ABSTRACT

GRIFFIN, JASON JAY. Interactive Effects of Environmental Stresses on Photosynthesis. (Under the direction of Dr. Thomas G. Ranney.)

Plants are frequently exposed to a variety of environmental stresses that occur separately or in combination. The independent, additive, and interactive effects of these stresses, and the ability of a plant to resist these stresses, can be important factors in plant growth, distribution, and survival.

Cercis canadensis L. (eastern redbud) has a broad range that includes diverse ecotypes. In this study eastern redbud and *Cercis canadensis* var. *mexicana* (Rose) M. Hopkins (mexican redbud) were examined for their tolerance to high temperature and drought stress. In another study, taxa of *Illicium* L. (flowering anise) were evaluated for their differential tolerance to high irradiance and underlying photosynthetic characteristics of different taxa grown in full-sun and 50% shade.

Thermotolerance and drought tolerance of eastern redbud and mexican redbud were studied by growing containerized plants under high temperatures and increasing drought. Although both ecotypes responded similarly, the mexican redbud maintained higher stomatal conductance and photosynthesis than eastern redbud as the root substrate dried. The mexican redbud also maintained greater instantaneous water use efficiency (WUE). At the optimum temperature for photosynthesis of all taxa (37 °C), mexican redbud had a greater maximum rate of photosynthesis than both watered and drought-stressed eastern redbud. The maximum rate of photosynthesis was greatest for watered plants, and the mexican redbud maintained a higher rate of assimilation than the eastern redbud. Tissue osmotic potential was more negative in the eastern redbud, but was

unaffected by drought stress in either ecotype. Soluble carbohydrate concentration was also higher in the eastern ecotype, perhaps contributing to differences in osmotic potential. In both ecotypes, pinitol was the major carbohydrate and its concentration was considerably higher in the drought-stressed plants. Total polyol accumulation was greater in the drought-stressed plants as well. Both ecotypes proved to be very tolerant of high temperatures and drought.

In addition to drought and heat stress, irradiance stress is another commonly encountered environmental stress. Species of *Illicium* are mostly understory species that are not well adapted to high irradiance. To investigate how light affects *Illicium*, 11 commercially available taxa were grown in full sun or under 50% shade. Light-tolerance was evaluated by measuring light-saturated photosynthetic capacity (A_{max}), dark-adapted quantum efficiency of photosystem II (Fv/Fm), and relative chlorophyll concentration. A_{max} indicated that three of the 11 taxa maintained similar rates of photosynthesis when grown in full-sun as when grown in 50% shade. All other taxa in full-sun experienced a significant reduction in A_{max}. Fv/Fm was similar between sun and shade plants for the same three taxa that were able to maintain A_{max},. Chlorophyll concentration was not significantly reduced in these taxa either, whereas the other taxa did experience a significant reduction. In fact, chlorophyll concentration was higher in *I. parviflorum* 'Forest Green' grown in full sun, which is trait typical of sun tolerant species. A deeper examination of *I. parviflorum* 'Forest Green' (high-light tolerance) and *I. floridanum* (low-light tolerance) demonstrated that *I. parviflorum* 'Forest Green' had a considerably higher A_{max}, a higher light saturation point, greater potential photosynthetic capacity,

reduced susceptibility to photoinhibition as indicated by superior PSII efficiency following light exposure, greater capacity for thermal de-excitation as indicated by a higher rate of non-photochemical quenching (NPQ) under full sun, greater apparent electron transport rate (ETR) at mid-day, and higher concentrations of the free-radical scavenger myo-inositol. All of these factors potentially contribute to a greater capacity to utilize light energy for carbon fixation while minimizing photodamage.

INTERACTIVE EFFECTS OF ENVIRONMENTAL STRESSES ON PHOTOSYNTHESIS

by
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Personal Biography

Jason J. Griffin was raised in the rural farming community of Hannibal in upstate New York. It was here that Jason acquired his love for the environment and an appreciation for everything in it. He attended primary and secondary schools in Hannibal where he met his future wife in 1989. Following graduation from Hannibal High School that same year, Jason enrolled in the Landscape Development Program at SUNY Cobleskill, Cobleskill, N.Y., and graduated with an AAS degree, Spring 1991. He then spent 3 years in a landscape business before deciding it was time to complete his formal education. In Fall 1994, Jason enrolled in the Department of Plant Science at Cornell University, Ithaca, N.Y. While at Cornell University, Jason participated in an undergraduate research project that stimulated his interest in research. In Spring 1996, Jason was awarded a BS degree with honors and distinction. In Fall 1996, Jason was admitted to the Graduate School at North Carolina State University to pursue a MS in Horticultural Science, and followed that with a PhD dual major in Horticultural Science and Plant Physiology at the same institution. Upon completion, Jason will begin his employment career as an Assistant Professor at Kansas State University, working at the John C. Pair Horticultural Center near Wichita, KS.

Jason's role models have been, and always will be, his family. From parents and grandparents, Jason has learned that with integrity, honesty, hard work, and determination, life's obstacles will be few and surmountable.

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On a personal note, friends and family have made this a very enjoyable chapter of my life. The acquaintances I have made will hopefully last throughout my career and

beyond. My family has stood behind me in support every day and my new wife has sacrificed greatly to provide for us both. Together, we have learned a great deal about life and each other. We will take very fond memories of North Carolina and North Carolina State University, the place and its people, with us.

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General Introduction

Plants are frequently exposed to a variety of environmental stresses that occur concurrently. These compounding stresses can be more detrimental to plant growth and survival than either stress when encountered alone. Drought and high temperature are two stresses that frequently co-occur annually in the southeastern United States. Summer droughts are usually accompanied by high air temperature. For example, during the summer of 1999, data collected from the National Weather Service in Raleigh, NC indicated that for the months of July and August the daily average high temperature was greater than 33 °C while the precipitation departure from normal was –14 cm (Capitol Broadcasting Company, 1999).

The ability of a plant to resist these stresses can be an important factor in plant growth, distribution, and survival. Overall, the wide-variety of stresses that plants are exposed to elicit relatively similar responses from plants (Lichtenhaler, 1996). In moderately drought-stressed plants, the first response typically seen is a reduction in stomatal conductance and a lowering of photosynthesis to a new sustainable level. This acclimatization of metabolic processes to a new 'rate' when subjected to non-lethal stress is necessary and beneficial for survival of terrestrial plants. In addition to stomatal characteristics, some plants increase the concentration of cellular solutes to adjust osmotic potential in an attempt to maintain turgor. This is well documented in some of the halophytes (Vernon and Bohnert, 1992). However, when the stress is severe enough to overwhelm the preconditioned defenses of the plant, long term damage can occur.

One of the common families of molecules that can cause significant damage is the reactive oxygen species. Singlet oxygen, superoxide, and hydroxyl radicals commonly occur in plants. However, in healthy plants, their concentration is managed by the quenching capabilities of the plant and any resulting damage can quickly be repaired. In stressed plants, the concentration of these reactive oxygen species can be increased, either by increased production or decreased quenching. In drought stressed plants the limited influx of CO_2 and general reduction in photosynthesis can lead to an increase in free energy in the cell. The loss of this sink for absorbed light energy can lead to an abundance of energy in the photosynthetic membranes. In these instances energy can be dissipated by reducing molecular oxygen.

High-temperature stress is often found in conjunction with drought. Elevated temperatures also affect photosynthesis. Moderate temperatures can reduce the activity of Rubisco activase and therefore limit the rate of carboxylation, thus resulting in reduced photosynthesis just as moderate drought-stress (Crafts-Brandner and Salvucci, 2000). The end-result can be the same; increased concentration of reactive oxygen species. High-temperature can be more damaging than moderate temperature. If sufficiently high, the temperature can cause disruption of membrane integrity and enzyme denaturation. In combination, it is easy to why these two stressed could have an additive effect on plant survival.

Many stresses can cause an increase in the concentration of and damage done by reactive oxygen species. However, the stimulus for the production of these compounds is light. Irradiance, therefore, can also be an environmental stress. While sun light is necessary for the survival of photoautotrophic organisms, it frequently can be in excess

of what is necessary for growth, and often results in the formation of reactive oxygen (Demmig-Adams et al., 1997). In a healthy plant that is fully acclimated to its current surroundings, excessive irradiance typically is not a problem. In many instances, the ability of carotenoids to dissipate energy prior to the reduction of oxygen is an effective defense. In other instances and elevated photosynthetic capacity can account for the additional energy. Although reactive oxygen species may be present, the plants quenching capability are usually sufficient to detoxify these molecules. However, a plant growing in full sun or a plant that has been moved to a high-light environment from a low-light environment is likely to experience a significant amount of photoinhibition, frequently expressed as photobleaching of foliage and a general reduction in photosynthesis.

The studies described in this dissertation attempt to examine the relative tolerance of different species, and varieties of the same species, to common environmental stresses. An attempt was made to explain the various mechanisms these plant utilize to tolerate or to avoid the harmful effects of the applied stress.

Literature Cited.

Capitol Broadcasting Company. 1999. WRAL.com-Weather [internet]. Internet Broadcasting Systems, Inc. Available from: <http://www.wral.com/weather> [Accessed 22 October 1999].

Crafts-Brandner, S.J. and M.E. Salvucci. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. PNAS 97(24):13430-13435.

Demmig-Adams, B., W.W. Adams III, and S.C. Grace. 1997. Physiology of light tolerance in plants. Hort. Rev. 18:215-246.

Lichtenthaler, H.K. 1996. Vegetation stress: an introduction to the stress concept in plants. J. Plant. Physiol. 48:4-14.

Vernon, D.M. and H.J. Bohnert. 1992. A novel methyl transferase induced by osmotic stress in the facultative halophyte *Mesembryanthemum crystallinum*. EMBO J. 11(6):2077-2085.

Chapter 1

Effects of Heat and Drought on Photosynthesis, Water Relations, and Soluble Carbohydrates of Two Ecotypes of Redbud (*Cercis canadensis*)

(In the format appropriate for submission to the

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Effects of Heat and Drought on Photosynthesis, Water Relations, and Soluble Carbohydrates of Two Ecotypes of Redbud (*Cercis canadensis*)

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Environmental Stress Physiology

Effects of Heat and Drought on Photosynthesis, Water Relations, and Soluble Carbohydrates of Two Ecotypes of Redbud (*Cercis canadensis*)

Additional index words. A/Ci curve, high temperature stress, ononitol, osmotic adjustment, D-pinitol, polyol, water deficit stress.

Abstract. Net photosynthesis (Pn) of two ecotypes of *Cercis canadensis* L. (redbud) was studied following growth under high temperatures and increasing drought. Although mexican redbud [*C. canadensis* var. *mexicana* (Rose) M. Hopkins] generally maintained greater Pn than eastern redbud (*C. canadensis* var. *canadensis*), Pn decreased at a similar rate with increasing water deficit stress for both ecotypes. Mexican redbud maintained greater instantaneous water use efficiency under increasing drought than eastern redbud. The response of Pn to increasing leaf temperature was also similar between the two ecotypes, though actual Pn at high temperatures (30 to 40 °C) varied. The optimum temperature for photosynthesis (37 °C) was unaffected by irrigation or ecotype. Tissue osmotic potential at full turgor was more negative and soluble carbohydrate content was higher in eastern redbud, but was unaffected by drought stress in either ecotype. Pinitol was the major soluble carbohydrate and was considerably more abundant in water-stressed plants. Total polyol content (myo-inositol + ononitol + pinitol) was also greater in water-stressed plants. Both ecotypes were tolerant of high temperatures and drought.

Introduction:

Plants are frequently exposed to a variety of environmental stresses that occur simultaneously. Drought and high temperature are two stresses that frequently co-occur. The additive and interactive effects of these stresses and the ability of a plant to resist multiple stresses simultaneously can be an important factor in plant growth and survival in stressful environments (Lichtenthaler, 1996).

Water deficit can trigger a variety of plant responses. One of the more immediate responses is a reduction of stomatal aperture (Dubey, 1997) that results in decreased gas exchange in addition to inhibiting the evaporative cooling potential of the leaf. In general, net photosynthesis decreases while leaf temperature increases as a drought event progresses. Many plants are capable of adjusting osmotically by accumulating cellular solutes so that greater turgor can be maintained as tissue water potential decreases. This osmotic adjustment is achieved through the accumulation of a variety of molecules whose concentrations have been positively correlated with increased turgor, stomatal conductance, transpirational cooling, net photosynthesis, and reduced leaf senescence (Berkowitz, 1998; Ranney et al., 1991; Thomas, 1997). Additionally, it has been suggested that the ability to acclimate and maintain positive net photosynthesis during a slow developing drought is directly related to survival and recovery following a drought (Krüger and van Rensburg, 1995). Many of the molecules involved in osmotic adjustment, the polyols in particular, also have the potential to scavenge reactive oxygen species (Guo and Oostrhuis, 1995; McManus et al., 2000; Popp and Smirnoff, 1995; Sheveleva et al., 1997; Smirnoff and Cumbes, 1989). Mannitol, for example was very

effective in scavenging reactive oxygen species when its production was targeted to the chloroplast in transgenic tobacco plants (Shen et al., 1997).

In addition to drought, high temperatures can also inhibit plant growth and development. Elevated temperatures increase respiration and therefore require greater carbon fixation for sustained growth and survival. However, carbon fixation can also be limited by high temperature. Rubisco activase, in particular, has been implicated as a major rate-limiting enzyme under elevated, but not excessive, temperatures (Crafts-Brandner and Law, 2000; Crafts-Brandner and Salvucci, 2000). Temperatures greater than 35 °C significantly decreased the ability of activase to maintain the activation state of Rubisco. The capacity of a plant to acclimate and maintain photosynthesis under high temperatures can be a critical factor in heat tolerance (Hale and Orcutt, 1987). This adaptation/acclimatization to temperature is considered one of the primary determinants of geographical distribution among agricultural crops (Mahan et al., 1997) and has been observed to differ among closely related species native to regions with different climates (Berry and Björkman, 1980; Ranney and Peet, 1994; Ranney and Ruter, 1997).

When combined, drought and high temperatures can have both additive and interactive effects on plant growth (Stoyanova and Yordanov, 1999). The inability to acclimate to either stress or the combined influence of both stresses can restrict the growth and survival of plants. Limited transpirational cooling can exacerbate the effects of already high air temperature (Hale and Orcutt, 1987) by causing leaf temperatures to rise above the air temperature. The resulting high leaf temperature can lead to a variety

of physiological aberrations in the photosynthetic membrane (Falk et al., 1996; Biswal, 1997; Dubey, 1997) that make the plant more susceptible to photoinhibition.

Cercis canadensis var. *canadensis* (eastern redbud) and *C. canadensis* var. *mexicana* (mexican redbud) are two varietal ecotypes of redbud. Eastern redbud grows throughout the eastern half of the United States and is generally found as a mesic, understory species. Mexican redbud grows from southwestern Texas, south through central Mexico to Mexico City, and is typically found in more xeric environments. Although both ecotypes can be found side-by-side in managed landscapes, they have very different native ranges and environments. Redbud seedlings collected over a range of native habitats and examined for morphological and physiological traits exhibited vast differences in leaf anatomy and growth patterns (Donselman and Flint, 1982). Leaf morphological characteristics typically associated with xeric conditions (increased pubescence, thicker and smaller leaves, and fewer and smaller stomata) were found to increase in the western populations. Mexican redbud has many features typical of drought tolerant species. It is a smaller tree than its eastern counterpart and has smaller leaves that are thicker and pubescent underneath. Thick leaves with a small surface area tend to reduce the area available for transpiration, while increasing the amount of water, and the number of cells per unit leaf, and therefore, may have an advantage during drought stress.

The objectives of this study were to, identify if water deficit stress compromises the thermotolerance of photosynthesis in redbuds, evaluate if this interaction varies by

ecotype, and examine specific limitations and mechanisms that influence resistance to these stresses.

Materials and Methods

Plant Material and Growing Conditions. Two-year-old seedlings of eastern redbud and mexican redbud were planted in each 38 L (10 gal) container (one of each ecotype per container). Container substrate was 4 pine bark : 2 peat : 1 perlite medium (by vol.) amended with 3 kg•m^{-3} (5 lbs•yd^{-3}) micronutrients (esmigran, The Scotts Co., Marysville, OH) and 5.3 kg•m^{-3} (9 lbs•yd^{-3}) dolomitic limestone. Final substrate pH was 6.2. Shared containers were used to ensure root systems of both taxa in a given container experienced very similar substrate conditions. Plants were grown in a glass greenhouse (24 °C day/21 °C night) and fertigated weekly with a complete water-soluble fertilizer [100 ppm N; 20N-8.3P-15K (Peters Peat-Lite, 20-19-18, The Scotts Co., Marysville, OH)] until treatment initiation. Irrigation was applied as necessary.

Plants were given two weeks to establish before greenhouse temperatures were raised to 35 °C day/25 °C night to acclimate all plants to high temperatures. Following 21 days of acclimation at this temperature regime, drought stress was initiated by withholding irrigation from half of the containers. At this stage all plants were healthy and growing rapidly. The large container volume and substrate composition ensured a gradual depletion of available water simulating a natural drought and allowing for acclimation and more uniform stomatal closure.

Gas exchange measurements as a function of substrate moisture content. Net photosynthesis (Pn), stomatal conductance (g_s), and instantaneous water use efficiency (WUE) were measured using a portable gas exchange system (CIRAS-1, PP Systems, Haverhill, MA). Data were collected on recently matured leaves at 30 °C, 350 $\mu l \bullet l^{-1}$ CO_2, and saturating light levels (2000 $\mu mol \bullet m^{-2} \bullet sec^{-1}$). WUE was calculated as the ratio of Pn to transpiration. Substrate water content was recorded in each container using a soil moisture probe (ThetaProbe, Dynamax Inc., Houston, TX) calibrated to substrate-specific water content. Probes were inserted horizontally into the root zone under each ecotype midway through the container profile.

Temperature response of photosynthesis. When substrate moisture reached 2-5% (corresponding to a pre-dawn water potential of approximately –2.0 MPa) plants were brought into the lab for data collection the evening before the measurements were to be taken. Similar to the method of Ranney and Ruter (1997), potential photosynthetic capacity was determined by measuring Pn during increasing leaf temperature under saturating CO_2 (2000 $\mu l \bullet l^{-1}$) and light (2000 $\mu mol \bullet m^{-2} \bullet sec^{-1}$). A recently matured leaf was placed in the cuvette at 20 °C and allowed 20 min to stabilize before the first measurement was taken. The temperature was then raised in 5 °C increments from 20 to 45 °C with a measurement taken at each temperature following a 20 min acclimation period.

A/Ci responses. Following temperature response measurements, A/Ci responses (Pn as a function of increasing intercellular CO_2 concentration) were measured on a

separate leaf to detect stomatal and non-stomatal limitations to Pn of well-watered and water-stressed plants. Again, a recently matured leaf was placed in the cuvette at a temperature of 30 °C and 2000 μmol•m^{-2}•sec^{-1} PAR, and CO_2 concentration of 350 μl•l^{-1}. The air within the cuvette was maintained at approximately 70% relative humidity to minimize stomatal heterogeneity. Following a 20 min acclimation period, Pn was recorded and the CO_2 concentration was reduced to 50 μl•l^{-1} where another reading was taken. A gradual increase in CO_2 to a final concentration of 1990 μl•l^{-1} in 10 increments occurred with a reading taken at each increment following a 10 min acclimation period. Data was then fit to the exponential model used by Jacob et al. (1995) and adopted by Reid and Fiscus (1998):

$$A = a(1 - e^{-bCi}) + c$$

where *a* is the maximum rate of carbon assimilation, *b* is the initial slope or carboxylation efficiency, and *c* is the intercept on the ordinate.

Leaf osmolality. Leaf osmolality at full turgor was determined on expressed sap using the procedures of Ranney et al. (1991). Recently matured leaves were harvested early in the morning and re-hydrated by submerging the cut petioles in deionized water and covering the leaf blades with a polyethylene bag for two hours. It has been shown that this length of time does not over-hydrate the tissue (Evans et al., 1990). Each leaf was then placed in a 3 ml plastic syringe (one leaf per syringe) and frozen at -80 °C. Upon removing the syringe from the freezer and allowing it to reach room temperature, sap was expressed by depressing the syringe. Osmolality of a 10 μl sample of expressed

sap solution was determined using a vapor pressure osmometer (Model 5500, Wescor, Logan, Utah). Two leaves from each plant were sampled and two sub-samples were tested from each leaf. Osmotic potential of the expressed sap was then calculated based on the van't Hoff relation reported by Nobel (1983). Although this method may result in some dilution of symplastic solutes due to apoplastic water, Lakso et al. (1984) found little difference in osmotic potential between expressed sap and the values determined using the pressure volume method.

Carbohydrate analysis. Recently matured leaves were removed from the plants in early morning and immediately frozen at -80 °C. Frozen leaf tissue was lyophilized and ground to pass a 40-mesh screen. For each plant sample 200 mg of ground tissue was placed in a 15 ml centrifuge tube and suspended in 3 ml of 80% aqueous ethanol. The slurry was incubated at 80 °C for 5 min then centrifuged at 1000 g_n for 10 min. The extraction was repeated two more times with the supernatant combined after each. Pooled extracts were evaporated at 45 °C under vacuum and oven dried over-night at 70 °C. The residue was dissolved in 1.0 ml of deionized, distilled water and soluble sugars analyzed by HPLC. The HPLC system (described by Prata et al., 1997) was equipped with a guard column (C-18 Corasil, Bio-Rad, Hercules, CA) and in-line cation and anion guards (Micro-Guard, Bio-Rad). Carbohydrates were separated on a column (Pb^{++} Carbohydrate column, Sierra Separations, Inc., Sparks, NV) with a flow rate of 0.4 ml water min^{-1} at 75 °C. Carbohydrate identity and quantity were analyzed using a differential refractometer (model 410, Waters/Millipore, Milford, MA) coupled to a

computing integrator (model SP4200, Spectra Physics, San Jose, CA), and compared with carbohydrate standards.

Experimental Design. The experimental design was a randomized complete block design with a split-plot arrangement of treatments, replicated seven times. Irrigation regimes were treated as whole plots, with sub-plots consisting of plant ecotypes. Pn, g_s, and WUE data during initial substrate dry-down were analyzed using regression analysis. Temperature response and A/Ci curves were analyzed as a split-split-plot with temperature and Ci as the sub-sub plots, respectively. The data was subjected to ANOVA and a cubic regression was fit to the temperature response curves. The A/Ci model was fit to the data using the Proc NLIN feature in SAS. The osmotic potential and the soluble carbohydrate data were also analyzed as a split-plot, and were subjected to ANOVA. Where appropriate, means were separated with a protected LSD ($P \leq 0.05$).

Results

After the final irrigation event, it took 14 days for the substrate water content of drought stressed plants to drop to 2 to 5%. The rate of net photosynthesis decreased linearly in both taxa, with a significant main effect of ecotype and substrate water content (Fig. 1). There was no interaction, suggesting the response to substrate drying is similar among the ecotypes. Stomatal conductance also decreased linearly in the eastern redbud; however, a quadratic response to soil drying was found for the mexican redbud (Fig. 2). Mexican redbud maintained conductance after the conductance in the eastern ecotype began to drop. The response of leaf WUE was also similar between the two ecotypes and

decreased linearly in response to drought. There was no interaction between the ecotypes, however, mexican redbud maintained a greater instantaneous WUE (Figure 3). Subsequent to collecting data, leaf discs the same size as the CIRAS leaf cuvette, were collected and weighted. It was found that the fresh weight of the mexican redbud leaves was approximately 30% greater than the eastern redbud, which may account for a majority of the difference in Pn rates between the two ecotypes.

To investigate how drought influenced the temperature optimum for potential photosynthetic capacity, temperature response curves were generated at saturating light and CO_2 concentration (Fig. 4). A three-way interaction occurred between the irrigation regime, ecotype, and temperature. The well-watered Mexican ecotype demonstrated greater photosynthetic capacity at nearly all temperatures. The only exception was the highest temperature (45 °C) where the rate of net photosynthesis drastically declined for all plants. The overall response to increasing temperature, however, was similar among the ecotypes and the treatments. The temperature optimum for maximum photosynthesis of both ecotypes was estimated at 37 °C and was unaffected by drought. The main effects of irrigation regime and ecotype, however, were significant for maximum photosynthetic capacity, but there was no interaction (Fig. 4). When ecotype and irrigation regime were compared at the optimum temperature, watered plants had higher rates of assimilation than the drought stressed plants (34.3 and 23.2 $\mu mol \bullet m^{-2} \bullet s^{-1}$, respectively), and the Mexican ecotype had a higher rate per unit area than the eastern ecotype (33.0 and 24.5 $\mu mol \bullet m^{-2} \bullet s^{-1}$, respectively).

The A/Ci response curves were used to evaluate the effects of drought on specific non-stomatal limitations to the carbon exchange rate. The estimated maximum rate of photosynthesis (Amax), carboxylation efficiency (CE), and respiration in the light (R_L) were determined for 30C. However, few measurements could be taken above saturating levels of CO_2 in the water-stressed plants due to low stomatal conductance. As a result the model provided a poor fit to the data and predicted much higher rates of Amax than were observed. A similar scenario was encountered by Escalona et al. (1999) who used the observed rate of photosynthesis at the highest level of CO_2 to represent Amax. Using this method we observed a significant main effect of irrigation and ecotype on Amax. Irrigated plants had a significantly higher Amax than the drought-stressed plants (24.9 and 16.4 $\mu mol \bullet m^{-2} \bullet s^{-1}$, respectively) and mexican redbud had a slightly higher Amax than eastern redbud (21.6 and 19.3 $\mu mol \bullet m^{-2} \bullet s^{-1}$, respectively). There were no differences in CE or R_L among the ecotypes or treatments (data not presented).

Tissue osmotic potential was significantly lower (more negative) in eastern redbud than mexican redbud (-1.4 and -1.0 MPa, respectively), and was unaffected by water regime (i.e., there was no osmotic adjustment). In both ecotypes, D-pinitol was the primary soluble carbohydrate occurring in the leaves (Table 1.). In fact, the sum of all other soluble carbohydrates was less than the content of D-pinitol alone, and in water stressed plants, the content of D-pinitol was more than 2-fold greater than all other soluble carbohydrates combined. The content of D-pinitol was greater in drought-stressed plants than well-watered plants and was higher in eastern redbud than mexican redbud. However, there was no interaction suggesting the changes in D-pinitol content

between the ecotypes responded similarly to water stress. If the polyols with putative roles as compatible solutes and free radical scavengers are summed (myo-inositol + ononitol + pinitol), their content was significantly increased from the watered plants to the water stressed plants (1605.4 vs. 2438.7 $\mu g \cdot g^{-1}$ dry weight, respectively). The percent increase, however, was greater for the Mexican ecotype than the eastern ecotype (62% and 40%, respectively) even though the absolute values were higher in the eastern redbud. The data was also analyzed on a fresh weight basis in case there were any differences in leaf water content. Similar treatment effects were observed with the same trends and same relative changes between treatments (data not presented).

Discussion

Species and varieties that exhibit greater drought resistance often have the capacity to maintain higher Pn rates during mild to moderate levels of water deficits, compared to drought sensitive plants (Krüger and van Rensburg, 1995). In this study, both ecotypes responded in a similar manner to increasing drought (Fig. 1). The mexican redbud did maintain greater net photosynthesis as substrate water content declined; however, leaf thickness and other morphological factors may have influenced the overall assimilation rate. Stomatal conductance was initially greater for mexican redbud which most likely contributed to the higher overall Pn (Fig. 2). Additionally, the mexican redbud had higher WUE throughout the drying cycle (Fig. 3), indicating that per unit leaf area, this ecotype can assimilate more carbon per mole of water transpired – a beneficial trait for plants growing in arid climates. WUE may have also been influenced by variations in leaf thickness.

Numerous studies have found that drought can compromise the thermotolerance of net photosynthesis and reduce the capacity of photosynthetic processes to acclimate to high temperatures (Briggs et al., 1986; Smolander and Lappi, 1984; Nobel et al., 1978). Redbuds, however, do not appear to follow that trend. In the current study, 37 °C was the optimum temperature for photosynthesis regardless of ecotype or water regime (Fig. 4). This data would suggest that the physiological and biochemical mechanisms of photosynthesis in these two ecotypes respond similarly to increasing temperature.

At the optimum temperature of 37 °C, both ecotype and irrigation affected the maximum rate of assimilation. Potential photosynthetic capacity (CO_2 saturated) was much greater in watered plants than drought stressed plants, indicating that water deficits inhibited photosynthetic processes independent of limitations due to stomatal conductance (Fig 4 and 5). Reduced photosynthetic capacity during drought can be an indication of photoinhibition. Drought stressed plants that are exposed to high-light and high temperatures are often more vulnerable to photoinhibition than well-watered plants (Osonubi and Davies, 1980). Conversely, higher rates of net photosynthesis under such conditions can indicate a tolerance to photoinhibition. This data may suggest that under high temperatures and drought, the mexican redbud provided some mechanism of photoprotection above and beyond that of the eastern redbud. However, the exact cause of the observed greater net photosynthetic rate in the mexican redbud requires further investigation. The data may have been influenced by numerous factors, morphological (e.g., greater leaf thickness), physiological, and/or biochemical.

Estimated potential photosynthetic capacity from A/Ci curves was greater for the watered plants and the mexican redbud, however there was no interaction (Fig. 5). Although the difference is small, the data does suggest that mexican redbud has a higher photosynthetic capacity than eastern redbuds and when faced with adverse environmental stresses, may provide some necessary carbohydrates for survival. However, whole-plant carbon budgets would be needed to better evaluate that potential. No significant differences were detected for CE either. One possible explanation is that the A/Ci curves were run at 30 °C whereas the optimum temperature for assimilation is 37 °C. At 30 °C the rates of assimilation across all treatments were more similar than they were at 37 °C (Fig. 4), and may therefore minimize any treatment differences.

The accumulation of various osmolytes in drought stressed plants has been reported frequently (see reviews by Hare et al., 1998; Popp and Smirnoff, 1995). In redbuds, however, the osmotic potential at full turgor was unaffected by drought conditions. Ecotype was the only factor influencing the osmotic potential at full turgor, with eastern redbud significantly lower than mexican redbud (-1.4 and -1.0 MPa, respectively). Despite this, both ecotypes accumulated considerable amounts of D-pinitol. The occurrence, accumulation, and potential physiological roles of D-pinitol have been well studied in *Glycine max* (L.) Merr. (soybeans) and pinitol has been found to be a common sugar alcohol in many legumes (Guo and Oostrhuis, 1995; Guo and Oostrhuis, 1997; Kuo et al., 1997; Smith and Phillips, 1982). The role of pinitol and its precursors (myo-inositol and ononitol) as osmoprotectants or compatible solutes has been

well studied in the halophyte *Mesembryanthemum crystallinum* L. (common ice plant), and its induction by salt and drought treatment has been well established (Paul and Cockburn, 1989; Vernon and Bohnert, 1992a, Vernon and Bohnert, 1992b). Deletion of these polyols through molecular techniques has proven to reduce the ability of the plant to tolerate halophytic conditions. Additionally, the putative antioxidant capabilities of these polyols may prove to have greater ecophysiological significance than the osmotic role. The amount of pinitol produced by redbuds may confer some level of tolerance to the production of free oxygen radicals. Additionally, the sum of all the polyols (myo-inositol + ononitol + pinitol) was increased in response to drought, with no indication of any osmotic adjustment at full turgor. Although the total accumulation was greater in the eastern redbud, the increase relative to the watered plants was greater in the mexican redbud. This accumulation of putative osmoprotectants and antioxidants represents a physiological response to drought and a mechanism to protect against photoinhibition.

Visual observations of the plants as the drought progressed were similar to water-stressed redbuds in the landscape. The leaf surface in both ecotypes lost its glaucus appearance and became dull, and the lamina was held vertically rather than horizontally. This is a common occurrence in drought stressed plants with a pulvinus. It was also observed that the mexican redbud tended to shed leaves in response to severe drought, a trait typical of drought deciduous plants. Rather than tolerating severe drought by protecting foliage from excessive dehydration and turgor loss, drought deciduous plants typically drop their leaves then re-foliate when water is present. Additionally, drought deciduous plants do not follow the typical trends during the initial stages of a drought.

Rather than reduce transpiration and slow photosynthesis, drought deciduous plants exploit the available water when it is available, but will abscise leaves if the stress becomes too severe. The mexican redbud fits this model. They had a rapid rate of net photosynthesis, did not immediately reduce stomatal conductance, and did not adjust the osmotic potential of the cell. In addition, when the drought became severe leaves of the Mexican ecotype began to abscise whereas leaves on the eastern ecotype did not. This characteristic may not be observed in more mesic environments, as soils rarely reach such a level of drought.

Both ecotypes proved to be extremely drought and heat tolerant in this study. It was not surprising that the Mexican ecotype was so tolerant, however the eastern redbud was far more tolerant than expected. A possible explanation was recently published by Davis et al. (2002) looking at the molecular taxonomy of redbuds. In their analysis, eastern redbud and mexican redbud are related through a common xerophytic ancestor, and eastern redbud represents a 'mesophytic reversion' of a xerophytic form. Thus, despite the fact that eastern redbud is typically found in more temperate, mesophytic environments, it may have retained certain xerophytic characteristics. These characteristics may provide competitive advantages in dry woodlands understory, ridge top, and rock outcrop habitats where eastern redbud is often found. If this scenario is in fact correct, it could explain why in the current study, the eastern redbud proved to be very heat and drought tolerant and responded similarly to the xerophytic mexican redbud in many respects.

Literature Cited.

Berkowitz, G.A. 1998. Water and Salt Stress, p. 226-237. In: A.S. Raghavendra (ed.). Photosynthesis: A Comprehensive Treatise. Cambridge University Press. Cambridge, UK.

Berry, J. and O. Björkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol. 31:491-553.

Biswal, B. 1997. Chloroplasts, pigments, and molecular responses of photosynthesis under stress, p. 877-885. In: Pessarakli, M (ed.). Handbook of Photosynthesis. Marcel Dekker, Inc., New York.

Briggs, G.M., T.W. Jurik, and D.M. Gates. 1986. Non-stomatal limitation of CO_2 assimilation in three tree species during natural drought conditions. Physiol. Plant. 66:521-526.

Crafts-Brandner, S.J. and R.D. Law. 2000. Effect of heat stress on the inhibition and recovery of the ribulose-1,5-bisphosphate carboxylase/oxygenase activation state. Planta 212:67-74.

Crafts-Brandner, S.J. and M.E. Salvucci. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proc. Natl. Acad. Sci. 97(24):13430-13435.

Davis, C.C., P.W. Fritsch, J. Li,and M.J. Donoghue. 2002. Phylogeny and biogeography of *Cercis* (Fabaceae): Evidence from nuclear ribosomal ITS and chloroplast *ndhF* sequence data. Sys. Bot. 27(2):289-302.

Donselman, H.M. and H.L. Flint. 1982. Genecology of Eastern redbud (*Cercis canadensis*). Ecology 63(4):962-971.

Dubey, R.S. 1997. Photosynthesis in Plants Under Stressful Conditions, p. 859-875. In: Pessarakli, M (ed.). Handbook of Photosynthesis. Marcel Dekker, Inc., New York.

Escalona, J.M., J. Flexas, and H. Medrano. 1999. Stomatal and non-stomatal limitation of photosynthesis under water stress in field-grown grapevines. Aust. J. Plant Physiol. 26:421-433.

Evans, R.D., R.A. Black, and S.O. Link. 1990. Rehydration-induced changes in pressure-volume relationships of *Artmisia tridentata* Nutt. Ssp. *tridentata*. Plant, Cell and Environ. 13:455-461.

Falk, S., D.P. Maxwell, D.E. Laudenbach, and N.P.A. Huner. 1996. Photosynthetic adjustment to temperature, p. 367-385. In: N.R. Baker (ed.). Advances in photosynthesis (vol. 5): Photosynthesis and the environment. Kluwer Academic Publishers. The Netherlands.

Guo, C. and D.M. Oosterhuis. 1995. Pinitol occurrence in soybean plants as affected by temperature and plant growth regulators. J. Exp. Bot. 46(283):249-253.

Guo, C. and D.M. Oosterhuis. 1997. Effect of water-deficit stress and genotypes on pinitol occurrence in soybean plants. Environ. Exp. Bot. 37:147-152.

Hale, M.G. and D.M. Orcutt. 1987. The Physiology of Plants Under Stress. John Wiley & Sons. New York.

Hare, P.D., W.A. Cress, and J. Van Staden. 1998. Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ. 21:535-53.

Jacob, J., C. Greitner, and B.G. Drake. 1995. Acclimation of photosynthesis in relation to Rubisco and non-structural carbohydrate contents and *in situ* carboxylase activity in *Scirpus olneyi* at elevated CO_2 in the field. Plant, Cell and Environ. 18:875-884.

Krüger, G.H.J and L. van Rensburg. 1995. Carbon dioxide fixation: Stomatal and non-stomatal limitation in drought-stressed *Nicotiana tabacum* L. cultivars, p. 505-510. In: Mathis, P (ed.). Photosynthesis: from Light to Biosphere, Vol IV. Kluwer Academic Publishers. Dordrecht, The Netherlands.

Kuo, T.M., C.A. Lowell, and T.C. Nelsen. 1997. Occurrence of pinitol in developing soybean seed tissues. Phytochem. 45(1):29-35

Lakso, A.N., A.S. Geyer, and S.G. Carpenter. 1984. Seasonal osmotic relations in apple leaves of different ages. J. Amer. Soc. Hort. Sci. 109(4):544-547.

Lichtenthaler, H.K. 1996. Vegetation stress: an introduction to the stress concept in plants. J. Plant. Physiol. 48:4-14.

Mahan, J.R., B.L. McMichael, and D.F. Wanjura. 1997. Reduction of high temperature stress in plants, p. 137-150. In: A.S. Basra and R.K. Basra (eds.). Mechanisms of Environmental Stress Resistance in Plants. Harwood Academic Publishers. The Netherlands.

McManus, M.T., R.L. Bieleski, J.R. Caradus, and D.J. Barker. 2000. Pinitol accumulation in mature leaves of white clover in response to a water deficit. Environ. and Exp. Bot. 43:11-18.

Nobel, P.S. 1983. Biophysical plant physiology and ecology. Freeman, New York.

Nobel, P.S., D.J. Longstreth, and T.L. Hartsock. 1978. Effect of water stress on the temperature optima of net CO_2 exchange for two desert species. Physiol. Plant. 44:97-101.

Osonubi, O. and W.J. Davies. 1980. The influence of water stress on the photosynthetic performance and stomatal behaviour of tree seedlings subjected to variation in temperature and irradiance. Oecologia 45:3-10.

Paul, M.J. and W. Cockburn. 1989. Pinitol, a compatible solute in *Mesembryanthemem crystallinium* L.? J. Exp. Bot. 40(219):1093-1098.

Popp, M. and N. Smirnoff. 1995. Polyol accumulation and metabolism during water deficit, p. 199-215, In: N. Smirnoff (ed.). Environment and plant metabolism: flexibility and acclimation. BIOS Scientific Publishers LTD. Oxford.

Prata, R.T.N., J.D. Williamson, M.A. Conkling, and D.M. Pharr. 1997. Sugar Repression of mannitol dehydrogenase activity in celery cells. Plant Physiol. 114:307-314.

Ranney, T.G., N.L. Bassuk, and T.H. Whitlow. 1991. Osmotic adjustment and solute constituents in leaves and roots of water-stressed cherry (*Prunus*) trees. J. Amer. Soc. Hort. Sci. 116(4):684-688.

Ranney, T.G. and M.M. Peet. 1994. Heat tolerance of five taxa of birch (*Betula*): Physiological response to supraoptimal leaf temperatures. J. Amer. Soc. Hort. Sci. 119:243-248.

Ranney, T.G. and J.M. Ruter. 1997. Foliar heat tolerance of three holly species (*Ilex* spp.): Responses of chlorophyll fluorescence and leaf gas exchange to supraoptimal leaf temperatures. J. Amer. Soc. Hort. Sci. 122:499-503.

Reid, C.D. and E.L. Fiscus. 1998. Effects of elevated [CO_2] and/or ozone on limitations to CO_2 assimilation in soybean (*Glycine max*). J. Exp. Bot. 49:885-895.

Shen, B., R.G. Jensen and H.J. Bohnert. 1997. Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. Pant Physiol. 113:1177-1183.

Sheveleva, E., W. Chmara, H.J. Bohnert, and R.G. Jensen. 1997. Increased salt and drought tolerance by D-ononitol production in transgenic *Nicotiana tabacum* L. Plant Physiol. 115:1211-1219.

Smirnoff, N. and Q.J. Cumbes. 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28(4):1057-1060.

Smith, A.E. and D.V. Phillips. 1982. Influence of sequential prolonged periods of dark and light on pinitol concentration in clover and soybean tissue. Physiol. Plant. 54:31-33.

Smolander, H. and J. Lappi. 1984. The interactive effect of water stress and temperature on the CO_2 response of photosynthesis in *Salix*. Silva Fennica. 18:133-139.

Stoyanova, D. and I. Yordanov. 1999. Influence of drought, high temperature, and carbamide cytokinin 4-PU-30 on photosynthetic activity of plants. 2. Chloroplast ultrastructure of primary bean leaves. Photosynthetica. 37(4):621-625.

Tipton, J.L. and M. White. 1995. Differences in leaf cuticle structure and efficacy among Eastern redbud and Mexican redbud phenotypes. J. Amer. Soc. Hort.Sci. 120(1):59-64.

Thomas, H. 1997. Drought resistance in plants, p. 1-42. In: A.S. Basra and R.K. Basra (eds.). Mechanisms of Environmental Stress Resistance in Plants. Harwood Academic Publishers. The Netherlands.

Vernon, D.M. and H.J. Bohnert. 1992a. A novel methyl transferase induced by osmotic stress in the facultative halophyte *Mesembryanthemum crystallinum*. EMBO J. 11(6):2077-2085.

Vernon, D.M. and H.J. Bohnert. 1992b. Increased expression of a *myo*-inositol methyl transferase in *Mesembryanthemum crystallinum* is part of a stress response distinct from crassulacean acid metabolism induction. Plant Physiol. 99:1695-1698.

Table 1. Soluble carbohydrate content of leaf tissue (µg•g^{-1} dry weight)

	Cercis canadensis var. *canadensis*		*Cercis canadensis* var. *mexicana*	
	Watered	Drought	Watered	Drought
Sucrose	950 a	551 b	481 a	183 b
Glucose	41	56	195	131
Fructose	111	201	201	222
Myo-inositol	57	91	63	89
Ononitol	103	45	75	76
Pinitol	1793 a	2603 b	1206 a	2011 b
Total	3055 a	35467 b	2222 a	2712 b

Mean separation within a row and within a taxon by Fisher's Protected LSD ($P \leq 0.05$). Numbers followed by a similar letter are not significantly different. If there are no letters in the row, ANOVA indicated no treatment effects.

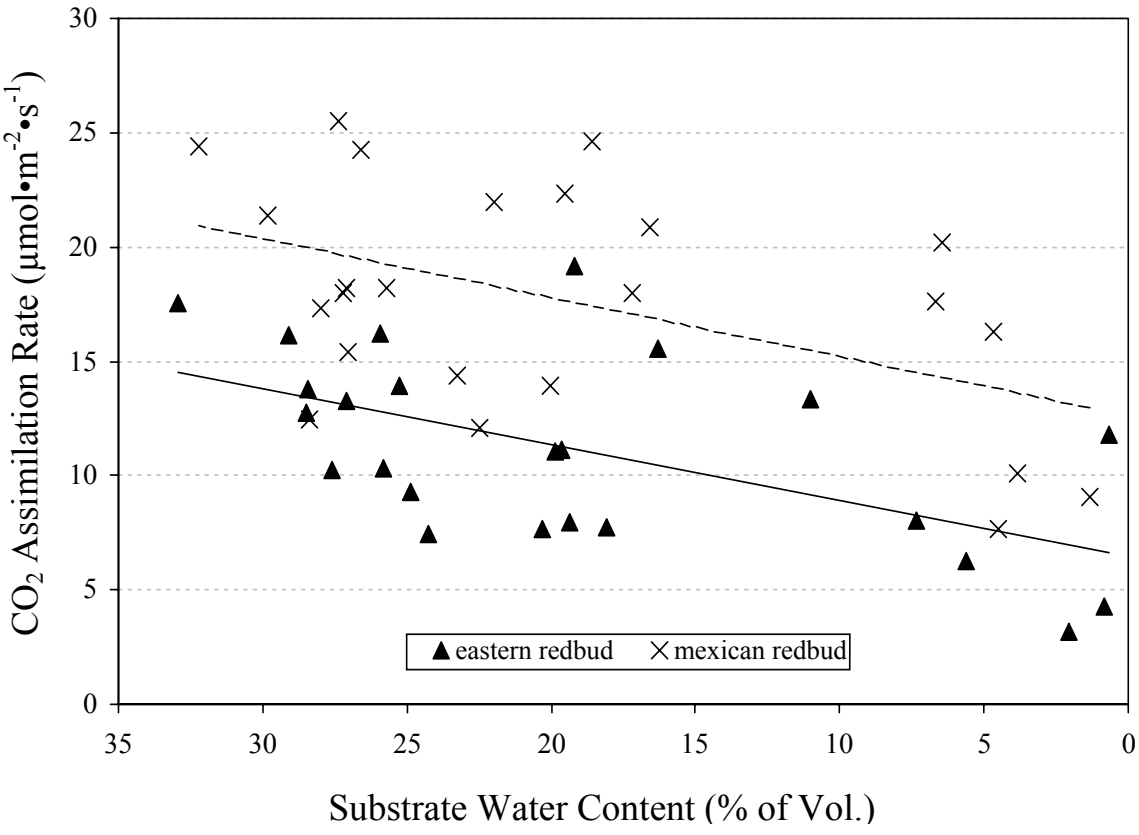

Figure 1. Photosynthesis of mexican redbud and eastern redbud during container substrate drying at 350 µL•L^{-1} CO_2 and 2000 µmol•m^{-2}•s^{-1} PAR. Linear regression fit to mexican redbud (broken line, $r^2 = 0.24$) and eastern redbud (solid line, $r^2 = 0.32$).

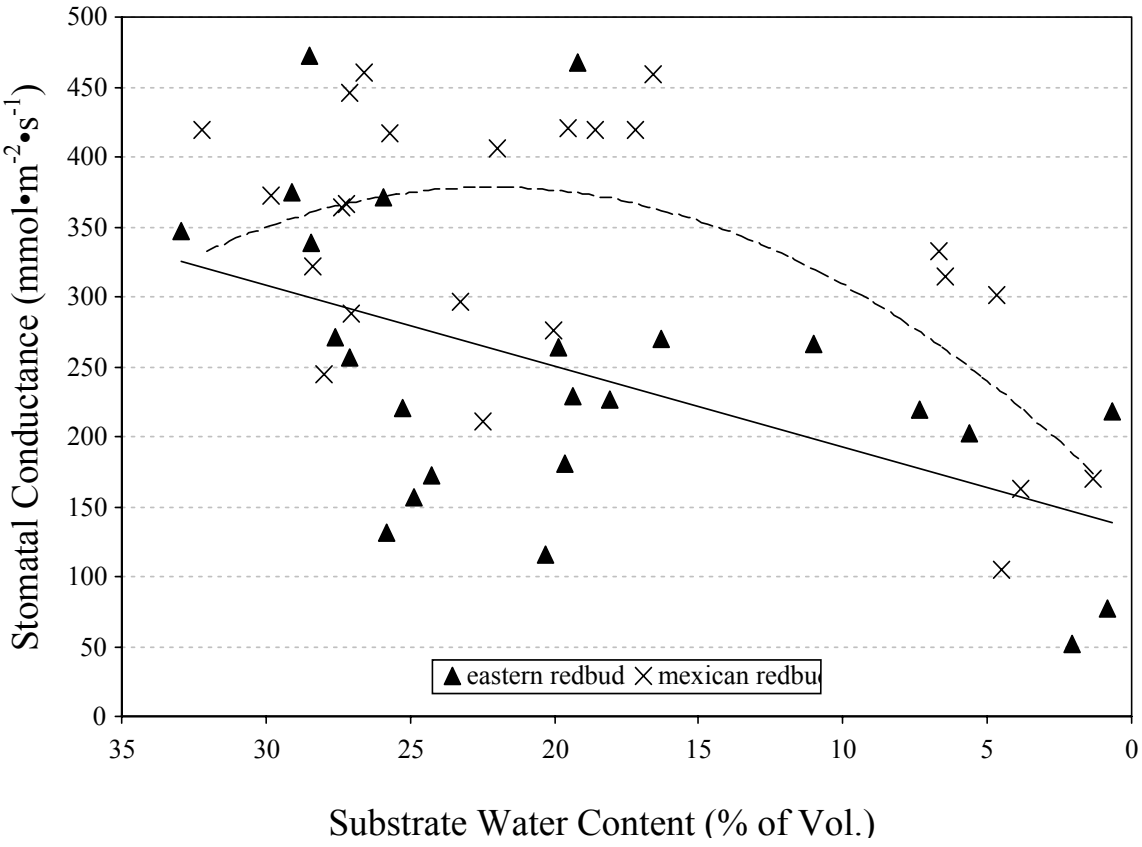

Figure 2. Stomatal conductance of mexican redbud and eastern redbud during substrate drying. Quadratic regression fit to mexican redbud (broken line, r^2 = 0.39) and linear regression fit to eastern redbud (solid line, r^2 = 0.27).

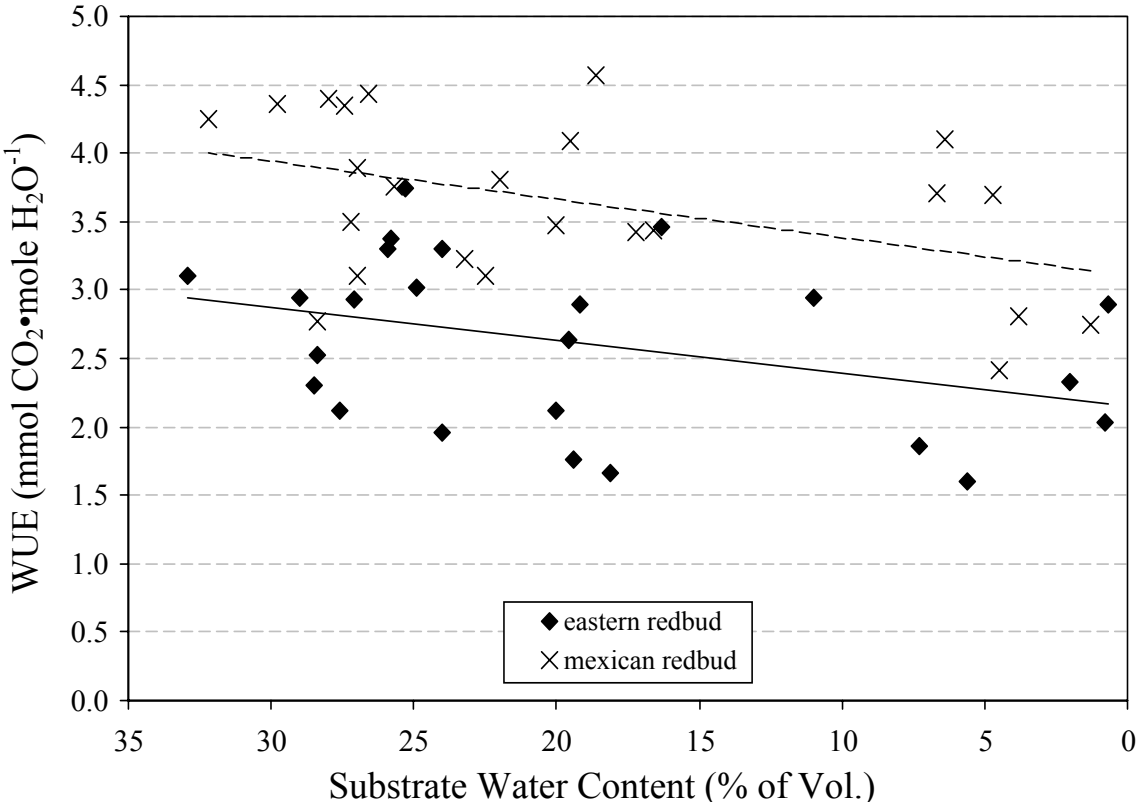

Figure 3. Instantaneous water use efficiency (WUE) (net photosynthesis/transpiration) of mexican redbud and eastern redbud during substrate drying. Linear regression fit to both ecotypes; mexican redbud (broken line, $r^2 = 0.19$) eastern redbud (solid line, $r^2 = 0.14$).

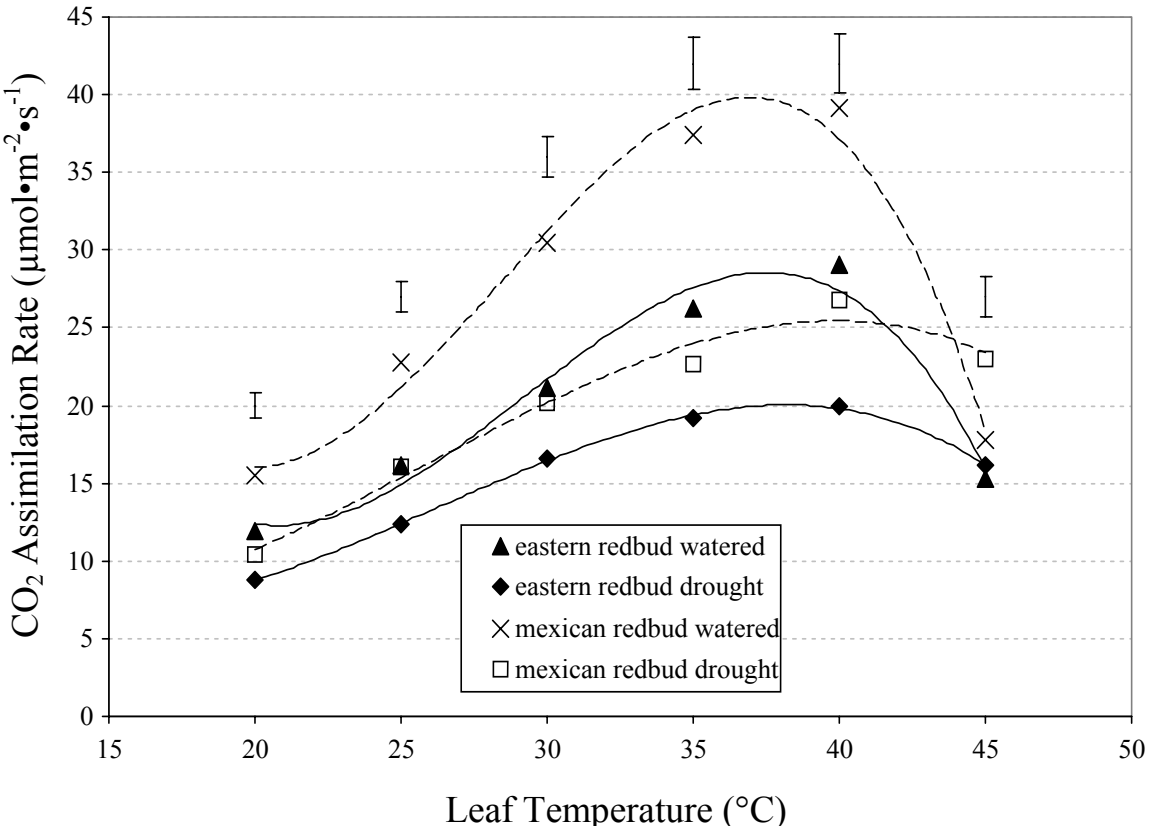

Figure 4. Photosynthesis with cubic regression fit to irrigated and drought stressed mexican redbud (broken line); $r^2 = 0.98$ and 0.97, respectively, and eastern redbud (solid line); $r^2 = 0.97$ and 0.99, respectively, during increasing temperature at 2000 $\mu L \cdot L^{-1}$ CO_2 and 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, n = 7. Error bars represent ± 1 standard error of the mean of all data collected at that temperature, n = 28.

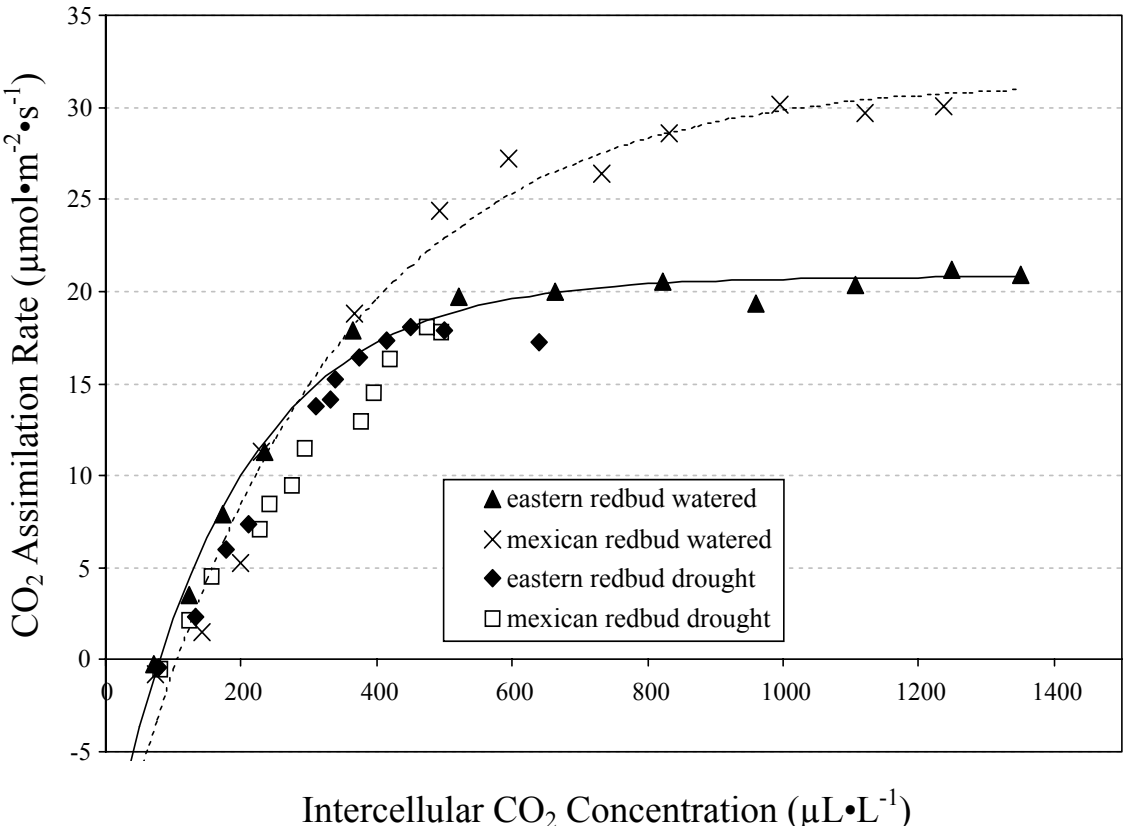

Figure 5. Photosynthesis of typical irrigated and drought stressed mexican redbuds and eastern redbuds during increasing intercellular CO_2 concentrations. Model fit to irrigated plants only; mexican redbud, broken line; eastern redbud, solid line.

Chapter 2

Photosynthetic Responses of *Illicium* Grown Under Varied Irradiance

(In the format appropriate for submission to the

Journal of the American Society for Horticultural Science)

Photosynthetic Responses of *Illicium* Grown Under Varied Irradiance

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Environmental Stress Physiology

Photosynthetic Responses of *Illicium* Grown Under Varied Irradiance

Additional index words. chlorophyll fluorescence, flowering anise, irradiance stress, light tolerance, mannoheptulose, nonphotochemical quenching, photoinhibition, photosynthetic capacity, quantum efficiency, soluble carbohydrates

Abstract. Tolerance to high solar irradiation can be an important aspect of stress tolerance for landscape plants, particularly for species native to understory conditions. The objective of this study was to evaluate differential tolerance to high solar irradiation and underlying photosynthetic characteristics of diverse taxa of *Illicium* L. grown under full sun or 50% shade. Eleven commercially available taxa of *Illicium* were evaluated for light tolerance by measuring light-saturated photosynthetic capacity (A_{max}), dark-adapted quantum efficiency of photosystem II (Fv/Fm), and relative chlorophyll content. Comparisons of A_{max} indicated that three of the 11 taxa (*I. anisatum* L., *I. parviflorum* Michx. ex Vent., and *I. parviflorum* 'Forest Green') maintained similar rates of light-saturated carbon assimilation when grown in either shade or full sun. All other taxa experienced a significant reduction in A_{max} when grown in full sun. Chlorophyll fluorescence analysis demonstrated that Fv/Fm was similar between sun and shade plants for the same three taxa that were able to maintain A_{max}. These taxa appeared to experience less photoinhibition than the others and maintained greater maximum photochemical efficiency of absorbed light. Chlorophyll content was not significantly

reduced in these three taxa either, whereas most other taxa experienced a significant reduction. In fact, chlorophyll content was significantly higher in *I. parviflorum* 'Forest Green' when grown under full sun. Furthermore, *I. parviflorum* 'Forest Green' maintained the highest A_{max} of all the taxa when grown under full sun. These results suggest that there is considerable variation in light tolerance among these taxa, with *I. parviflorum* 'Forest Green' demonstrating superior tolerance to high-light among the plants compared. A deeper examination of *I. parviflorum* 'Forest Green' (high-light tolerance) and *I. floridanum* (low-light tolerance) demonstrated that *I. parviflorum* 'Forest Green' had a considerably higher A_{max}, a higher light saturation point, greater potential photosynthetic capacity, reduced susceptibility to photoinhibition as indicated by superior PSII efficiency following light exposure, greater capacity for thermal de-excitation as indicated by a higher rate of non-photochemical quenching (NPQ) under full sun, greater apparent electron transport rate (ETR) at mid-day, and higher concentrations of the free-radical scavenger myo-inositol. All of these factors potentially contribute to a greater capacity to utilize light energy for carbon fixation while minimizing photodamage.

Introduction

Despite the necessity of light for autotropic organisms, no plant is capable of utilizing 100% of maximum solar irradiation for photosynthesis (Demming-Adams et al., 1997). When irradiance exceeds that which can be used for photochemistry, other protective mechanisms must be utilized to dissipate excess excitation energy. If these mechanisms are inadequate, then damage will occur.

Most plants have the ability to acclimate to a specific light environment. Chlorophyll concentration, Calvin cycle intermediates, chloroplast density, and carotenoid composition have all been reported to fluctuate during the acclimation process (Demmig-Adams et al., 1997). Understory plants grown in high, but not excessive, light typically have the ability to increase maximum, light-saturated, photosynthetic capacity (A_{max}) as well as other energy dissipating mechanisms. Likewise, A_{max} of pioneer species grown under a low light environment can be suppressed (Kitao, 2000). Ramalho et al. (2000) demonstrated that a 15 day exposure to high-light slightly elevated (9% increase) A_{max},, and more than doubled the irradiance required to reach A_{max} in CO_2 saturated leaves of *Coffea arabica* L. 'Catuaní' (coffee) indicating the presence of alternative dissipation mechanisms.

The capacity of a plant to utilize and dissipate light energy is a function of both genotype and environmental conditioning. Pioneer species and species that typically grow in exposed locations usually have a greater light-saturated A_{max} than understory species (Lambers et al., 1998). Therefore, a larger percent of absorbed energy is used in

photosynthesis, reducing the need for alternative dissipating mechanisms, and minimizing the risk of photodamage. However, when grown in shade, understory species typically have a higher quantum yield (Φ) and greater A_{max} than pioneer species (Kitao, 2000).

Shade-grown plants often have relatively large antenna complexes for maximum light capture (Lambers et al., 1998). When exposed to high irradiance, the energy absorbed by these relatively large light-capturing complexes can be detrimental to the plant if not efficiently dissipated. Even plants that have been grown under high-light conditions can experience supraoptimal irradiance at times. When this occurs, photoinhibition, or a decrease in quantum efficiency of photosystem II (PSII), can be significant (Demmig-Adams et al., 1998; Kitao et al., 2000; Schindler and Lichtenthaler, 1996). Therefore, the ability of a plant to acclimate to high irradiance and elevate A_{max} could be a highly successful mechanism to hasten non-damaging energy dissipation.

The ratio of variable fluorescence to maximal fluorescence (Fv/Fm) of dark-adapted leaves is commonly used to assess the relative state of photosystem II. Fortunately, Fv/Fm, which represents the maximum quantum efficiency of PSII, can be determined rapidly by chlorophyll fluorescence and is used frequently as an expression of photoinhibition (Critchley, 1998, Kitao et al., 2000; Krause et al., 1999; Schansker and van Rensen, 1999). Additionally, photoinhibition is typically associated with, but not necessarily accompanied by, chlorophyll degradation and a reduction of A_{max} (Bumann and Oesterhelt, 1995; Critchley, 1998; Demmig-Adams et al., 1998). Excess absorbed

energy can result in destruction of chlorophyll causing an overall yellowing of the foliage. A rapid method to evaluate relative chlorophyll concentrations in leaves of plants grown under varying light regimes might be an effective method to separate those plants based on light tolerance. Species tolerant of high-light are able to maintain high concentrations of chlorophyll when grown in a high-light environment. Chlorophyll meters (e.g., SPAD-502, Minolta Corp., Ramsey, N.J.) can be useful tools for estimating chlorophyll content and monitoring foliar nitrogen content (Monje and Bugbee, 1992; Rodriguez and Miller, 2000; Sibley et al., 1996; Yadava, 1986). In addition, chlorophyll meters can be used to provide an objective value of leaf greenness compared to a subjective visual rating. When interpreted in combination with light saturated A_{max} and quantum efficiency of PSII, the relative chlorophyll concentration (or greenness) of leaves could provide supporting evidence in assigning light tolerance to various species.

The genus *Illicium* (anise tree) consists of broad-leaved evergreen shrubs and small trees that are native to tropical and sub-tropical regions primarily of Asia and southeastern North America (Smith, 1947). Plants generally exist as part of the understory on moist sites, and as such have become a popular landscape plant, particularly for low-light situations. However, when planted in an inappropriate location where solar irradiance is high, many taxa of *Illicium* show symptoms of stress, such as leaf chlorosis (possibly photobleaching) and in extreme situations, necrotic regions appear on the leaves. Although typically not lethal to the plant, the aesthetic value is severely reduced and thus the plant's value in the landscape is diminished. A better

understanding of light tolerance among these taxa would aid in the selection and improvement of these plants for varied light environments.

The first objective of this research was to evaluate and compare a diversity of taxa of *Illicium* for light tolerance by evaluating $A_{max,}$ dark-adapted Fv/Fm, and relative chlorophyll content of plants grown under different irradiance. The second objective was to compare and contrast the photochemical light utilization of two selected taxa. One species was chosen for its ability to successfully grow in a high-light environment and one species was selected for its apparent inability to grow in high-light.

Materials and Methods

Experiment 1.

Rooted cuttings of all taxa listed in Table 1 were transplanted into 18 L containers filled with a substrate of 8 pine bark : 1 sand (by volume) amended with 1.2 kg dolomitic limestone and topdressed with 127 g of Osmocote Pro 23-4-8 (23N-1.7P-6.6K) controlled release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH). On 25 May 2000, containers were placed on the nursery production pad at the Horticultural Field Laboratory, North Carolina State University, Raleigh, NC. Plants were placed in full sun or under shade structures that excluded 50% incident irradiance. All plants were irrigated daily with sufficient water to maintain a minimum leaching fraction of 25%.

On 14 July 2000, measurements were begun. This date allowed sufficient time for acclimation and production of new leaves in their respective light environment. By this date, some of the full-sun plants were beginning to show symptoms of stress. Leaves chosen for all measurements were newly formed, fully expanded, and representative of the given plant.

Net photosynthesis. Net photosynthesis measurements were conducted on a block-by-block basis on sunny days between the hours of 9:00 AM and 12:00 PM using a CIRAS-1 (PP Systems, Haverhill, Mass.) infrared gas analyzer modified with a temperature and light controlled cuvette. Preliminary measurements indicated that before 9:00 AM and after 12:00 PM stomatal conductance was limited and carbon assimilation was unusually low across all taxa. All plants were exposed to 15 min of full sunlight to allow for stomatal opening prior to taking measurements. The sample leaf was then placed in the cuvette that was maintained at 27 °C and exposed to 2000 $\mu mol \bullet m^{-2} \bullet s^{-1}$ PAR and an ambient CO_2 concentration of 350 $\mu L \bullet L^{-1}$. Once stomatal conductance and CO_2 assimilation stabilized, data were recorded. When inside the cuvette, all sampled leaves were exposed to identical environmental conditions. Allowing sufficient time to pass in the cuvette, ensured photosynthetic rates were more related to physiological factors and plant acclimation than metabolic differences related to varying leaf temperatures associated with sun and shade leaves.

Chlorophyll fluorescence. Dark-adapted quantum efficiency of open PSII reaction centers (Fv/Fm) was measured on 20 July 2000 after sun down on each plant.

Chlorophyll fluorescence was measured using a pulse-modulated fluorometer (OS-500, OptiScience, Tyngsboro, Mass) providing a modulating light intensity of 3.6 μmol•m^{-2}•s^{-1} PAR followed by a 0.8 s saturating pulse of 1800 μmol•m^{-2}•s^{-1} PAR.

Relative chlorophyll content. Relative chlorophyll content of each taxa was determined using a chlorophyll meter (Minolta SPAD-502). One SPAD meter reading was taken on each of three fully expanded leaves that were typical in appearance for that plant. These three readings were averaged for a single SPAD reading for that plant. This procedure was repeated three consecutive days during the first week of July 2000, and the three averaged SPAD readings were used for statistical analysis.

The experimental design was a randomized complete block (n=6) with a split plot arrangement of treatments. Two light regimes (100% and 50% of full sunlight) represented the whole plots, while 11 taxa (Table 1) made up the split plot units. Each shade structure was large enough to provide adequate shade for an entire block of 11 shaded plants. Data was subjected to ANOVA and, where appropriate, means were separated with a protected LSD ($P \leq 0.05$).

Experiment 2.

In the fall of 2000, plants were pruned to remove the most recent flush of growth and over-wintered, outside, under a layer of protective white plastic. In the spring of 2001, the containerized plants were again randomly placed in full sun or under 50% shade as previously described. Growing conditions were similar to those described in Exp. 1. Beginning in early July, after plants had produced new growth and acclimated to their environment, analyses were conducted on *I. parviflorum* 'Forest Green' and *I. floridanum* only. These two taxa were selected for further analyses based on their relative tolerance and intolerance to exposure to full sun, respectively.

Light response curves. Light response curves were generated on a block-by-block basis for each plant. An entire block of plants was moved into a greenhouse the evening prior to the day measurements were to be taken. Light response curves were generated the following day for each plant in random order. A recently matured leaf was placed in a dark cuvette at 30 °C supplied with 350 $\mu L \cdot L^{-1}$ CO_2 and given 15 min to stabilize. Dark respiration was recorded and irradiance was increased to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR in nine increments (50, 100, 150, 200, 500, 800, 1000, 1500, and 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Assimilation was recorded at each light level following a 10 min acclimation period. Data was fit to the model equation:

$$A = \frac{\Phi \bullet Q + A_{max} - \sqrt{(\Phi \bullet Q + A_{max})^2 - 4 \bullet \Phi \bullet Q \bullet k \bullet A_{max}}}{2 \bullet k} - R_{day}$$

as given by Lambers et al. (1998) where A is the rate of assimilation, Φ is the apparent quantum efficiency, Q is the incident irradiance, A_{max} is assimilation rate at light saturation, k is the parameter for convexity, and R_{day} is the rate of dark respiration.

A/Ci curves. Following light response curves, A/Ci curves (photosynthesis plotted against intercellular CO_2 concentration) were generated to investigate specific characteristics of photosynthesis and to estimate potential photosynthetic capacity. Another recently matured leaf was placed in the cuvette at a CO_2 concentration of 370 $\mu L \bullet L^{-1}$, 30 °C, and 1000 $\mu mol \bullet m^{-2} \bullet sec^{-1}$ PAR. The air within the cuvette was maintained at approximately 70% relative humidity to minimize stomatal heterogeneity. Following a 15 min acclimation period, Pn was recorded and the CO_2 concentration was reduced to 50 $\mu L \bullet L^{-1}$ where another reading was taken. A gradual increase in ambient CO_2 to a final concentration of 2000 $\mu L \bullet L^{-1}$ in 10 increments occurred with a reading taken at each increment following a 10 min acclimation period. Data was then fit to the exponential model used by Jacob et al. (1995) and adopted by Reid and Fiscus (1998):

$$A = a(1 - e^{-bCi}) + c$$

where a is the potential photosynthetic capacity, b is the initial slope or carboxylation efficiency, c is the intercept on the ordinate, and Ci is the estimated intercellular CO_2 concentration.

Chlorophyll fluorescence. Dark-adapted Fv/Fm measurements were taken as described in Experiment 1, only this time measurements were predawn. Shade grown

plants were then moved to full sun for the remaining measurements to monitor how they responded to excessive irradiance. Light adapted chlorophyll fluorescence measurements were taken at 8:00 AM, 10:00 AM, 12:00 PM, 2:00 PM, and 4:00 PM. The fluorometer fiberoptic was placed at a 60° angle to the leaf blade and positioned so as not to shade the leaf surface. Following exposure to a modulating light of 3.6 $\mu mol \bullet m^{-2} \bullet s^{-1}$ PAR a 0.8 s saturating pulse of 1800 $\mu mol \bullet m^{-2} \bullet s^{-1}$ PAR was applied to the leaf.

Chlorophyll content. Leaf discs were collected in early morning from the lamina of recently matured leaves with a 1.0 cm diameter cork borer. Chlorophyll was extracted by placing three discs (2.36 cm^2) in 8.0 ml N,N'-dimethylformamide (DMF) in the dark at room temperature on a rotary shaker for 48 hr. Chlorophyll a and b were determined spectrophotometrically using the wavelengths and equations of Porra et al. (1989).

Carbohydrate analysis. Recently matured leaves were removed from the plants in early morning and immediately frozen at -80 °C (5 or 6 leaves per plant). Frozen leaf tissue was lyophilized and ground to pass a 40-mesh screen. For each plant sample 200 mg of ground tissue was placed in a 15 ml centrifuge tube and suspended in 3 ml of 80% aqueous ethanol. The slurry was incubated at 80 °C for 5 min then centrifuged at 1000 g_n for 10 min. The extraction was repeated two more times with the supernatant combined after each. Pooled extracts were evaporated at 45 °C under vacuum and oven dried over-night at 70 °C. The residue was dissolved in 1.0 ml of deionized, distilled water and soluble sugars analyzed by HPLC. The HPLC system (described by Prata et al., 1997) was equipped with a guard column (C-18 Corasil, Bio-Rad) and in-line cation and anion

guards (Micro-Guard, Bio-Rad). Carbohydrates were separated on a column (Ca^{++} Carbohydrate column, Sierra Separations, Inc., Sparks, NV) with a flow rate of 0.4 ml water min^{-1} at 75 °C. Carbohydrate identity and quantity were analyzed using a differential refractometer (model 410, Waters/Millipore) coupled to a computing integrator (model SP4200, Spectra Physics, San Jose, CA) and compared with carbohydrate standards.

The experimental design was a randomized complete block (n=6) with a split plot arrangement of treatments. Two light regimes (100% and 50% of full sunlight) represented the whole plots, while *I. floridanum* and *I. parviflorum* 'Forest Green' made up the split plot units. The light response curves, A/Ci curves, ΔF/Fm', and ETR were analyzed as a split-split-plot with incident light (light response curves), intercellular CO_2 concentration (A/Ci), or time of day (ΔF/Fm' and ETR) as the sub-sub-plot. Data was subjected to ANOVA and, where appropriate, means were separated with a protected LSD ($P \leq 0.05$). Appropriate models were fit to the data using the PROC NLIN command in SAS.

Results

Experiment 1

Net photosynthesis. An interaction was observed between light environment and taxa for A_{max}. Net photosynthesis of plants grown in full sun was significantly reduced in all but three taxa (Table 1), two of which were cultivars of the same species (*I. anisatum* L., *I. parviflorum* Michx. ex Vent, and *I. parviflorum* 'Forest Green'). A_{max} for sun and shade grown plants of *I. parviflorum* and *I. parviflorum* 'Forest Green' were nearly identical (approx. 14.2 and 17.6 $\mu mol \bullet m^{-2} \bullet s^{-1}$, respectively). Assimilation rates for all other taxa were reduced by 2 $\mu mol\ CO_2 \bullet m^{-2} \bullet s^{-1}$ or more when grown in sun.

Among the shade grown plants, *I. parviflorum* 'Forest Green' (17.6 $\mu mol \bullet m^{-2} \bullet s^{-1}$) and *I.* × 'Woodland Ruby' (16.0 $\mu mol \bullet m^{-2} \bullet s^{-1}$) had the highest A_{max}, demonstrating the greatest capacity to utilized incident light energy for carbon accumulation. Conversely, *I. lanceolatum* Smith (9.6 $\mu mol \bullet m^{-2} \bullet s^{-1}$) and *I. henryi* Diels (10.9 $\mu mol \bullet m^{-2} \bullet s^{-1}$) had the lowest A_{max} when grown in shade, and therefore had the least capacity to utilize the absorbed light energy to assimilate carbon. Of all the *I. floridanum* Ellis taxa, *I. floridanum* 'Halley's Comet' had the highest A_{max} .

A_{max} among sun grown plants followed a similar trend with a few exceptions. *Illicium parviflorum* 'Forest Green' (17.7 $\mu mol \bullet m^{-2} \bullet s^{-1}$) still had the greatest assimilation rate, but was followed by the unnamed clone of *I. parviflorum* (14.1 $\mu mol \bullet m^{-2} \bullet s^{-1}$). While A_{max} of shade grown *I.* × 'Woodland Ruby' was relatively high, assimilation in the sun grown plants was reduced considerably (16.0 and 10.5 $\mu mol \bullet m^{-2} \bullet s^{-1}$, respectively).

Although the relative decrease in A_{max} was greatest for this taxon, it was still the median value for all sun grown plants, indicating some level of tolerance. As was true with the shade plants, *I. floridanum* 'Halley's Comet' retained the highest A_{max} for the taxa of *I. floridanum*.

Chlorophyll fluorescence. There was an interaction between taxa and exposure for dark-adapted Fv/Fm measurements taken after sundown between taxa and light environment. Plants grown under conditions of high-light had reduced Fv/Fm in some, but not all, taxa. In fact, the effect of light was rather specific. With the exception of *I. mexicanum* Smith 'Aztec Fire', where Fv/Fm was reduced by high-light, the only taxa that were affected significantly by light were clones of *I. floridanum.* Quantum efficiency of dark-adapted leaves of *I. floridanum, I. floridanum* 'Head's Compact', and *I. floridanum* 'Semmes' were all reduced by the high-light environment. The only taxon of *I. floridanum* that was not affected was *I. floridanum* 'Halley's Comet', the same taxon that was able to maintain a relatively high A_{max}. Overall, *I. floridanum* was affected the greatest by high-light, indicating some degree of photoinhibition in high-light.

Relative chlorophyll content. Relative chlorophyll content indicated that many of the taxa with a low A_{max} also had low chlorophyll content (Table 1). *Illicium floridanum* had the lowest relative chlorophyll content. The three taxa that were able to maintain a high A_{max} also maintained high chlorophyll content. Of all the taxa, only *I. parviflorum* 'Forest Green' significantly increased chlorophyll content when grown under high-light.

Plants grown in full sun were visually distinct from those grown in 50% shade. Although data was not collected, sun plants appeared to have shorter internodes, reduced leaf size, and leaves were frequently chlorotic with marginal necrosis. Leaves of *I. parviflorum* and *I. parviflorum* 'Forest Green', however, displayed no symptoms of chlorosis or necrosis, assumed a vertical orientation on the sun plants, and a horizontal orientation on the shade plants. A similar trend was observed with *I. anisatum*. However, none of the other taxa displayed this morphological characteristic.

Experiment 2

Light response curves. The light response curves revealed no difference in photosynthesis between plants of the same species grown in full sun or under shade, so only the main effects of species are presented (Fig. 1). The data clearly revealed a fundamental difference between these two species. *Illicium parviflorum* 'Forest Green' had a substantially higher light-saturated photosynthetic rate than *I. floridanum.* Predicted A_{max} of *I. parviflorum* 'Forest Green' and *I. floridanum* was 23 and 7 µmol $CO_2 \bullet m^{-2} \bullet s^{-1}$, respectively. Surprisingly, no differences between the two species were detected for quantum efficiency, the light compensation point, or the rate of respiration. However, the light saturation point was more than two fold greater for *I. parviflorum* 'Forest Green' than *I. floridanum* (1170 and 200 µmol$\bullet m^{-2} \bullet s^{-1}$ PAR, respectively).

A/Ci curves. The data from the A/Ci curves suggests that the difference in photosynthetic capacity between the two species is not entirely due to stomatal aperture. Although stomatal conductance was less in *I. floridanum* (data not presented), it was still

sufficient to reach a saturating internal CO_2 concentration (Fig. 2). At a saturating concentration of CO_2, *I. parviflorum* 'Forest Green' had a potential photosynthetic capacity of 56 μmol $CO_2 \bullet m^{-2} \bullet s^{-1}$ compared to 24 μmol $CO_2 \bullet m^{-2} \bullet s^{-1}$ for *I. floridanum*. Although the relative difference between the calculated potential photosynthetic capacities of these two species is not as great as the light response data, it still suggests that *I. parviflorum* is simply capable of fixing more CO_2 per unit leaf area given the same growing conditions. Similar to the light response curves, the maximum rate of photosynthesis was the only parameter measured that differed between the two species. Neither carboxylation efficiency nor the rate of respiration differed.

Chlorophyll fluorescence. Dark-adapted Fv/Fm of *I. parviflorum* 'Forest Green' in sun or shade grown plants was found to be in a range typical of many species that are not stressed (0.82 and 0.80, respectively) (Bjorkman and Demmig, 1987). However, Fv/Fm of shade grown *I. floridanum* was significantly less (0.71) and was even further reduced in the sun grown plants (0.47). Actual PSII efficiency (ΔF/Fm') was also effected by the increasing solar intensity throughout the day (Fig. 3). In both species, ΔF/Fm' was significantly reduced during the middle part of the day followed by a slight recovery in afternoon. However, the decrease for *I. floridanum* was much greater than it was for *I. parviflorum* 'Forest Green', suggesting greater damage to PSII as duration and intensity of irradiance increased. Of greater significance, perhaps, is the response of non-photochemical quenching (NPQ). In *I. parviflorum* 'Forest Green', an 88% increase in NPQ was observed from shade- to sun-grown plants (0.51 and 0.96, respectively), compared to a 94% decrease when that same comparison is made in *I. floridanum* (0.51

and 0.03, respectively) (Fig. 4). The effect of time of day was similar for the two species and light treatment, so the values were averaged and only the main effect of time was presented. NPQ was relatively low at 10:00 AM (0.18), rising to a high at 2:00 PM (0.69), before falling off at 4:00 PM (0.44). Light treatment did not affect apparent photosynthetic electron transport rate (ETR) within a species; however, there were significant differences between the two species (Fig. 5). While ETR for both species was relatively similar through 10:00 AM, at 12:00 PM ETR of *I. floridanum* had reached its maximum observed rate. The rate for *I. parviflorum* 'Forest Green', however, continued to increase and reached its maximum at 2:00 PM before beginning to decline. The maximum rate was approximately 100 $\mu mol \bullet m^{-2} \bullet s^{-1}$ greater in *I. parviflorum* 'Forest Green' than *I. floridanum.*

Chlorophyll content. Total chlorophyll content, chl a, and the ratio chl a:b did not significantly change among the species or treatments (Table 2). However, chl b, the primary chlorophyll constituent of the light harvesting antennae, was significantly higher in shade-grown *I. floridanum* than all other plants and treatments. It is common for shade plants to have more chl b than sun-grown plants, and is therefore surprising that there are no differences among the sun- and shade-grown *I. parviflorum* 'Forest Green'.

Soluble carbohydrate concentration. Carbohydrate analysis revealed different patterns of sugar production in these two species (Table 3). While both species produced the raffinose family oligosaccharides (RFOs) stachyose and raffinose, *I. floridanum* contained twice as high a concentration of RFOs as *I. parviflorum* 'Forest Green'. The

proportion of those two sugars in relation to each other was different also. Raffinose is the primary oligosaccharide in *I. floridanum*, whereas the difference in content between the two sugars practically disappears in *I. parviflorum* 'Forest Green'. Sucrose appears to be the primary sugar in leaves of *I. parviflorum* 'Forest Green', and its concentration was unaffected by sun or shade in either species. Both galactinol and glucose were absent in *I. floridanum* but present in *I. parviflorum* 'Forest Green'. As with sucrose, their concentration was unaffected by sun or shade treatments. The 7-carbon sugar, mannoheptulose was also detected in these plants. Interestingly, it was a dominant soluble carbohydrate in *I. floridanum*, yet a small component in *I. parviflorum* 'Forest Green'. Additionally, its concentration was higher in shade-grown *I. floridanum*, whereas the growing environment did not affect concentration in *I. parviflorum* 'Forest Green'. The remaining soluble carbohydrate detected in these plants was the polyol, myo-inositol. Its content was higher in I. parviflorum, and sun-grown plants contained greater amounts than shade-grown plants.

Discussion:

Sun-tolerant and light-acclimated plants typically have a higher A_{max} when exposed to high-light than do sun-intolerant plants (Lambers et al., 1998). The results presented here, with high-light acclimated and low-light acclimated plants, indicate that among the taxa tested, *I. anisatum*, *I. parviflorum*, and *I. parviflorum* 'Forest Green' were the most sun tolerant. A_{max} for these taxa was unaffected when grown under full solar irradiance, whereas A_{max} for all other taxa was significantly reduced in the high-light environment, which is characteristic of photoinhibition (Table 1). The majority of tested

taxa were not able to acclimate and increase A_{max} in the high-light environment. Previous research found that *I. anisatum* did have a significant ability to increase its light saturated A_{max} rate in response to high-light while growing in its natural environment (Kusumoto 1957). It was also reported that respiration and the light compensation point were significantly greater in the high-light acclimated plants, another characteristic of plants that have acclimated to a high-light environment (Lambers et al., 1998).

Further investigation of *I. parviflorum* 'Forest Green' and *I. floridanum* revealed substantial differences in photosynthetic characteristics. As Fig. 1 shows, *I. parviflorum* 'Forest Green' is able to assimilate three times more CO_2 than *I. floridanum* at a saturating irradiance. This ability to utilize a greater portion of the absorbed light energy to assimilate a significantly greater amount of carbon may lower the amount of excess energy and thereby reduce the extent of photoinhibition. In these plants, however, A_{max} did not vary as a result of growth in sun or shade. This may suggest that these species have little ability to acclimate to higher levels of irradiance. It is worth noting that these plants were grown outside, where 50% shade can still exceed 1000 $\mu mol \bullet m^{-2} \bullet s^{-1}$ PAR during early afternoon. This level is rarely achieved in growth chambers, yet often a lesser irradiance is used as a 'high-light' treatment throughout the literature. In the current study, 50% shade may have been sufficient to elicit a high irradiance acclimation without inducing photoinhibition from excess irradiance, thereby masking many of the differences between sun- and shade-grown plants. Unlike A_{max}, there were no differences in quantum efficiency or the light compensation point between these two species, suggesting that at low irradiance the two are equally efficient in utilizing the absorbed

light. The light saturation point, however, was significantly higher for *I. parviflorum* 'Forest Green'. Again, this is another indication that *I. parviflorum* 'Forest Green' is simply able to use a greater portion of sunlight in photosynthesis than *I. floridanum*.

The higher photosynthetic capacity of *I. parviflorum* 'Forest Green' was confirmed by the A/Ci curves (Fig. 2). Even at saturating concentrations of CO_2, *I. parviflorum* 'Forest Green' assimilated twice as much carbon as *I. floridanum*. Again, there was no difference between the sun- and shade-grown plants. This would indicate that the physiological capacity to assimilate CO_2 is not affected by the different light environments in which the plants were grown. The data also suggests that at low concentrations of CO_2 there was no difference in the efficiency or the rate that carbon was assimilated, only that *I. floridanum* becomes CO_2 saturated at a lower ambient concentration. As CO_2 concentration increases, the higher photosynthetic capacity allows *I. parviflorum* 'Forest Green' to continue assimilating carbon whereas *I. floridanum* cannot.

Chlorophyll fluorescence is frequently used to determine the state of energy distribution in the thylakoid membrane, the quantum efficiency of PSII, and the extent of photoinhibition (Björkman and Demmig-Adams, 1994; Critchley, 1998; Maxwell and Johnson, 2000). Plants subjected to high-irradiance stress, typically have lower Fv/Fm values than non-stressed plants (Björkman and Demmig, 1987). The data presented here indicates when Fv/Fm was significantly reduced by the high-light environment, a reduction in A_{max} was also observed in that taxon (Table 1). Likewise, the three taxa that

maintained A_{max} in high-light, also maintained similar values of Fv/Fm. Under high irradiance, the transfer of absorbed energy becomes particularly important as excess energy can lead to the formation of reactive oxygen species, a prime cause of photoinhibition (Critchley, 1998; Schansker and van Rensen, 1999). Quantum efficiency of four taxa in this study was decreased by the high-light (Table 1). These taxa also displayed symptoms associated with photoinhibition such as reduced chlorophyll content and necrotic regions of the leaf.

In the second phase of this research, apparent quantum efficiency of PSII in *I. parviflorum* 'Forest Green' was unaffected by growing environment, indicating no significant damage to the PSII complex in this species. In *I. floridanum*, however, Fv/Fm of the shade-grown plants was significantly lower than *I. parviflorum* 'Forest Green', and exposure to full sun caused a further reduction in Fv/Fm. This is similar to the response that was observed in phase one of this study. Such a response is frequently reported on stressed plants. It is often interpreted that the applied stress has caused significant damage to PSII, and reduced the efficiency that absorbed light energy is passed from the light-harvesting complex to PSII (Bilger and Björkman, 1990; Maxwell et al., 2000; Ranney and Peet, 1994). Light adapted ΔF/Fm' was also unaffected by growth in full sun or shade in both species (Fig. 3). However, the two species differed throughout the course of the day. PSII efficiency decreased as the day progressed in both species eventually reaching a minimum at approximately 2:00 PM, where photoinhibition would be most likely. However, the decrease in *I. floridanum* was more severe suggesting inferior tolerance to high irradiance. A similar trend was also noted by Demmig-Adams

et al. (1998) who reported a severe decrease in PSII efficiency as the day progressed in shade leaves of *Shefflera arboricola* (Hayata) Merrill. This data combined with the Fv/Fm, A/Ci response curves, and light response curves once again leads to the conclusion that *I. floridanum* is unable to process as much absorbed light energy into photosynthesis as *I. parviflorum* 'Forest Green'. Species that are adapted to or acclimated to high irradiance have a greater level of non-photochemical quenching (NPQ) than species that are considered light sensitive or not acclimated to high irradiance. There were no statistical differences in NPQ through the course of the day. However, total NPQ averaged over all times was significant (Fig. 4). Whereas NPQ in *I. parviflorum* 'Forest Green' increased from shade- to sun-grown plants, NPQ in the sun-grown plants of *I. floridanum* was significantly less than the shade-grown plants indicating a significant inability to dissipate excess energy thermally. Additionally, the higher ETR in *I. parviflorum* 'Forest Green' is further evidence that this species has a higher photochemical capacity in high-light conditions (Fig. 5). This information would indicate the potential for considerable photoinhibition in the leaves of *I. floridanum*.

Typically, shade-grown plants have higher chlorophyll concentrations per unity area than sun plants. Relative chlorophyll content of *I. anisatum*, *I. parviflorum*, and *I. parviflorum* 'Forest Green', the same three taxa that were able to maintain A_{max} in high-light, was not significantly reduced by the high-light. In fact, *I. parviflorum* 'Forest Green' actually increased the amount of chlorophyll in response to the increased irradiance. This characteristic is common among light tolerant species (Lambers et al., 1998). *Illicium floridanum* 'Halley's Comet' and *I. floridanum* 'Heads Compact'

maintained similar chlorophyll content in shade and high-light. That *I. floridanum* 'Halley's Comet' was not affected is not surprising since it was the most tolerant of all the *I. floridanum* taxa and Fv/Fm in this taxon was not affected by the high-light. Although chlorophyll content in *I.* × 'Woodland Ruby' and *I. lanceolatum* were both significantly reduced by the high-light, the diminished level was still similar to those plants where no reduction was observed. Interestingly, these two taxa showed no decrease in Fv/Fm due to the high-light either, indicating the reduced A_{max} may be due to some other physiological factor.

When actual chlorophyll content was measured in the second phase of this study, only chl b was effected by treatments, and it was highest in *I. floridanum* grown in the shade. Chl b is most abundant in the antennae of the light harvesting complex, whereas chl a is concentrated around PSII. In order to capture as much light as possible, shade-grown plants typically have more light-harvesting complexes per unit area than do sun-grown plants that typically receive more light than needed. Therefore, it is not surprising that chl b is higher in the shade-grown plant. What is surprising is that shade-grown *I. parviflorum* 'Forest Green' did not have more chl b than the sun-grown plants. In light of the lack of physiological differences between sun- and shade-grown plants with regards to photosynthesis and chlorophyll fluorescence, perhaps species of *Illicium*, similar to *I. parviflorum* 'Forest Green', do not normally adjust chlorophyll content in response to irradiance. Or perhaps, 50% shade was sufficient to elicit a high-light acclimation. If this is the case, the decrease in chl b content in *I. floridanum* could be an indication of chlorophyll destruction by excess irradiance. This theory is reinforced by

the dramatic reduction of NPQ in sun-grown *I. floridanum*, a process that occurs in the light-harvesting complex. These two sets of data suggest that in sun-grown *I. floridanum* there is a dramatic reduction in the concentration of light harvesting complexes and thus a reduction of chl b.

The soluble carbohydrate profile of these plants was both complex and unusual. Both species contained a substantial amount of RFOs, but *I. floridanum* contained significantly more. Although *I. floridanum* contained abundant RFOs, there was no free glucose, galactinol, or fructose present in the leaves, suggesting that the galactosyl transferase in this species was sufficient to prevent an accumulation of the substrates. Sucrose was the soluble sugar of greatest content in *I. parviflorum* 'Forest Green' and was significantly greater than in *I. floridanum*. This is a logical sequence given the higher level of RFOs in *I. floridanum*. If *I. parviflorum* 'Forest Green' produced a similar amount of RFOs, the sucrose content would have to fall to a similar level as *I. floridanum*. Myo-inositol was present in both species, was greater in content in *I. parviflorum* 'Forest Green'. This carbohydrate had previously been implicated in scavenging reactive oxygen species, which is a critical defense against excess irradiance. Additionally the amount of myo-inositol was greatest in *I. parviflorum* 'Forest Green' that had been grown in full sun, suggesting that its content may be affected by sun exposure. Perhaps most intriguing was the presence of the seven carbon sugar, mannoheptulose. While the production of this carbohydrate is well documented in *Persea americana* Miller (avocado) its occurrence elsewhere is not well known. The presence of mannoheptulose in these two species of *Illicium* varies considerably. In *I. floridanum*,

mannoheptulose contributed to 33% and 43% of soluble carbohydrates in sun- and shade-grown plants, respectively. That compared to only 2% in leaves of *I. parviflorum* 'Forest Green', regardless of sun exposure. However, mannoheptulose is a competitive inhibitor of hexokinase, an enzyme that represses gene expression. Therefore, the physiological significance of mannoheptulose as well as its cellular localization in *Illicium* remains to be seen. The production of mannoheptulose in avocado occurs by the condensation of dihydroxyacetone phosphate (3-carbon) with erythrose-4-phosphate (4-carbon) to form sedoheptulose-1,7-bisphosphate (7-carbon) which is then isomerized to a phosphorylated D-mannoheptulose (Liu et al., 2002). All the above are intermediates in the photosynthetic carbon reduction cycle and raise the question as to whether the accumulation of mannoheptulose acts as a sink interfering with adequate recycling of ribulose-1,5-bisphosphate in *I. floridanum*.

These results demonstrate that there is a wide range of tolerance to high irradiance among taxa of *Illicium*. Light saturated A_{max} varied considerably among the taxa and, in general, was lower in sun plants than shade plants. However, not all taxa experienced a decreased A_{max} in the sun. Of the tested taxa, *I. parviflorum* and *I. parviflorum* 'Forest Green' appear to be the most light tolerant. Their superior rate of photosynthesis in both high irradiance and 50% shade suggests that photoinhibition was minimal in this species. *Illicium anisatum* also maintained a statistically similar light-saturated A_{max} in both sun- and shade-grown plants. Other taxa such as *I. floridanum*, *I. henryi*, and *I. lanceolatum*, with lower rates of light-saturated photosynthesis, were more

likely to encounter excess irradiance and therefore experience significant over-excitation of the photosystem.

Although *I. floridanum* is one of the more popular taxa in commercial production, these plants were intolerant of the high-light environment. When compared to *I. parviflorum* 'Forest Green', chlorophyll fluorescence analysis revealed *I. floridanum* had inferior ΔF/Fm', NPQ, and ETR, suggesting photoinhibition during peak daily irradiance, may play a role in the reduced light-saturated A_{max} and PPC. Although most of the taxa did not perform well in full sun, there is some tolerance to high irradiance in this genus. Both clones of *I. parviflorum* withstood full sunlight with no signs of photoinhibition or any ill effects of the high irradiance.

Literature Cited

Bilger, W. and O. Björkman. 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosyn. Res. 25:173-185.

Björkman, O. and B. Demmig. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170:489-504.

Björkman, O. and B. Demmig-Adams. 1994. Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants, p. 17-47. In: E.D. Schulze and M.M. Caldwell (eds.). Ecophysiology of photosynthesis. Ecological Studies, vol. 100. Springer-Verlag, NY.

Bumann, D. and D. Oesterhelt. 1995. Destruction of a single chlorophyll is correlated with the photoinhibition of photosystem II with a transiently inactive donor side. Proc. Natl. Acad. Sci. 92:12195-12199.

Critchley, C. 1998. Photoinhibition, p. 264-272. In: A.S. Raghavendra (ed.). Photosynthesis: A comprehensive treatise. Cambridge Univ. Press. Cambridge, UK.

Demmig-Adams, B., W.W. Adams III, and S.C. Grace. 1997. Physiology of light tolerance in plants. Hort. Rev. 18:215-246.

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

Jacob, J., C. Greitner, and B.G. Drake. 1995. Acclimation of photosynthesis in relation to Rubisco and non-structural carbohydrate contents and *in situ* carboxylase activity in *Scirpus olneyi* at elevated CO_2 in the field. Plant, Cell and Environ. 18:875-884.

Kitao, M., T.T. Lei, T. Koike, H. Tobita, and Y. Maruyama. 2000. Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. Plant, Cell Environ. 23:81-89.

Krause, G.H., N. Carouge, and H. Garden. 1999. Long-term effects of temperature shifts on xanthophyll cycle and photoinhibition in spinach (*Spinacia oleracea*). Aust. J. Plant Physiol. 26:125-134.

Kusumoto, T. 1957. Physiological and ecological studies on the plant production in plant communities. Bot. Mag. 70:299-304.

Lambers, H., F.S. Chapin III, and T.L. Pons. 1998. Plant physiological ecology. Springer-Verlag New York Inc. NY.

Liu, X., J. Sievert, M. Arpaia, and M.A. Madore. 2002. Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. J. Amer. Soc. Hort. Sci. 127(1):108-114.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence-a practical guide. J. Exp. Bot. 51:659-668.

Monje, O.A. and B. Bugbee. 1992. Inherent limitations of nondestructive chlorophyll meters: A comparison of two types of meters. HortScience 27:69-71.

Porra, R.J., W.A. Thompson, and P.E. Kriedemann. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll

standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta 975:384-394.

Prata, R.T.N., J.D. Williamson, M.A. Conkling, and D.M. Pharr. 1997. Sugar Repression of mannitol dehydrogenase activity in celery cells. Plant Physiol. 114:307-314.

Ranney, T.G. and M.M. Peet. 1994. Heat tolerance of five taxa of birch (*Betula*): Physiological responses to supraoptimal leaf temperatures. J. Amer. Soc. Hort. Sci. 119(2):243-248.

Ramalho J.C., T.L. Pons, H.W. Groeneveld, H.G. Azinheira, and M.A. Nunes. 2000. Photosynthetic acclimation to high-light conditions in mature leaves of *Coffea arabica* L.: role of xanthophylls, quenching mechanisms and nitrogen nutrition. Aust. J. Plant Physiol. 27:43-51.

Reid, C.D. and E.L. Fiscus. 1998. Effects of elevated [CO_2] and/or ozone on limitations to CO_2 assimilation in soybean (*Glycine max*). J. Exp. Bot. 49:885-895.

Rodriguez, I.R. and G.L. Miller. 2000. Using a chlorophyll meter to determine the chlorophyll concentration, nitrogen concentration, and visual quality of St. Augustinegrass. HortScience 35:751-754.

Schansker, G. and J.J.S. van Rensen. 1999. Performance of active photosystem II centers in photoinhibited pea leaves. Photosyn. Res. 62:175-184.

Schindler, C. and H.K. Lichtenthaler. 1996. Photosynthetic CO_2-assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field grown maple trees in the curse of a sunny and a cloudy day. J. Plant Physiol. 148:399-412.

Sibley, J.L., D.J. Eakes, C.H. Gilliam, G.J. Keever, W.A. Dozier, Jr, and D.G. Himelrick. 1996. Foliar SPAD-502 meter values, nitrogen levels, and extractable chlorophyll for red maple selections. HortScience 31:468-470.

Smith, A.C. 1947. The families Illiciaceae and Schisandraceae. Sargentia. 7:1-224.

Yadava, U.L. 1986. A rapid and nondestructive method to determine chlorophyll in intact leaves. HortScience 21:1449-1450.

Table 1. Light saturated photosynthetic capacity (A_{max}), quantum efficiency of dark-adapted leaves (Fv/Fm) and relative chlorophyll content (SPAD units) of 11 different taxa of *Illicium* grown under 50% shade (shade) or full solar irradiance (sun).

	A_{max} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		Fv/Fm		Chlorophyll (SPAD Units)	
Taxa	Sun	Shade	Sun	Shade	Sun	Shade
I. anisatum *L.*	11.4a[z]	13.4a	0.82a	0.84a	58a	60a
I. floridanum Ellis	8.6a	12.3b	0.62a	0.78b	33a	42b
I. floridanum 'Halleys Comet'	12.6a	15.0b	0.79a	0.82a	54a	59a
I. floridanum 'Head's Compact'	10.1a	13.5b	0.72a	0.79b	44a	48a
I. floridanum 'Semmes'	11.3a	14.b	0.73a	0.80b	49a	57b
I. henryi Diels	8.5a	10.9b	0.73a	0.76a	42a	52b
I. lanceolatum Smith	6.6a	9.6b	0.81a	0.83a	54a	60b
I. mexicanum Smith 'Aztec Fire'	9.4a	13.3b	0.76a	0.81b	48a	59b
I. parviflorum Michx. ex Vent.	14.1a	14.3a	0.82a	0.83a	58a	58a
I. parviflorum 'Forest Green'	17.7a	17.6a	0.82a	0.83a	63a	56b
I. × 'Woodland Ruby'	10.6a	16.0b	0.79a	0.82a	53a	64b

[z]Mean separation between light regimes within taxa and measured variable, numbers followed by the same letter are not significantly different at the *P*=0.05 level; n=6.

Table 2. Chlorophyll content of *I. floridanum* and *I. parviflorum* 'Forest Green' grown in full solar irradiance (sun) or under 50% shade (shade)

	Chlorophyll Content (µg•cm^{-2})			
Species / treatment	Chl a	Chl b	Total	a:b
I. floridanum / shade	64	24 a	88	2.8
I. floridanum / sun	51	16 b	67	3.3
I. parviflorum / shade	54	18 b	72	3.0
I. parviflorum / sun	52	17 b	69	3.1
Significance	ns	**	ns	ns

Means were separated within a column by Fisher's Protected LSD where appropriate. Number with the same trailing letter are not significantly different at the *P*= 0.05 level; n=6. **Significant at *P*=0.01.

Table 3. Leaf tissue soluble carbohydrate content (µg•g^{-1} dry weight) of *Illicium floridanum* and *I. parviflorum* 'Forest Green' grown under full solar irradiance (sun) or 50% shade (shade).

	I. floridanum		*I. parviflorum* 'Forest Green'	
Soluble carbohydrate	Sun	Shade	Sun	Shade
Stachyose	85 a	73 a	313 b	259 b
Raffinose	1417 a	1252 b	458 c	368 c
Sucrose	947 a	918 a	1304 b	1153 b
Galactinol	0 a	0 a	407 b	351 b
Glucose	0 a	0 a	212 b	185 b
Myo-inositol	151 a	171 a	284 b	213 c
Mannoheptulose	1271 a	1838 b	71 c	47 c
Total	3872 a	4252 a	3050 b	2576 c

Means were separated within a row by Fisher's Protected LSD. Number with the same trailing letter are not significantly different at the *P*= 0.05 level; n=6.

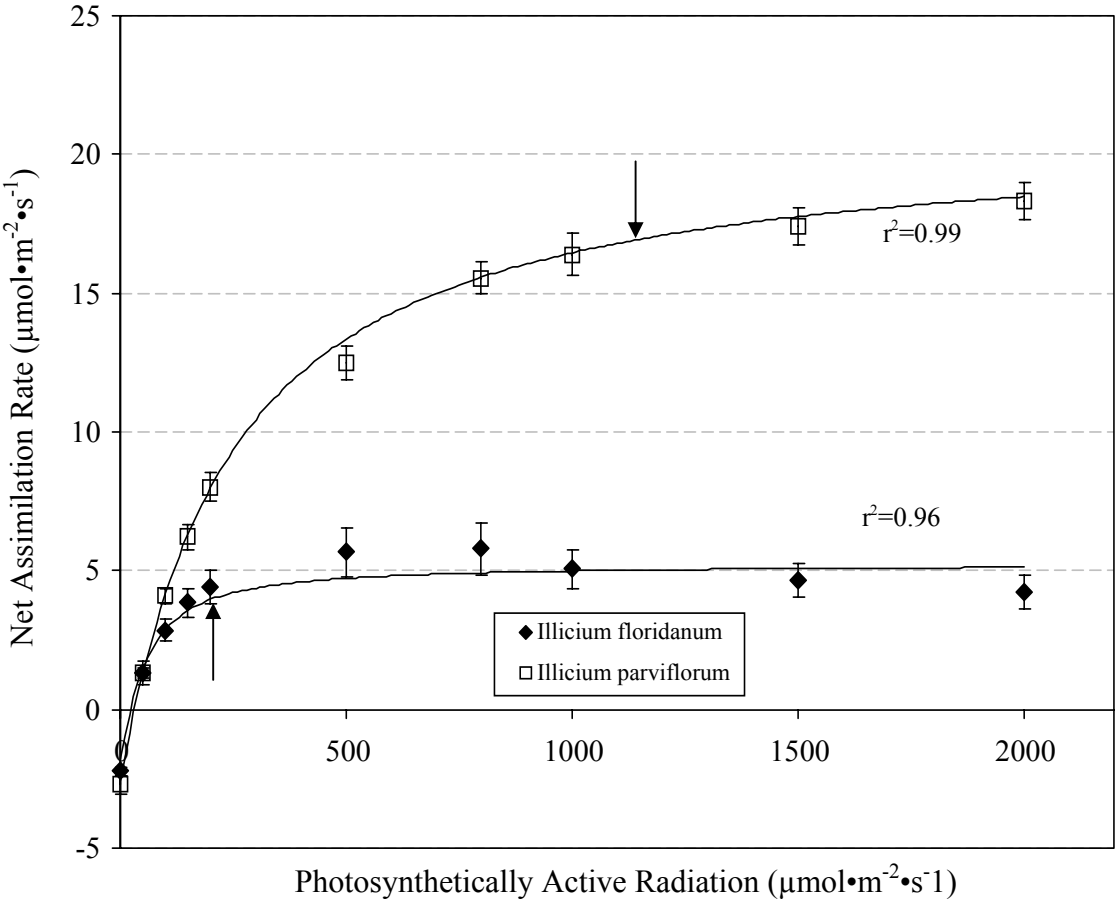

Figure 1. Light response curves for *I. floridanum* and *I. parviflorum* 'Forest Green'. Data averaged over light environment during growth within a taxon. CO_2 concn = 350 µL•L^{-1}, leaf temperature = 30 ºC. Arrows represent light saturation point for *I. floridanum* and *I. parviflorum* 'Forest Green' (200 and 1170 µmol•m^{-2}•s^{-1} PAR, respectively). Error bars = ±1 standard error of the mean, n = 12. Data fit to the model proposed by Lambers et al., (1998).

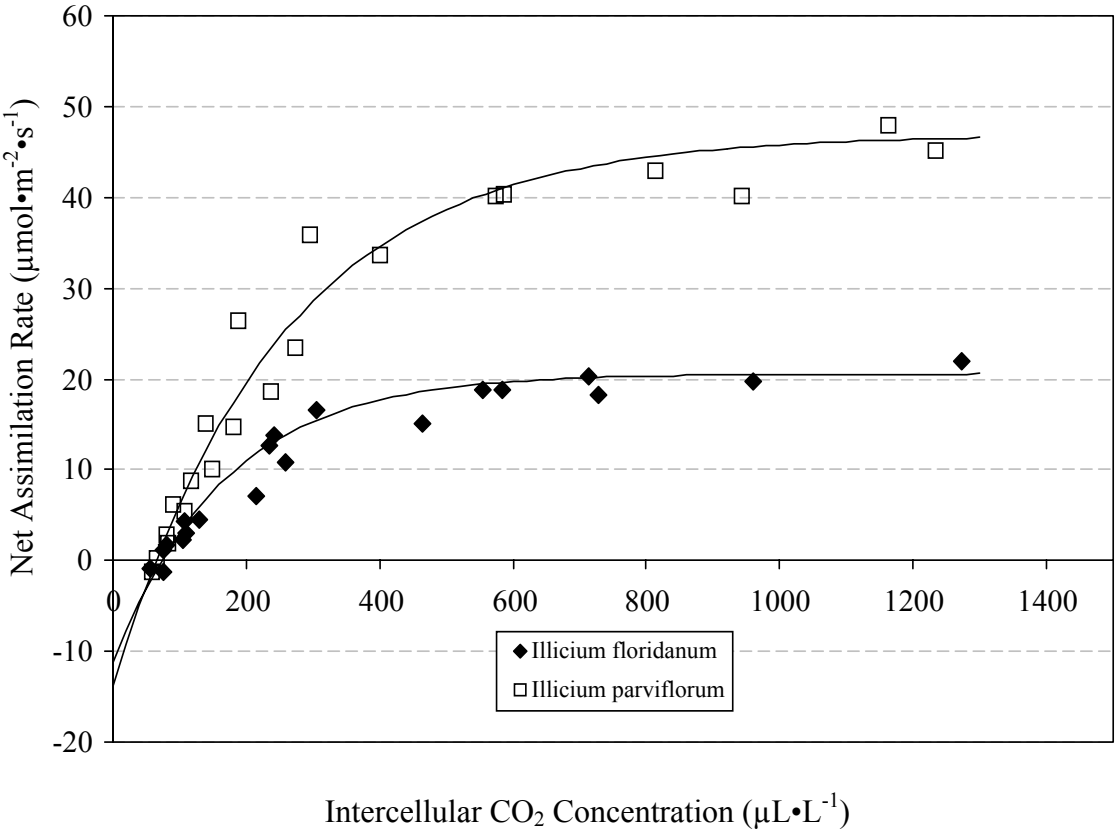

Figure 2. A/Ci curves for *I. floridanum* and *I. parviflorum* 'Forest Green'. Data averaged over light environment within a species during growth and fit to the model proposed by Jacob et al., (1995). Leaf temperature = 30 °C, irradiance = 1000 µmol•m^{-2}•s^{-1}.

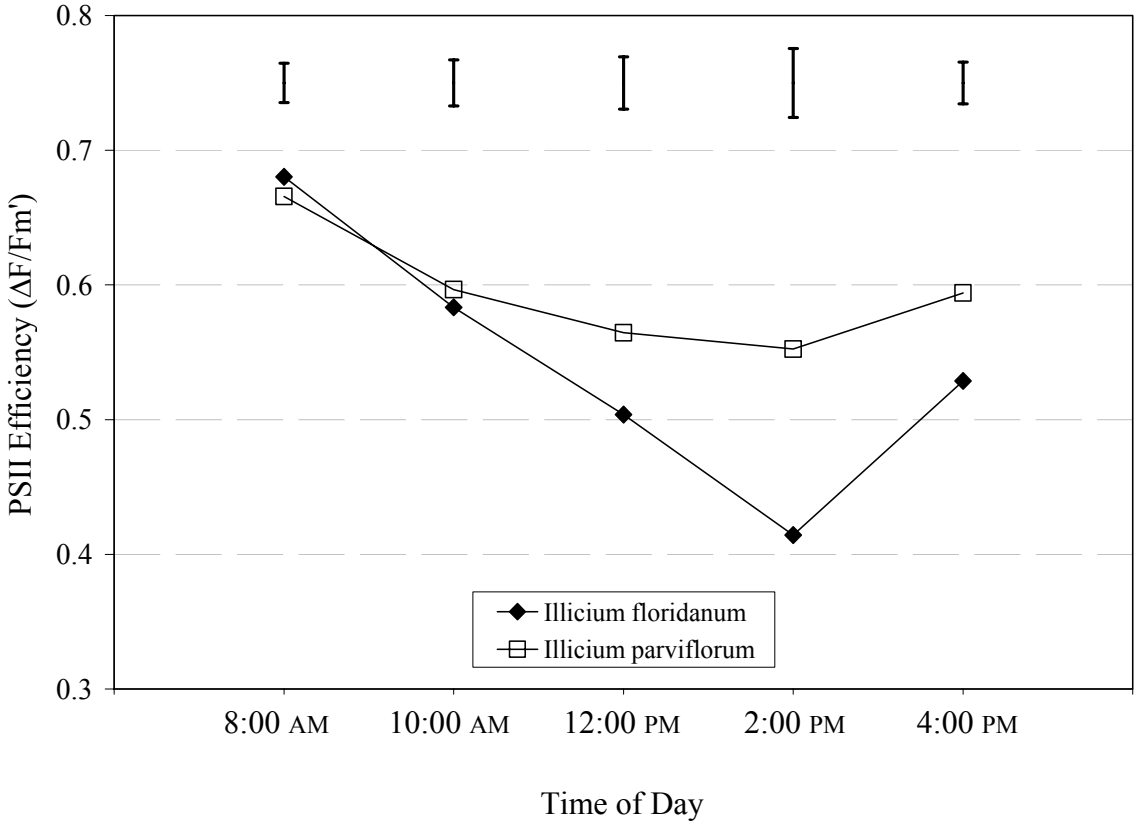

Figure 3. Actual PSII efficiency (ΔF/Fm') of *I. floridanum* and *I. parviflorum* 'Forest Green' during the course of a sunny day. Data averaged over light environment during growth within a taxon. Error bars = ±1 standard error of the mean of all data collected at that time, n = 12.

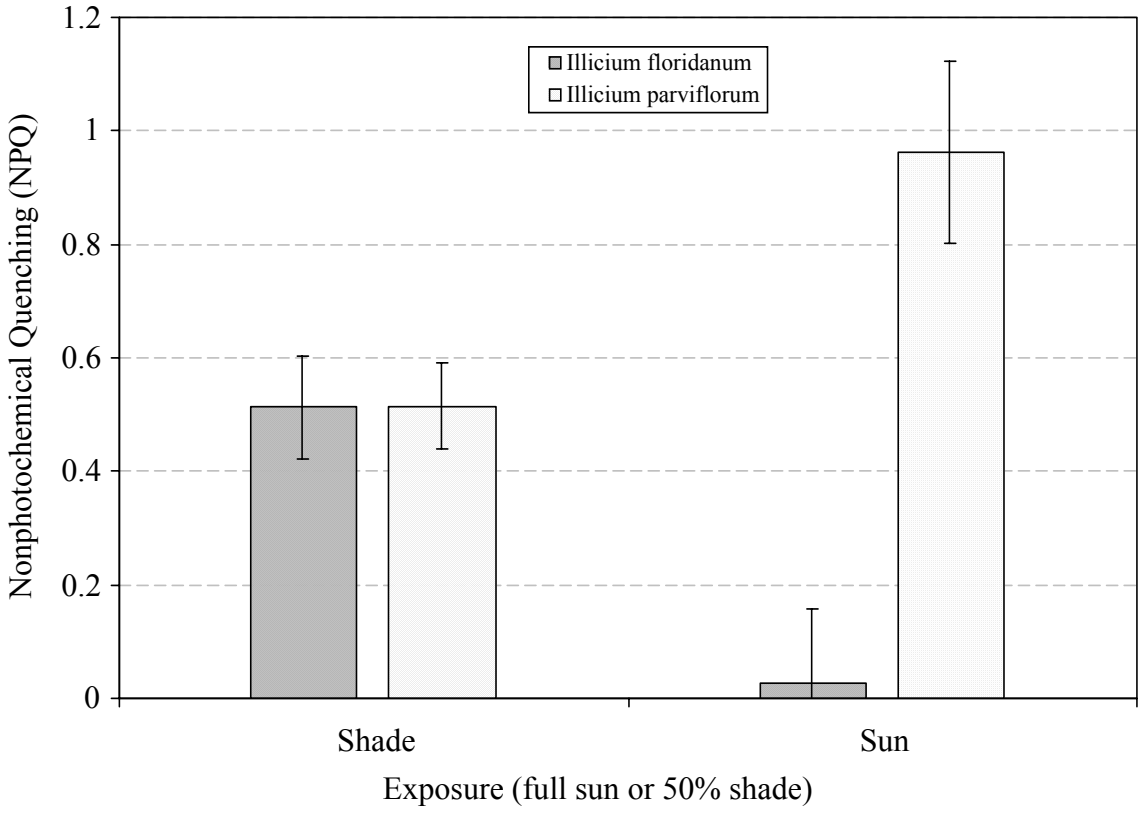

Figure 4. Nonphotochemical quenching of *I. floridanum* and *I. parviflorum* 'Forest Green' averaged over the course of a day. Error bars = ±1 standard error of the mean, n = 30.

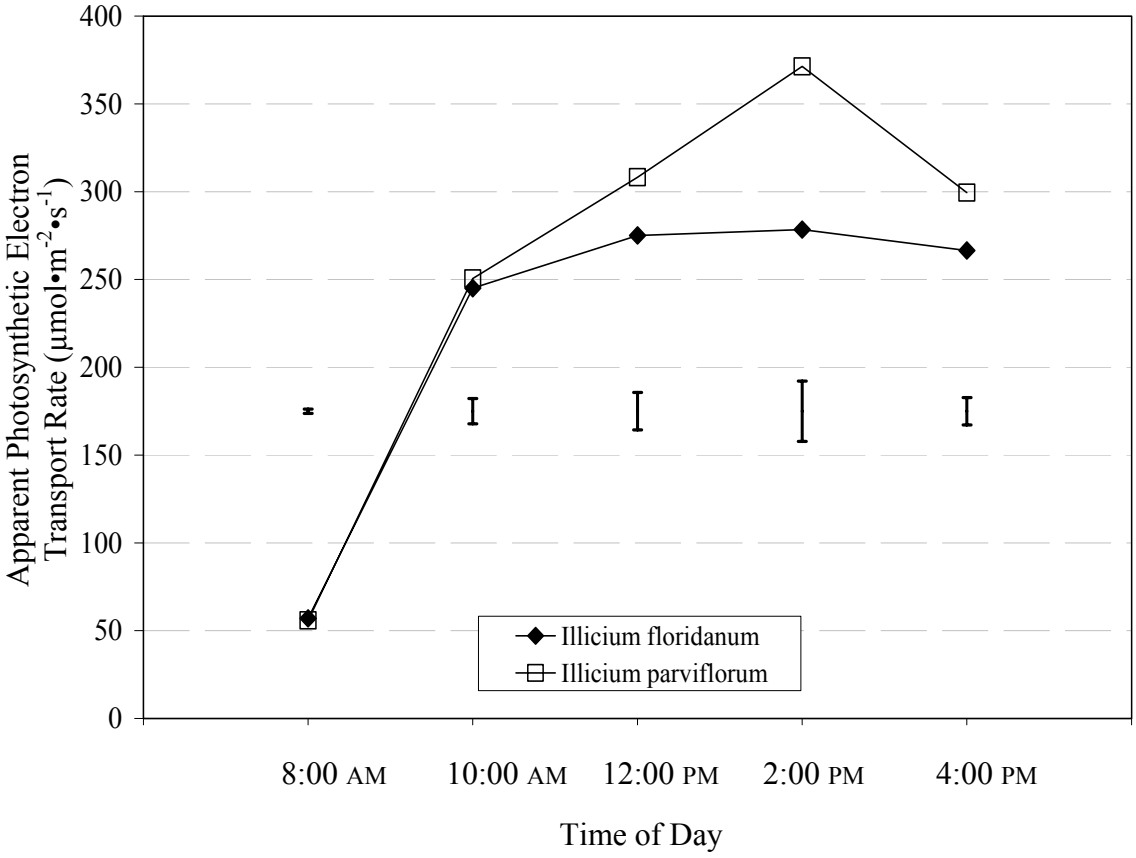

Figure 5. Apparent photosynthetic electron transport rate (ETR) of *I. floridanum* and *I. parviflorum* 'Forest Green'. Data averaged over light environment during growth. Error bars = ±1 standard error of the mean of all data collected at that time, n = 12.