The photoprotective role of thermonastic leaf movements in *Rhododendron maximum*: potential implications to early spring carbon gain

Raymond B. Russell

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

in

Biological Sciences

Committee:
E.T. Nilsen, Chair
R.H. Jones
J.R. Seiler

July 19, 2006

Blacksburg, Virginia

KEY WORDS: Photoprotection, Photoinhibition, *Rhododendron maximum*, Early Spring Photosynthesis, Fluorescence, Gas exchange

The photoprotective role of thermonastic leaf movements in *Rhododendron maximum*: potential implications to early spring carbon gain

Raymond Russell

ABSTRACT

Rhododendron maximum L. is a dominant subcanopy species in the southern Appalachian Mountains. *R. maximum* undergo distinct thermonastic leaf movements (TLM). The purpose of these movements has not yet been determined. Previous studies have suggested TLM are a photoprotective mechanism for the dynamic light environment of the subcanopy in a deciduous forest during winter. The present study aimed to determine the effects of restricting TLM on photoinhibition, net photosynthesis, and other gas exchange parameters, particularly during the early spring. After restricting TLM on certain leaves, we observed the above parameters from autumn 2005 to late spring 2006. Our results indicated that photoinhibition increased (lower F_v/F_m) in treatment leaves over reference leaves throughout the winter. The difference became greater during the early spring, when reference leaves began to return to normal levels of photochemical efficiency and treatment leaves sustained low F_v/F_m. Net photosynthesis was lower for treatment leaves than reference leaves. This became most significant during the early spring, when maximum carbon gain is possible. Finally, gas exchange parameters as measured by light and CO_2 response curves did not indicate any significant difference between treatment and reference leaves post canopy closure. Out results suggest that TLM are an important mechanism for photoprotection, allowing leaves of *R. maximum* to recover quickly during the early spring and maximize their early spring carbon gain.

ACKNOWLEDGEMENTS

I would like to thank Erik Nilsen for his guidance throughout this project. My other committee members, Robert Jones, and John Seiler, also deserve a large amount of credit for their insight and help over this process. Thanks to Bobbie Niederlehner for her assistance developing an HPLC protocol and during my attempts at pigment analysis. I extend my thanks to Volker Ebbert from the University of Colorado at Boulder for assistance in developing the attempted HPLC protocols. This project was funded by the Graduate Research Development Program and the Virginia Tech department of Biological Sciences. Lastly I would like to express my gratitude to Amanda Laz for her guidance and Meredith Benedict for her encouragement and support.

TABLE of CONTENTS

List of Figures

List of Tables

Chapter 1: Literature review

The photoprotective role of thermonastic leaf movements in *Rhododendron maximum*: potential implications to early spring carbon gain

1.1 Environmental Conditions Faced by Evergreens

Temperate forests are areas of broad seasonal and diurnal environmental changes. These areas often have high temperatures and rainfall during summers and low rainfall and temperatures during the winter. Plants may be faced with severe drought and freezing stress during the winter. In fact, plants are often subject to potentially damaging freeze-thaw cycles. Furthermore, in deciduous forests, canopies open in the winter and allow prolonged high light intensities into the subcanopy. Over-wintering evergreen plants of the subcanopy are, therefore, faced with the difficult situation of surviving high light, cold temperatures, and low moisture availability. Conditions such as these can cause leaf physiological problems such as photoinhibition. It may be for this reason that there are relatively few subcanopy evergreen taxa throughout temperate regions (Quigley and Platt 2002).

1.2 Photoinhibition

1.21 Definition

Photoinhibition is the decrease in photosynthetic ability of plants subject to high light intensities (Adir et al. 2002). This decrease can be brought about by oxidative damage to photosystem II by reactive oxygen species (ROS) and the subsequent removal of the D1 protein from the PSII reaction center (Schnettger *et al.* 1994). Also, photoinhibition is observed when an increase in photoprotective pigments quenches the light thereby reducing photosynthetic capacity (Taiz and Zieger 2002). Previous work has shown an increase in photoinhibition during the winter when genes encoding photosynthetic proteins, including light-harvesting chlorophyll a/b binding protein, are down regulated (Wei *et al.* 2005).

1.22 Causes and Problems

Under high light intensities, electrons could enter the photosystems at a higher rate than they can be passed on to the final acceptor of the transport chain. Under these conditions, the photosystems are readily over-reduced resulting in the formation of ROS (Buchanan *et al*. 2000). This problem is worsened considerably by water stress, which causes the stomata to close and then the CO_2 concentration drops inside the leaf due to carboxylation of Rubp by RUBISCO. This drop of internal CO_2 concentration in the leaves causes a reduction in photosynthesis. There will be progressively less CO_2 for RUBISCO to use, causing the Calvin cycle to slow and photorespiration to occur. With the slowing of the Calvin cycle, the photosynthetic machinery is no longer a dominant sink for electrons (Taiz and Zeiger 2002). Also, photosynthesis is slowed during times of drought by inhibited ATP synthase, causing a build up of ADP+ and RuBP, and resulting in the inability of photosynthesis to pass on electrons (Tezera et al. 1999). The build up of this reduction potential can result in electrons being passed on to form ROS (Adams et al. 2004). When oxygen interacts with the now over-reduced photosystem directly, singlet oxygen (a type of ROS) can be formed. When oxygen is the final electron acceptor in the electron transport chain, superoxide radicals (a type of ROS) are formed (Demmig-Adams and Adams 1993). ROS can damage membranes and proteins causing damage to cellular and organelle function. Potential membrane damage by ROS is a particular problem for subcanopy evergreen plants in the winter during conditions of high light, water limitation and slow enzymatic rates due to cold temperatures.

The D1 protein is a core protein in the PSII reaction center. Removal of the D1 protein is a mechanism for plants to avoid severe damage to PSII. The D1 protein is continually removed and replaced at normal irradiances. At high light intensities or during periods of stress the rate of damage and removal of the D1 protein may exceed the rate of repair (Adir *et al*. 2003). If this occurs then the efficiency of electron transfer will be greatly reduced. Photoinhibition, as measured

by chlorophyll fluorescence, increases as the rate of recovery of the D1 protein decreases (Ebbert *et al.* 2005). This will occur if plants lose the ability to degrade the damaged D1 protein, resynthesize it, and return it to the PSII reaction center (Aro *et al.* 1993). Once plants are returned to normal light levels, the D1 protein can be repaired and reinserted into the PSII reaction center. However, it may be more energy efficient to prevent the degradation of the D1 protein by photoprotective mechanisms than to operate repair mechanisms (Adams *et al*. 2004). Also, these repair mechanisms may not be functional during periods of cold temperature or drought stress (Logan *et al.* 1998b).

Many enzymes lose their functionality when temperatures drop, including photosynthetic enzymes and those which scavenge ROS. Also, when temperatures fall, there is often a lack of moisture availability to the plant, causing stomatal closure. During the winter in deciduous forests of temperate regions, cold temperatures combine with low moisture availability and high light intensities. Plants that retain their leaves are subjected to all of these conditions, and so they often experience photoinhibition (Long et al. 1994). The reduced photosynthetic capacity of these plants likely cannot return to normal until the temperature rises and moisture again becomes available (Neuner et al. 1999). This delay may be due to the decreased ability to repair the photosystems under cold conditions.

1.23 Quantifying Photoinhibition

Photosynthetic Photo Flux Density (PPFD) absorbed in the light harvesting apparatus can have three fates. Absorbed photo energy can be used for photochemistry, radiated as heat, or fluoresced as light (Maxwell and Johnson 2000). By measuring the fluorescence parameter F_v/F_m it is possible to examine PSII photochemical efficiency and to quantify photoinhibition. To gather these data, leaves are dark adapted for 15 minutes and then fluorescence measurements are taken. Values of F_v/F_m below approximately 0.8 indicate photoinhibition that is not reversible after at least

15 minutes of dark adaptation (Einhorn *et al.* 2004). Measuring Fv/Fm has become a common tool for assessing photoinhibition (Gouallec *et al.* 1991). However, a reduction of F_v/F_m can be due to both photoprotection and photo-damage (Maxwell and Johnson 2000). Often a portion of any reduction in F_v/F_m is due to thermal energy dissipation via an increase in photoprotective xanthophyll pigments (Demmig-Adams 1998). Many studies quantify photoprotective mechanisms in order to separate them from damage.

1.3 Study Species, *Rhododendron maximum* L.

1.31 Ecology and Habit

Rhododendron (Ericaceae) is a diverse genus of over 1000 species including many evergreen shrubs in alpine, temperate, and tropical environments. *Rhododendron maximum* L. is a dominant subcanopy evergreen of the southern Appalachian forest. A mosaic of dense *R. maximum* thickets can be found throughout the subcanopy of both deciduous and coniferous areas of the southern Appalachian Mountains. *R. maximum*, a large multi-branched shrub (to 4m in height), is most vigorous in moist areas, especially around forest streams and on north slopes. Leaves are large (length=20cm width=7cm area=92cm^2), 4-7 merous, lanceolate to oblong lanceolate, and found on the terminal 2-7 nodes of each branch. Leaves survive up to 7 years in shaded environments, 4 years in intermediate light, and 2 years in full sun (Nilsen 1986). *R. maximum* is an ideal model for studying photoinhibition because leaves experience several winter seasons when temperatures are often below freezing and light intensities are the highest of the year because the forest canopy opens at this time. The large entire leaves, their persistence through the winter, and their distinct yearly growth make working with leaves on *R. maximum* appropriate for studies of photoprotection mechanisms.

1.32 Distribution

The range of *R. maximum* is from Central Georgia to southern Canada in the Appalachian and Adirondack Mountains. This broad latitude and elevation range causes the species to encounter a wide variety of environmental conditions. Thus, *R. maximum* must be well adapted for many combinations of cold and drought that would predispose leaves to a high risk of photoinhibition.

1.33 Leaf Movements

Several species of *Rhododendron*, including *R. maximum*, exhibit winter thermonastic leaf movements (TLM) in response to cold temperature and drought stress (Nilsen 1992). The thermonastic movements of *Rhododendron* species include two distinct processes. Leaves fold downward at the petiole in response to petiole turgor pressure, and leaves roll around their central lengthwise axis in response to cold temperatures. The functional significance of TLM is a subject of debate. However, the most supported hypothesis is that TLM functions as a mechanism to protect leaves of subcanopy evergreen plants from photo-damage due to high light and cold temperatures (Nilsen 1992). Leaves that undergo TLM may reduce the level of sunlight directly incident upon the leaf surface and therefore reduce the potential for photo-oxidative damage.

1.34 Early Spring Carbon Gain

The subcanopy of a temperate deciduous forest is an area of dynamic seasonal light intensities. The growing season is dominated by heavy shading with intermittent sunflecks reaching the understory (Chazdon 1988). When the deciduous upper canopy opens in the autumn, the forest understory is subjected to the highest light intensities of the year, yet temperatures are generally too low during the winter to allow high levels of photosynthesis (Adams *et. al.* 2004). The possible exceptions to these conditions may be during the early spring and late autumn when temperatures

can be warm enough and light intensities high enough to allow significantly increased photosynthetic rates (Lei and Koike 1998). These fairly short time periods of favorable conditions may allow for a significant amount of the annual carbon gain for understory plants (Rothstein and Zak 2001). Some species may use this increase in carbon gain to allocate increased resources to storage (Walters and Reich 1999) or to budding, shoot growth, and root growth (Wang and Zwiazek 1999). Understory plants able to increase their photosynthetic capacity have the best chance of utilizing this brief and rapid increase in favorable conditions (Rothstein and Zak 2001). Intermittent periods of freezing temperatures can greatly reduce the ability of plants to maximize their early spring carbon gain (Ensminger *et. al.* 2004). Since these time periods are important to annual carbon gain for many plants, any interruption in photosynthetic rates and capacity could be detrimental to the fitness of understory plants. It is important for many understory plants to obtain maximal photosynthetic rates during the early spring to maximize annual carbon gain before the canopy closes and they return to typical, shade tolerant photosynthetic rates (Lei and Koike 1998).

1.4 Photoprotection

1.41 ROS Scavenging

Plants have many methods of dealing with the deleterious effects of extreme light intensities. When ROS are formed, the plant must scavenge these in order to prevent damage to the photosystems, cell membranes, or proteins. The water-water cycle is one such mechanism to control ROS. This process utilizes superoxide dismutase to convert the ROS, superoxide, into H_2O_2 and O_2 at which point ascorbate peroxidase can convert H_2O_2 back into water (Asada 1999). Plants also contain other enzymes to prevent damage from ROS such as glutathione reductase and catalase that both help by reducing H_2O_2 to H_2O. In high light environments, plants often increase levels of ascorbate and other antioxidants in order to scavenge ROS that will inevitably be produced (Logan

et al. 1996, 1998a). For the understory evergreen, *Mahonia repens*, growing in a temperate climate, high light environments during winter increased levels of ascorbate and other antioxidants (Logan *et al.* 1998b).

1.42 Other Protective Mechanisms

There are many tools that plants posses to fix damage caused by ROS and to scavenge these reactive species, however it is more energy efficient for the plant to prevent such damage (Adams *et al.* 2004). Photorespiration is a potential mechanism of diverting excess energy from being passed into ROS. Some plants utilize the movement of chloroplasts to avoid direct sunlight and thereby reduce the amount of light energy absorbed (Kasahara *et al.* 2002). Other species utilize coverings on the leaf surface such as waxes to reflect damaging light (Barker *et al*. 1997) *R. maximum* may use leaf movements as a means of avoiding direct sunlight during times or high light, cold stress, and water stress (Nilsen 1992). Also, the core protein of PSII, D1, becomes phosphorylated during extended periods of high light and will remain in that state even once light levels return to normal. The exact purpose of this is still unclear, but it may play a role in winter acclimation, preventing damage to PSII core proteins by keeping them removed during times of increased light and low temperatures (Ebbert *et al.* 2005).

A recent study suggests that *Rhododendron catawbiense* may potentially reduce the formation of ROS in cold acclimated leaves by down-regulating the genes encoding NADH dehydrogenase (Wei *et al*. 2005). Wei *et al*. (2005) also found that *R. catawbiense* up-regulated the genes encoding early light induced proteins in cold acclimated leaves (ELIPs). ELIPs are proteins that accumulate under high light and may dissipate excess light energy to prevent photo-damage (Hutin *et al.* 2003).

Most plants likely utilize multiple mechanisms to protect leaves from damaging light

intensities. However, it is commonly accepted that the largest sink for light energy, particularly in high light conditions and drought stress, is thermal dissipation (Osmond *et al.* 1997). Unlike chlorophyll, which will readily pass energy to O_2 creating ROS, carotenoids absorb excess energy and can dissipate it as heat (Demmig-Adams and Adams 1996).

1.43 Biochemistry of the Xanthophyll Cycle

Carotenoids are a family of pigments that contain the xanthophylls. There are three states of xanthophyll pigments in chloroplast lamellae. Violaxanthin dominates in low light conditions and under high light intensities it is converted to zeaxanthin through the antheraxanthin intermediate (Yamamoto *et al*. 1962). The three states of xanthophyll in chloroplast lamellae undergo a balanced shift between the different forms that can be detected by several analytical means (Yamamoto 1985). The shift from violaxanthin to zeaxanthin via antheraxanthin is a de-epoxidation reaction, whereas the reverse is completed by epoxidation (Demmig-Adams 1993). The epoxidation occurs in the stroma of the chloroplast, while de-epoxidation occurs in the loculus (Yamamoto 1979). These reactions are catalyzed by the enzyme violaxanthin de-epoxidase (VDE) (Yamamoto 1978). The de-epoxidation of violaxanthin to zeaxanthin is driven by the build up of ascorbate and the creation of a pH gradient across the thylakoids membrane during high light (Yamamoto 1979). The creation of this pH gradient not only drives the de-epoxidation of violaxanthin, but may aid directly in quenching of excess light (Gilmore and Yamamoto 1993).

The structure of these xanthophyll molecules, particularly zeaxanthin, allows for light energy to be dissipated harmlessly as heat through non-radiative energy dissipation (Adams *et al.* 2004). These pigments are in close relation to the light harvesting complex (LHC) of PSI and PSII, and have been shown to up-regulate and become more strongly correlated with PSI and PSII in plants gown in high light conditions (Verhoeven *et al*. 1999b). Furthermore, the expression of

VDE is up-regulated in plants grown in high light environments (Bugos *et al.* 1999). The size of the xanthophyll pool is substantially up-regulated during winter months in many plants (Demmig-Adams and Adams 1996). Specifically, levels of zeaxanthin and antheraxanthin increase during high light and cold temperatures (Logan *et al.* 1998b). A study with *R. catawbiense* has shown a 2.3-fold increase in xanthophyll pigments during the winter strongly correlated with a decrease in chlorophyll fluorescence (F_v/F_m), an indicator of photoinhibition (Harris *et al.* 2006).

1.44 Ecology of the Xanthophyll Cycle

During winter months, plants are often faced with cold temperatures and high light conditions. Up-regulation of the xanthophyll pool during the winter is clearly a mechanism to protect the plant from these harsh conditions (Verhoeven *et al.* 1998, 1999a; Adams *et al.* 2001). The xanthophyll cycle is the primary mechanism of non-radiative dissipation of light energy in many plants (Demmig-Adams and Adams 1996). The xanthophyll cycle protects the photosynthetic machinery particularly during these periods of stress when other protective machinery may not be functioning (Verhoeven 2005). There is a strong relationship between photoinhibition and increases in zeaxanthin and antheraxanthin, such that during times of prolonged excess light zeaxanthin and antheraxanthin are retained and PSII photochemistry will remain low (Demmig-Adams *et al.* 1998). This slowly relaxing non-photochemical quenching of light may allow plants to cope with excess light while not able to undergo sufficient levels of electron transport to handle the incident light energy (Horton *et al*. 1996). Such conditions occur during winters in temperate deciduous forests where temperature and water availability are low while having increased sunlight reaching the subcanopy. The dissipation of excess light energy that the xanthophyll cycle allows may permit many plants to survive through cold winters in high light.

1.5 Rationale for the Current Study

R. maximum is ideal for studying photoinhibition because it is exposed to many combinations of cold and drought that would predispose leaves to a high risk of photoinhibition. Furthermore, the TLM that *R. maximum* leaves undergo may play an important role in preventing severe photo-oxidative damage. A previous study has suggested that photo-protection is the functional significance of TLM (Bao and Nilsen 1988); however the exact role remains unclear. In order to fully understand how *R. maximum* leaves survive the winter, it is critical to know the significance of TLM to preventing photo-damage and maximizing carbon gain in the early spring. Our study is designed to measure chlorophyll fluorescence as an indicator of photoinhibition and to test the seasonal effect of TLM on photoinhibition through the winter and into the spring. Also, we will measure seasonal net photosynthesis to discover if a potential increase in photoinhibition will negatively affect net carbon gain. Most importantly, we examine the significance of TLM to early spring carbon gain and the extent of recovery by the late spring (after canopy closure). Since the early spring is of such importance to subcanopy plants (Lei and Koike 1998), it is important to recover from winter photoinhibition before canopy closure. Previous work has shown that the capacity for carbon gain in *R. catawbiense* did not fully recover from winter acclimation until 14 days after temperatures were increased (Harris *et al*. 2006). Similar delays in *R. maximum*, if they occur, may have large impacts on annual carbon gain.

Chapter 2: The photoprotective role of thermonastic leaf movements in *Rhododendron maximum*: potential implications to early spring carbon gain

2.1 INTRODUCTION

Temperate forests are areas of broad environmental changes that have high temperatures and rainfall during summers and low temperatures and reduced water availability during the winter. Plants are likely to experience severe drought and freezing stress during the winter. Furthermore, in deciduous forests of these regions, the canopy opens in the winter and allows prolonged high intensity light to reach the subcanopy. Therefore, over-wintering leaves on evergreen plants in the subcanopy must survive high light, freezing temperatures, and low moisture availability. Conditions such as these can cause damage to evergreen leaves including photoinhibition. In mountain regions, such as the Appalachian Mountains of the USA, winter climatic conditions are temporally heterogeneous. Some days may have freezing conditions all day, other days may reach thawing temperatures, and yet other days have remarkably warm conditions all day. This temporal heterogeneity may allow subcanopy evergreen leaves to temporarily recover from photoinhibition during the winter.

Photoinhibition is the decrease in photosynthetic ability of plants subject to high light intensities (Adir et al. 2002), which can be brought about by oxidative damage to photosystem II by reactive oxygen species (ROS) and the removal and reduced turnover of the D1 protein from PSII reaction centers (Schnettger *et al.* 1994). This oxidative damage can be worsened considerably by water stress, which causes the stomata to close, reduces intercellular CO_2, and increases intercellular O_2. The build up of reduction potential in the electron transport system, caused by low electron flow into the Calvin cycle intermediates, can result in electrons being passed to oxygen to form ROS and increased photoinhibition (Adams et al. 2004). However, some plants have developed photoprotective mechanisms to prevent such oxidative damage.

Rhododendron (Ericaceae) is a diverse genus of over 1000 species including many evergreen shrubs in alpine, temperate, and tropical environments. Several species of *Rhododendron*, including *R. maximum* and *R. catawbiense*, exhibit winter thermonastic leaf movements (TLM) in response to cold temperature and drought stress (Nilsen 1991). TLM in *Rhododendron* species include two distinct processes. Leaves fold downward at the petiole in response to petiole turgor pressure, and leaves curl around their central lengthwise axis in response to cold temperatures. The functional significance of TLM is equivocal. However, it is most likely that TLM functions as a mechanism to reduce exposure to light, which may protect leaves of subcanopy evergreen plants from photo-damage due to winter time high light and cold temperatures (Nilsen 1992). Photoprotection through TLM may be critical for allowing recovery during the winter and maximal early spring carbon gain.

Early spring photosynthesis can contribute significantly to the annual carbon gain of subcanopy species (Lei and Koike 1998). Just before upper canopy leaf-out, temperatures and moisture availability become favorable for photosynthesis while still in a high light environment. During this period it is important for subcanopy evergreen plants to maximize photosynthesis in order to take advantage of the high light intensities and warm temperatures (Rothstein and Zak 2001). Any disturbance to the plants ability to utilize these favorable conditions could have harmful effects on annual carbon gain and fitness (Enminger *et al.* 2004).

Rhododendron maximum L. is a dominant subcanopy overwintering evergreen of the southern Appalachian forest. A mosaic of dense *R. maximum* thickets can be found throughout the subcanopy of both deciduous and coniferous areas of the southern Appalachian Mountains. *R. maximum*, a large multi-branched shrub (to 4m in height), is most vigorous in moist areas, especially around forest streams and on north slopes. Leaves are large (length=20cm width=7cm area=92cm^2), 4-7 merous, entire, lanceolate to oblong lanceolate, and found on the terminal 2-7

nodes of each branch. Leaves survive up to 7 years in shaded environments, 4 years in intermediate light, and 2 years in full sun (Nilsen 1986). There is clear anatomical evidence for photo-damage of leaves in high light environments, potentially leading to shorter survivorship (Nilsen *et al*. 1988).

R. maximum is ideal for studying photoinhibition because it is exposed to many combinations of cold and drought that would predispose leaves to a high risk of photoinhibition. Furthermore, leaves experience several winter seasons during their life time when temperatures are often below freezing and light intensities are the highest of the year. The large entire leaves, their persistence through multiple winters, and their distinct yearly growth (even aged leaf cohorts) make working with leaves on *R. maximum* appropriate for experiments on photoprotection mechanisms.

Previous work on TLM in *R. maximum* has shown that restricting these movements can cause a decrease in chlorophyll content in the spring and have a long term impact on photosynthetic rates and quantum yield (Bao and Nilsen 1988). This study indicated that there may be a lasting effect of restricting TLM over the winter when conditions are most favorable for photo-damage. However, this effect was found to be minimal and thought to be of little ecophysiological significance to plants in a low light, subcanopy environment. Initial studies of the functional significance of TLM have not demonstrated an effect on winter photoinhibition, the ability to recover from photoinhibition in the winter, or the importance of TLM to early spring carbon gain. This information is critical to understand the functional significance of TLM and to fully understand the mechanisms by which evergreen leaves survive winter conditions.

The overall objective of this project is to provide evidence to address the question:
Do TLM in *Rhododendron maximum* provide enough protection from photoinhibition to allow a full recovery of photosynthesis for maximal early spring carbon gain?
Evidence in support of this objective would suggest that TLM are an effective means of reducing the negative impacts of photoinhibition on *Rhododendron maximum* leaves. Furthermore,

supporting evidence would suggest that photoprotection by TLM may be an important means for allowing *R. maximum* to maximize early spring carbon gain.

In this study, we focused on the presence and absence of TLM, evidence for photoinhibition, and consequences to gas exchange for *R. maximum* from the early autumn to the late spring. We based our study on the assumption that TLM is a photoprotective mechanism that will prevent photoinhibition and allow rapid recovery of gas exchange during favorable temperature conditions. We then tested the following hypotheses:

1) Leaves prevented from TLM will experience greater photoinhibition as compared to reference leaves.
2) During the early spring (before upper canopy leaf-out), photosynthetic rates and PSII photochemical efficiency (F_v/F_m) in leaves prevented from TLM will exhibit reduced recovery from winter conditions when compared to reference leaves.
3) During the late spring (after canopy closure), light saturated photosynthetic rates (A_{max}), Quantum efficiency (QE), maximum electron flow capacity (J_{max}), and CO_2 saturated photosynthesis (A_{sat}) will be lower in leaves prevented from TLM than in reference leaves.

This research was designed to build on previous studies of TLM and to further elucidate the functional significance of TLM for understory evergreen plants. Moreover, this study provides further understanding of mechanisms for photoprotection of evergreen leaves in temperate environments. We approached this study by designing an experiment to investigate the consequences of TLM to gas exchange properties. We implemented treatments restricting TLM to asses their effects on fluorescence, gas exchange, and early spring carbon gain.

2.2 METHODS

2.21 Study Location

This study was conducted in the Jefferson National Forest of southwest Virginia approximately 8 kilometers northwest of Blacksburg, VA (37°17'N, 80°27'W, 675m elevation). Average temperature in winter is approximately 2°C (commonly below 0°C) with average precipitation below 70cm/month. During the summer, the average temperature is approximately 20°C with precipitation above 110cm/month (Nilsen 1992) Deciduous trees *Quercus alba, Quercus rubra,* and *Acer rubrum* dominate the canopy. *Rhododendron maximum* is the dominant subcanopy plant.

2.22 Experimental Design

In October 2005, we arbitrarily identified and labeled 15 individual *R. maximum* plants. We assigned two outer canopy branches on each plant either as treatment or reference (n=15 for treatment and reference). On both treatment and reference branches, the second year leaves were individually labeled for study. *R. maximum* leaves grow in distinct annual whorls which allows for accurate determination of leaf age (Nilsen 1986). Second year leaves were selected to ensure full physiological and morphological maturity of the leaves (Nilsen *et al*. 1988) and hardening of the stem required to support the treatment.

All second year leaves were restricted from thermonastic leaf movements on treatment branches. We prevented leaf folding by twisting insulated solid copper wire (14 gauge) around the stem on the internode between the second and third year leaves. Above the twisted section, the wire was bent out into a large circle (approximately 30cm in diameter) just under the second year leaves in order to support them. We cut the middle of double barreled plastic coffee stirrers to approximately the width of each leaf, then placed these divided stirrers around the lamina of each leaf on all treatment branches to prevent curling. We placed wire around the stems of reference

branches and made a loop that extended above the topmost leaves to control for possible treatment damage.

2.23 Photosynthesis Survey

We measured the rate of leaf net photosynthesis with a steady-state, portable photosynthesis system (model 6400, LI-COR Inc., Lincoln, NE, USA) using the standard transparent leaf chamber. Initially we measured net photosynthesis over the course of a day to determine times of maximum photosynthetic activity. CO_2 concentrations were held to about 380ppm using the LI-COR 6400 CO_2 mixer. Survey photosynthesis measurements were collected over one day for all plants between 1000 and 1500, as was determined to be the time of maximum daily photosynthesis by diurnal cycle measurements. Measurements were taken during the fall and winter of 2005 into the spring of 2006. Data were collected twice in November 2005 once in January and February 2006 and then every two weeks during March, April, and ending in May 2006. No measurements were taken during December 2005 because the reference leaves generally remained curled and preliminary data (not shown) indicated the leaves were photosynthetically inactive.

2.24 Chlorophyll Fluorescence

Dark adapted, PSII photochemical efficiency (F_v/F_m) was measured with a modulated fluorometer (model OS 500, Opti-Sciences, Hudson, NH, USA)). These data were collected over one day for all plants between 1000 and 1500. At least one leaf from each treatment and reference branch (30-45 leaves) was sampled once a month starting in October 2005 until March 2006 when measurements were taken once every two weeks until completion of the study in May 2006. Leaves were dark adapted for at least 15 minutes prior to measurements using dark adapting clips (Opti-Sciences,

Hudson, NH, USA). Values below approximately 0.8 indicate photoinhibition that is not reversible after at least 15 minutes of dark adaptation (Einhorn *et al.* 2004).

2.25 Photosynthetic Response Curves

We measured photosynthetic response to intercellular CO_2 (A-C_i curve) and light at three different times during the study. A-Ci and light response curves were measured on 6 plants (12 branches, 6 treatment, 6 control) between 1000 and 1500 over a span of 3 days. We measured response curves during early November before the canopy opened, again in late April before the canopy closed, and finally in early May, after the canopy closed.

The A-C_i response of leaves was measured with a portable photosynthesis system at 1000 μmol m^{-2} s^{-1} using the LI-6400-02B light source attachment (LI-COR Inc., Lincoln, NE, USA). Block temperature was held to approximately ambient and relative humidity (RH) was held at ambient levels. An auto program was used to collect A-C_i data. During the program, the CO_2 concentration in the cuvette (C_a) was initially held at 400ppm and, over 12 steps, decreased to 30ppm then increased to 1200ppm with measurements taken at each step (once stable for 15 seconds). We analyzed the data using Photosynthesis Assistant (Dundee Scientific, Ver. 1.1.2, Dundee, UK) to calculate CO_2 compensation point, CO_2 and light saturated photosynthesis (A_{sat}), carboxylation efficiency (CE), maximum rate of carboxylation by RUBISCO (V_{Cmax}), and maximal electron transport (J_{max}).

Light response data were collected using a LI-COR 6400 portable photosynthesis system. The concentration of CO_2 was held at 400ppm and light was controlled by the attached 6400-02B light source. Because darkness causes the stomata to close, the light response program began at high light (1200 μmol m^{-2} s^{-1}) and then stepped down over 13 intervals to 0 μmol m^{-2} s^{-1}. Seven of the 13 steps in the program were between 100 and 0 μmol m^{-2} s^{-1}. We used Photosynthesis Assistant to

calculate light saturated rate of photosynthesis (A_{max}), light compensation point, light saturation point, quantum efficiency (QE), and dark respiration rate.

2.26 Statistical Analysis

Survey net photosynthesis measurements and fluorescence data were analyzed using two methods with branches as the basic unit of measurement. First, a repeated measures paired analysis using a mixed procedure in SAS 9.1 (SAS Institute, Cary, NC, USA) was used to determine if there were any differences in treatment and reference leaves over the course of the study. This analysis was done with the help of the Virginia Tech Statistical Consulting Center (Blacksburg, VA). Secondly, paired t-tests were run on data from each sampling date to look for individual differences separate from other dates. Means of gas exchange data for treatment and reference leaves from A-C_i and light response analyses were evaluated by paired t-tests.

2.3 RESULTS

2.31 Survey Measurements

Pn began relatively high (compared to the rest of our measurements) in early November as the canopy began to open and declined in the winter for both treatment and reference leaves (Fig. 1). This decline coincided with lower temperatures after canopy opening. Pn rates remained low throughout the winter with treatment leaves regularly lower than reference leaves. Treatment leaves remained an average of 17% lower than reference leaves during the winter and early spring. Pn remained low until temperatures became warmer in March. Net photosynthesis did not recover during warm periods during the winter (Fig. 1). In early March, Pn began to recover in reference leaves yet was significantly lower in treatment leaves and the difference between treatment and reference increased during this time and became significantly different (Fig. 2). Reference leaves fully recovered by mid-April when temperatures increased while treatment leaves continued to have lower Pn. Net-photosynthesis of treatment leaves remained significantly lower than reference leaves until late April as the canopy began to close. During March and April mean treatment leaf Pn was consistently 14% lower that that of reference leaves. After canopy closure in May, both treatment and reference Pn decreased similarly due to lower light availability and treatment levels were no longer lower than reference.

F_v/F_m began high for treatment and reference leaves and quickly declined as the canopy opened and temperatures fell in November (Fig. 3). F_v/F_m of treatment leaves remained an average of 10% lower than treatment leaves from November 19th, until the end of the study. Changes in F_v/F_m were regular due to moderate winter weather, and significant recoveries of F_v/F_m occurred during the winter when the temperature was warmer. In fact, the patterns of temperature and Fv/Fm are highly associated throughout the study. The difference between treatment and reference reached its greatest value on the March 19 sampling date and during the rest of the early spring (Fig. 4). In

the early spring when temperatures warmed, F_v/F_m of treatment leaves were an average of 13% lower than reference leaves. Fluorescence (F_v/F_m) measured on treatment leaves remained significantly below reference leaves until after full canopy closure. Furthermore, F_v/F_m measured on treatment leaves was significantly lower than that of reference leaves on the final date of data collection (Jun 3rd).

2.32 Gas Exchange Response Curves

CO_2 and light response curves could only be collected when conditions were favorable enough for the plants to have conductance values above 0.01μmol/m^2/s. The generally low conductance values of *R. maximum* and the difficulty to induce stomata to open once closed prevented the measurement of any response other than survey measurements during the winter. For this reason, CO_2 and light response curves were only collected in October (before canopy opening), April (before canopy closure), and late May (after canopy closure).

CO_2 response curves of treatment and reference leaves (Fig. 5) allowed for the calculation of several physiological parameters. Respiration (Resp), maximum rate of RUBISCO carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}), CO_2 compensation point, carboxylation efficiency (CE), and light and CO_2 saturated rate of photosynthesis (A_{sat}) were all derived from CO_2 response curves sampled during October, April, and May (Table 1). Statistical analysis of treatment and reference values from May indicated no significant difference for any of the parameters (paired t-test, all p-values greater than 0.1). Sample sizes were too small during October and April to allow reliable statistical analysis. However, there appears to be a slight trend in the data, CE is slightly higher in both treatment and reference during May than the corresponding values in April.

Treatment and reference values, from light response curves (Fig. 6), of dark respiration (Resp), quantum efficiency (QE), light saturated photosynthetic rate (A_{max}), the light compensation

point, and the light saturation point showed no statistically significant difference in May or April (Table 2) (paired t-test, all p-values greater than 0.1). Due to the difficulty in collecting reliable response curves from *R. maximum* leaves during times of environmental stress, the sample size in October was too small to allow a reliable statistical analysis. However, there appear to be several slight trends in the data. Respiration rates of treatment and reference increased during April from October values and then decreased again in May, after canopy closure. The same trend was seen in light compensation points and light saturation levels as in respiration rates.

2.4 DISCUSSION

TLM have been proposed to be a photoprotective mechanism in *Rhododendron maximum* (Nilsen 1992). However, little work has been done to directly support this hypothesis. The proposed method by which TLM may help to prevent photoinhibition is by controlling leaf temperatures and avoiding direct sunlight (Bao and Nilsen 1988). Until this point, no work has observed the effects of restricting TLM on photoinhibition over the course of the winter into the spring. Furthermore, we have collected data on the effect that restricted TLM has on seasonal photosynthesis, since increases in photoinhibition may have a significant effect on photosynthesis.

2.41 Seasonal Photoinhibition

Fluorescence data collected from October 2005 to June 2006 indicate that treatment leaves, restricted from TLM, experienced greater photoinhibition than reference leaves (Fig. 3). These findings support Hypothesis 1. These data support other findings that fluorescence, as measured by F_v/F_m, decreases during the winter with low temperatures and increased light availability to subcanopy plants (Oberhuber and Bauer 1991; Verhoven *et al.* 1998). Since F_v/F_m is a powerful indicator of photoinhibition (Maxwell and Johnson 2000), our data suggest that *R. maximum* leaves are strongly photoinhibited during the winter. Furthermore, treatment leaves are subjected to even higher levels of photoinhibition.

We found that there were increased differences in F_v/F_m between treatment and reference leaves at times of warming during the winter and recovery during the spring. The largest difference between treatment and reference leaves was during early March (Fig. 4). This was a date when the coldest temperatures of winter abated. This finding suggests that TLM may allow for quick recovery times when conditions allow the plant to increase photochemical capacity. Other studies have shown that on warm winter days, evergreen species in sun exposed sites had sustained low

F_v/F_m, but in shaded sites did not have sustained low F_v/F_m (Adams *et al.* 2001). Treatment leaves in our study, had significantly lower F_v/F_m compared reference leaves on warm winter days. This suggests that TLM decreased the incident light on leaves which allowed some recovery of electron transport on warm winter days. We also found that treatment leaves had significantly lower F_v/F_m than reference leaves in the early spring when temperatures increased. These findings suggest an inability for treatment leaves to recover effectively from photoinhibition and lend support to Hypothesis 2.

Since recovery from photoinhibition involves the regeneration and replacement of the D1 protein (Alfonso 2004), we speculate that treatment leaves experienced further damage to photosynthetic machinery. Increased photoinhibition may be due to several factors. Down-regulation of photosynthetic machinery (Harris *et al.* 2006) as well as chronic removal and degradation of the D1 protein from PS II (Alfonso 2004) both result in increased photoinhibition. Photo-damage to machinery and degradation of PSII reaction centers rather than effective D1 removal and replacement would result in sustained photoinhibition in treatment plants, not allowing them to recover effectively. Furthermore, increased levels of xanthophyll pigments, particularly antheraxanthin and zeaxanthin will result in lower F_v/F_m (Adams *et al.* 2004; Harris *et al.* 2006). Based on these findings, we speculate that treatment leaves in our study may have been exposed to higher light levels for extended periods of time and therefore may have increased levels of xanthophylls. We propose the possibility that there may be a tradeoff between the use of TLM for photoprotection and the use of xanthophylls. These increased pigment levels could remain high during favorable conditions in the winter resulting in lower F_v/F_m. However, we were not able to directly address this issue and it would take further work to elucidate any biochemical changes in these leaves.

2.42 Photosynthesis and Early Spring Carbon Gain

It is likely that Pn declined in November because of low temperatures and low moisture availability. However, F_v/F_m probably remained high in November because of shading from the remaining canopy. Our results indicate that during the early spring, before canopy closure, photosynthetic rates and photochemical efficiency (F_v/F_m) in leaves prevented from TLM exhibited reduced recovery from winter conditions compared to reference plants. This supports Hypothesis 2. From January through late April, photosynthetic rates were always lower in treatment leaves than in reference leaves. The largest difference was during March and early April as the temperature increased and conditions became favorable (Figs. 1 & 2). The winter-long trend of lower photosynthetic rates in treatment leaves than in reference leaves is further evidence for Hypothesis 1, suggesting that treatment leaves are subject to more photoinhibition (here indicated by lower net photosynthetic rates) than reference leaves.

The most significant finding from our photosynthetic measurements is the larger decrease in treatment photosynthetic rates during the early spring (Fig. 2). This result supports other findings that *R. maximum* leaves restricted from TLM exhibit lower chlorophyll levels, during the spring, than unrestricted leaves (Bao and Nilsen 1988). Decreased photosynthetic capacity and net photosynthesis during the early spring could be damaging to the annual carbon gain of a plant (Rothstein and Zak 2001). Our findings suggest that TLM allow *R. maximum* to quickly recover its photosynthetic ability during the early spring in order to maximize carbon gain. This result may be the most important ecophysiological role of TLM, since early spring carbon gain is of such importance to plants under a deciduous canopy (Lei and Koike 1998). Our results indicated that photosynthetic rates in treatment leaves recovered to the level of reference leaves after canopy closure. This suggests that there is no long term impact on photosynthetic rates from restricting

TLM, but over several seasons we speculate that the loss of carbon gain from reduced photosynthesis in the early spring may be a more serious consequence.

2.43 Residual Impacts on Gas Exchange

Due to the logistical difficulty of collecting reliable light and CO_2 response curves during the winter, we were unable to observe detailed changes in gas exchange parameters from winter to spring. However, we were able to compare treatment with reference leaves and look for trends between October, April, and May. During the late spring (after canopy closure), light saturated photosynthetic rates (A_{max}), Quantum efficiency (QE), maximum electron flow capacity (J_{max}), and CO_2 saturated photosynthesis (A_{sat}) did not show any statistical difference between treatment and reference leaves. Nor we did not find any significant differences between other gas exchange parameters (Tables 1 and 2). These data do not support Hypothesis 3. This suggests that restricting TLM causes no lasting impact on gas exchange after canopy closure; both treatment and reference leaves were able to fully recover after canopy closure. These data contradict other findings which showed a significant reduction in maximum photosynthesis and quantum efficiency in leaves restricted from TLM (Bao and Nilsen 1988).

We observed several trends in out data over the three sampling dates. Light response curves indicated that respiration, light compensation point, and light saturation point were all slightly higher in April than in either October or late May. These data support other findings that show increased photosynthetic activity during early spring in subcanopy plants (Lei and Koike 1998). The increase in light compensation and saturation points may be due to the acclimation of leaves to higher light conditions. Our study exhibited very low light compensation points (3-14 $\mu mol/m^2/s$). These data reflect the shade tolerant nature of *R. maximum*, where plants adapted to use of low light levels may have very low light compensation points, near 5 $\mu mol/m^2/s$ (Lei *et al.* 2005). During the

early spring, plants may become acclimated to more favorable conditions and light compensation points would increase.

From CO_2 response curves we observed a trend indicating an increase in V_{cmax} and A_{sat} during April compared to October and late May. This seems to suggest acclimation to warmer temperatures favorable for photosynthetic activity. These trends, although not statistically significant, support other findings that plants can acclimate to utilize favorable conditions in the early spring (Rothstein and Zak 2001). The trend of increases in respiration and light saturation from light response curves and increased V_{cmax} and A_{sat} from CO_2 response curves may be indicative of the acclimation of plants to more favorable conditions (higher light intensity) for growth and light utilization in early spring.

2.44 Conclusions

The purpose of this study was to investigate the consequences of TLM to gas exchange properties and photoinhibition. By restricting TLM we were able to asses their effects on fluorescence, gas exchange, and early spring carbon gain. We found supporting evidence for Hypothesis 1, by restricting TLM we found a significant increase in photoinhibition throughout the winter and into the spring. Also, we found supporting evidence for Hypothesis 2; during the early spring, photosynthetic rates and PSII photochemical efficiency (F_v/F_m) in leaves prevented from TLM exhibited reduced recovery from winter conditions compared to reference plants. Finally, we were unable to support Hypothesis 3. We detected no long term impact on gas exchange between treatment and response curve data. However, we found several trends from response curves indicating an increase in photosynthetic acclimation during the early spring. This study has supplied evidence to support the hypothesis that TLM play an important photoprotective role for *R.*

maximum. Furthermore, TLM appear to allow *R. maximum* to recover quickly in the early spring to obtain maximal carbon gain.

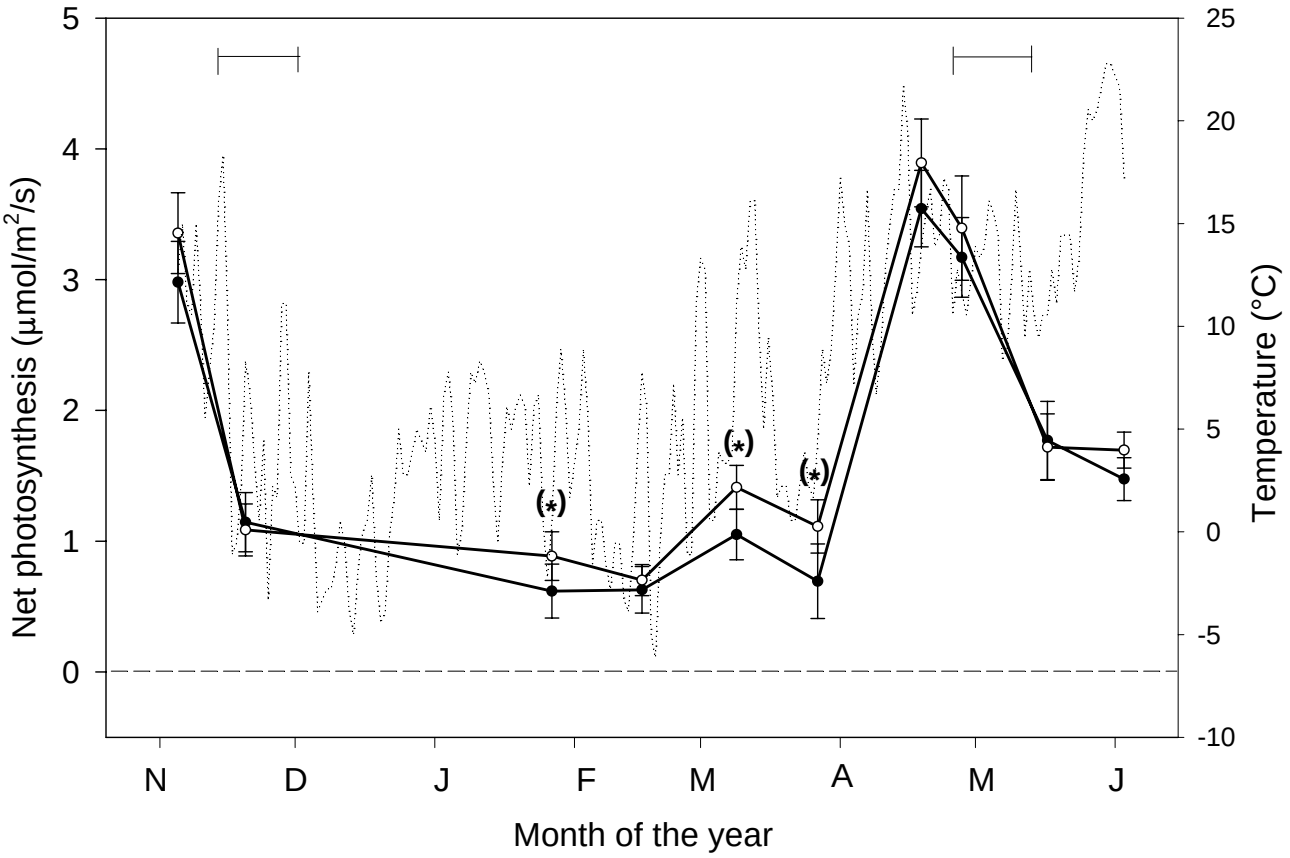

Figure 1. Seasonal changes in net photosynthetic rates (Pn) for *Rhododendron maximum* under a deciduous canopy from November 5, 2005 to June 3, 2006. Open circles are reference branches (Thermonastic leaf movements (TLM) allowed) and closed circles are treatment branches (TLM restricted). Average daily temperature data (^{o}C) shown by superimposed dotted line. Brackets (|--|) represent canopy opening and closing in November and April, respectively. Values are means +/- one standard error. (*) indicates significant differences in Pn from paired t-test ((*)$p<0.1$, (**)$p<0.05$).

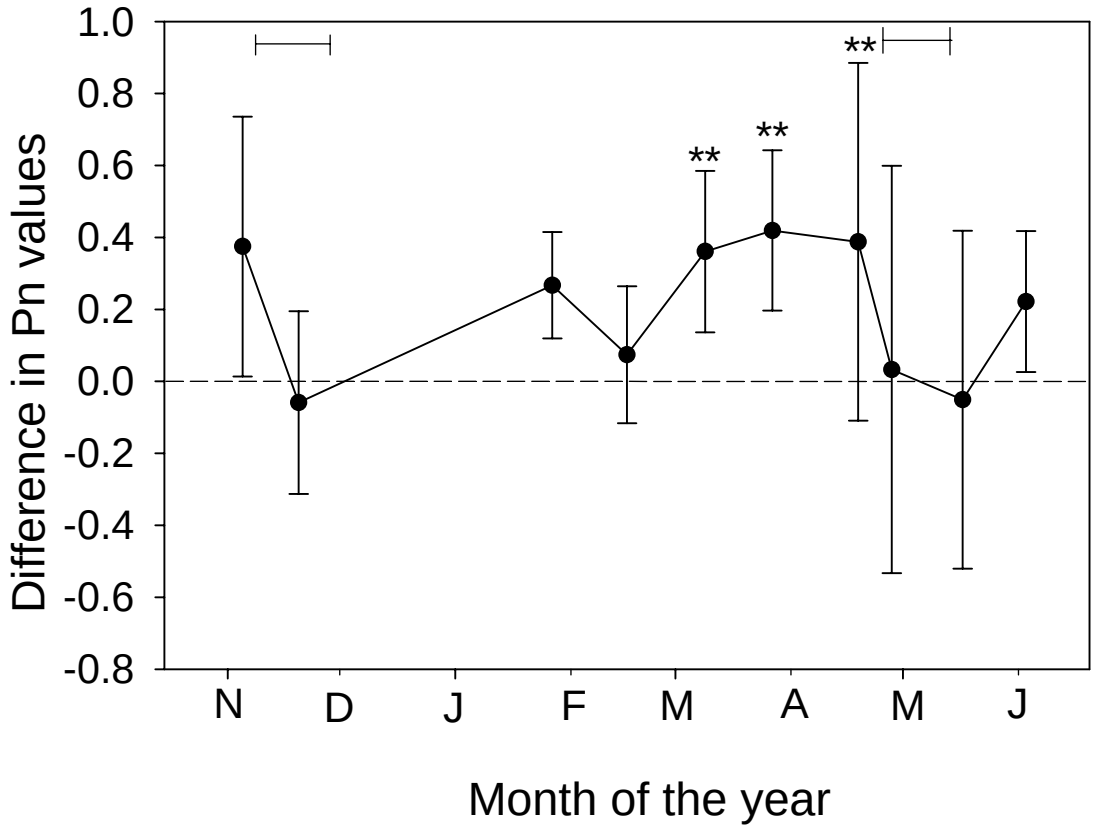

Figure 2. Differences between reference and treatment values of Pn for *Rhododendron maximum* under a deciduous canopy from October 20, 2005 to June 3, 2006. Positive values indicate reference values higher than treatment and negative values indicate higher treatment levels. Brackets (|--|) represent canopy opening and closing in November and April, respectively. Values are means +/- SE for each sampling date. * indicates significant differences from zero in Pn from repeated measures analysis ($_{*}p<0.1$, $_{**}p<0.05$).

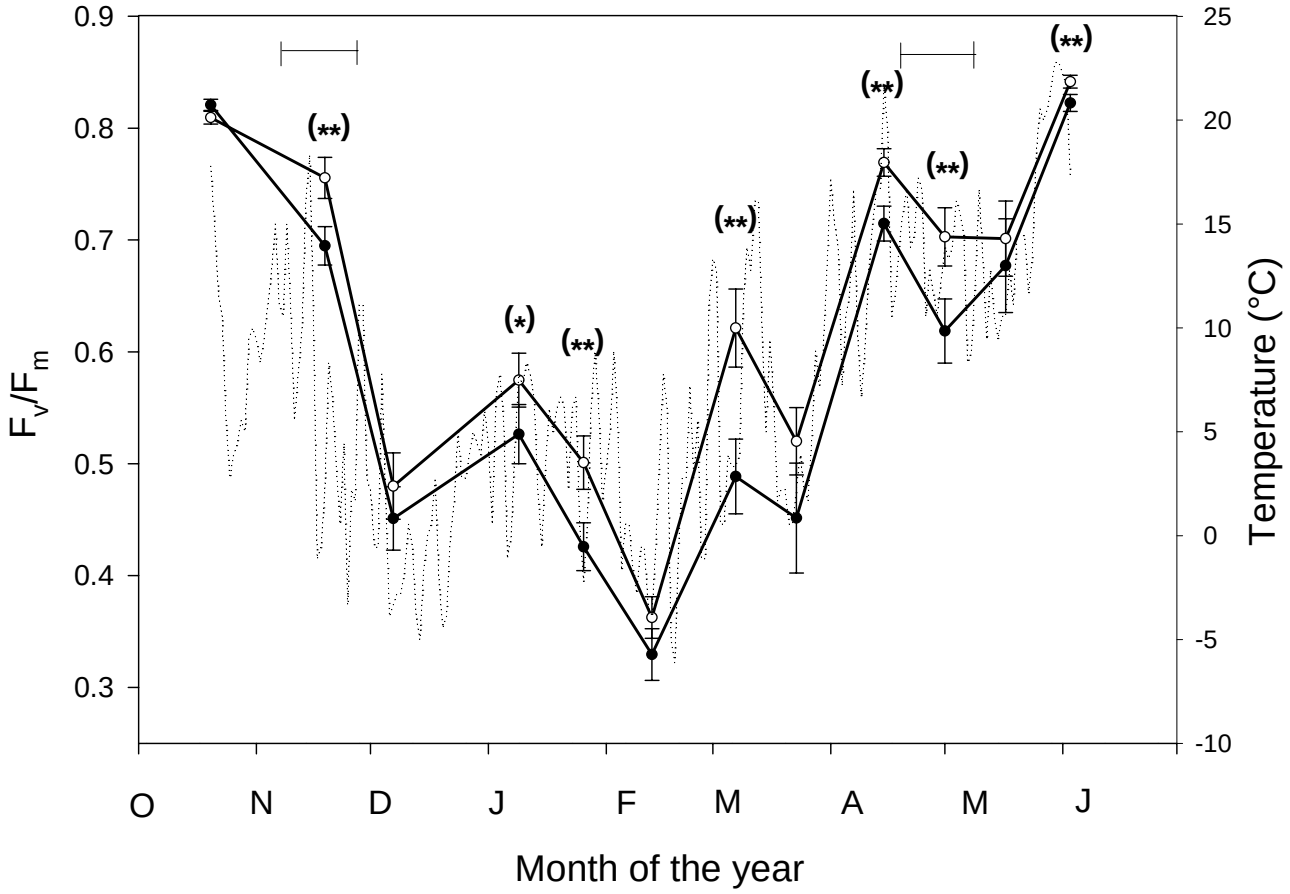

Figure 3. Seasonal changes in dark adapted fluorescence (solid lines), measured as photochemical efficiency of PSII (F_v/F_m) for *Rhododendron maximum* under a deciduous canopy from October 20, 2005 to June 3, 2006. Open circles are reference branches (Thermonastic leaf movements (TLM) allowed) and closed circles are treatment branches (TLM restricted). Average daily temperature data (^oC) shown by superimposed dotted line. Brackets (|--|) represent canopy opening and closing in November and April, respectively. Values are means +/- one standard error. (*) indicates significant differences in F_v/F_m from paired t-test ((*)$p<0.1$, (**)$p<0.05$).

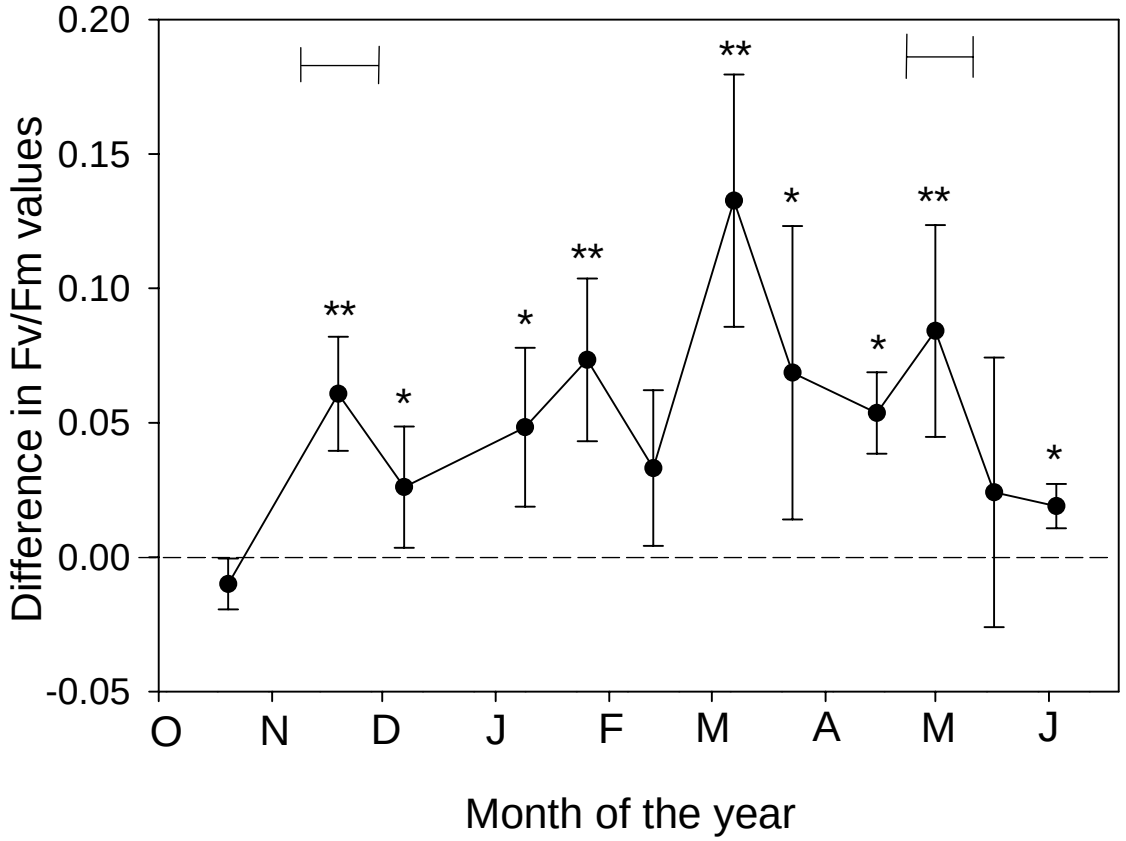

Figure 4. Differences between reference and treatment values of F_v/F_m for *Rhododendron maximum* under a deciduous canopy from October 20, 2005 to June 3, 2006. Positive values indicate reference values higher than treatment and negative values indicate higher treatment levels. Brackets (|--|) represent canopy opening and closing in November and April, respectively. Values are means +/- SE for each sampling date. * indicates significant differences from zero in F_v/F_m from repeated measures analysis ($_*p<0.1$, $_{**}p<0.05$).

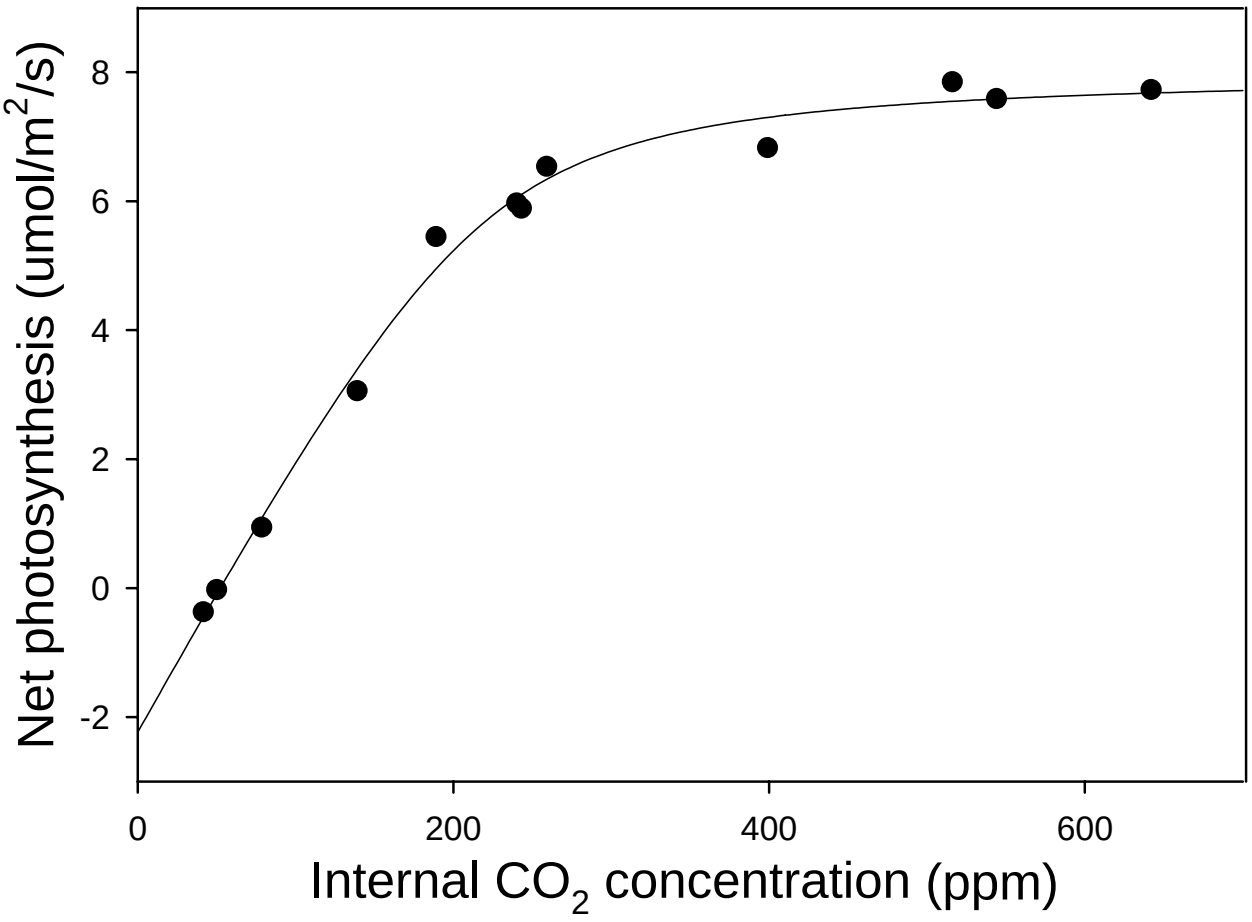

Figure 5. Representative CO_2 response curve from *Rhododendron maximum* showing the relationship between net photosynthetic rate (Pn) and internal CO_2 concentration (C_i). Line fit by regression in SigmaPlot (Richmond, California, USA, Systat Software Inc).

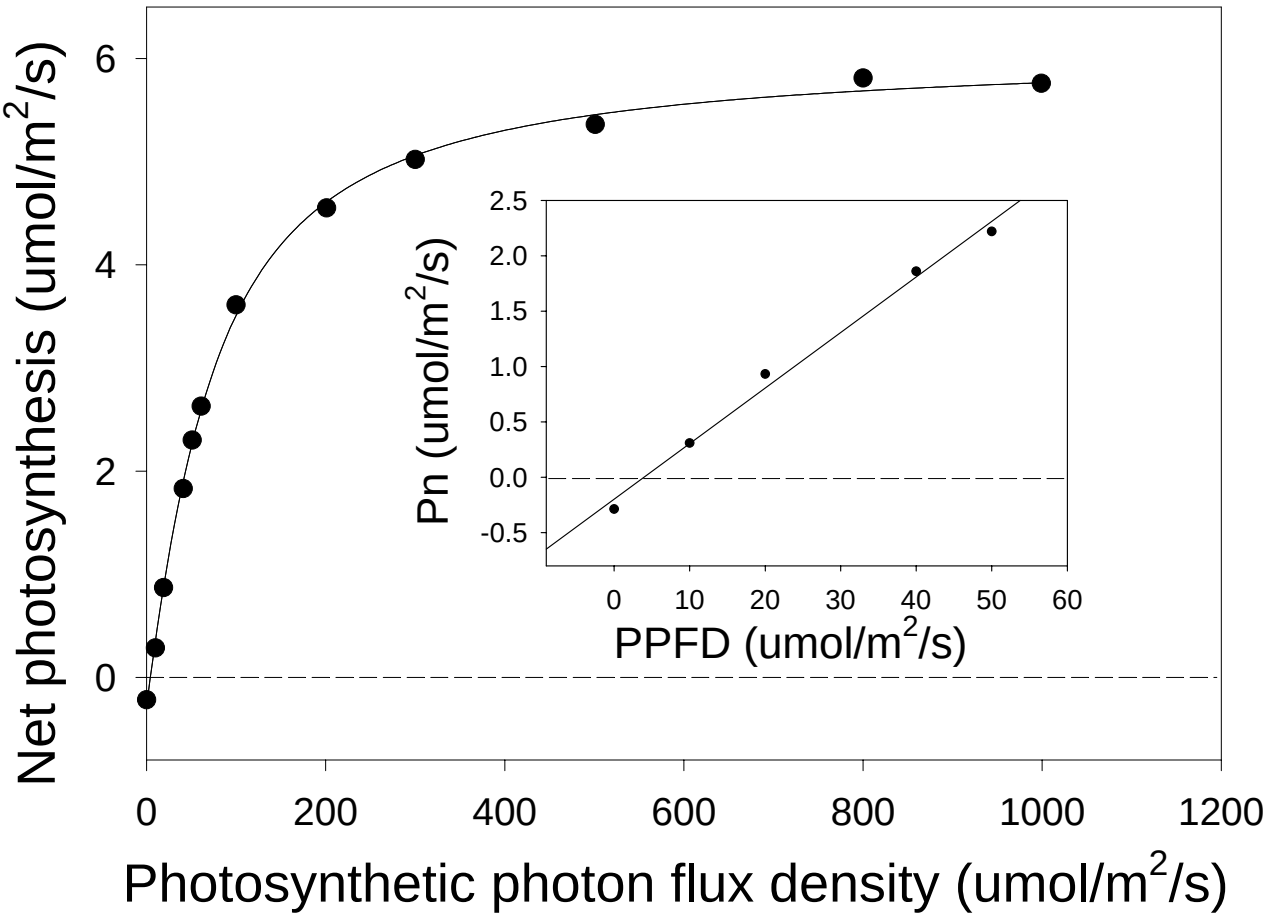

Figure 6. Representative photosynthetic light response curve from *Rhododendron maximum* showing the relationship between net photosynthetic rate (Pn) and increasing PPFD. Insert shows expanded view of the area used to calculate quantum efficiency (first 60 umol/m^2/s). Line fit by regression in SigmaPlot (Richmond, California, USA, Systat Software Inc).

Table 1. CO_2 response curve data. Values are means (±SE) of CO_2 response curve data of leaves from treatment (T) and reference (R) branches of *Rhododendron maximum* in November 2005, April 2006, and May 2006; Resp = dark respiration, V_{cmax} = maximum rate of RUBISCO carboxylation, J_{max} = maximum rate of electron transport, CO_2 Comp = CO2 compensation point, CE = Carboxylation efficiency, and A_{sat} = light saturated photosynthetic rates. No significant differences were found between mean values of treatment and reference (paired T-test P = 0.05)

	October		April		May	
	n=1	n=2	n=1	n=3	n=5	n=6
	R	**T**	**R**	**T**	**R**	**T**
Resp ($umol/m^2/s$)	-1.25	-1.85	-1.48	-1.73 ±0.39	-1.20 ±0.27	-1.51 ±0.28
Vcmax ($umol/m^2/s$)	15.30	16.80	17.00	30.27 ±6.3	19.12 ±3.39	21.43 ±1.96
Jmax ($umol/m^2/s$)	49.60	53.90	47.40	55.63 ±4.1	41.72 ±2.79	44.60 ±2.38
CO_2 comp (ppm)	5.16	6.32	5.64	6.92 ±0.51	6.69 ±0.7	7.73 ±0.95
CE	0.14	0.11	0.09	0.12 ±0.63	0.26 ±0.14	0.22 ±0.07
Asat ($umol/m^2/s$)	17.05	18.75	19.35	23.91 ±4.4	17.77 ±3.29	18.25 ±1.36

Table 2. Light response curve data. Values are means (±SE) of light response curve data of leaves from treatment (T) and reference (R) branches of *Rhododendron maximum* in November 2005, April 2006, and May 2006; Resp = dark respiration, QE = quantum efficiency, A_{max} = light saturated photosynthetic rate, Light Comp = Light compensation point, light sat = light saturation point. No significant differences were found between mean values of treatment and reference (paired T-test P = 0.05).

	Oct		April		May	
	n=2	n=2	n=3	n=3	n=6	n=7
	R	**T**	**R**	**T**	**R**	**T**
Resp ($umol/m^2/s$)	-0.19	-0.26	-0.59 ±0.07	-0.57 ±0.24	-0.13 ±0.05	-0.15 ±0.07
QE	0.05	0.07	0.03 ±0.01	0.04 ±0.01	0.05 ±0.01	0.04 ±.01
Amax ($umol/m^2/s$)	2.36	4.28	4.73 ±0.87	4.84 ±1.08	4.55 ±0.58	3.72 ±0.63
Light comp. ($umol/m^2/s$)	3.97	4.55	14.10 ±3.35	7.82 ±4.15	3.78 ±0.52	6.38 ±1.19
Light sat. ($umol/m^2/s$)	53.45	68.25	106.90 ±22.15	175.10 ±41.49	91.55 ±9.23	126.51 ±28.6

Literature Cited

Adams, W.W. III, Demmig-Adams, B, Rosentiel, T.N., and Ebbert, V. 2001. Dependence of photosynthesis and energy dissipation activity upon growth form and light environment during the winter. Photosynthesis Research 67: 51-62.

Adams, W.W. III, Zarter, R., Ebbert V., and Demmig-Adams, B. 2004. Photoprotective Strategies of Overwintering Evergreens. BioScience 54(1): 41-49.

Adir, N., Zer H., Shochat, S., and Ohad, I. 2003. Photoinhibition – a historical perspective. Photosynthesis Research 73: 343-370.

Aro, E., McCaffery, S., and Anderson, J.M. 1993. Photoinhibition and D1 protein degradation in peas acclimated to different growth irradiances. Plant Physiology 103: 835-843.

Asada, K. 1999. The water-water cycle in chloroplasts: Scavenging of active oxygen and dissipation of excess photons. Annual Review of Plant physiology and Molecular Biology 50: 601-639.

Bao, T. and Nilsen, E.T. 1988. The ecological significance of leaf movements in *Rhododendron maximum*. Ecology 69(5): 1578-1587.

Barker, D.H., Seaton, G.G.R., and Robinson, S.A. 1997. Internal and external photoprotection in developing leaves of the CAM plant *Cotyledon orbiculata*. Plant Cell & Environment 20: 617-624.

Buchanan, B. B., Gruissem, W. and Jones R.L. 2000. Biochemistry and Molecular Biology of Plants. The American Society of Plant Physiologists, Rockville, MD.

Bugos, R.C., Chang, S., and Yamamoto, H.Y. 1999. Developmental expression of violaxanthin de-epoxidase in leaves of tobacco growing under high and low light. Plant Physiology 121: 207-213.

Chazdon, R.L. 1988. Sunflecks and their importance to forest understory plants. Advances in Ecological Research 18: 1-63.

Demmig-Adams, B. and Adams, W.W. III. 1993. The Xanthophyll Cycle. In *Antioxidants in Higher Plants*. Eds. Alscher, R.G and Hess, J.L. CRC Press, Boca Raton, LA.

Demmig-Adams, B. and Adams, W.W. III 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends in Plant Science 1(1): 21-26.

Demmig-Adams, B. 1998. Survey of thermal energy dissipation and pigment composition in sun and shade leaves. Plant Cell Physiology 39(5): 474-382.

Demmig-Adams, B., Moeller, D.L., Logan, B.A., and Adams, W.W. III. 1998. Positive correlation between levels of retained zeaxanthin + antheraxanthin and degree of photoinhibition in shade leaves of *Schefflera arboicola* (Hayata) Merrill. Planta 205: 367-374.

Ebbert, V., Adams, W.W., Mattoo, A.K., Sokolenko, A., and Demmig-Adams, B. 2005. Up-regulation of a photosystem II core protein phophatase inhibitor and sustained D1 phosphorylation in zeaxanthin-retaining, photoinhibited needles of overwintering Douglas fir. Plant, Cell and Environment 28: 232-240.

Einhorn, K.S., Rosenqvist, E., and Leverenz, J.W. 2004. Photoinhibition in seedlings of *Fraxinus* and *Fagus* under natural light conditions: implications for forest regeneration? Oecologia 140:241-251.

Ensminger, I., *et al.* 2004. Intermittent low temperatures constrain spring recovery of photosynthesis in boreal Scots pine forests. Global Change Biology 10: 1-14.

Gilmore, A.M. and Yamamoto, H.Y. 1993. Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching. Evidence that antheraxanthin explains zeaxanthin-independent quenching. Photosynthesis Research 35: 67-78.

Gouallec, J.L., Cornic, G., and Briantais, J. 1991. Chlorophyll fluorescence and photoinhibition in a tropical rainforest understory plant. Photosynthesis Research 27: 135-142.

Harris, G.C., Antoine, V., Chan, M., Nevidomskyte, D., and Koniger, M. 2006. Seasonal changes in photosynthesis, protein composition and mineral content in *Rhododendron* leaves. Plant Science 170:314-325.

Horton, P., Ruban, A.V., and Walters, R.G. 1996. Regulation of Light Harvesting in Green Plants. Annual Review of Plant Physiology and Molecular Biology 47: 655-84.

Hutin, C., Nussaume, L., Moise, N., Moya, I., Kloppstech, K., and Havaux, M. 2003. Early light-induced proteins protect *Arabidopsis* from photooxidative stress. Proceedings of the National Academy of Science 100: 4921-4926.

Kasahara, M. Kagawa, T, Oikawa, K., Suetsugu, N., Miyao, M., Wada, M. 2002. Chloroplast avoidance movements reduce photodamage in plants. Nature 420: 829-832.

Lei, T.T. and Koike, T. 1998. Some observations on phenology and ecophysiology of *Daphne kamtschatica* Maxim. var. *jezoensis* (Maxim.) Ohwi, a shade deciduous shrub, in the forest of northern Japan. Journal of Plant Research 111: 207-212.

Logan, B.A., Barker, D.H., Demmig-Adams, B., and Adams, W.W. III. 1996. Acclimation of leaf carotenoid composition and ascorbate levels to gradients in the light environment within an Australian rainforest. Plant, Cell and Environment 19:1083-1090.

Logan, B.A., Demmig-Adams, B., Adams, W.W.III, and Grace, S.C. 1998a. Antioxidants and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. acclimated to four growth PPFDs in the field. Journal of Experimental Botany 49(328):1869- 1879.

Logan, B.A., Grace, S.C., Adams, W.W. III, Demmig-Adams, B. 1998b. Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. Oecologia 116:9-17.

Long, S.P., Humphries, S., and Falkowski, P.G. 1994. Photoinhibition and photosynthesis in nature. Annual Review of Plant Physiology and Plant Molecular Biology 45: 633-662.

Maxwell, K. and Johnson, G.N. 2000. Chlorophyll fluorescence—a practical guide. Journal of Experimental Botany 51(345): 659-668.

Neuner, Gilbert, Ambach, Dorothea, and Aichner, Klara. 1999. Impact of snow cover on photoinhibition in evergreen *Rhododendron ferrugineum* leaves during subalpine winter. Tree Physiology 19: 725-732.

Nilsen, E. T. 1986. Quantitative phenology and leaf survivorship of *Rhododendron maximum* L. in contrasting irradiance environments of southern Appalachian Mountains. American Journal of Botany 73: 822-831.

Nilsen, E.T., Stetler D.A., and Gassman, C.A. 1988. Influence of age and microclimate on the photochemistry of *Rhododendron maximum* leaves II. Chloroplast structure and photosynthetic light response. American Journal of Botany 75(10): 1526-1534.

Nilsen, E.T. 1991. The relationship between freezing tolerance and thermotropic leaf movement in five *Rhododendron* species. Oecologia 87(1):63-71.

Nilsen, E.T. 1992. Thermonastic leaf movements: a synthesis of research with *Rhododendron*. Botanical Journal of the Linnaean Society 110: 205-233.

Oberhuber, W. and Bauer, H. 1991. Photoinhibition of photosynthesis under natural conditions in ivy (*Hedera helix* L.) growing in an understory of deciduous trees. Planta 185: 545-553.

Quigley, M.F. and Platt, W.J. 2002. Composition and structure of seasonally deciduous forests in the Americas. Ecological Monographs 73(1): 87–106.

Rothstein, D.E. and Zak, D.R. 2001. Photosynthetic adaptation and acclimation to exploit seasonal periods of direct irradiance in three temperate, deciduous-forest herbs. Functional Ecology 15: 722-731.

Schnettger B., Critchley C., Santore U.J., Graf M., and Krause G.H. 1994. Relationship between photoinhibition of photosynthesis, D1 protein turnover, and chloroplast structure: effects of protein synthesis inhibitors. Plant Cell and Environment 17: 55-64.

Taiz, L. and Zeiger, E. 1999. Plant Physiology: Third edition. Sinauer Associates, Sunderland, MA.

Tezara, W., V. J. Mitchell, S. D. Driscoll, and D. W. Lawlor. 1999. Water stress inhibits plan photosynthesis by decreasing coupling factor and ATP. Nature 401: 914-917.

Verhoeven, A.S., W.W. Adams III, B. Demmig-Adams. 1998. Two forms of sustained xanthophyll cycle-dependent energy dissipation in overwintering *Euonymus kiautschovicus*. Plant, Cell and Environment 21: 893-903.

Verhoeven, A.S., W.W. Adams III, B. Demmig-Adams. 1999a. The xanthophyll cycle and acclimation of *Pinus ponderosa* and *Malva neglecta* to winter stress. Oecologia 118: 277-287.

Verhoeven, A.S., Adams, W.W. III, Demmig-Adams, B., Croce, R., and Bassi, R. 1999b. Xanthophyll cycle pigment localization and dynamics during exposure to low temperature and light stress in *Vinca major*. Plant Physiology 120: 727-737.

Verhoeven, A.S, Swanberg, A., Thao, M., and Whiteman, J. 2005. Seasonal changes in leaf antioxidant systems and xanthophyll cycle characteristics in *Taxus x media* growing in sun and shade environments. Physiologia Plantarium 123: 428-434.

Walters, M.B. and Reich, P.B. 1999. Low-light carbon balance and shade tolderance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? New Phytologist 143: 143-154.

Wang, Y. and Zwiazek, J.J. 1999. Effects of early spring photosynthesis on carbohydrate content, bud flushing and root and shoot growth in *Picea glauca* barefoot seedlings. Scandinavian Journal of Forest Research 14: 295-302.

Wei, Hui, Dhanaraj, N.L., Rowland, L.J., Fu, Y., Krebs, S.L., and Arora, R. 2005. Comparative analysis of expressed sequence tags from cold-acclimated and non-acclimated leaves of *Rhododendron catawbiense* Michx. Planta 221: 406-416.

Yamamoto, H.Y., Nakayama, T.O.M.n and Chichester, C.O. 1962. Studies on the Light and Dark Interconversions of Leaf Xanthophylls. Archives of Biochemistry and Biophysics 97: 168-173.

Yamamoto, H.Y. and Higashi, R.M. 1978. Violaxanthin de-epoxidase: lipid composition and substrate specificity. Archives of Biochemistry and Biophysics 190(2): 514-522.

Yamamoto, H.Y. 1979. Biochemistry of the violaxanthin cycle in higher plants. Pure and Applied Chemistry 51: 639-648.

Yamamoto, H.Y. 1985. Xanthophyll Cycles. Methods in Enzymology 110: 303-312.