

ECOPHYSIOLOGICAL ADAPTATIONS OF *GALAX APHYLLA* TO THE
UNDERSTORY OF SOUTHERN APPALACHIAN FORESTS

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A Thesis

by

James Kingston McCarron III

Submitted to the Graduate School

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in partial fulfillment of the requirements for the degree of

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December 1995

Major Department: Biology

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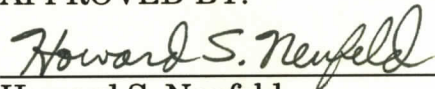
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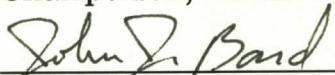
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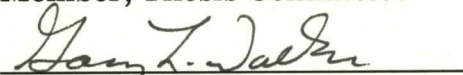
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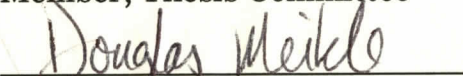
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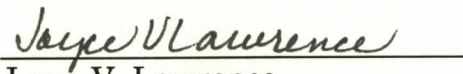
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ABSTRACT

ADAPTED MORPHOLOGICAL ADAPTATIONS OF *GALLIA APTELLA* TO THE
UNDERSTORY OF SOUTHWESTERN APPALACHIAN FORESTS

December 1995

James Kieran McCarron III, B.S., University of Charleston

M.S., Appalachian State University

Thesis Committee: Eugene E. Snodgrass

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ABSTRACT

ECOPHYSIOLOGICAL ADAPTATIONS OF *GALAX APHYLLA* TO THE UNDERSTORY OF SOUTHERN APPALACHIAN FORESTS.

(December 1995)

James Kingston McCarron III, B.S., University of Charleston

M.S., Appalachian State University

Thesis Chairperson: Howard S. Neufeld

Galax aphylla is a common, evergreen, clonal herb found in the understory of forests in the southern Appalachian mountains. Little is known of the ecophysiology of *Galax* or its adaptations to growing in the forest understory. The objectives of this study were to determine how leaf gas exchange and water relations change seasonally, and the influence of leaf age on these processes. In addition, we looked at the effects of additional nitrogen, water, and shade to determine their effects on leaf demography and gas exchange. *Galax* maintains leaves for at least two growing seasons, with two and sometimes three age classes present at any given time. During the summer, photosynthesis was reduced by light levels that often remained below light saturation ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$) except during light flecks. Lower light levels helped to minimize water deficits through reduced solar heating, which allowed for higher mid-day stomatal conductances ($0.14 \text{ mol m}^{-2} \text{s}^{-1}$). Spring and fall had higher light levels coupled with increased evaporative demands that may have lessened water availability. During this time, *Galax* was able to attain higher

photosynthetic rates ($4.0 \mu\text{mol m}^{-2} \text{s}^{-1}$), although stomatal conductances and water potentials could be reduced. During the winter, low air and soil temperatures combined to limit gas exchange. However, Galax was able to photosynthesize during warm periods, particularly if soil temperatures were above freezing. Colder soils reduced water availability, causing reduced pre-dawn and mid-day water potentials (-0.9 MPa and -1.6 MPa , respectively). In the fall leaves exposed to high light conditions showed increased anthocyanin content which may serve to protect leaves from photoinhibition during the winter. Photosynthesis and stomatal conductance decreased as leaves age, with the greatest reductions occurring during the second growing season. By remaining metabolically active throughout the year, Galax may have a competitive advantage over neighboring non-evergreen species, and can pay back the resources invested in producing evergreen leaves. Galax was generally unaffected by experimental treatments such as increased shading, irrigation and fertilizing with nitrogen. This reflects a stress-tolerant strategy that allows Galax to persist amid the changing environmental conditions of the forest floor, yet tolerate resource limitations common in these habitats. A trade-off exists where Galax is able to cope with limited resources, but not able to take advantage of increased resources.

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Many thanks to my good friends that have encouraged me along the way and made sure that I finished: Susan Wright Cochran, for helping me collect plants and giving me much needed advice on native plants. Dr. Donald Imm, thanks for your help with collecting and your many suggestions. Dr. Irvin Schultz, OK, I did not get to Peru this time, but I finished; I will be there for the next adventure. Todd Radenbaugh, many thanks for your moral support (and insight on the best beer and wines in Boone - Cheers!). To all those in the Biology department at Appalachian State University and at the Savannah River Ecology Laboratory, thank you all.

To Dr. Kenneth W. McLeod, I appreciate all that you have done for me, your insight, knowledge, and advice has made me a better scientist and in many ways a better person. You have taught me what it is to be an ecologist.

DEDICATION

This thesis is dedicated to the memory of my parents, James K. McCarron Jr. and Joyce G. McCarron. We will never be masters of this world; only momentary guests.

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Introduction

Forests of the southern Appalachian Mountains are well known for having a rich diversity of herbaceous species. Competition for light, nutrients, water and space, as well as, seasonal climatic changes have led to many phenological and physiological adaptations (Reich et al., 1992; Muller, 1978; Bratton, 1976). In particular, the annual development of the overstory canopy results in substantial changes in light reaching the forest floor during the growing season. Many species avoid the shade of the forest floor by completing their life cycle before the canopy closes, while others persist throughout the summer, tolerating the forest shade (Kikuzawa, 1995; Mahall and Borman, 1978). These seasonal changes in forest structure and climate have created adaptive opportunities for plants to survive on the forest floor, in which avoidance and tolerance are the most general of these strategies (Hicks and Chabot, 1985).

Sparling (1964, 1967) and other authors have attempted to classify these strategies into phenological groups based on the timing of growth and leaf longevity relative to the phenology of the canopy species (Kikuzawa, 1989; Hicks and Chabot, 1985; Mahall and Bormann, 1978). In general these groups are: vernal and autumnal ephemerals, plants that complete their life cycle during the periods between winter and canopy closure or between canopy abscission and winter; summergreens (shade tolerants), those plants that start to develop leaves about the same time as canopy closure and complete their life cycles beneath the closed canopy; wintergreens, which maintain leaves for one year and then replace them

with a new cohort; and evergreens, which maintain their leaves for more than one year. These categories separate plants by their abilities to tolerate or avoid shaded conditions within the forest. Ephemerals avoid the lower light conditions of summer by completing their life cycles before canopy closure, while summergreens, wintergreens, and evergreens have developed mechanisms to tolerate lower light levels (Hicks and Chabot, 1985; Mahall and Bormann, 1978)

Light is one of the main limiting resources during the growing season, where as little as 1 to 5% of the incident radiation penetrates to the forest floor following canopy closure (Anderson, 1963). Grime (1979) suggests that there are two types of plants found in light limited environments: plants that are in the process of competing for light (light competitors) and plants that tolerate shaded conditions (shade-tolerant). Light competitors are found in lightly-shaded environments where light filters through the canopy in the form of light flecks or tree gaps. These plants respond positively to increases in the light environment but do poorly as the light levels decrease. Shade-tolerators are found in more deeply-shaded environments and have evolved to perform efficiently at reduced light levels, but are less able to take advantage of temporary increases in light (Grime, 1979; Boardman, 1977; Loach, 1970).

Grime (1979) also suggests that natural selection in deeply-shaded habitats has been associated with the evolution of mechanisms for conserving energy rather than those which increase the quantity of energy capture. Plants that occupy light limited environments are frequently characterized as having reduced photosynthetic rates that become light saturated at lower light levels compared to plants from higher light

environments (Ludlow, 1983; Grime, 1979; Boardman, 1977; Böhning and Burnside, 1956). Lower saturation rates allow the plant to reach maximum photosynthetic rates at lower light levels, thus making it more efficient under shaded conditions. However, in many cases these adaptations also limit the ability of the plant to utilize increased light levels (Boardman, 1977).

Bratton (1976) also indicates the importance of canopy structure and light for influencing the survival of forest herbs, but adds that microtopography, nutrient availability and soil hydrology are also important factors. Nutrient content and water-holding capacities of soils can change over short distances, allowing for a broad diversity of species within a small area.

Water availability within the deciduous forest community can be affected by seasonal precipitation, topography, water holding capacity of the soil, and competition among species (Stephenson, 1990; Young, 1987; Meiners et al., 1984; Bratton, 1976). For the Appalachians, water availability is frequently highest during the early spring because of snow melt and seasonal rainfall patterns and lowest in the late summer months (Helvey and Patric, 1988). Vernal ephemeral species are often less drought tolerant and complete their life cycles during the period of highest water availability (Muller, 1978). The summer months are characterized by lower relative humidity and higher evapotranspiration, which result in lower water availability (Roberts *et al.*, 1980). Although the shaded conditions of the forest floor often protect the herbaceous layer from high transpiration rates, the high demands for water by canopy trees may cause localized soil water deficits (Young, 1987; Muller, 1978). However,

evergreens are typically more water use efficient and thus can withstand longer periods of water deficit. As a result, they are often associated with more xeric habitats (Goldberg, 1982; Hinckley et al., 1979).

Within the Appalachians, herb species richness increases with the nutrient content of the soil (Greller, 1993; Gilliam and Turrill, 1993). In areas where nutrients are more available, ephemeral and summergreen species often dominate on the forest floor. These species have shorter life cycles and are usually better adapted at obtaining resources quickly (Bazzaz, 1984; Muller, 1978). In less nutrient rich soils evergreen species are more common (Chapin, 1980; Schlesinger and Chabot 1977; Monk, 1966). The ability to retain leaves for more than one year is a selective advantage in nutrient-poor environments, because invested nutrients can be utilized longer (Chabot and Hicks, 1982). This gives the plant a better carbon return per unit of nutrients taken up, i.e., a better nutrient use efficiency (Vitousek, 1982; Small, 1972). However, this competitive advantage decreases as nutrient availability increases, since evergreen species often have slower nutrient uptake rates compared to faster growing deciduous species (Shaver and Chapin, 1980).

Chabot and Hicks (1982) suggest that leaf life spans are linked to their ability to pay back construction and maintenance costs. The cost of producing new leaves is greater when resources (i.e. light, nutrients, and water) are limited and this necessitates a longer leaf longevity, whereas when resources are not limited the cost of new leaves is less and leaf longevities are shorter. Thus shorter leaf longevities (deciduous) are a selective advantage in resource rich environments, where higher photosynthetic capabilities can pay back invested carbon faster, while

increased leaf longevities (evergreens) are favored in resource limited environments where poor resource availability can limit photosynthetic rates. This also implies that plants growing in environments with high resource availability produce leaves that are able to rapidly acquire resources but are less adept at retaining them (Kikuzawa, 1995, 1991; Chabot and Hicks, 1982), whereas in resource limited environments, plants produce leaves that are more efficient at utilizing lower resources which prolongs retention (Chapin, 1980).

Among the phenological strategies found on the forest floor, evergreens have evolved to survive in the most stressful conditions. As resources become more limiting within the forest community, evergreens increase in importance (Greller, 1993). Extensive research has focused on survival strategies of evergreens in the shrub layer, such as *Rhododendron* and *Kalmia* (Muller, 1991; McGraw, 1989; Nilsen, 1987, 1986; Monk et al., 1985; Della-Bianca and McGee, 1972), but little-to-no research has investigated herbaceous evergreens, such as *Epigaea*, *Gaultheria*, *Hexastylis*, and *Galax*. Therefore, we decided to investigate the ecophysiological adaptations of one evergreen herb to the understory environment.

One of the more common evergreen herbs in the southern Appalachians is *Galax aphylla*, a monotypic member of the family Diapensiaceae. *Galax* is often associated with the xeric environments of heath balds and the nutrient poor soils of chestnut-oak forests (Whittaker, 1966, 1963), and is frequently found growing with *Kalmia* and *Rhododendron* (Baldwin, 1941). To date only three papers have been published on *Galax* (Baldwin, 1941, 1939; Ward, 1919) of which none dealt

with the phenology or physiology of *Galax*. Baldwin looked at the geographical distribution of *Galax* in relation to two chromosome races (diploid and tetraploid race), while Ward commented on the introduction of *Galax* in Massachusetts for commercial use. Otherwise, little is known about the ecology of *Galax aphylla*.

The purpose of this study was to look at the ecophysiology of *Galax aphylla* in hopes of better understanding its role in understory environment of the southern Appalachian forests. Since *Galax* is often found in association with other stress-tolerant (*sensu* Grime, 1979) evergreens, we hypothesized that *Galax* would exhibit similar physiological characteristics as these species, such as low gas exchange rates, limited responses to resource fluxes, and the ability to take advantage of favorable growing conditions in the spring, fall and winter. In order to test these ideas, we proposed a two part study: (1) to gain baseline knowledge concerning seasonal patterns in the ecophysiology of *Galax*, and (2) leaf physiological responses to resource augmentation.

Materials and Methods

SITE DESCRIPTION

This study was conducted near the Blue Ridge Parkway in the Appalachian Mountains approximately four kilometers northwest of Blowing Rock, North Carolina (36°08' N 81°47' W) (Figure 1). The site was located in a southeastern facing alcove with an approximate slope of 20° and an average elevation of 1155 m. An oak-maple community dominated this forested site, with *Quercus alba*, *Quercus rubra* and *Acer rubrum* as canopy dominants, and *Rhododendron maximum*, *Cornus florida*, and *Betula alleghaniensis* comprising the major portion of the sub-canopy. *Galax aphylla* was the predominant perennial in the herbaceous layer. The alcove had well drained soils on the upper

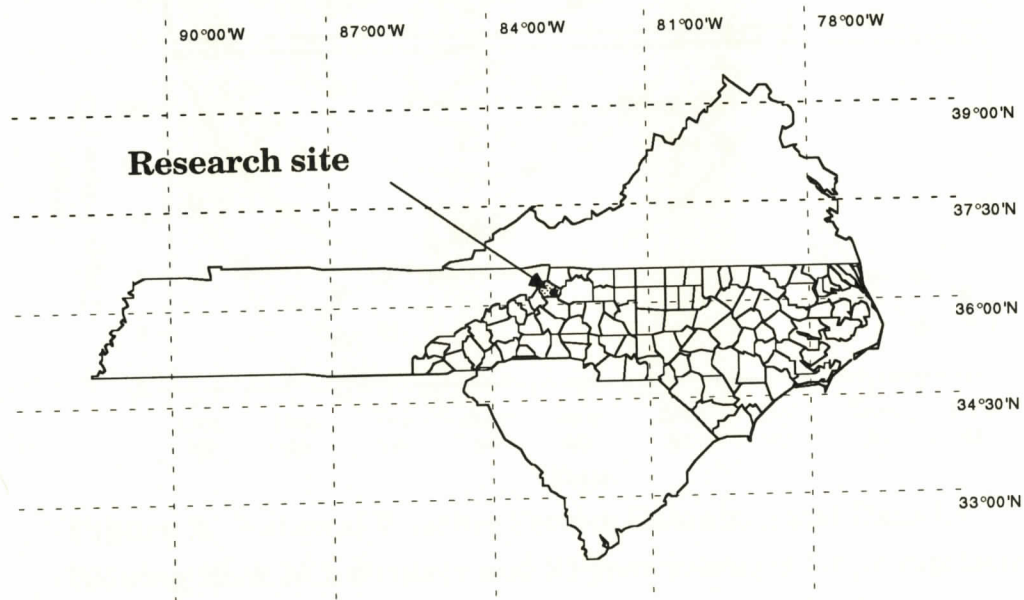


Figure 1. Study site located in Watauga county, North Carolina.

slopes and more mesic soils towards the lower elevations, due to a small, ephemeral stream.

The closest weather station to the study area was located approximately 2.5 km southeast in Blowing Rock, North Carolina. Twenty year averages indicate annual precipitation of 116.8 cm with 45% occurring during the growing season (May to September) and annual temperatures of 18.9°C in summer and 1.9°C during the winter (NOAA / National Weather Service, 1990) (Figure 2).

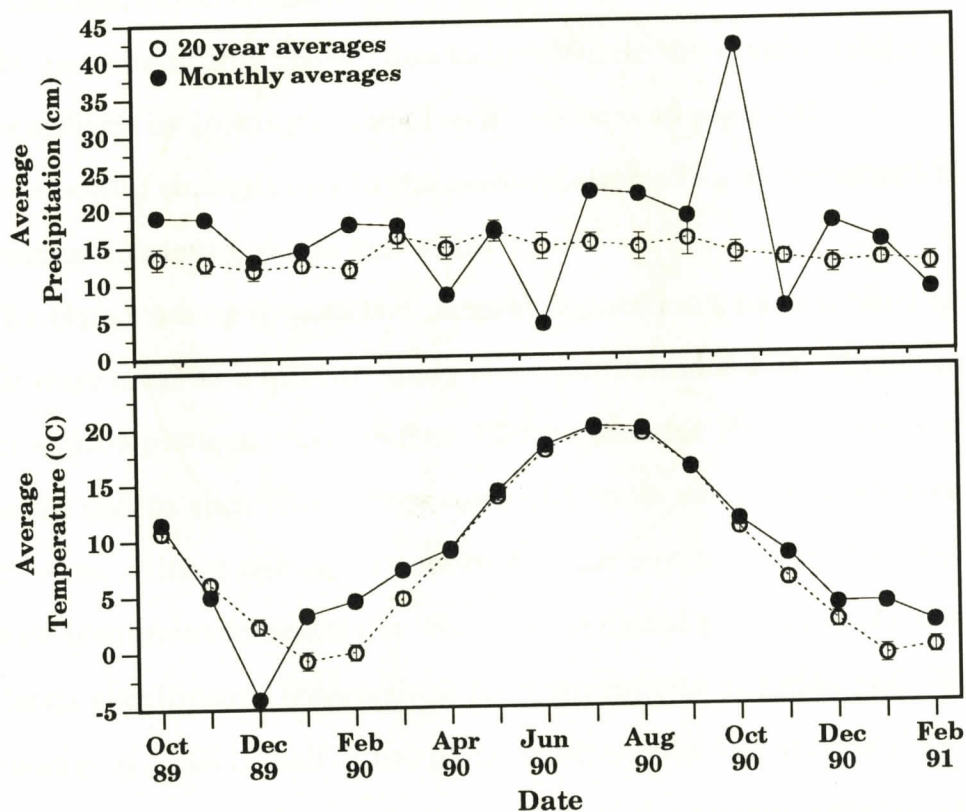


Figure 2. National Weather Service Climatological Data for Blowing Rock NC; monthly and 20 year averages for precipitation and temperature. Points represent means \pm standard errors.

The site was chosen because two large colonies of *Galax aphylla* occupied the upper and lower portions of the area. The first colony was located on the upper slope and covered an area of approximately 40 m² (site A), while the second colony (site B) was on the lower portion of the site and measured approximately 25 m². Site A and B were separated by about 20 m and there were no visible signs that they were connected by rhizomes.

EXPERIMENTAL DESIGN

The purpose of this project was to investigate the ecophysiology of *Galax aphylla* by looking at the plants' response to seasonal environmental changes and to the manipulation of its environment by increasing nutrients, shade and water.

The *Galax* colony formed two natural populations, as described earlier, which were used as duplicate study sites; site A and site B. Eight one meter square plots, marked by four 2.5 cm diameter PVC stakes, were arranged within each site. Large trees and rocks were excluded from the plots. Also, at least one meter separated each plot to insure that there were no treatment interactions. Since some clonal plants can translocate nutrients via rhizomal interactions between ramets (Magda *et al.*, 1988; Ashmun *et al.*, 1981), half of the plots had trenches dug around them to eliminate any connections with plants outside the experimental plots. Trenches were 45 cm deep and approximately 30 cm wide. Polyethylene plastic sheeting (thickness of 0.6 mm) was placed within each trench to insure that rhizomal interactions did not re-establish. The soil was then replaced leaving approximately 5 cm of plastic protruding from the soil.

Three environmental conditions were studied in the field: increased nitrogen, water and shade. Two plots were assigned to each experimental treatment within each site; one trenched and one without a trench (non-trenched). Since the sites were situated on a slope, plots with added nitrogen fertilizer and water were given additional consideration in that no plots were placed within three meters downslope of these experiments. This eliminated the chance that any leaching of nitrogen fertilizer or runoff would enter other plots.

All statistical analyses performed on the data from this study were analyzed using SuperANOVA and StatView general linear modeling statistical packages (Abacus Concepts, 1989) unless otherwise noted. Treatment differences were usually determined among age cohorts within and among dates. A one-way analysis of variance (ANOVA) was used to determine within date differences among age cohorts, while among date differences were determined using a two-way ANOVA and comparing age cohort vs. date.

LEAF PHENOLOGY

To differentiate among age classes of leaves, 30 leaves per age class from each plot were labeled with plastic colored tags (PolyPaper[®] Carolina Biological Supply Co., Burlington NC.) placed around the petiole of each leaf. Leaves that were present at the beginning of the study (April 1989) were given blue tags. These leaves are referred to as first year leaves (*FYL*), although the actual age of these leaves was unknown. Leaves that emerged in the spring of 1989, second year leaves (*SYL*), were given white tags, and those that emerged in the spring of 1990, third year leaves

(*TYL*), were given orange tags. In addition, one hundred reserve leaves per age class were tagged outside the plots to be used for various experiments. Within plots, tags were checked every two months during the winter and once a month during the growing season to determine leaf survival. To determine if the application of treatments affected leaf survival, only a 10% or greater change in population from the control plots was considered significant.

Growth measurements were made on newly developing leaves to determine leaf growth rates and to ascertain if experimental treatments affected leaf development. These measurements were made at the beginning of the 1990 growing season, at which time the plots had been exposed to their treatments for at least one year. Starting in May 1990, observations were made biweekly to determine when leaves emerged. At first, when new leaves emerge they are tightly rolled and very fragile. Any attempt to unroll the leaf to measure its size caused tearing in many of the leaves. Because of this, growth measurements were delayed until leaves had fully unrolled. Two measurements were made for each leaf: from the petiole to the tip of the blade and from each side at the point where the petiole connects to the blade. Ten leaves in each plot were measured weekly until there were no significant changes in leaf size. Follow-up measurements were made two and four weeks after the last weekly measurement to establish that leaf growth had been completed.

Leaf areas of an additional 50 leaves collected from the site were estimated from their width and length measurements. Petioles were removed from the leaves and a leaf area meter (Delta-T Devices, Cambridge England) was used to determine actual leaf area. A regression

analysis was used to determine the relationship between actual leaf area and the product of the width and length measurements (Figure 3). This regression equation was then used to calculate leaf areas for those leaves tallied in the field.

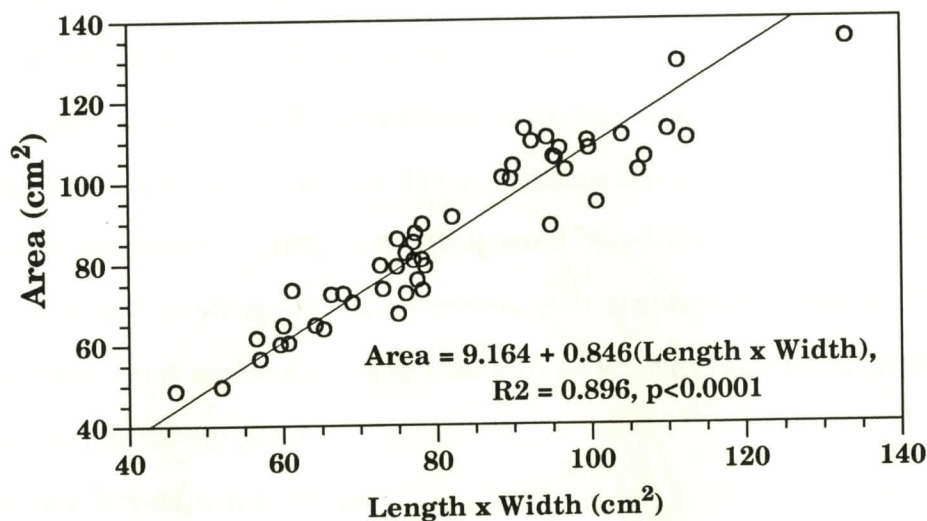


Figure 3. Regression analysis to determine the relationship between actual leaf area and the product of the width and length measurements.

TREATMENT PLOTS

Treatments applications began on April 26, 1989, and continued for two growing seasons until the end of October 1990. Each treatment was applied once per week during the growing season (April to October) but not during the winter months.

Nitrogen Treatment Plots:

Soil samples were taken on February 21, 1989 to determine the available soil nitrogen in the study sites. Samples were oven dried at 65 C° for one week and then mailed to the Institute of Ecology, University

of Georgia for analysis. $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations were colorimetrically determined using a Technicon Auto Analyzer (Technicon Industrial Systems, NY). Nitrogen levels were found to be $0.036 \text{ mg NO}_3\text{-N Kg}^{-1}$ dry soil and $1.56 \text{ mg NH}_4\text{-N Kg}^{-1}$ dry soil. Using a soil bulk density of 0.9 g cm^{-3} (Montagnini 1986) this converts to 0.22 g N m^{-2} in a 15 cm depth of soil (the effective soil depth).

Nitrogen treatment plots received twice the measured available nitrogen each month in weekly doses, tripling the amount of nitrogen normally available. Nitrogen was supplied by adding $0.31 \text{ g NH}_4\text{NO}_3$ (0.054 g N m^{-2}) powder sprinkled evenly over a plot once a week. The leaves were then shook to insure that the powder did not stick to the plant but settled on the ground.

Shade Treatment Plots:

Shade was increased by constructing a PVC frame ($1.0 \text{ m} \times 1.0 \text{ m} \times 0.5 \text{ m}$) over the plot and then covering this with 50% shade cloth (Chicopee Manufacturing Company, Cornelia, GA). Photosynthetically active radiation (PAR) readings were taken underneath and outside the shade cloth, using a Li-185B Quantum Meter with a Li-190SB Quantum Sensor (Li-Cor Inc., Lincoln, Nebraska). Shade levels were increased by 53%. Periodically during the study these levels were measured again to see if shade levels remained constant. Also, all debris was cleared from on top of the shade cloth once a week; snow however was not removed.

Water Treatment Plots:

Water treatment plots received twice the average monthly rainfall each month during the growing season. Because rainfall did not vary

greatly during the growing season, monthly rainfalls were averaged. This value, 150.2 L per month, was applied to the water treatment plots in weekly increments of 37.6 L of deionized water.

PHYSIOLOGICAL RESPONSES TO TREATMENTS

Gas exchange measurements were made on leaves in each of the treatment plots using a Li-Cor 6200 Portable Photosynthesis System and a one liter chamber (Li-Cor Inc., Lincoln, Nebraska). The system was calibrated each morning before its use following procedures provided by Li-Cor.

Measurements were taken in the first two weeks of September 1990, between the hours of 11:00 A.M. and 2:00 P.M. EST. Preliminary experiments indicated that *Galax aphylla* had its highest photosynthetic rates during this time period. Photosynthesis (A), and stomatal conductance (g_s) were measured from 10 randomly chosen leaves in each experimental plot. Only *TYL* were used due to the lack of availability of *SYL*. On the day a given treatment was measured, measurements were made on leaves from both the trenched and non-trenched plots, as well as from the control trenched and non-trenched plots. During each measurement care was taken to avoid sunflecks when possible. Because of the number of readings and short time in which to measure gas exchange, only one treatment group was measured on any one day. For each treatment and control group a two-way ANOVA was used to compare treatment vs. site. If site was significantly different or there was a significant interaction term, each site was treated as a separate

experiment and compared using a one-way ANOVA for treatment within each site.

SEASONAL DIURNAL RELATIONSHIPS

Diurnal gas exchange and leaf water potentials (Ψ_l) were measured in *Galax aphylla* on a seasonal basis. Measurements were made approximately every month during the growing season and every other month during the rest of the year.

Photosynthesis was measured every two hours starting at dawn and continuing until dusk. All measurements were taken on leaves in the non-trenched control plot of site A. Five leaves from each age class were randomly chosen at the beginning of the study and used repeatedly on all diurnal field days. As new cohorts developed five more leaves were randomly chosen and added to the study. If a leaf was damaged or died it was replaced with another randomly chosen leaf of the same age class.

Water potential was measured using a leaf pressure chamber (constructed at Appalachian State University) following procedures similar to Hinkley *et al.* (1980). Measurements were made every two hours on five leaves from each age class present, with the first set of readings made before sunrise (pre-dawn). Because this method destroys the leaf, samples were taken from the surplus tagged leaves outside the experimental plots. A measurement was taken by cutting a leaf from the rhizome and then inserting the petiole into a rubber stopper. The leaf and stopper were then placed into the pressure chamber and pressure applied to the leaf until sap extruded from the petiole. All measurements were

made within one minute of the time the leaf was removed from the rhizome.

Environmental conditions were monitored hourly by recording PAR, relative humidity, air temperature at leaf level, and soil temperatures at 12 cm and 25 cm. To determine the amount of natural shade of the site, two PAR readings were made: one outside the canopy in a nearby field and nine additional readings spaced evenly over the sample plot. Relative humidity was measured using the humidity sensor in the Li-Cor 6200 system. Ambient air was allowed to pass through the open system for 30 seconds, after which time the relative humidity reading was made. Air and ground temperatures were measured using 24 gauge thermocouples (Omega Engineering Inc., Stanford, Conn.) attached to a Campbell 21X Data Logger (Campbell Scientific Inc., Logan, Utah).

For each set of diurnal measurements the time of day that maximum photosynthesis rates (A_{max}) were reached was noted. All measurements made during this time were tallied and used to compare among age cohorts and dates. Differences among age cohorts within a date and age cohorts among dates were determined using a one-way ANOVA.

SEASONAL PRESSURE-VOLUME CURVES

Seasonal patterns for water potential components were measured periodically throughout 1990. The pressure-volume technique has been shown to be one of the best methods to derive estimates of water potential components (Ritchie and Roden, 1985; Richter *et al.*, 1979; Tyree *et al.*, 1978b). Osmotic potential at full turgor (Ψ_{so}), osmotic potential at turgor loss (Ψ_{tl}), relative water content at turgor loss (RWC_{tl}), and symplastic

water content (*SWC*) and apoplastic water content (*AWC*) were derived using this method as described by Turner (1981).

Five leaves from each age class present were collected from the surplus tagged leaves. The petiole of each leaf was cut from the rhizome at ground level and placed in a pan of deionized water. The petiole was then recut underwater, to ensure a clean cut surface and to remove embolisms that may have developed while collecting. Samples were quickly transferred to a beaker of deionized water and transported back to the lab. The beaker and leaves were placed in a sealed plastic bag and allowed to rehydrate overnight in the dark. This allowed the plants to equilibrate to full turgor before testing.

After rehydrating for one day, a leaf was removed from the beaker and the petiole inserted into a pre-weighed rubber stopper. The leaf and stopper were weighed using a microbalance (Model L-01029-42, Sartorius, Goettingen, West Germany), then rapidly inserted into the pressure chamber. Increasing pressure was applied to the leaf using a tank of compressed nitrogen at a rate of 0.01 to 0.02 MPa s⁻¹ until xylem sap appeared at the cut surface. The leaf and stopper were then removed from the pressure chamber and re-weighed. Between readings the leaf was allowed to transpire freely outside the pressure chamber. This procedure was repeated until the water potential reached -2.5 MPa. The interval between readings varied with the rate at which water was lost from the leaf. Water loss was greatest in the first 10 minutes with readings approximately 45 to 55 seconds apart. As the rate of water loss slowed the time between water potential readings was increased.

An additional sample of 50 plants was collected on July 16, 1990 from

the surplus tagged plants, sealed in a plastic bag, and sent to Dr. Frederick C. Meinzer (Hawaiian Sugar Planters' Association, Aiea, HI) to be analyzed by an alternative method of generating pressure volume curves. This involved using over-pressure instead of free transpiration to cause water loss (Tyree *et al.*, 1978b; Ritchie and Roden, 1985). His results were used for comparison with the seasonal pressure volume data collected by the method used at Appalachian State University.

CHLOROPHYLL AND ANTHOCYANIN CONTENT

Twenty leaf samples (each 31.2 mm²) were taken from both the surplus tagged leaves and from the non-trenched shaded plots using a hole punch. Samples were placed in 20 ml polypropylene scintillation vials and put in a styrofoam cooler on ice and brought back to the lab. Half the samples from each plot were used to determine chlorophyll concentrations and the other half were used to determine relative anthocyanin amounts. Collections were started in mid-November, before leaves showed any visible signs of color change, and continued until mid-January. Only SYL leaves were present at the time of this study.

To extract leaf chlorophyll, 5 ml of N,N-dimethylformamide were added to 2 leaf samples within 40 minutes of collection. Samples were then stored at 4°C for 48 hours (Moran and Porath, 1980; Moran, 1982; Inskeep and Bloom, 1985). Chlorophyll *a*, *b* and total chlorophyll content were determined using a UV-visible spectrophotometer (Perkins-Elmer, model 3840) following procedures of Inskeep and Bloom (1985). Absorption was measured at 664 and 647 nm with a resolution of 0.25 nm at an average of

16 cycles per sample. The following formulae were used to determine chlorophyll concentrations (mg ml^{-1}):

$$C_T = 8.08 A_{664.5} + 17.90 A_{647}$$

$$C_a = 12.70 A_{664.5} - 2.79 A_{647}$$

$$C_b = -4.62 A_{664.5} + 20.70 A_{647}$$

where C_a = chlorophyll *a* concentration, C_b = chlorophyll *b* concentration, C_T = total chlorophyll concentration, $A_{664.5}$ = absorbance at 664.5 nm and A_{647} = absorbance at 647 nm (Inskeep and Bloom, 1985).

Leaf samples for anthocyanin analysis were collected in the same manner as those for chlorophyll analysis, and from the same leaves. Samples were placed in test tubes with 5 ml of deionized water and 1.3 ml of 6 M hydrochloric acid, and then were heated for 15 minutes in a water bath. The solution was placed in a graduated cylinder and enough deionized water added to bring the total volume back to 5 ml. This was then poured into a polypropylene scintillation vial and refrigerated for 48 hours at 4°C. Anthocyanin absorption was measured using a Perkin-Elmer model 3840 UV-visible spectrophotometer set at 514 nm with a resolution of 0.25 nm and an average of 16 cycles per sample. Preliminary experiments showed that the maximum absorption for anthocyanins in *Galax aphylla* occurred at approximately 514 nm. Since anthocyanin composition was not determined, absorption was used as a means of determining relative total anthocyanin concentration. It was assumed that the higher the percent absorption, the greater the anthocyanin concentration, and that differences in absorption were due to changes in anthocyanin amount and not type.

LIGHT RESPONSE CURVES

Rooted *Galax aphylla* plants were collected from the research site on April 17, 1990 and placed in 30 by 60 cm plastic tubs. These were transported back to the lab and placed in a greenhouse under light levels similar to those in the field in the summer (100 to 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$). *FYL* did not survive transplant to the greenhouse, thus only *SYL* and *TYL* were used in this study.

Before an experiment began, a tub was moved from the greenhouse to the lab and placed beneath a 300 Watt quartz halogen lamp at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for two hours. A glass container of recirculated fresh water was placed between the light source and tub to reduce heating effects. The maximum light level that was obtained with this design was 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Preliminary experiments showed that leaf temperatures above 30° C adversely affected photosynthesis in *Galax aphylla* in the lab, so the maximum light intensity that could be used without a significant increase in leaf temperature above 30° C was 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

After two hours of acclimation at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, a single attached leaf was placed in a quarter liter chamber of a Li-Cor 6200 Portable Photosynthesis system. Fresh air was continuously pumped through the chamber between readings. Within the chamber relative humidity and temperature were constantly monitored. Relative humidity was kept between 30 to 40% while the chamber temperature was kept between 25 and 30°C. After ten minutes the system was closed and A , g_s , and internal CO_2 (C_i) were measured. The light intensity was then increased to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and another measurement was taken after 10 minutes acclimation. Thereafter, light levels were decreased by 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$

every 10 minutes with a reading taken at the end of each change. The last reading was done in complete darkness. The light level was then brought back up to $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ and after 10 minutes one last reading was made. This was to determine how responsive the leaf was to light after being exposed to decreased light levels and to see if any permanent reduction in gas exchange parameters occurred due to the experimental protocol.

A second set of light response curves were constructed from seasonal diurnal data collected in the field. Because these data were not originally collected for light curves they were edited to fit parameters similar to those of the leaves measured in the lab. Measurements were tallied from readings taken between May 1, 1990 and September 31, 1990, with leaf temperatures above 25°C and below 30°C , and relative humidity between 30 and 40%. Light response curves were fitted using a modified Weibull function (Figure 4) with parameters estimated by non-linear regression (Proc NLIN; SAS Institute, 1989). From this model the light compensation point was estimated by solving the equation for $A = 0$, which is the light level at which photosynthesis balances respiration. The point at which light saturates the photosynthetic apparatus is called the light saturation point, and all rates measured above this point are considered to be light saturated photosynthesis (A_{Ls}). This was estimated by taking 80% of the asymptote and solving the equation for PAR. To compare the light saturation data between the three cohorts and the lab generated data, pairwise t-tests were used to find significant differences.

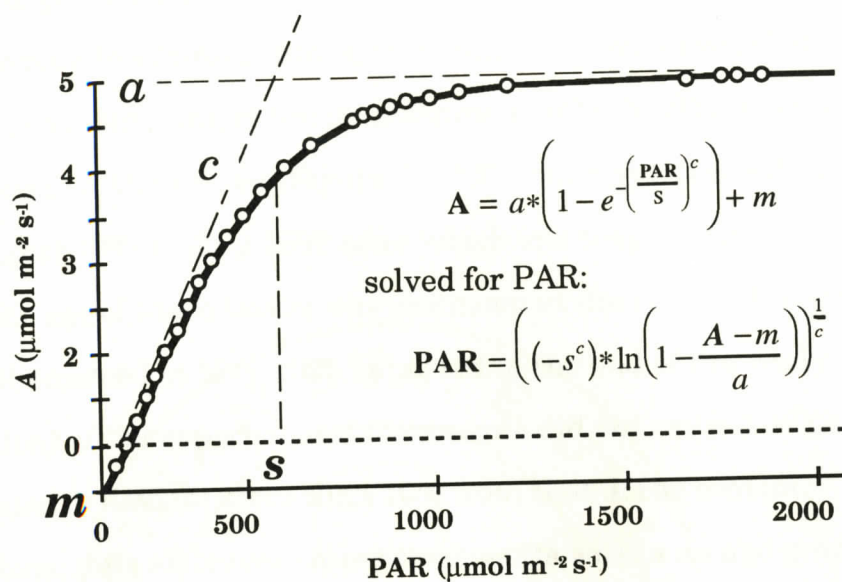


Figure 4. Weibull function fit to response of photosynthesis (A) to photosynthetically active radiation (PAR): a is estimation of maximum rate of A ; c and s are parameters used to determine shape and scale of curve; m was added to the Weibull function to allow the line to extend beyond the x -axis (also equal to the y -intercept when x is zero).

Results

LEAF PHENOLOGY

The length of time that *Galax* leaves persist on the plant could not be unequivocally determined during this study. Of the leaves that were tagged at the beginning of the investigation, only the *SYL* survived the entire length of the survey (Figure 5). *FYL* rapidly decreased in numbers from August 1989 to July 1990 after which one tagged leaf remained. Again, the age of these leaves was unknown at the start of the study. The *TYL* that emerged in May 1990 persisted to the end of the study. The applications of the experimental treatments did not cause leaf persistence to consistently deviate more than 10% from that of the control populations, thus all leaves across treatments within an age group were grouped together.

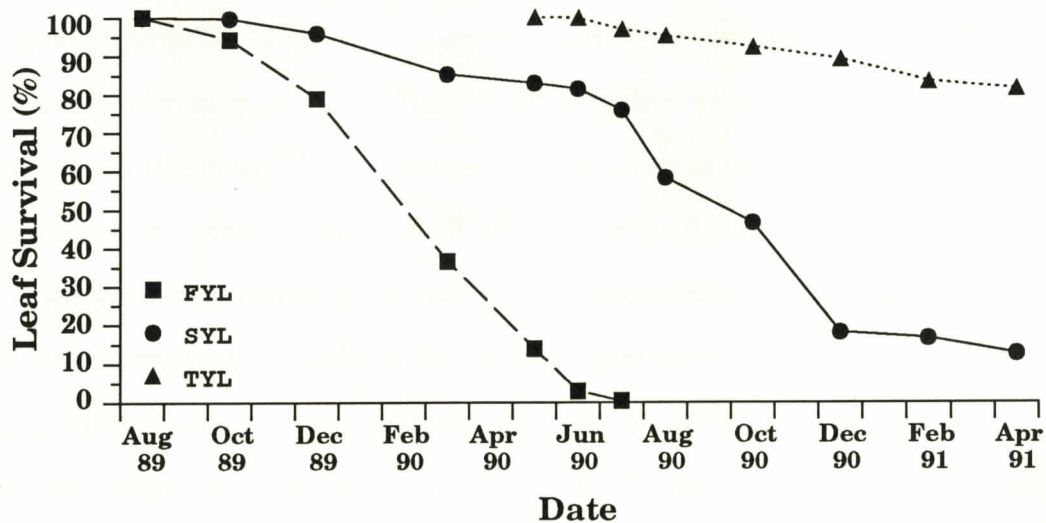


Figure 5. Percent leaf survival of *Galax aphylla* for first (*FYL*), second (*SYL*) and third year leaves (*TYL*).

In the first year of the study (August 1989 to August 1990) *FYL* numbers decreased by 100% while *SYL* decreased by 24.4%. For both leaf age classes, the greatest decrease in survival was during the winter months, from December 1989 to March 1990 (52.4% and 11.4% decreases, respectively). In the spring when the next new cohort was developing (April to July) *FYL* completely died out while *SYL* had a mortality rate of 8.7%. The newly developed *TYL* had a mortality rate of 4.0% for the first three months of their growth. The majority of this mortality seemed to be due to insect damage on the young leaves.

During the second year of the census, mortality increased greatly for *SYL*. From August 1990 (58.4% survival) to December 1990 (17.5% survival) *SYL* decreased by 72% from the original population while *TYL* only declined by 10.8%. By the end of the survey only 12.7% of the original *SYL* and 81.6% of *TYL* were still present.

Leaf expansion for the 1990 growing season, was completed by the fourth set of measurements (June 6, 1990); this was approximately six weeks from the time of emergence. After this date there were no significant changes in leaf size. Because of significant differences in sites for both growth rate and final leaf area, sites were not grouped together.

Growth rates during the time of expansion were not affected by the application of treatments, although there were isolated treatment differences for a few dates in sites A and B (Figure 6). In site A, the nitrogen plot leaves grew significantly faster than the control plot ($p=0.0394$) up to May 24, 1990. Nitrogen-trenched plot leaves grew considerably faster than both the control and control trenched plots ($p=0.0002$ and $p=0.0003$, respectively) up to June 14, 1990. The

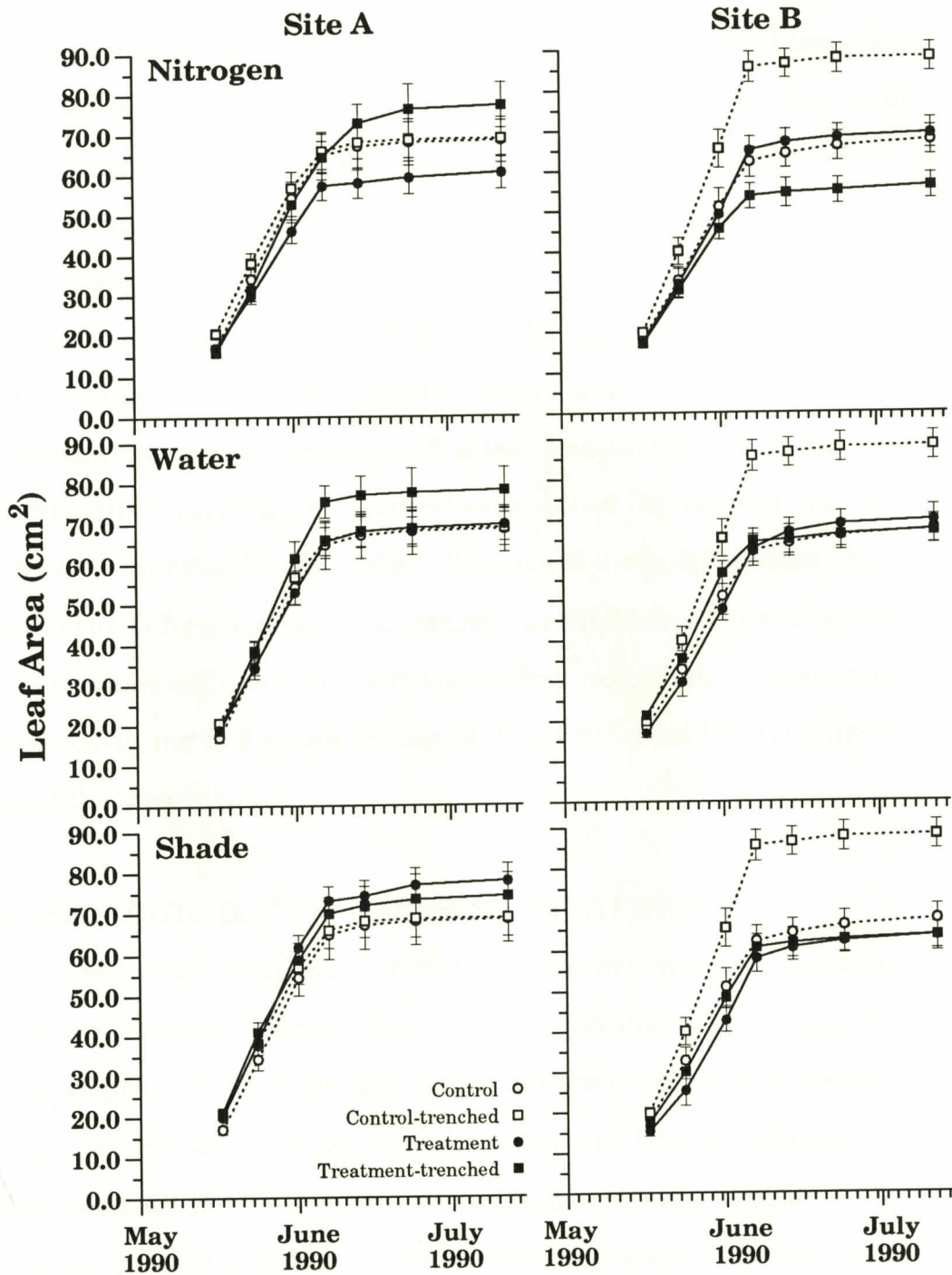


Figure 7. Change in leaf area for *Galax aphylla* in both sites and for all treatments during the 1991 growing season. Points are means \pm standard errors.

water-trenched leaves grew significantly faster than the control-trenched leaves ($p=0.0239$) up to June 6, 1990. For site B, the control-trenched leaves grew significantly faster for the first three weeks relative to all other treatments (all comparisons had $p<0.0400$), except the water-trenched leaves on June 1, 1990. All other treatments in site B were not significantly different from each other.

Final leaf areas were not affected by the application of treatments in site A, but in site B, control-trenched leaves were larger, causing some treatment differences (Figure 7). Control-trenched leaves in site B were significantly larger than the control-untrenched leaves (88.1 cm^2 vs. 67.5 cm^2 , respectively; $p=0.0015$). Because of these differences, there were significant differences between control-trenched leaves and treatment leaves within each plot (all comparisons had $p<0.0300$). However, the three remaining plots were not significantly different from one another for any of the treatments.

PHYSIOLOGICAL RESPONSES TO TREATMENTS

Gas exchange measurements made on the experimental treatment plots were not significantly affected by the addition of trenching. Thus, the trenched and non-trenched plot values were combined to increase the sample size for all three experiments. However, because of significant differences in light levels between sites which directly affected A, sites were not grouped together. Photosynthetically active radiation, A, and g_s , for all treatments in both sites are summarized in Figure 8.

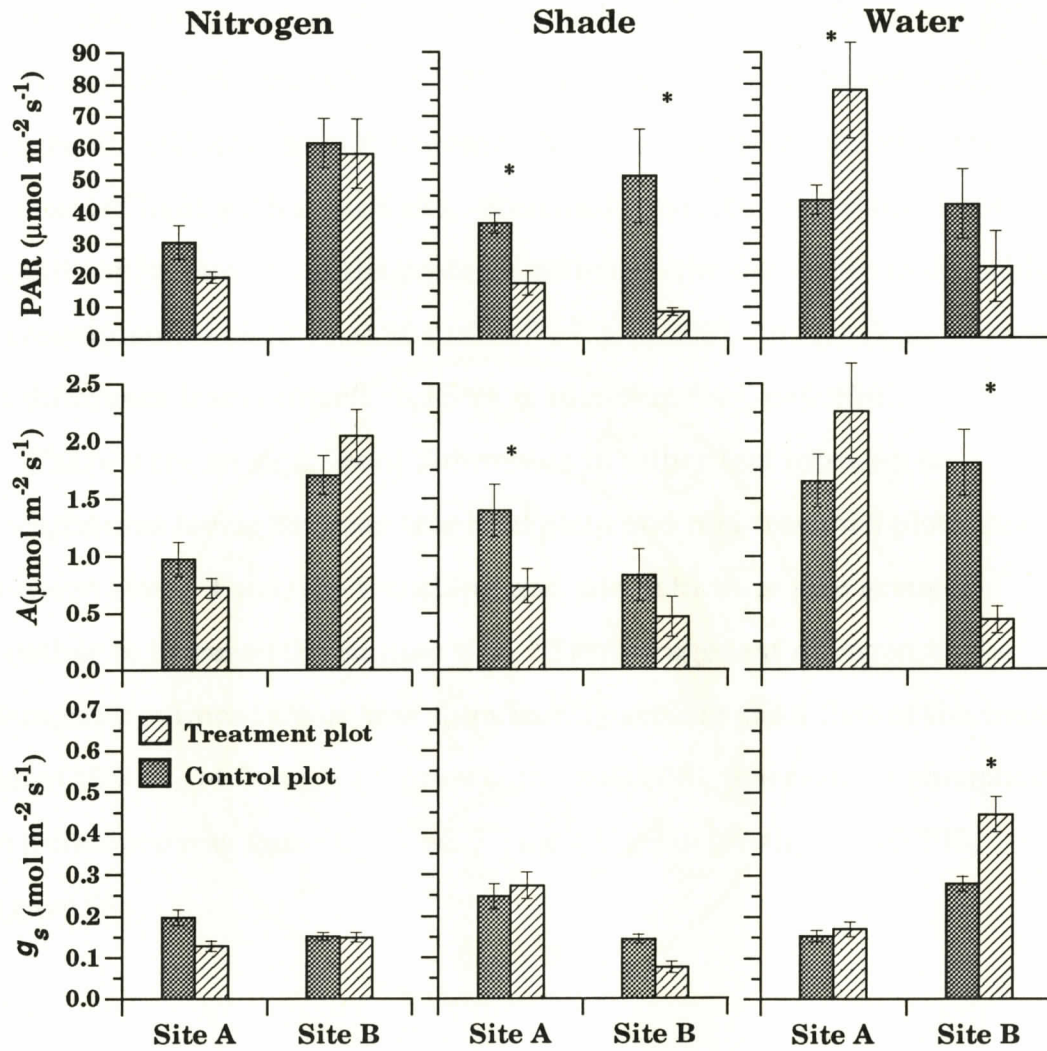


Figure 8. Photosynthetically active radiation (PAR), photosynthesis (A), and stomatal conductance (g_s) for the nitrogen, shade, and water treatment plots and control plots in sites A and B. Bars represent means \pm standard errors. (*) denotes significant ($p \leq 0.05$) difference between treatments in the designated sample.

Nitrogen Treatment Plots:

The addition of nitrogen to the treatment plots did not significantly affect A in either site A or site B when compared to their controls (0.72 vs. 0.97 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 2.10 vs. 1.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, Figure 8). Differences in A between sites were directly related to the amount of light within each site. Stomatal conductance for site A was significantly lower than the controls, although the difference is probably inconsequential (0.13 vs. 0.20 $\text{mol m}^{-2} \text{s}^{-1}$, $p=0.006$). In site B, g_s showed no difference from controls (0.15 vs. 0.15 $\text{mol m}^{-2} \text{s}^{-1}$, $p=0.956$).

There were no significant differences in either leaf nitrogen or phosphorous levels between trenched plots and non-trenched plots, nor between sites. Thus, the trenching and site data were also grouped together to increase the sample size. The average leaf nitrogen in the nitrogen treatment plots was significantly greater than that of the control plots (15.3 vs. 14.0 mg N g^{-1} dry weight, $p=0.003$), whereas for phosphorus no difference was found (1.13 vs. 1.14 mg P g^{-1} dry weight, $p=0.795$, Figure 9).

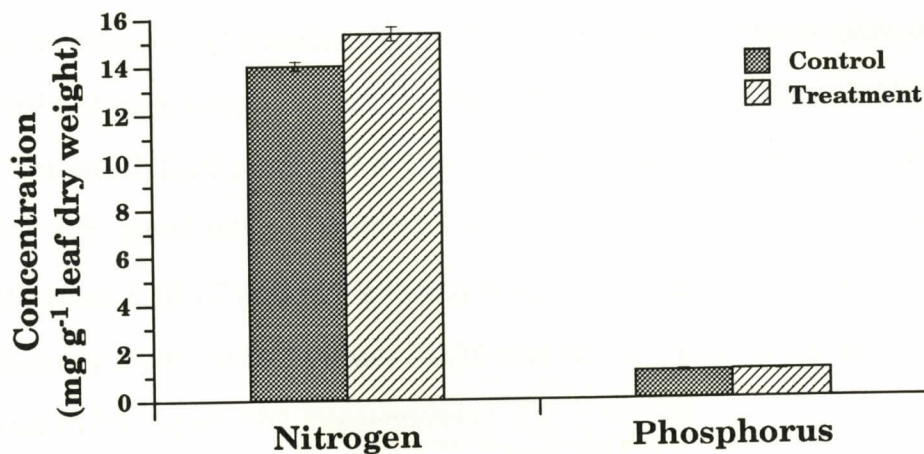


Figure 9. Leaf nitrogen and phosphorus concentrations in the control and nitrogen treatment plots. Bars represent means \pm standard errors.

Shade Treatment Plots:

Shading significantly reduced PAR levels in both Sites A and B below those of the control plots (site A; 18 vs. 37 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $p=0.001$ and site B; 8 vs. 51 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $p=0.009$, respectively). Photosynthetic rates in the shaded plots were significantly lower for site A, but not for site B when compared to their controls (site A; 0.74 vs. 1.39 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $p=0.026$ and site B; 0.46 vs. 0.82 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $p=0.219$, respectively). Stomatal conductance was not affected by shade in site A, but was significantly lower in site B (site A; 0.27 vs. 0.25 $\text{mol m}^{-2} \text{s}^{-1}$, and site B; 0.08 vs. 0.14 $\text{mol m}^{-2} \text{s}^{-1}$, $p=0.003$, respectively).

Water Treatment Plots:

Levels of PAR in the water treatment plots were significantly different for site A (control: 44 $\mu\text{mol m}^{-2} \text{s}^{-1}$, treated: 78 $\mu\text{mol m}^{-2} \text{s}^{-1}$; $p=0.042$), while levels for site B were not (control: 42 $\mu\text{mol m}^{-2} \text{s}^{-1}$, treated:

23.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$; $p=0.226$). Differences in PAR levels in site A were caused by canopy light flecks. The addition of water to the treatment plots did not statistically affect the rate of *A* in site A (control: 1.65 $\mu\text{mol m}^{-2} \text{s}^{-1}$, treated: 2.26 $\mu\text{mol m}^{-2} \text{s}^{-1}$; $p=0.216$) but did lower rates in site B (control: 1.81 $\mu\text{mol m}^{-2} \text{s}^{-1}$, treated: 0.44 $\mu\text{mol m}^{-2} \text{s}^{-1}$; $p=0.0003$). The g_s for both sites A (0.17 $\text{mol m}^{-2} \text{s}^{-1}$) and B (0.44 $\text{mol m}^{-2} \text{s}^{-1}$) did not deviate significantly from control values (0.15 $\text{mol m}^{-2} \text{s}^{-1}$, $p=0.487$ and 0.51 $\text{mol m}^{-2} \text{s}^{-1}$, $p=0.770$, respectively).

SEASONAL DIURNAL RELATIONSHIPS

Seasonal fluctuations in average midday temperatures at leaf and ground level, and average midday PAR at leaf level (Figure 10) influenced seasonal trends in *A*, g_s , and Ψ_l (Figure 11). During the spring (May 1990) when the canopy leaves were beginning to develop, PAR levels were 33.5% of full sunlight at leaf level (about 15 cm above ground). *FYL* and *SYL* tended to have slightly lower A_{max} rates (1.1 and 3.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ respectively, Figure 11A) and higher g_s (0.06 and 0.09 $\text{mol m}^{-2} \text{s}^{-1}$ respectively) as compared to values in late winter (March 1990) (1.5 and 3.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ respectively, 0.03 and 0.05 $\text{mol m}^{-2} \text{s}^{-1}$, respectively, Figure 11B). Newly developed *TYL* had statistically similar A_{max} rates to *SYL* ($p=0.707$) whereas *FYL* were significantly lower ($p=0.003$). *TYL* had higher g_s than both *FYL* and *SYL* ($p=0.001$ and $p=0.019$).

Pre-dawn Ψ_l for *FYL* (-0.07 MPa) and *SYL* (-0.08 MPa) in the spring were not significantly different from late winter values (-0.06 MPa and -0.03 MPa, respectively, Figure 11C). However, mid-day Ψ_l for both *FYL*

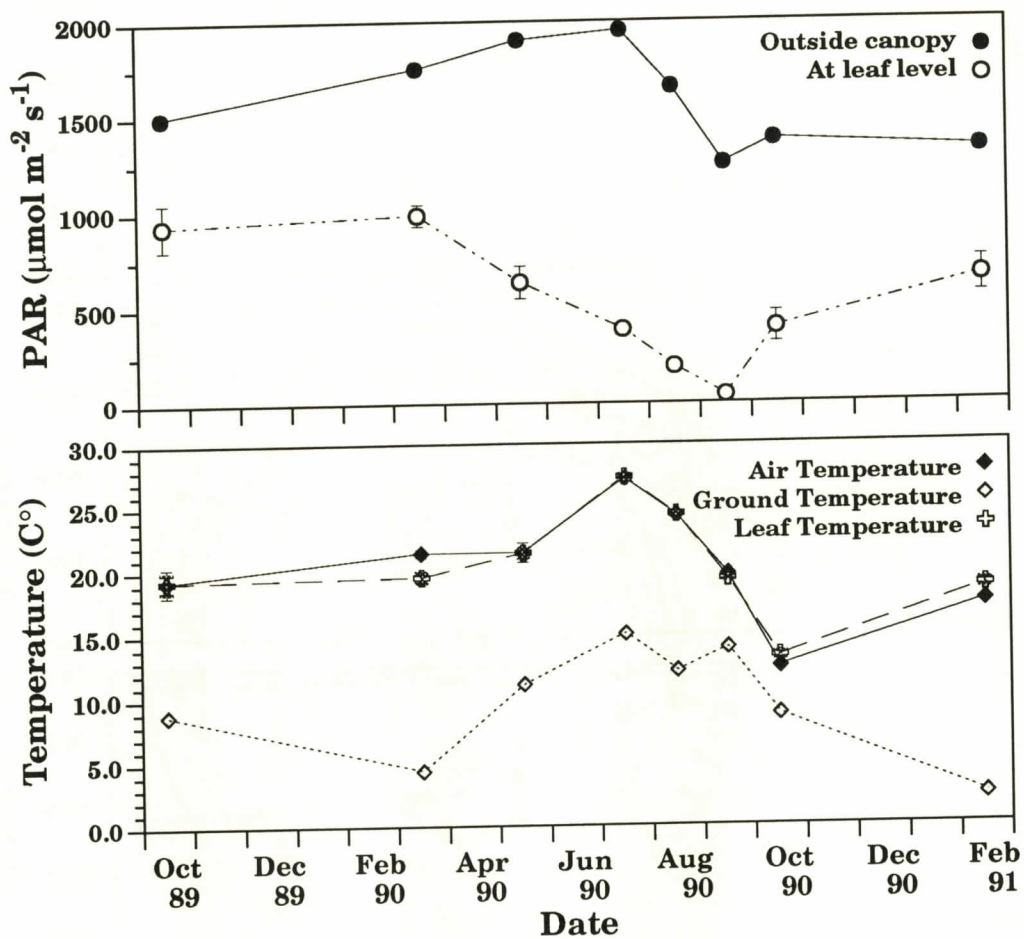


Figure 10. Midday measurements for seasonal photosynthetically active radiation (PAR) outside the canopy and at leaf level, and temperature at leaf level and 6 cm below soil level. Points represent means \pm standard errors.

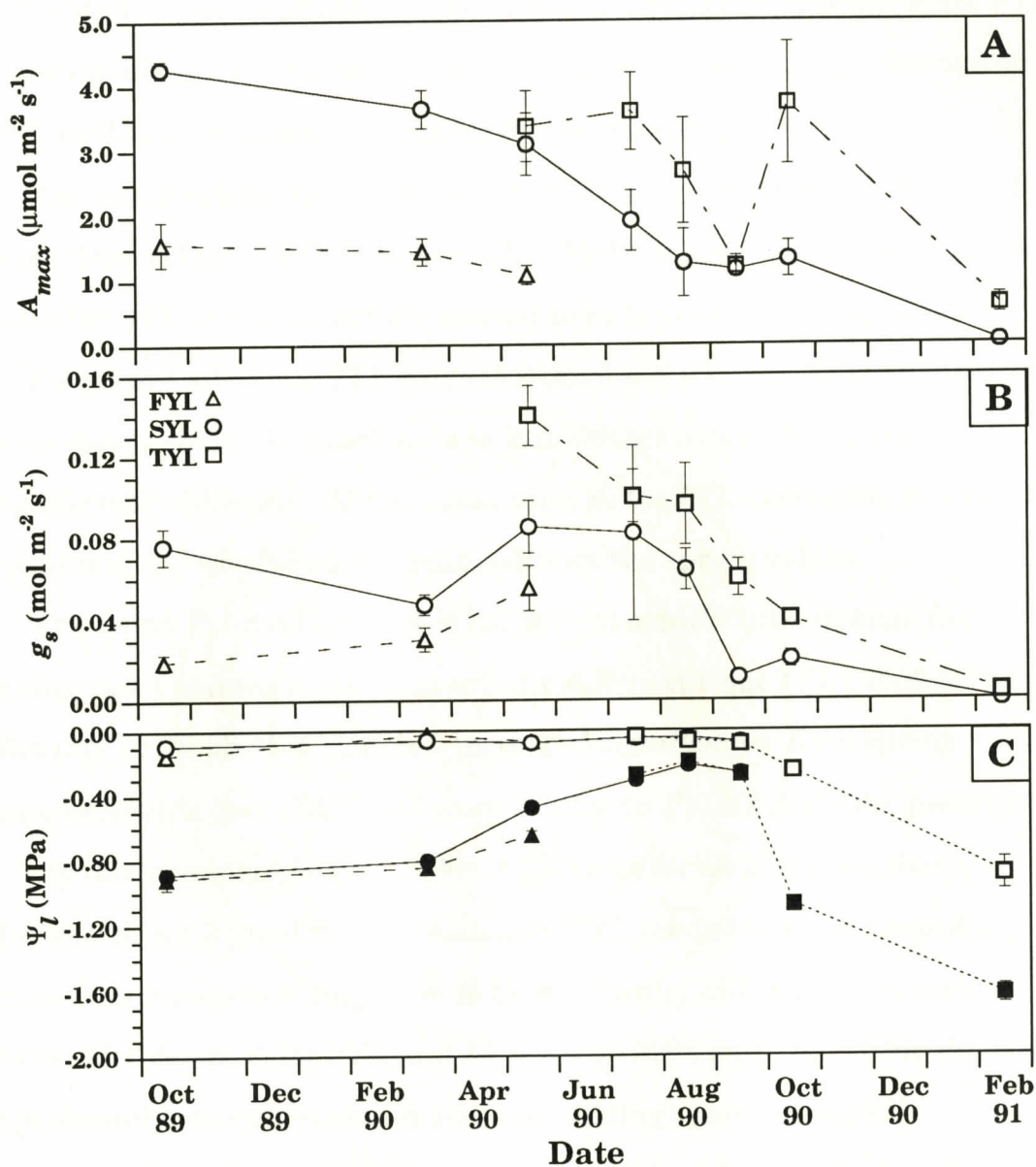


Figure 11. (A) Seasonal maximum photosynthesis (A_{max}), (B) stomatal conductance (g_s), and (C) water potentials (Ψ_l) for first (FYL), second (SYL) and third year leaves (TYL). Open symbols in Ψ_l graph represent predawn values, closed symbols midday values. Points represent means \pm standard errors.

and *SYL* were notably lower in late winter (-0.80 MPa, $p=0.0041$ and -0.85 MPa, $p=0.0037$; respectively). During the spring mid-day Ψ_l for *FYL* were significantly lower than *SYL* ($p=0.0054$). Ψ_l were not performed on *TYL* at this time because young leaves were too fragile.

As the canopy completed development during the early summer, PAR rates dropped to 11.5% of full sunlight. Mid-summer (July 1990) A_{max} rates for *SYL* ($1.9 \mu\text{mol m}^{-2} \text{s}^{-1}$) tended to be lower than spring rates ($3.1 \mu\text{mol m}^{-2} \text{s}^{-1}$) while *TYL* rates ($3.6 \mu\text{mol m}^{-2} \text{s}^{-1}$) increased slightly from spring rates ($3.3 \mu\text{mol m}^{-2} \text{s}^{-1}$), but neither comparisons were significantly different. At the same time, g_s for *TYL* decreased to a point equal to *SYL*, which had not changed from the spring values.

Pre-dawn Ψ_l for *SYL* (-0.04 MPa) did not significantly change from spring rates and were not substantially different from *TYL* (-0.05 MPa). Mid-day Ψ_l (-0.28 MPa) for *SYL* increased significantly from spring measurements ($p=0.0022$) and were similar to *TYL* (-0.31 MPa, $p=0.0614$).

By late summer (August 1990), A_{max} rates for *SYL* had further decreased to $1.3 \mu\text{mol m}^{-2} \text{s}^{-1}$. Although *TYL* tended to have lower A_{max} rates in late summer, they were not significantly different from spring rates. Pre-dawn Ψ_l for *SYL* and *TYL* (-0.05 MPa and -0.07 MPa) did not significantly change from mid-summer readings and were not substantially different from each other ($p=0.71$). Mid-day Ψ_l for *SYL* and *TYL* were statistically similar and slightly higher than mid-summer measurements (July 1990), though, not statistically so ($p=0.0840$).

In the fall (1989 and 1990) PAR levels at leaf level averaged about 45% of full sunlight. This percentage does not reflect the PAR data collected in September 1990, which was an unusually wet and cloudy month

(Figure 2). A_{max} rates tended to be higher for all age classes of leaves during the fall compared to late summer. When A_{max} was compared between the two fall readings of 1989 and 1990, *SYL* had a higher average A_{max} rate ($4.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) in 1989 than the same cohort one year later in 1990 ($1.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) ($p=0.002$). *FYL* did not survive till the second fall of the study (October 1990), although, it is interesting to note that *FYL* ($4.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) in October 1989 and *SLY* ($3.7 \mu\text{mol m}^{-2} \text{s}^{-1}$) in October 1990 had similar average A_{max} rates ($p=0.365$). Also, *SYL* ($4.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) in October 1989 and *TYL* ($4.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) in October 1990 also had similar average A_{max} rates ($p=0.602$).

Pre-dawn and mid-day Ψ_l for *SYL* and *TYL* in September (1990) were not significantly different from late summer rates. However, by October (1990) both pre-dawn (-0.25 MPa) and mid-day (-1.1 MPa) Ψ_l for *TYL* were significantly lower than September values ($p=0.015$ and $p=0.0001$, respectively). By October 1990 all *SYL* that had been tagged for water relations studies had died.

During the winter PAR values increased to 53.0% of full sunlight. Mid-winter readings (February 1991) had significantly lower A_{max} than any previous measurements (*SYL*; $0.04 \mu\text{mol m}^{-2} \text{s}^{-1}$ and *TYL*; $0.62 \mu\text{mol m}^{-2} \text{s}^{-1}$). At this time soil temperatures remained below 0°C for most of the day, while maximum air temperatures were higher than those recorded during the fall of the same year. The late winter A_{max} readings (March 1990) were similar to the fall and spring measurements, but significantly greater than mid-winter A_{max} measurements (*SYL*; $p=0.001$, *TYL*; $p=0.002$). Soil temperatures for March 1990 did not fall below 4.0°C .

Pre-dawn (-0.90 MPa) and mid-day (-1.63 MPa) Ψ_l were also at their

lowest during the winter and were significantly lower than fall values ($p=0.043$ and $p=0.002$, respectively) and late winter values in March (1990) ($p=0.001$ for both).

SEASONAL INTERNAL LEAF WATER RELATIONS

Ψ_{so} and Ψ_{tl} values fluctuated seasonally, with highest values occurring between May and September and lowest values between October and April (Figure 12A & 12B). *TYL* had the highest readings in June 1990 (-0.08 and -1.02 MPa, respectively), the first month they were large enough for testing. By mid-summer their values decreased to nearly match those of *SYL* for Ψ_{so} in July and August and for Ψ_{tl} in August. *TYL* in January 1991 were statistically similar to *SYL* in January 1990 for Ψ_{so} and Ψ_{tl} ($p=0.75$ and $p=0.39$, respectively). *FYL* had the lowest values for Ψ_{so} and Ψ_{tl} in January 1990 (-1.85 and -2.27 MPa). In contrast *SYL* had relatively high values (-1.05 and -1.56 MPa) at that same time.

RWC_{tl} values were generally lowest between February and June and highest between July and December (Figure 12C). *FYL* had the lowest average RWC_{tl} of 93.5% (SE=0.4%), with *SYL* having significantly higher averages of 95.1% (SE=0.2%) and 95.8% (SE \pm 0.3%).

The seasonal pressure volume data (Figure 13) and that collected by Dr. Meinzer were similar for Ψ_{so} , Ψ_{tl} , and RWC_{tl} , but differed considerably for *SWC* and *AWC* (personal communication). Seasonal *SWC* and *AWC* averaged 24.8% (SE= \pm 1.9%) and 75.2% (SE= \pm 1.9%), respectively, using the method described in this paper. Symplastic water contents were considerably higher than *AWC* when calculated using the over-pressure method (61.0% and 39.0%, respectively).

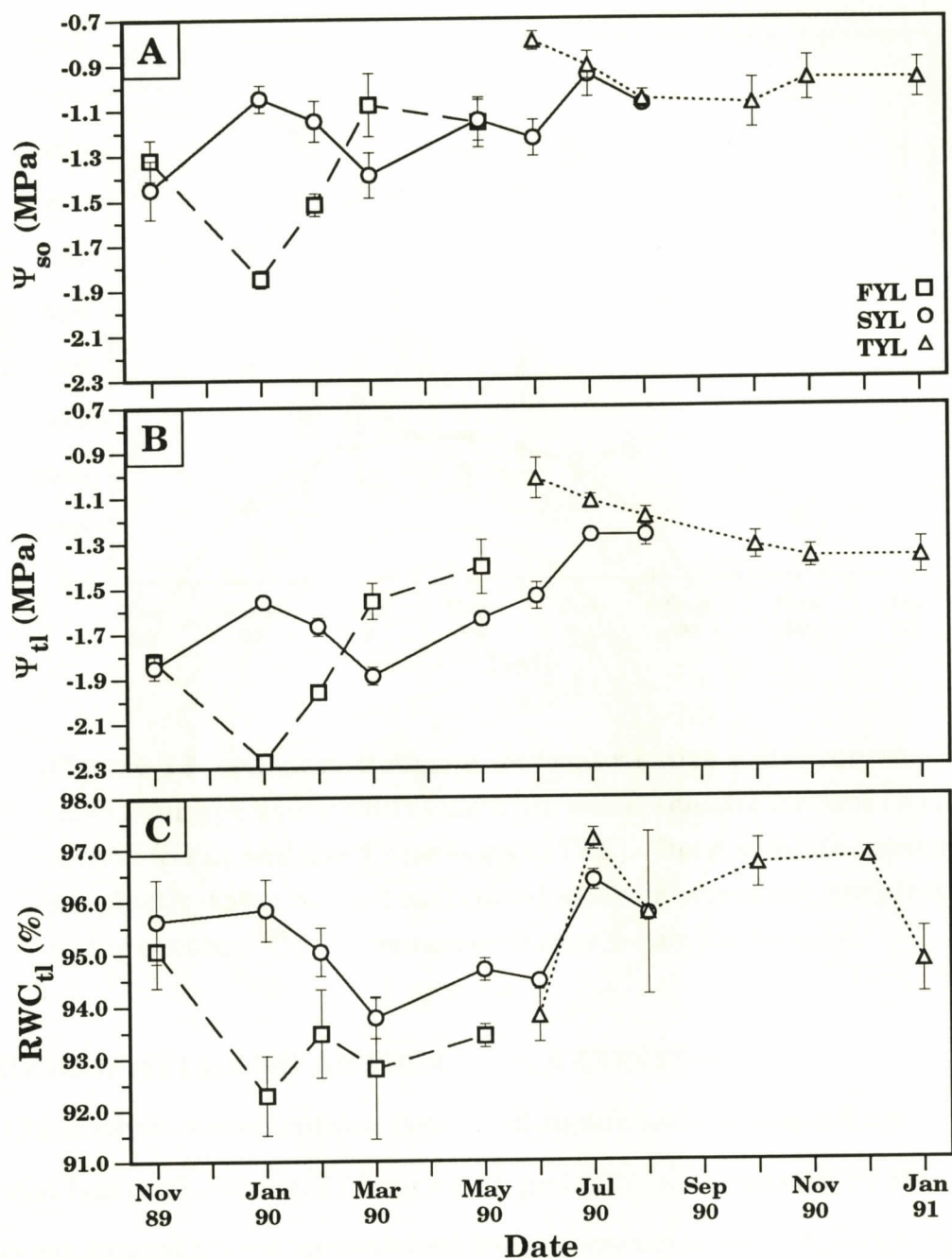


Figure 12. Seasonal changes in water potential components, (A) osmotic potential at full turgor (Ψ_{so}), (B) water potentials at turgor loss (Ψ_{tl}), and (C) relative water content at turgor loss (RWC_{tl}) for first (FYL), second (SYL) and third year leaves (TYL). Points represent means \pm standard errors.

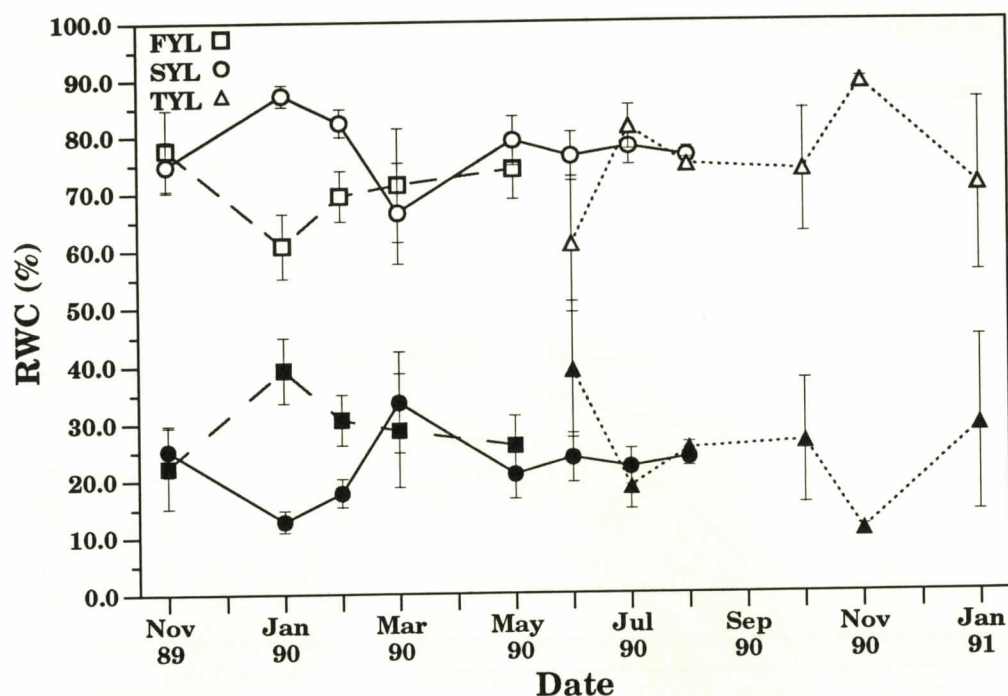


Figure 13. Seasonal change in percent relative water content (*RWC*) for apoplastic and symplastic water content for first (*FYL*), second (*SYL*) and third year leaves (*TYL*). Open symbols represent apoplastic water content and closed symbols represent symplastic water content. Points represent means \pm standard errors.

CHLOROPHYLL AND ANTHOCYANIN CONTENT

Chlorophyll concentrations decreased significantly in the fall for shaded leaves (0.255 to 0.172 mg mm⁻², $p=0.003$), a decrease of 32.9%, whereas control leaves (not shaded) had a decrease of only 18.5% (0.285 to 0.232 mg mm⁻², $p=0.025$) (Figure 14). Control leaves tended to have lower chlorophyll concentrations than shaded leaves but these differences were only statistically different for the last two readings in January.

Anthocyanin levels in both shaded and control leaves increased

significantly during the fall ($p=0.0001$ for both sets of leaves). Shaded leaves tended to have lower anthocyanin levels than control leaves but the differences were only significant for the last two readings in January ($p=0.033$ and $p=0.028$, respectively).

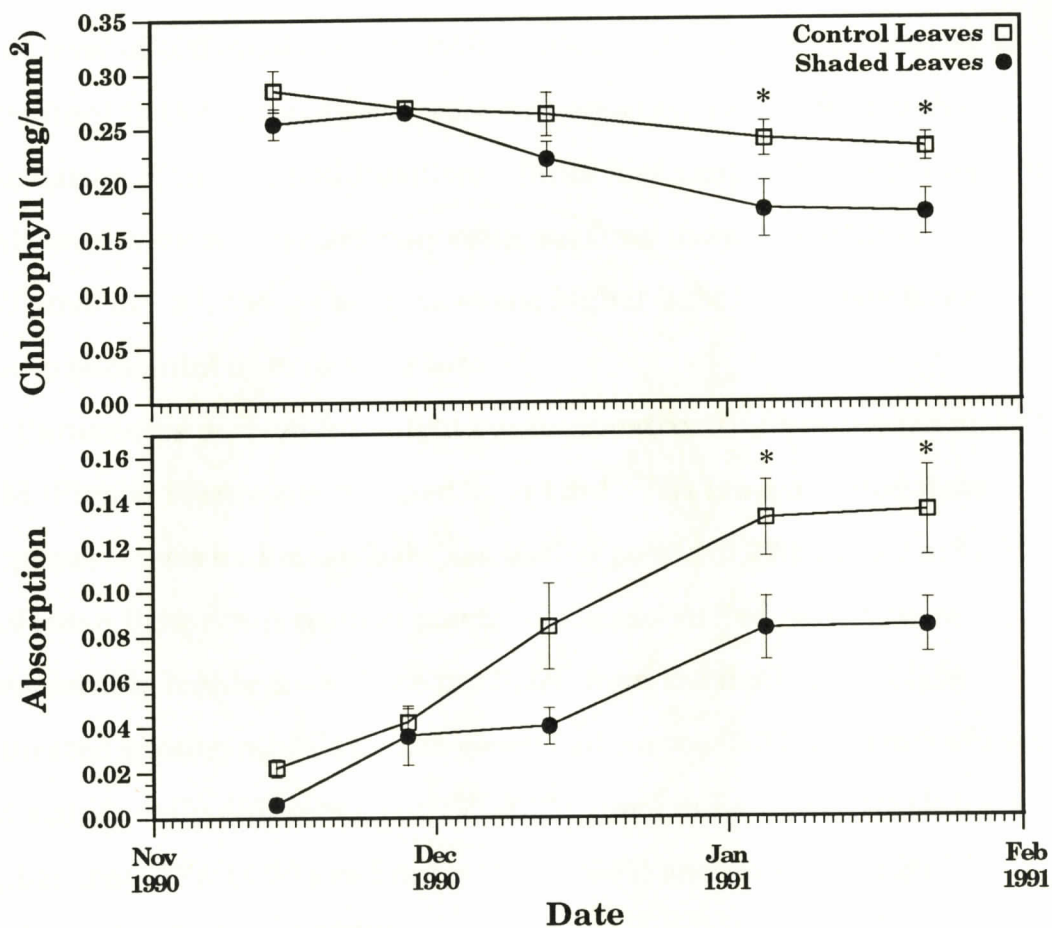


Figure 14. Chlorophyll content and relative anthocyanin accumulation in control and shaded *Galax aphylla* leaves during the winter of 1990 and 1991. Points represent means \pm standard errors. (*) denotes significant difference ($p \leq 0.05$) between treatments in the designated sample.

LIGHT RESPONSE CURVES

There were no significant differences, in the lab, between *SYL* and *TYL* ($p=0.1100$) for light saturated photosynthesis (A_{ls}), thus the data were combined to form a larger sample size (Figure 15). Leaves reached their maximum A of $3.33 \mu\text{mol m}^{-2} \text{s}^{-1}$ (with a g_s of $0.034 \text{ mol m}^{-2} \text{s}^{-1}$) at a PAR level of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. PAR levels of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ appeared inhibitory to *Galax* leaves and were subsequently removed from the calculation of the Weibull functions. Since leaf temperatures at this high PAR level were not significantly different from those at a PAR of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$, the lower rates at the higher light levels may have been due to photoinhibition of some sort.

Parameters derived from light curves constructed from the lab and the field (Figure 16) are summarized in Table 1. The lab generated light response curves had mean light saturation points of $220.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ and mean light compensation points of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$, which were considerably higher than those predicted from the field data. Light saturated photosynthesis for lab generated curves ($2.94 \mu\text{mol m}^{-2} \text{s}^{-1}$) was not significantly different from *TYL* ($3.18 \mu\text{mol m}^{-2} \text{s}^{-1}$, $p=0.318$) but was higher than *FYL* ($1.50 \mu\text{mol m}^{-2} \text{s}^{-1}$, $p=0.0001$) and lower than *SYL* ($3.80 \mu\text{mol m}^{-2} \text{s}^{-1}$, $p=0.0024$).

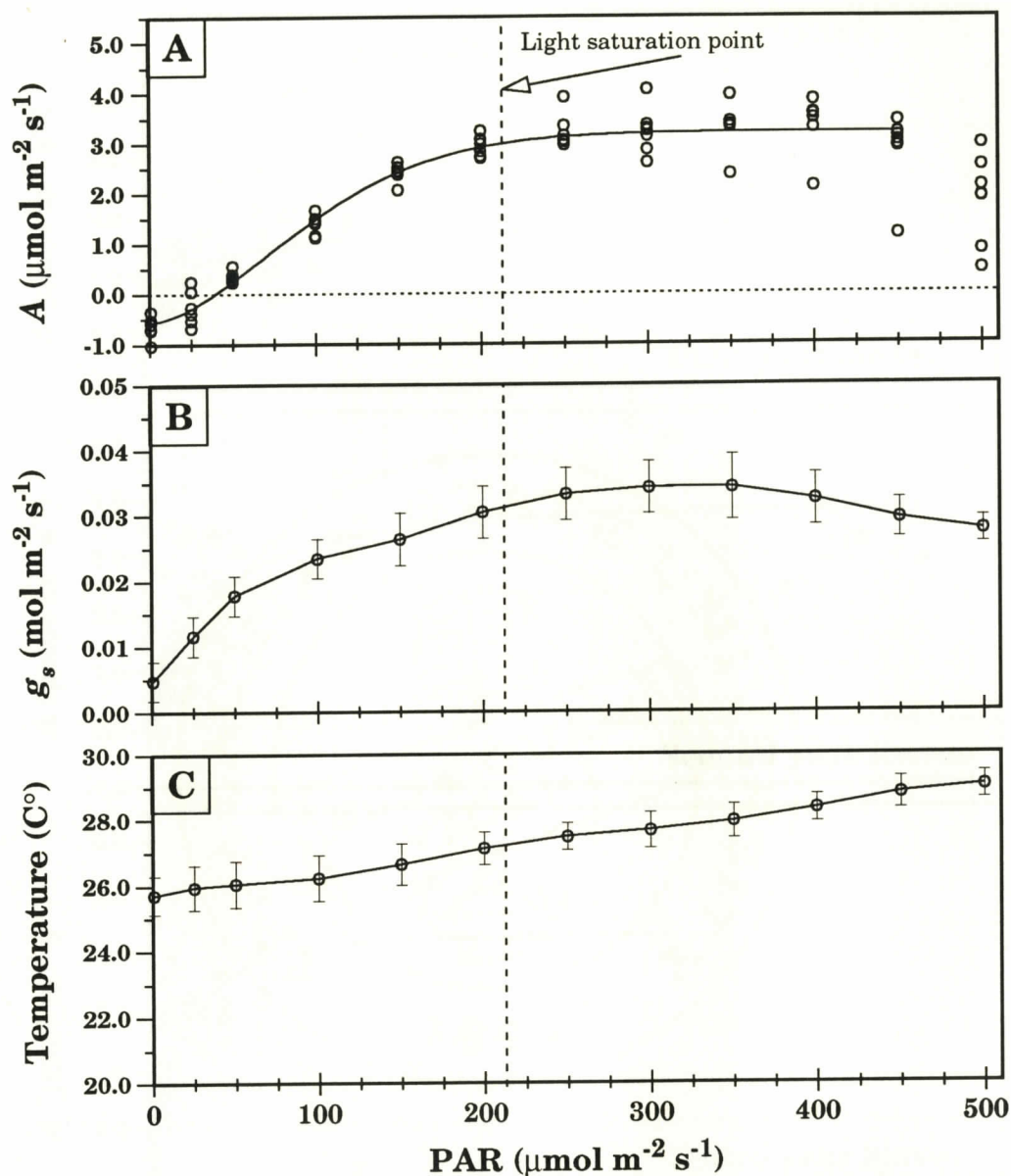


Figure 15. Lab generated light response curves for *Galax*; with (A) photosynthesis (A), (B) stomatal conductance (g_s) and (C) leaf temperature versus photosynthetically active radiation (PAR). Line for A was fitted using a modified Weibull function (see Figure 4). Points represent means \pm standard errors.

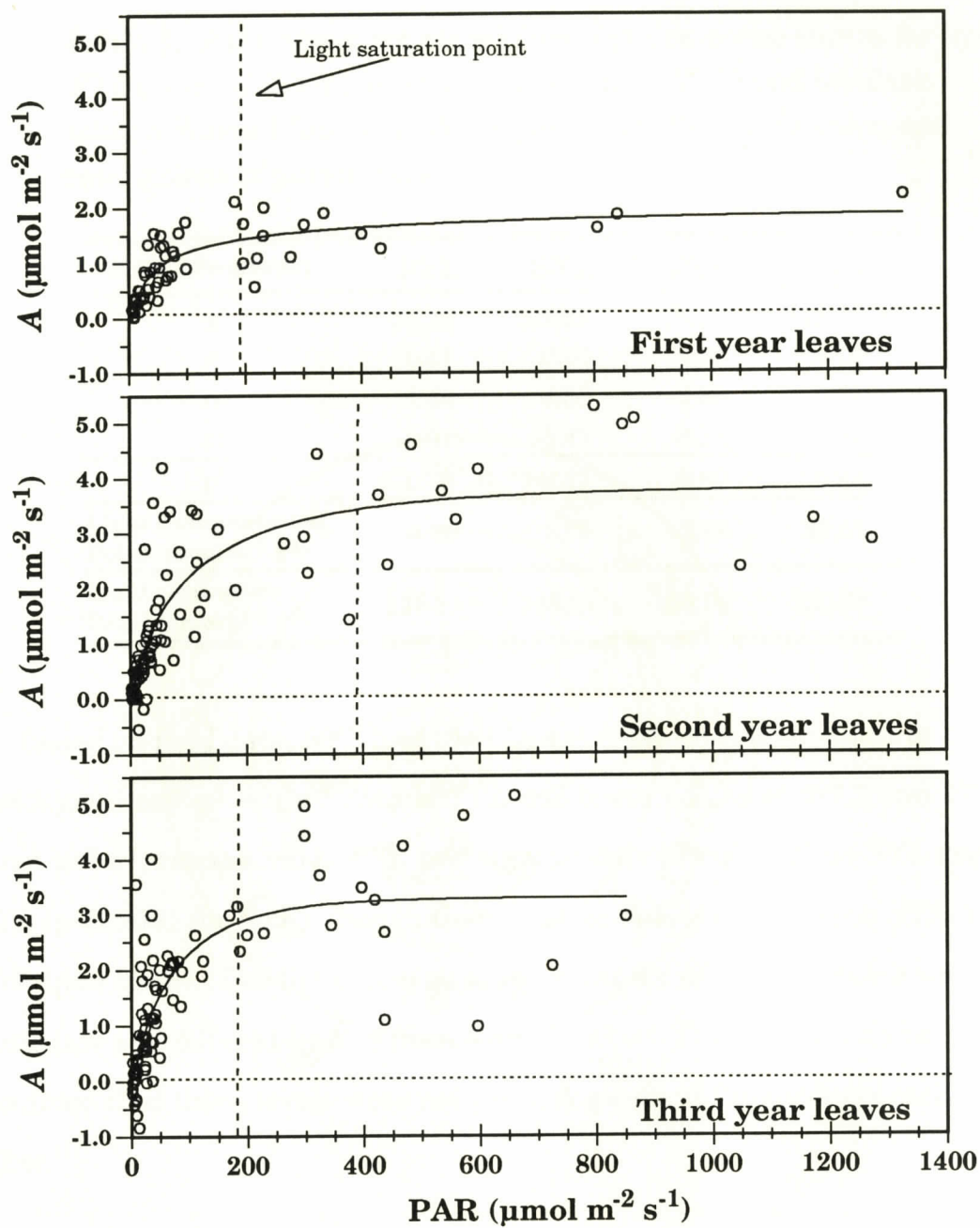


Figure 16. Light response curves for first, second, and third year leaves, with photosynthesis (A) versus photosynthetically active radiation (PAR). These curves were constructed from seasonal data collected from the field. Lines were fitted with a modified Weibull function (see Figure 4).

Table 1. Parameters estimated from light response curves for first (*FYL*), second (*SYL*), and third year leaves (*TYL*) and lab data using a Weibull function. See Figure 4 for Weibull function and descriptions of parameters.

| Weibull Parameters | FYL | SYL | TYL | Lab |
|---|--------|--------|--------|--------|
| <i>s</i> | 44.29 | 103.68 | 75.22 | 115.31 |
| <i>c</i> | 0.81 | 0.65 | 0.82 | 1.70 |
| <i>a</i> | 1.85 | 4.30 | 3.50 | 3.77 |
| <i>m</i> | -0.31 | -0.47 | -0.22 | -0.57 |
| <i>r</i> ² | 0.68 | 0.69 | 0.60 | 0.93 |
| Light Compensation Point ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 5.36 | 3.89 | 2.64 | 39.83 |
| Light saturation Point ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 197.10 | 398.22 | 172.96 | 220.95 |

Based on field data, *SYL* had the highest light saturation point at $398.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ while *FYL* and *TYL* had lower points at 197.1 and $172.9 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. *FYL* had significantly lower A_{ls} than *SYL* and *TYL* ($p=0.0001$ for both), though there was no difference between *SYL* and *TYL* ($p=0.0948$). The light compensation point for all three cohorts was quite low and differed by less than $3 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 1). It can be assumed that these levels were not physiologically different from each other.

Discussion

LEAF PHENOLOGY

Galax produces a new cohort of evergreen leaves during the spring of every year. These leaves start to develop about mid-May, appearing on the rhizome as a new petiole in which the lamina is curled around the apex. As the petiole elongates, the lamina unrolls within the first two weeks of emergence. New leaves are light in color, extremely fragile, and easily damaged. The majority of insect damage occurs during this time. As leaves mature and expand, they become stiffer and darken with age, reaching full size about four weeks after emergence.

It has been suggested that *Galax* leaves can survive as long as four years (Holloway, personal communication). However, in this study, most leaves survived only about two years. Second year leaves were the only cohort to survive the total length of the study; from May 1989 to about June 1991. First year leaves survived only the first year of the study and third year leaves, which emerged in May 1990, were still present the last year of the study. The length of time that evergreen leaves can persist depends on many factors. Leaf longevity increases as resources become more limited, and is related to a balance between leaf costs and benefits (Chabot and Hicks, 1982). In environments where resources are limited, leaf longevity would be increased because of the longer time needed to repay leaf construction costs.

Leaf mortality in *Galax* was greatest during the winter months, with very few leaves dying during the summer. Assuming that first year leaves

were at the end of their second growing season when this study began, mortality was greatest for all cohorts during their second winter.

Evergreen leaves are often retained only as storage organs during the winter, but as photosynthetic rates decline with age, these resources are used up, and the leaf dies (Shaver, 1983; Reader, 1978). Also, by shedding leaves during the winter, the carbon costs of winter-hardening and leaf maintenance are avoided (Hadley and Bliss, 1964).

The results of this study indicate that *Galax* has at least two age classes of leaves at any given time, and potentially three age classes during early spring. New leaves are produced about the same time the previous year's leaves start to show decreased photosynthetic rates. This progression of new and old cohorts probably allows *Galax* to maximize whole plant carbon gain, while still benefiting from the carbon produced in older leaves.

SEASONAL TRENDS

Temperature and moisture were the two dominant external influences on *Galax* leaf physiology. In the summer, canopy closure reduces light, solar heating and evaporative demands for plants on the forest floor. Less light leads to lower photosynthetic rates, and at the same time, decreased water loss in the plant by reducing leaf temperatures. *Galax* reacted to lower evaporative demands by increasing stomatal conductance, which increased the potential for greater gas exchange. This strategy works well only under conditions of limited evaporative demand or when water is not limiting. If water does become limiting, stomatal conductance would

decrease to reduce water loss, which in turn would impede photosynthesis (Young, 1987).

Since water stress was not detected during the summer months, light was probably more limiting to photosynthesis than water availability. For the majority of the time, due to the angle of the sun, dense canopy, or cloudy conditions, light levels were below light saturation. But, during light flecks, light levels could rise above light saturation ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$). *Galax*, like many plants found on the forest floor, is characterized as having low light saturation points and low light compensation points compared to plants that occupy higher light habitats (Böhning and Burnside 1956, Boadman 1977, Ludlow 1983). *Galax* can thus efficiently utilize the lower light during the growing season.

In forest environments where light can be limiting, many plants are able to increase carbon gain 30 to 60% by utilizing sunflecks (Chazdon, 1988; Weber *et al.*, 1985). Sunflecks were not directly studied, but it is likely that photosynthesis in *Galax* responds to sunflecks. However, it is not known how efficiently *Galax* can utilize sunflecks to achieve higher photosynthetic rates. In some plants there is an induction period required before the plant will react to the sunfleck. Once achieved, plants can respond in less than 30 seconds (Gildner and Larson 1992; Tinoco-Ojanguren and Pearcy 1992; Pearcy *et al.* 1985). Some of the variation seen in the field light saturated photosynthetic rates could be related to the length of time and frequency that a *Galax* leaf was exposed to sunflecks. The induction period for *Galax aphylla* is not known at this time.

Younger leaves were less affected by reduced light than older leaves. This reduction of photosynthetic capacity in aging leaves has been well documented in a number of studies (Sobrado, 1994; Kikuzawa, 1990; Teskey et al., 1983; Chabot and Hicks, 1982; Reader, 1978; Johnson and Tieszen, 1976). Ludlow and Jarvis (1971) suggest that as leaves age, photosynthesis declines because of increased stomatal and mesophyll resistances, which are brought on in part by the accumulation of waxes within the ante-chamber of the stomata. With *Galax*, new leaves produced at the beginning of the summer were able to maintain higher photosynthetic and stomatal rates than older leaves, equaling levels obtained by previous cohorts during the spring and fall when light was less limiting. One advantage of producing new leaves at the beginning of the summer is that younger leaves are more efficient at utilizing available light and thus more productive. They can help maintain whole plant carbon uptake as older leaves start to decrease photosynthetic rates in response to age and the low light of summer.

Overall light levels were higher in the spring and fall than in the summer, due to the lack of leaves in the canopy at these times. Although light became less limiting for *Galax*, solar heating and evaporative demand increased. Additionally, the open canopy may have allowed for greater air circulation in the understory, further reducing relative humidity and increasing soil water evaporation (Hicks and Chabot, 1985). Since *Galax* produces most of its roots within the top 20 cm of soil (personal observation), the reduction of soil water and the additional evaporative demand may have increased the chance of water limitation for *Galax*. Evidence for water limitation could be seen by the lower pre-dawn

and mid-day water potentials during the spring and fall.

As plants become water stressed, the stomata begin to close to conserve water within the plant. If stomatal closure is extreme, gas exchange can be impeded, resulting in a reduction in photosynthesis (Kramer, 1983; Hinckley *et al.*, 1980). Younger leaves were able to maintain photosynthetic rates equal to maximum rates obtained during the summer, even with a reduction in stomatal conductance. Older leaves had reduced photosynthetic rates coupled with decreased stomatal conductances, perhaps reflecting an inability of older leaves to adjust to mild water stress. Jordan *et al.* (1975) found as leaves age, their ability to cope with water stress decreases, due to increased stomatal resistance. Thus, there is a trade-off with water availability; increased light can increase carbon gain, but also increases evaporative demand, lessening water availability and reducing stomatal conductance, which in turn reduces photosynthesis.

One advantage of being evergreen is that there is the potential to gain carbon during all seasons. During the winter, understory light levels remained above the saturation point for photosynthesis, but low leaf and soil temperatures combined to limit gas exchange. *Galax* did have some positive photosynthesis rates during the winter months, although these events were primarily limited to warmer days when mid-day air temperatures were at least above 5°C and soil had begun to thaw. In this study, it was shown that younger *Galax* leaves can obtain positive photosynthetic rates at soil temperatures as low as 2.5°C. However, when soil temperature increased to 5°C, photosynthetic rates equaling summer rates were obtained. The colder soil temperatures may have limited water

availability by causing a higher resistance to water flow within the root systems (Teskey *et al.*, 1984). During periods of warming, air and leaf temperatures can change more rapidly than soil temperatures, potentially causing water to become limiting for the first few days of warming (Turner and Jarvis, 1975). For *Galax* this was apparent with the February 1991 measurements, which had soil temperatures only a few degrees above freezing. As the plants responded to the increased air and leaf temperatures the lack of available ground water caused lower pre-dawn and mid-day water potentials. This induced stomatal closure, impeding gas exchange. After a few more days of warm weather soils will thaw and increase water availability. The higher winter gas exchange found in March 1990 may reflect *Galax's* response to a longer period of soil warming.

The response of photosynthesis to warm winter days has been shown in a number of evergreen species (*Juniperus virginiana*: Lassoie *et al.*, 1983; *Pseudotsuga menziesii*: Waring and Franklin, 1979; *Picea sitchensis*: Turner and Jarvis, 1975). Not all evergreens take advantage of these warm trends though. Nilsen (1986) found that *Rhododendron maximum*, which occupies similar habitats to that of *Galax*, did not have any photosynthetic capability during winter months. He found that once soil temperatures started to decrease, stomates closed and remained closed until the spring. He suggested that this is a mechanism to avoid potential water stress during the winter. Some species, such as *Picea sitchensis*, are able to maintain photosynthesis at soil temperatures below 0°C, but at greatly reduced rates (Turner and Jarvis, 1975). Larger evergreens have the advantage of deeper root systems that can extend

below the frost line where water may not be frozen. Since the root system of *Galax* is found in the upper 20 cm of soil, it can become frozen more quickly, and it is doubtful that *Galax* carries on photosynthesis when soils are frozen. However, a shallow root system can ultimately be beneficial because top soils thaw rapidly allowing photosynthesis to rapidly respond to short-term warming trends.

SEASONAL TRENDS IN TISSUE WATER RELATIONS

Mechanisms by which plants stabilize their internal water environment against a changing external water regime have great adaptive significance (Roberts *et al.*, 1980). Fluctuating patterns of osmotic potentials at full turgor, at turgor loss, and relative water content at turgor loss for *Galax* indicated seasonal changes within the leaf. Lower osmotic potentials (more negative) are associated with increases in solutes within the cell and with increased water deficits. In this study, water potentials tended to be highest at the start of the growing season and lowest during the winter months. Similar seasonal trends have been reported for other evergreens (*Rhododendron*: Nilsen, 1987; *Abies*: Teskey *et al.*, 1984; *Pseudotsuga*: Ritchie and Shula, 1984; *Tsuga*: Tyree *et al.*, 1978b).

As reported in the previous section winter poses the greatest threat for water deficits in *Galax*. Since the plant remained active during the winter lower osmotic potentials at full turgor and at turgor loss may indicate an adaptive feature to maintain water balance during periods of water stress. In some evergreens lower water potentials during winter reflect an elevated concentration of foliar sugars (Ritchie and Shula, 1984). Levitt

(1980) suggests that the increase in sugars in the fall and winter is a mechanism for cold hardiness, and that this accumulation retards ice crystal formation within the cells by lowering the protoplasmic water freezing point. Increased sugars may result from photosynthesis during the fall and winter months when day temperatures are warm enough, even when soil temperatures remain low. Since cold soils cause a high resistance to water movement within the root system (Teskey *et al.*, 1984), any photosynthates produced may not be able to be translocated to the roots, resulting in increased foliar solute concentration, which subsequently lowers water potentials. Increased solute concentrations may also allow the plant to remain active during times of water stress by increasing the water potential gradient from soil to plant, maintaining water uptake and allowing turgor to be maintained at lower leaf water potentials and lower relative leaf water content (Roberts *et al.*, 1980).

Lower leaf water potentials during the winter may also be the result of "winter desiccation" (Kramer and Kozlowski, 1960). Evergreen leaves can lose water through the stomata or by cuticular transpiration, even while the ground water remains frozen (Tranquillini, 1982), resulting in conditions similar to that of a drought. With no way to overcome this deficit, foliar solutes become more concentrated, and slowly increase until the ground water thaws and the plant can take up new water by the roots.

Older leaves had lower osmotic potentials at full turgor and at turgor loss during the winter than younger leaves. Similar reports have been found in other species where older leaves had lower osmotic potentials than younger leaves on the same plant (Tyree *et al.*, 1978a). This may relate to the aging of the stomatal mechanism and the inability of the

older leaf to maintain water content during winter drought-like conditions. Decreased efficiency in the stomatal mechanism associated with accumulation of waxes in the stomata and age related changes in the guard cell (Teskey *et al.*, 1984), could contribute to decreased water content during stressful periods. Lower osmotic potentials indicate that solutes may be accumulating in the protoplast of older leaves more readily than new leaves. It is uncertain at this time if this is an adaptive feature to help increase water uptake in older leaves, or the inability of older plants to translocate photosynthates efficiently during cold periods.

Water potentials started to increase in the spring, reaching a maximum about mid-summer and then slowly decreased in the fall. The increase in the spring may result from the development of new leaves and a decrease in water stress as soils thaw and new root growth is initiated. When new leaves begin to develop there is an increased demand for carbon within the plant, and sugars may be metabolized faster than the plant can produce them, causing the solute concentrations in the symplast to decrease, which in turn causes an increase in osmotic potentials (Abrams and Menges, 1992; Ritchie and Shula, 1984). Once the new leaves reach maturity the high demand for carbon is lessened and solute concentrations can begin to increase again. Similar patterns of osmotic fluctuations in new foliage have been reported by others (Muller, 1991; Svejcar, 1986; Teskey *et al.*, 1983; Roberts *et al.*, 1981).

There seemed to be some inconsistencies in the determination of relative apoplastic and symplastic water contents when compared to Dr. Meinzer's estimations. Normally, symplastic water makes up anywhere from 75% to 80% of total leaf water, while apoplastic water accounts for

only about 20 to 25% (Kramer, 1983). In this study, apoplastic water accounted for the major fraction of leaf water, opposite to what was expected. When the overpressure method (Dr. Meinzer's protocol) was used, more typical symplastic and apoplastic estimates were achieved (61.0% and 39.0%, respectively). Kubiske and Abrams (1991) have shown that artificially rehydrating the leaf can cause an uptake of excess apoplastic water, resulting in a larger than expected increase in relative water content, and which may affect the estimate of osmotic potentials and relative water content at full turgor (Abrams and Menges, 1992). When Dr. Meinzer artificially rehydrated the leaves in the over-pressure method, he did not find excessive apoplastic water, and his estimations of the other pressure volume parameters were similar to those found in this study. At this time it is unclear why these two different methods vary in the estimation of the apoplastic and symplastic water contents.

ANTHOCYANIN AND CHLOROPHYLL CONTENT

Galax leaves normally turn red during the winter when upper canopy leaves have fallen and air temperatures start to drop to near 0°C. This ability to turn red may result from the accumulation of anthocyanins during periods of prolonged exposure to high light. Though some leaves may show signs of reddening with increased age (greater than 1.5 years), or damage after maturity, young leaves do not (personal observation). Levin (1971) indicates that anthocyanins may be produced in response to a variety of environmental stresses, including damage.

The winter increase in anthocyanins was greatest in those leaves which were not shaded. It has been shown in a number of studies that

anthocyanin production increases when leaves are exposed to high light stress (Demmig-Adams and Adams, 1992a; Lee *et al.*, 1987; Lindoo and Caldwell, 1978; Macinelli *et al.*, 1976). Lee and Lowery (1980) suggest that this could be a protective mechanism against high light stress, as it is often seen in young leaves. During leaf development the epidermal and cuticular layers are underdeveloped, and may not be able to protect the leaf from damaging ultraviolet radiation. The presence of anthocyanins in these young leaves increases the reflectivity of the leaf surface relative to ultraviolet radiation and also absorbs some radiation before it can damage internal tissues (Lee and Lowery, 1980). The production of anthocyanins in *Galax* may therefore act as a protective measure against high light stress during the winter.

Galax starts to produce new leaves at about the same time the canopy closes and light levels begin to decline. Shade tolerant plants usually invest a greater amount of energy in the light harvesting mechanism and relatively less energy in protective structures than do plants grown in higher light conditions (Boardman, 1977). This allows shade tolerant plants to be more efficient in a light-limited environment, but less able to withstand higher light conditions. In a number of experiments where shade tolerant plants were exposed to increased solar radiation, photoinhibition resulted at first, followed after prolonged exposure by a degradation of the photosynthetic apparatus (Critchley 1981; Powles 1984; Demmig-Adams and Adams 1992b;). *Galax* leaves may not be able to withstand the high light levels during winter, and the accumulation of anthocyanins at this time may serve as a protective measure.

PHENOLOGICAL AND PHYSIOLOGICAL RESPONSES TO TREATMENTS

Leaf phenology and physiology were unaffected by the addition of supplemental nitrogen, water and increased shade. *Galax*, like many evergreens, seems to be conservative in its response to additional resources. This may reflect a trade-off that allows evergreens to occupy resource limited areas, but renders them less able to utilize resources when they are not limiting (Chapin *et al.*, 1986). For *Galax* this conservative strategy may allow the plant to better survive changing environmental conditions, but less able to take advantage of increased resource fluxes.

When this study began it was assumed that *Galax* might be growing in a nutrient limited environment. But, when supplemental nitrogen was added to the *Galax* plots, there were no changes in growth or physiology. However, there was a significant increase in leaf nitrogen. It has been suggested that since evergreen species are often found in nutrient-poor habitats, they may not be capable of uptake of excess nutrients, i.e. "luxury consumption" (Small, 1972). In a number of studies it has been found that evergreens do respond to increases in nitrogen but not as rapidly or to the extent of deciduous species (Arets, 1990; Simms, 1987; Shaver, 1983; Gray and Schlesinger, 1983). For *Galax* the increases in leaf nitrogen indicate a response to supplemental nitrogen, but not enough to effect growth or physiology.

Simms (1987) found that when additional nitrogen was placed on evergreen and deciduous species in nutrient-poor conditions that both species showed increased growth. The lack of response in growth may

indicate that growth was not nitrogen limited for *Galax*. In some evergreen species, nutrients taken up in excess of immediate growth requirements during nutrient flushes may be reserved to support growth after soil reserves are exhausted or stored in leaves to be used for new growth in the spring (Chapin, 1980; Reader, 1980).

There is some controversy over the effects of increased nitrogen on leaf longevity and growth in evergreens. Shaver (1981) found that the artificial addition of nitrogen shortened leaf longevity. He suggested that when nutrients are non-limiting the costs of maintaining older leaves may outweigh their usefulness, and they are allowed to die. In similar studies Arets (1989) and Reader (1980) found that increased nitrogen did not significantly effect leaf longevity. Shaver (1983) felt that Reader did not continue his studies long enough, and that evergreens do respond to fertilization, but because of their slower metabolism need to be studied for longer periods. In this study, the effect of nitrogen was examined for only two growing seasons, with three different age classes of leaves. Both the first and second year leaves reached maturity before the application of nitrogen had begun. If Shaver is correct, then these generations of leaves may not have had time to show a response to the increased nitrogen. The third year leaves were the only generation to develop while treatments were in progress, but the study ended before their second growing season and effects on their longevity could not be determined.

Controversy also exists over the effects of increased leaf nitrogen on photosynthesis in evergreens. Several studies have shown that as foliar nitrogen increases, photosynthesis also increases until nitrogen becomes non-limiting (a curvi-linear relationship) (Evans, 1983; Dejong and Doyle,

1985; Olesinski *et al.*, 1989). This relationship has been well documented with regards to deciduous species (Longstreth and Nobel, 1980; Gulmon and Chu, 1981; Evans, 1983; Olesinski *et al.*, 1989), but results vary from positive, neutral, to even negative for evergreen species (Field *et al.*, 1983; Sheriff *et al.*, 1986; Reich and Schoettle, 1988; Lajtha and Whitford, 1989). Chapin *et al.* (1986) suggest that there are possible limitations for nutrient studies in nutrient-poor plant communities. Competition for nutrients is so great in these environments that plants are usually better competitors for retaining nutrients but are less adapted at taking advantage of temporary nutrient additions. Also, supplemental nutrients often get tied up by fast growing species that are better able to take advantage of these increases. Thus, the addition of nutrients to natural plant communities needs to be sufficient enough to saturate environmental processes (chemical and microbial) while still meeting plant nutrient requirements. In the case of *Galax*, the nitrogen levels were high enough that foliar nitrogen increased, but perhaps not high enough to cause physiological responses.

The low metabolic responses of *Galax* were also apparent when leaves were grown in the light limited conditions of the shade plots. When *Galax* was placed under half the light that it would normally receive, leaf size and expansion were unaffected, although there were some indications of decreased photosynthetic rates. Plants that occupy shaded habitats often have larger leaves than the same species grown at higher light levels (Dale, 1988; Ashmun and Pitelka, 1984; Boardman, 1977; Grime, 1966). In studies where plants are grown in one light environment and then placed in a lower light environment, photosynthetic rates initially

decreased, and as the plants acclimate over time compensation and saturation points also decrease (Boardman, 1977; Ludlow and Ng, 1976). This apparently helps to increase the light gathering capabilities under lower light conditions. Dale (1988) suggested that leaves are primarily photosynthetic organs that need to be large enough to optimize this function while at the same time balancing water availability and loss from transpiration. A plant can only produce leaves large enough to optimize light gathering efficiency while at the same time not overextending the other metabolic processes in a given light environment. *Galax* may not have responded to the decreased light because leaves had already reached an optimum size for shaded conditions on the forest floor.

Increased shade also did not change mortality rates or leaf longevity in *Galax*. In various studies, shading has been shown to cause a reduction in leaf longevity when plants that developed in non-shaded conditions were moved to shaded conditions (Ashmun and Pitelka, 1984; Reader, 1980; Bazzaz and Harper, 1977). However, for those leaves that develop under shaded conditions, longevity is usually increased compared to leaves from higher light environments. Kikuzawa (1982) concluded that leaf longevity is short when the photosynthetic capacity of the leaf is high and leaf construction costs are small. But when photosynthetic rates are low more time is needed to pay back construction costs and leaf longevity is increased. It is likely that *Galax* is well adapted to surviving in shaded conditions and the change in light conditions due to the shade treatment was not enough to cause a change in mortality or longevity. During the course of this study and through personal observations, other populations of *Galax* have been found growing in light conditions equal to or lower

than the shade plots. It may be more enlightening to see if *Galax* would respond to *increased* light conditions during summer months.

The addition of supplemental water to the *Galax* plots was designed to eliminate water stress during the growing season. However, during the summer that phenological and physiological measurements were made precipitation for the site was above normal. This may have nullified the impact of additional water, since it is unlikely that any of the plants experienced severe water stress during the growing season. Another unforeseen complication was that the increased water caused an ephemeral stream near the bottom of site B to become a temporary wetland, which at times engulfed the water treatment on this site. While this eliminated any water deficits, it may have caused complications due to flooding of the roots. All other plots, including the controls, were above this wet area and remained well drained.

The physiological measurements on *Galax* were also hampered by the above average precipitation. The flooding of the water plots in site B did give some insight into *Galax's* response to flooding. When plants are exposed to extended periods of flooding photosynthetic rates drop in response to partial stomatal closure (Bradford and Yang, 1981; Bradford and Hsiao, 1979; Kozlowski and Pallardy, 1979). With *Galax* photosynthetic rates were decreased but stomatal conductance rates were increased. Jackson and Drew (1984) suggest that in some plants that do not normally experience flooding, hormone imbalances may cause stomata to remain open, resulting in wilting. This response to flooding may indicate a lack of tolerance to flooding and a limitation to *Galax's* ability to survive in wet environments.

There is little experimental data in this study to help evaluate the adaptive significance of clonality in *Galax*, since trenching did not affect plants in any of the treatments plot. Although leaf nitrogen concentrations increased in the added nitrogen plots compared to control plots, there were no significant differences in nitrogen concentrations between trenched and non-trenched nitrogen plots. One adaptive feature in some clonal plants is the ability to translocate nutrients from one ramet to another (Headley *et. al.*, 1988; Pitelka and Ashmun, 1985; Ashmun *et. al.*, 1981). It would seem that nitrogen was taken up by *Galax* but not translocated to neighboring ramets in the non-trenched nitrogen plot. Translocation of nutrients is usually studied using stable isotopes and in situations where a nutrient gradient exists between ramets. There is no way to determine conclusively if *Galax* does or does not translocate nitrogen, since it is unknown if nitrogen was limiting in the surrounding environment, and, simply measuring leaf nitrogen contents is not a direct indication of whether translocation occurred or not. Further experiments should be done using isotopes and experimental gradients among connected ramets.

CONCLUSION

Galax aphylla, like many evergreens, remains metabolically active throughout the year. This allows the plant to potentially obtain carbon during times other than the growing season, which can be a competitive advantage over neighboring non-evergreen species. To help pay back the resources invested in producing evergreen leaves, *Galax* maintains its leaves for a least two growing seasons, with two and sometimes three age

classes of leaves present at any given time. Since gas exchange rates are higher in younger leaves and decrease as the leaves age, this allows the plant to benefit from the more efficient younger leaves while still utilizing the carbon being produced by older leaves.

As seasons change throughout the year, light, temperature and moisture conditions regulate the ability of *Galax* to obtain resources. During the summer, lower light levels reduce photosynthesis, while minimizing water deficits through reduced solar heating. Spring and fall have higher light levels coupled by increased evaporative demands that may lessen water availability. During the winter, low air and soil temperatures combine to limit gas exchange, although it seems that *Galax* can photosynthesize during warm periods, particularly if soil temperatures are above freezing. The plasticity of its physiology potentially allows *Galax* to photosynthesize during each season, despite the fact that these conditions may not be *ideal*. Resources gained throughout the year may help to sustain the plant during stressful conditions.

Galax is generally unaffected by increased shade and the addition of supplemental nitrogen and water. This reflects a stress-tolerant strategy (*sensu* Grime, 1979) that allows *Galax* to persist amid the changing environmental conditions of the forest floor, yet tolerate resource limitations common in these habitats. A trade-off exists where *Galax* is able to cope with limited resources, but its conservative nature lessens its ability to take advantage of increased resources.

Given its stress tolerant growth habit and low metabolic rates, future studies should be conducted long enough to detect potential responses of the plant to experimental conditions. Since this is one of the first studies

to look at the ecophysiology of *Galax*, there are many aspects of *Galax*'s ecology remaining to be investigated, such as drought and cold tolerance, the significance of clonality, and responses to rapidly changing light (i.e., sunflecks).

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