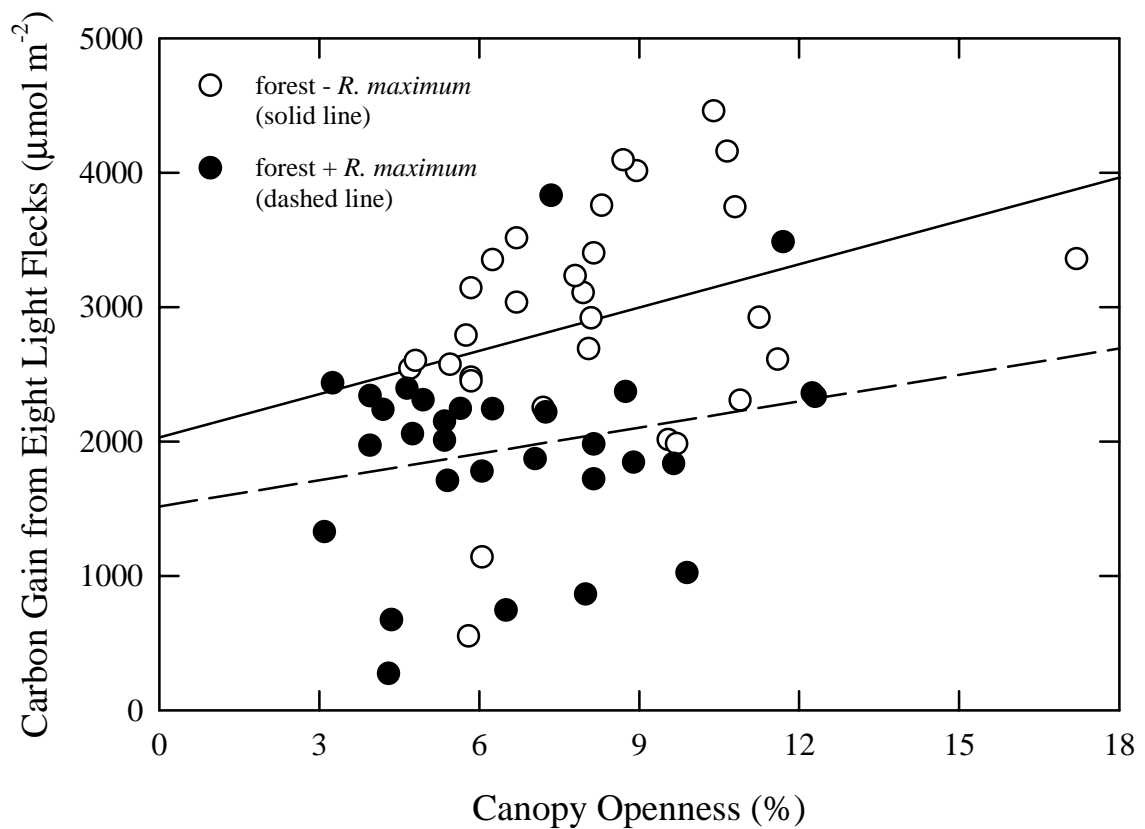


Figure 4.2: Positive regressions of total carbon gain from eight consecutive light flecks versus canopy openness above seedlings. Solid line ( $y = 2030 + 107x$ ;  $R_{sqr} = 0.11$ ;  $P = 0.07$ ). Dashed line ( $y = 1515 + 65.5x$ ;  $R_{sqr} = 0.05$ ;  $P = 0.221$ ). Each symbol represents data from a single seedling.

### *Analyses of other parameters derived from each individual fleck*

Plants in the forest understory often receive their daily allotment of sunflecks as short duration pulses of direct light, grouped together in a confined period of time during the day (Pearcy 1990). Short duration sunflecks grouped together in time can have the same effect on leaf photosynthetic induction as one long sunfleck. As a result, with each successive sunfleck, the leaf is better prepared to take full advantage of the next sunfleck as long as the space between flecks is not so long to cause loss of photosynthetic induction. Parameters from each individual light fleck in the eight fleck simulation were compared for seedlings in both forest with and without *R. maximum*. This was done to determine the effect of light flecks grouped temporally close together on parameters of fleck use efficiency, and to determine whether seedlings in forest with *R. maximum* might utilize short duration flecks of light in succession as effectively or efficiently as seedlings in forest without *R. maximum*.

Carbon gained during each fleck of the eight fleck simulation was derived to determine whether plants in forest with *R. maximum* have the capacity to assimilate as much carbon as plants in forest without *R. maximum* when given eight light flecks in succession. The data show that carbon gain from each light fleck + 30s of post illumination CO<sub>2</sub> assimilation was significantly greater for seedlings located in forest without *R. maximum* across all eight flecks (Figure 4.3). Although not shown, there was no significant interaction effect as a result of site specific differences on carbon gain. For seedlings in both forest conditions, carbon gained during flecks 1-8, including a post

illumination period for each fleck, increased in an exponential fashion toward an upper asymptote (Figure 4.3)

With successive light flecks the mean rate of photosynthesis at low light (i.e., between successive flecks), and maximum rate of CO<sub>2</sub> assimilation at high light should increase, because of increased induction with successive flecks. Ultimately, these parameters will have a direct effect on carbon gained during a fleck and possibly the photosynthetic efficiency with which a seedling utilizes a particular fleck. As expected and coinciding with higher carbon gain for seedlings in forest without *R. maximum*, mean steady state low light CO<sub>2</sub> assimilation and maximum high light photosynthesis were different between seedlings located in the two forest conditions (Figures 4.4 & 4.5). Maximum photosynthesis recorded during each fleck, during the entire simulation was always significantly higher for seedlings in forest without *R. maximum* across all eight flecks (Figure 4.4). There was no effect of site location on the treatment effect of subcanopy cover in the form of a significant interaction between subcanopy and site. Maximum photosynthesis, like carbon gain, increased in an exponential fashion and the curves became more divergent with increasing fleck number (Figure 4.4). Mean steady state photosynthesis at low light was consistently higher for plants in forest without *R. maximum* (Figure 4.5) than forest sites with *R. maximum*. Steady state CO<sub>2</sub> assimilation at low light was significantly higher for seedlings in forest without *R. maximum* for flecks 2-8 out of the simulation. Average low light photosynthesis was not different between the two forest conditions prior to the first light fleck of the simulation (Figure 4.5).

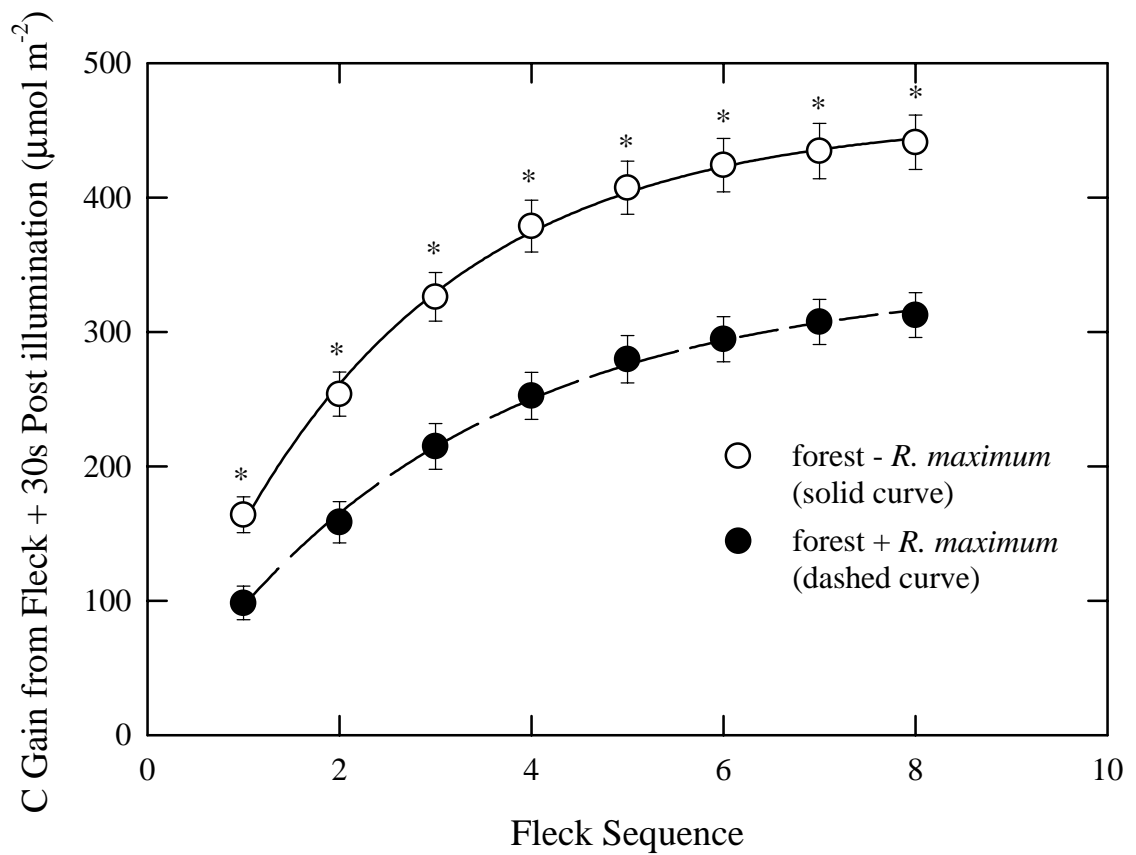


Figure 4.3: Graph illustrating carbon gain from individual flecks + 30s post-illumination CO<sub>2</sub> assimilation versus fleck sequence for seedlings located in forest with without *R. maximum*. Each point is a mean  $\pm$  1 standard error (n = 30 plants). Asterisks indicated means that are significantly different ( $\alpha = 0.05$ ) for each individual fleck. Curves represent best fit non-linear regression of the form  $f(y) = a(1-b^x)$ . Solid curve:  $y = 460(1-0.657^x)$ ;  $R_{sqr} = .99$ ;  $P < 0.0001$ . Dashed curve:  $y = 34(1-0.717^x)$ ;  $R_{sqr} = 0.99$ ;  $P < 0.0001$ .

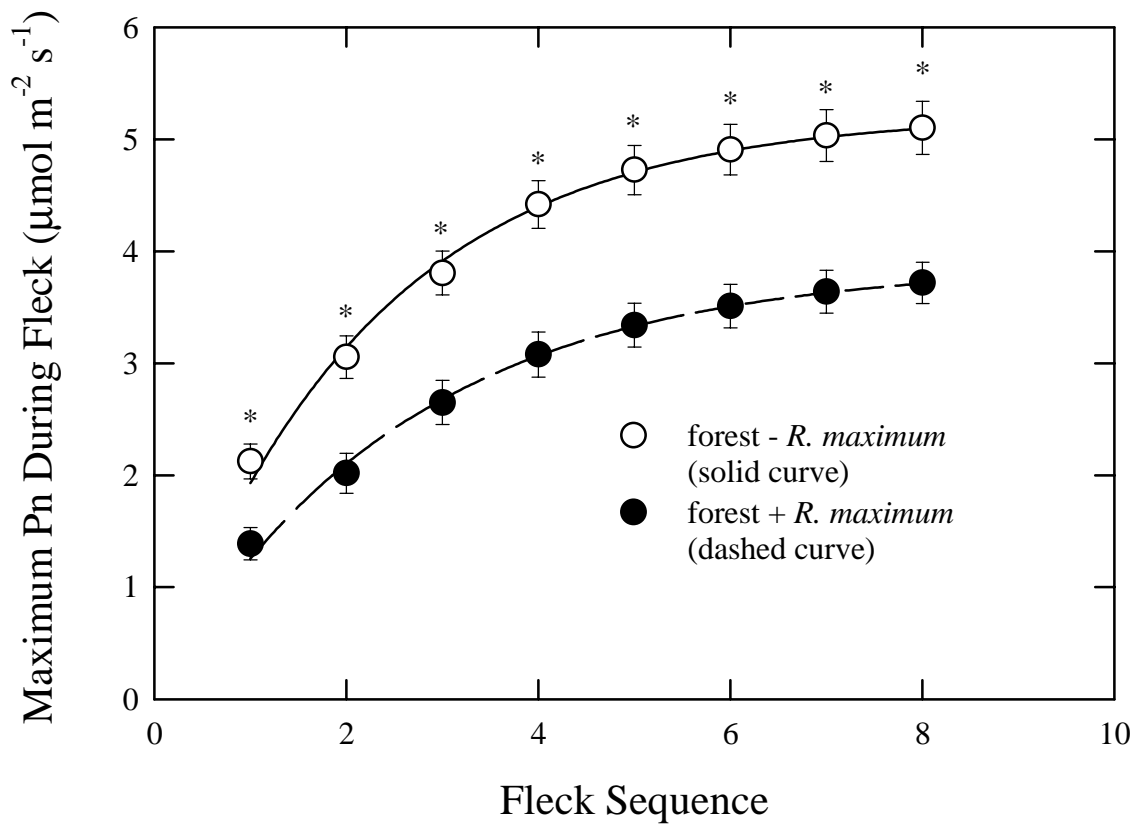


Figure 4.4: Graph of maximum photosynthesis during each light fleck at  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR versus fleck sequence for seedlings located in forest with and without *R. maximum*. Symbols are means  $\pm 1$  standard error. Asterisks indicated significantly different means ( $\alpha = 0.05$ ) for each individual fleck. Curves represent best fit non-linear regressions of the form  $f(y) = a(1-b^x)$ . Solid line:  $y = 5.23(1-0.631^x)$ ;  $\text{Rsqr} = 0.99$ ;  $P < 0.0001$ . Dashed curve:  $y = 3.88(1-0.677^x)$ ;  $\text{Rsqr} = 0.99$ ;  $P < 0.0001$ .

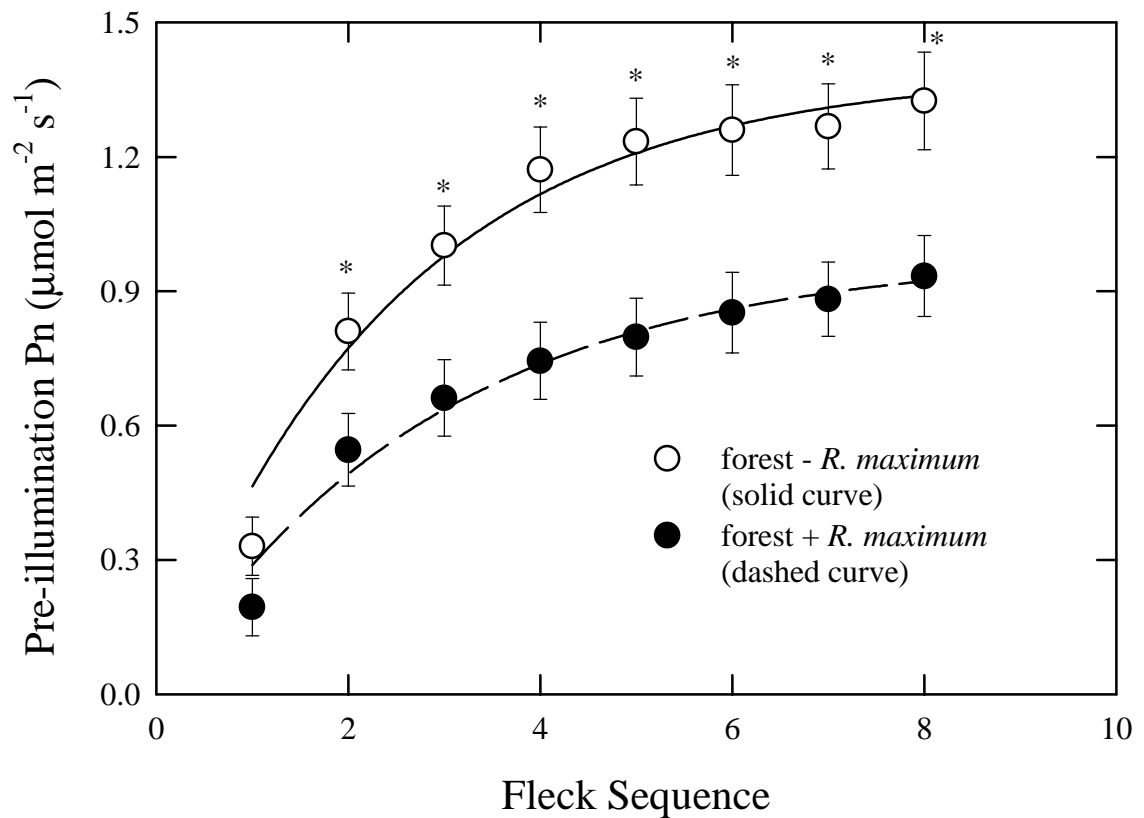


Figure 4.5: Graph of pre-illumination photosynthesis at  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  versus fleck sequence for seedlings located in forest with and without *R. maximum*. Symbols are means  $\pm$  1 standard error. Asterisks indicated significantly different means ( $\alpha = 0.05$ ) for individual each fleck. Curves represent best fit non-linear regressions of the form  $f(y) = a(1-b^x)$ . Solid curve:  $y = 1.39(1-0.667^x)$ ;  $\text{Rsqr} = 0.97$ ;  $P < 0.0001$ . Dashed curve:  $y = 0.984(1-0.707^x)$ ;  $\text{Rsqr} = 0.97$ ;  $P < 0.0001$ .

Again there were no significant interactions to indicate that treatment effects behaved differently based upon site location.

#### *Light fleck use efficiency and leaf induction state*

Light use efficiency is a measure of relative photosynthetic efficiency and was used to compare photosynthetic response of seedlings in forest with and without *R. maximum* to successive light flecks (Figure 4.6). Again this parameter was used to assess the efficacy with which seedlings utilize sunflecks and to determine if seedlings located in forest with *R. maximum* have the capacity to utilize sunflecks as efficiently as plants in forest without *R. maximum*. Photosynthetic efficiencies of greater than 100% are possible if measured carbon gain is greater than the predicted value. For this study, carbon gain attributed to the fleck + 30s of post illumination CO<sub>2</sub> assimilation was used as the measured carbon gain to take into account potential post illumination CO<sub>2</sub> assimilation. Seedling light use efficiency increased exponentially toward an upper asymptote in both forest types. Across all eight light flecks, seedlings in forest without *R. maximum* had higher LUE's than seedlings in forest with *R. maximum* (Figure 4.6). Differences were significant for the first five successive light flecks. There were no significant interactions between the treatment effect and site location for all eight flecks comprising the complete simulation.

Leaf induction status at 0, 60 and 90 seconds was calculated for each light fleck across the entire simulation (figure 4.7). Induction status was used to compare the relative state of readiness, or percent of maximum assimilation, that seedlings under



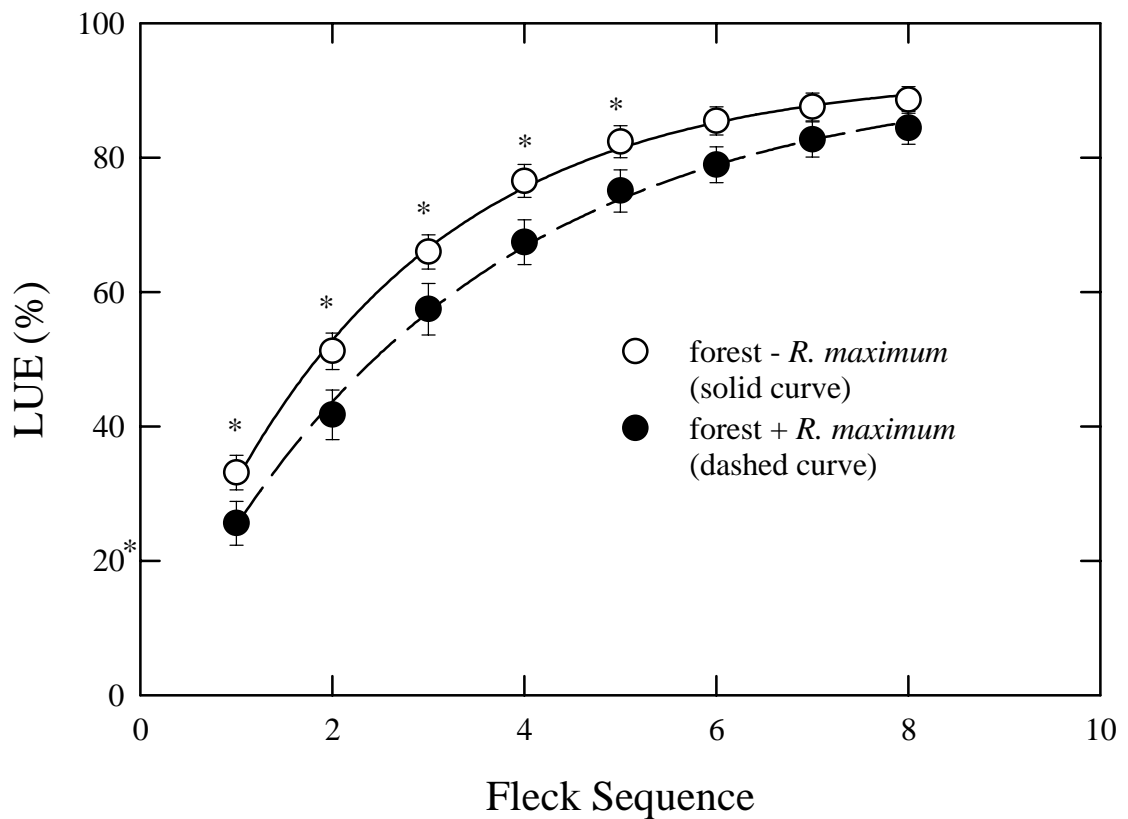


Figure 4.6: Graph of light use efficiency versus fleck sequence for seedlings located in forest with and without *R. maximum*. Symbols are means  $\pm$  1 standard error. Asterisks indicated significantly different means ( $\alpha = 0.05$ ) for each individual fleck. Curves represent best fit non-linear regressions of the form  $f(y) = a(1-b^x)$ . Solid curve:  $y = 92.5(1-0.650^x)$ ;  $R_{sqr} = 0.99$ ;  $P < 0.0001$ . Dashed curve:  $y = 92.6(1-0.726^x)$ ;  $R_{sqr} = 0.99$ ;  $P < 0.0001$ .

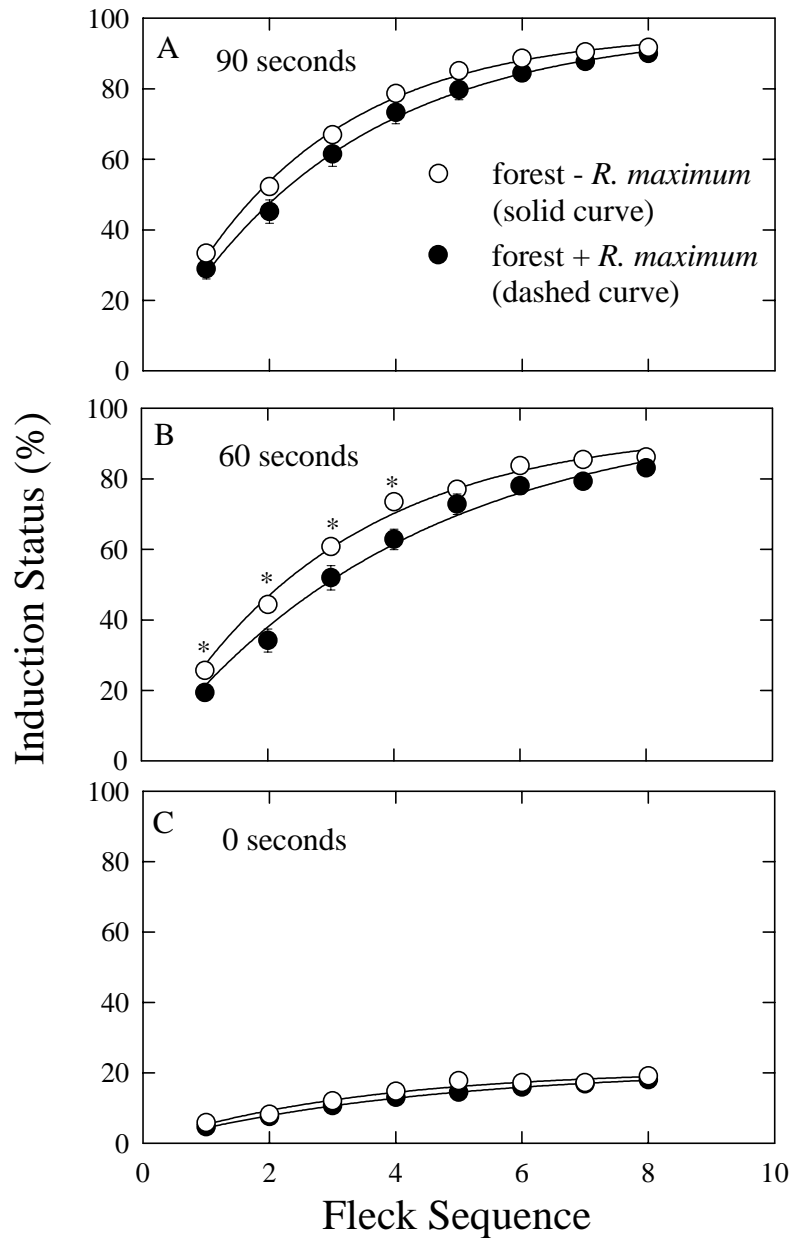


Figure 4.7: Graph illustrating induction states at 90, 60, and 0 seconds versus fleck sequence for seedlings located in forest with and without *R. maximum*. Symbols represent means ( $n = 30$  seedlings)  $\pm 1$  standard error. Asterisks indicate means that are significantly different ( $\alpha = 0.05$ ) for each individual fleck. Curves are the best fit non-linear regression of the form  $f(y) = a(1-b^x)$ . (A). Solid curve:  $y = 96.3(1-0.665^x)$ ;  $R_{sqr} = 0.99$ ;  $P < 0.0001$ . Dashed curve:  $y = 97.3(1-0.715^x)$ ;  $R_{sqr} = 0.99$ ;  $P < 0.0001$ . (B). Solid curve:  $y = 94.5(1-0.710^x)$ ;  $R_{sqr} = 0.99$ ;  $P < 0.0001$ . Dashed curve:  $y = 99.5(1-0.786^x)$ ;  $R_{sqr} = 0.99$ ;  $P < 0.0001$ . (C). Solid curve:  $y = 21.3(1-0.794^x)$ ;  $R_{sqr} = 0.99$ ;  $P < 0.0001$ . Dashed curve:  $y = 20.9(1-0.745^x)$ ;  $R_{sqr} = 0.97$ ;  $P < 0.0001$ .

either forest condition obtain at distinct time intervals. With successive light flecks, the induction status at a given time period should increase, but do seedlings in forest with *R. maximum* have lower induction states at given time periods relative to those plants in forest without *R. maximum* when exposed to successive light flecks? These data were more similar for seedlings in both forest conditions than all other parameters measured. Induction status at all three time intervals increased exponentially with increasing fleck number (Figure 4.7). In all cases, seedlings located within forest without *R. maximum* maintained higher induction states relative to seedlings within thickets of *R. maximum*. The mean differences in  $IS_0$  were low and not significantly different between canopy types with or without *R. maximum* regardless of fleck number. At 60s leaf induction states for seedlings located in forest without *R. maximum* were significantly greater than for seedlings located in forest with *R. maximum* across flecks 1-4 (Figure 4.7); however, there was no significant differences in  $IS_{60}$  for flecks 5-8. A similar pattern held true at 90s, because leaf induction states were slightly higher for seedlings outside of thicket influence, but in only one case (Fleck 2) was the difference significant. There were no significant interactions between site and subcanopy for any of the induction states.

## **Discussion**

There is increasing evidence that the percent cover of subcanopy evergreen shrubs (e.g., *Rhododendron maximum* and *Kalmia latifolia*) is increasing in deciduous forests of the southeastern United States (Dobbs 1995; Clinton and Vose 1996). This trend is alarming because of reports that identify these Ericaceous shrubs as “weeds” that interfere with the regeneration and growth of important hardwood timber species in the

southern Appalachian Mountains (Smith 1963; Romancier 1971; Martinez 1975; Elliot and Vose 1995; Baker and Van Lear 1998). The question remains however, why are these shrub thickets inhibitory to recruitment and regeneration of canopy tree seedlings? Is light limitation the sole mechanism of inhibition, or do factors other than light contribute to the inhibitory nature of thickets?

A major mechanism of thicket inhibition could be the reduction of ambient diffuse PAR not only below saturating levels for seedling photosynthesis but very often below the light compensation point for many of the hardwood tree species such as *Q. rubra* (Semones et al. manuscript submitted). As a result, it is possible that for large periods of the day (e.g., early in the morning and late in the afternoon) when diffuse light levels drop below  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ , canopy tree seedlings within thickets experience reduced net photosynthesis because of excessive respiratory loss. Such reductions in net photosynthesis could result in reduced daily carbon gain and ultimately adversely affect the survivorship of these seedlings. Differential survivorship of woody species within forest sites with *R. maximum* compared to forest without *R. maximum* could influence forest community composition. If this scenario holds true then efficient and maximal use of direct light in the form of sunflecks would be critical for seedling survival in these forests

Approximately, 20- 90% of the total daily PAR that forest understory plants receive is in the form of sunflecks (Pearcy 1990; Pfitsch and Pearcy 1989). As a result, it has been estimated that sunflecks can account for 20-80% of the total daily carbon gain for understory plants in various forests (Pearcy 1987; Pearcy 1990; Pfitsch and Pearcy

1989; Weber et al. 1985). Because light limitation is thought to play a role in inhibiting seedling regeneration within thickets of *R. maximum*, effective utilization of incident PAR during sunflecks should be critical for seedling, daily net carbon gain. Semones et al. manuscript submitted, found that with increasing fleck length (especially over 120s), across a range of fleck intensities, seedlings located in forest with *R. maximum* accumulated significantly less carbon than plants located within forest without *R. maximum*. This effect was not as pronounced for short duration flecks (i.e., <90s) or for seedlings with no prior induction. It was the aim of this project to determine if plants located within subcanopy evergreen thickets experience constrained light fleck responses, and if so is this response controlled predominantly by PAR availability to the seedlings or does another resource influence the light fleck response of plants growing *in situ*. Several important findings developed from this project.

There is conclusive evidence that across paired locations at our study site, canopy openness is significantly lower under thickets of *R. maximum* (Tables 4.1 & 4.2). Percent canopy openness is an indirect measure of light availability. If it corresponds to actual light availability then it supports prior research showing that PAR is significantly attenuated in forest sites with *R. maximum* (Clinton and Vose 1996; Nilsen et al. 1999; Lei et al. unpublished data). Furthermore it could be evidence that seedlings in thicket conditions receive fewer sunflecks of potentially lower intensity during the day or across the growing season. Despite significantly greater mean WCO in forest sites without *R. maximum* it is important to mention that there is considerable variation in the range of WCO within both forest conditions (i.e., with and without *R. maximum*). Hence, there

are forest sites covered by *R. maximum* that have WCO values as high as those measured in forest without *R. maximum*, and the opposite also holds true. As a result, mean WCO may not represent what the majority of plants are actually experiencing. In addition, weighted canopy openness might not be the best indicator of light availability because canopy openings are weighted according to zenith angle. Consequently, openings overhead are most heavily weighted and this parameter is therefore sensitive to canopy gaps more directly overhead (Nicotra et al. 1999). Perhaps a measure of PAR availability that weights all openings evenly would be a better indicator of PAR availability, especially for plants within thickets where there is pronounced edge to the subcanopy. Still if WCO provides a relatively accurate measure of true PAR availability, then this lends support to the notion that light is a limiting resource for seedling carbon gain in sites with *R. maximum*.

The results of this project also lead to the conclusion that when given light flecks of similar duration and intensity, seedlings located within forest with *R. maximum* assimilate significantly less carbon than similar plants located in forest without *R. maximum* (Figure 4.2). This result is likely a direct effect of the fact that seedlings located within thickets of *R. maximum* have significantly lower rates of maximum CO<sub>2</sub> assimilation (Figure 4.4). Plus it is apparent from the pre-illumination CO<sub>2</sub> assimilation data that seedlings in forest without *R. maximum* maintain significantly higher steady state photosynthetic rates at 10 μmol m<sup>-2</sup> s<sup>-1</sup> between repetitive light flecks (Figure 4.5). Although this does not necessarily correlate to a significantly higher induction state, which is a relative value, it does contribute to a higher overall carbon gain during a series

of eight consecutive light flecks (Figure 4.7). These data lead to the conclusion that seedlings located in forest sites with *R. maximum* do not benefit from PAR available during sunflecks as much as those seedlings not within a thicket perimeter. More importantly, the difference in carbon gain becomes more pronounced with increasing numbers of flecks in succession resulting in a significantly lower overall carbon gain for a series of sunflecks. Also, this result provides further support to the concept that light is the overriding limiting resource for tree seedling carbon gain in forest with *R. maximum*. Semones et al. manuscript submitted, found similar results when they exposed seedlings to a range of light flecks varying in intensity and duration, except at lower fleck durations and when seedlings experienced complete loss of induction.

Another objective of this project was to determine if plants that grow in the shadier environment of a *R. maximum* thickets can utilize sunflecks as efficiently as plants growing in forest sites without *R. maximum*. There is evidence that shade adapted leaves can utilize PAR from sunflecks as efficiently as sun adapted leaves (Chazdon and Pearcy 1986b; Küppers and Schneider 1993; Tang et al. 1993). The results do not indicate this is the case. At 0, 60 and 90s, seedlings in both forest conditions have very similar induction states regardless of the number of previous light flecks. Photosynthetic response is relatively similar. Induction status at 60s was significantly higher for plants located in forest without *R. maximum* for the first four light flecks in a row (Figure 4.6). This is likely due to a quicker initial photosynthetic response to an increase in light. This effect becomes dampened following the fourth consecutive light fleck. Seedlings in *R.*

*maximum* do have significantly lower LUE however when exposed to at least 5 similar light flecks consecutively.

One of the key questions of this project was whether a factor other than light limits the efficacy by which seedling photosynthesis responds to sunflecks particularly in forest sites with *R. maximum*. In other words, can seedlings located in brighter sites with *R. maximum* thickets acclimate to the prevailing PAR conditions such that they might still utilize sunflecks more efficiently or reach higher rates of maximum assimilation and therefore not experience significantly reduced carbon gain compared to plants in forest without *R. maximum*? Do seedlings in forest sites with *R. maximum*, that receive similar quantities of PAR to forest sites without *R. maximum*, respond to sunflecks in an equivalent fashion to seedlings not within a thicket perimeter? The answers to these questions are critical to the analysis of mechanisms responsible for depressed seedling survivorship in these forests. Regression analysis indicated that plants in thicket environments tended to assimilate less carbon than seedlings in forest without *R. maximum* at similar canopy openness. However, the relationship was not significant nor did the variable canopy openness explain much of the variation in the dependent variable total carbon gain.

There are a couple of reasons why this might be the case. First, as mentioned previously, canopy openness as determined from hemispherical canopy photographs may not be the best indicator of light availability. This might be especially important for seedlings located along the edges of thickets that are exposed to a greater number of canopy openings that are not directly overhead. Second, something other than PAR



availability could be regulating the photosynthetic response of seedlings in these forests. However, other characteristics of leaf photosynthesis indicate that seedlings in thickets have photosystems that are more acclimated to take advantage of the lower diffuse light levels not direct light in the form of sunflecks. For instance, at low light there was no difference in the photosynthetic rate of seedlings irrespective of their position in the forest. In a prior study, Semones et al. (manuscript submitted) found that steady state photosynthesis at low light was actually significantly higher for plants located within thickets. Second, there was little difference in the induction states of seedlings from either forest condition at 0 or 90s indicating that they reach relatively the same induction status. The predominant difference was that maximum photosynthetic rates were significantly greater for plants located in forest without *R. maximum*.

### *Conclusion*

Our study supports light limitation as a major mechanism of seedling inhibition within thickets of *R. maximum*. The general reduction in light availability under thickets of *R. maximum* causes an adjustment in the photosynthetic apparatus of seedlings within thickets such that they have lower maximum rates of photosynthesis at similar PAR intensities when compared to plants not growing under *R. maximum*. Second, most plants located within thickets do not receive as much PAR, especially in the form of sunflecks. Furthermore, seedlings in forest with *R. maximum* do not always utilize sunflecks with equal efficiency as those seedlings outside of thickets. This holds true even though induction status at 0, 60 and 90s for at least eight consecutive sunflecks is not different

for seedlings within thickets when compared to plants outside of thickets. Moreover, seedlings located within thickets do not optimize carbon gain during flecks when compared to seedlings in forest sites without *R. maximum*. Consequently, seedlings growing under *R. maximum* will not gain an optimal benefit from sunflecks and thereby they will suffer from carbon limitation.

### **Acknowledgments**

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## Conclusion

Thickets of *R. maximum* are expanding in the forest understory of the southern Appalachian Mountains. Shrub thickets inhibit the regeneration of understory plants including the seedlings of most canopy tree species. In addition, shrubs also lower the production of canopy trees within thicket perimeters. Until recently, the mechanisms of inhibition were mostly conjecture. The studies presented in this dissertation help clarify several mechanisms of inhibition for tree seedlings growing within thickets.

There is evidence that soil resources can be significantly impacted in thicket soils when compared to soils in forest without *R. maximum*. Particularly, soil water content, cation concentration, and nitrogen mineralization rates are all lower in thicket soils when compared to forest soils without *R. maximum*. However, by far the most dramatic impact of *R. maximum* thickets is the attenuation of below canopy PAR. Using direct and indirect measures of light availability we found PAR availability to be significantly lower in forest areas covered by shrub thickets. However, it should be noted that there is considerable variation in canopy openness such that there are locations within shrub thickets with similar or higher percent canopy openness as sites in forest without *R. maximum* and vice or versa.

*Quercus rubra* and *P. serotina* seedlings growing within *R. maximum* thickets have lower mid-day photosynthetic rates and significantly lower maximum photosynthesis at saturating PAR than seedlings growing in forest without shrubs. Furthermore, diffuse PAR within thickets is often near or below the light compensation

point for these woody species as indicated by light response curves. As a result, direct PAR in the form of sunflecks should be critical for net daily carbon gain of tree seedlings located within thickets when compared to forest sites without *R. maximum*.

Tree seedlings growing in forest with *R. maximum* apparently have optimized light harvesting capabilities to take advantage of the low quantities of diffuse PAR present in the forest understory the majority of the time. Plants within thickets often have significantly higher net photosynthesis at low light (e.g.,  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than seedlings in brighter forest sites without *R. maximum*. Also, when exposed to light flecks of varying duration and intensity, seedlings growing in thickets assimilate significantly less carbon than plants located in forest sites without *R. maximum*. This reduced carbon gain during light flecks is the direct result of lower maximum  $\text{CO}_2$  assimilation for seedlings growing in forest sites with *R. maximum*. In addition, the results fail to indicate that seedlings utilize light flecks of similar duration and intensity as efficiently as seedlings growing in forest without *R. maximum*. In fact, for the first five light flecks in succession, seedlings growing in forest without shrubs had significantly greater photosynthetic efficiencies than seedlings located within a thicket perimeter. Moreover, tree seedlings located in forest sites without *R. maximum* reach higher states (often significantly higher) at 0, 60, and 90 seconds when compared to plants under thickets. Hence, tree seedlings in forest sites without *R. maximum* utilize sunflecks more effectively and efficiently than seedlings growing in the deep shade of a thicket.

Do factors other than PAR exposure during leaf development constrain the carbon gain during a sunfleck for tree seedlings growing within a shrub thicket? This question

could not be answered conclusively from the results presented in this dissertation. It is entirely possible that some other resource limitation (e.g., abiotic or biotic edaphic resource) prevents optimal utilization of direct PAR during sunflecks for tree seedlings growing in thickets relative to plants located in forest sites where shrubs are absent. This could be a particular problem for understory plants surrounded by a thicket that has a predominant gap in the subcanopy.

Light is often the most limiting resource for plant carbon gain in the understory of many forests. Now there is compelling evidence that light could be the predominant limiting resource for seedling carbon gain when growing within thickets of *R. maximum*. Consequently, dramatic PAR attenuation resulting in rapid carbon limitation could be the mechanism underlying the inhibitory nature of *R. maximum* thickets on understory plants. These results are important because expanding thickets of *R. maximum* could influence the structure and composition of temperate deciduous forests by differentially affecting the survival of regenerating woody species.

## **Vita**

Shawn Wayne Semones was born on September 22, 1968 in Roanoke, Virginia. Nelson Wayne Semones and Louanne Abercrombie Semones are his parents. He attended Jeffersonville High School in Jeffersonville, Indiana where he graduated in 1986. Shawn began his undergraduate education at Wabash College in Crawfordsville, Indiana where he stayed until his junior year. He then transferred to Virginia Commonwealth University in Richmond, Virginia spring semester 1989 and went on to earn a B.S in biology. In 1991, Shawn began working on a Masters degree under the advisement of Dr. Donald R. Young. His thesis work focussed on the ecology of barrier islands, and over the next three years Shawn spent a considerable amount of time on the Virginia barrier islands. While in Richmond, Virginia, Shawn met his future wife, Deborah Purvis Semones. On July 23, 1993, Deborah gave birth to Shawn's son Jacob Alexander Semones. Shawn and Deborah were married July 15, 1994. Shortly thereafter Shawn earned his M.S. in biology. With degree in hand, Shawn moved his family back to Roanoke, Virginia to start a career. The career was developing slowly but in 1995 Dr. Erik Nilsen offered Shawn an opportunity to attend Virginia Tech and work on a Ph.D. Hence, in September 1995, Shawn began working on his doctoral dissertation. Shawn currently lives in Christiansburg, Virginia with his wife and son. Following his doctoral work, Shawn will be employed at Sybron Biochemicals Inc. located in Salem, Virginia.