

Diurnal regulation of photosynthesis in understory saplings

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SUMMARY

Photosynthetic rates of plants grown in natural systems exhibit diurnal patterns often characterized by an afternoon decline, even when measured under constant light and temperature conditions. Since we thought changes in the carbohydrate status could cause this pattern through feedback from starch and sucrose synthesis, we studied the natural fluctuations in photosynthesis rates of plants grown at 36 and 56 Pa CO₂ at a FACE (free-air-CO₂-enrichment) research site. Light-saturated photosynthesis varied by 40% during the day and was independent of the light-limited quantum yield of photosynthesis, which varied little through the day. Photosynthesis did not correspond with xylem water potential or leaf carbohydrate build-up, but rather with diurnal changes in air vapor-pressure deficit and light. The afternoon decline in photosynthesis also corresponded with decreased stomatal conductance and decreased Rubisco carboxylation efficiency which in turn allowed leaf-air-space CO₂ partial pressure to remain constant. Growth at elevated CO₂ did not affect the afternoon decline in photosynthesis, but did stimulate early-morning photosynthesis rates relative to the rest of the day. Plants grown at 56 Pa CO₂ had higher light-limited quantum yields than those at 36 Pa CO₂ but, there was no growth-CO₂ effect on quantum yield when measured at 2 kPa O₂. Therefore, understory plants have a high light-limited quantum yield that does not vary through the day. Thus, the major diurnal changes in photosynthesis occur under light-saturated conditions which may help understory saplings maximize their sunfleck-use-efficiency.

Key words: photosynthesis, diurnal variation, elevated CO₂, FACE, understory, *Acer rubrum*, *Liquidambar styraciflua*, *Cercis canadensis*.

INTRODUCTION

Under natural conditions, photosynthesis is biochemically regulated to maintain a balance between the rates of its component processes and the concentrations of metabolites (Geiger & Servaites, 1994) while environmental variables such as sunlight, water availability, and atmospheric conditions continuously change. Regulatory mechanisms can respond either to environmental stimuli (exogenous factors) or to biochemical limitations of the mesophyll cells (endogenous factors). Photosynthesis responds to exogenous controls such as excess photon flux density (PFD) (Correia *et al.*, 1990; Ögren & Sjöström, 1990; Watling *et al.*, 1997), drought (Valentini *et al.*, 1995; Basu *et al.*, 1998), and leaf-air vapor pressure difference (VPD; Tenhunen *et al.*, 1984; Raschke & Resemann, 1986; Pathre *et al.*, 1998), and to endogenous controls such

as feedback limitation by end-product synthesis (Goldschmidt & Huber, 1992; Pammenter *et al.*, 1993; Büssis *et al.*, 1997; Greer, 1998). These controls are not independent. For example, photosynthesis responds to changes in leaf-air VPD by depressing the biochemistry of photosynthesis in the mesophyll cells (Sharkey, 1984; Wise *et al.*, 1991), and to excess PFD, by decreasing the efficiency of electron transport (Ögren & Sjöström, 1990; Watling *et al.*, 1997), an endogenous process. In this way, interactions between exogenous and endogenous factors determine the diurnal pattern of photosynthesis.

This regulation is important for plants growing in a forest understory, where periods of non-saturating PFD are punctuated by sunflecks that can provide nearly full sunlight. This condition presents a problem and an opportunity for photosynthesis in the understory. If sunfleck PFD is greater than the photosystems can use, nonphotochemical quenching reduces the quantum yield of photosynthesis

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(Watling *et al.*, 1997). On the other hand, plants that can rapidly induce photosynthesis during a sunfleck can make optimal use of the high PFD. The efficiency with which sunflecks are used is important in determining the daily carbon gain of plants in understory environments (Sims & Pearcy, 1993; Pearcy & Yang, 1998). Therefore, the diurnal regulation of photosynthesis can potentially benefit plants most by increasing their sunfleck-use-efficiency.

Photosynthesis is also sensitive to changes in atmospheric CO₂ partial pressure. Elevated CO₂ effects on photosynthesis are well documented, and often include increased maximum photosynthesis rate, reduced leaf Rubisco content, reduced chlorophyll content, increased leaf carbohydrate content, increased quantum yield, and increased water-use and light-use efficiencies (Van Oosten *et al.*, 1994; Drake & Gonzalez-Meler, 1996; Nakano *et al.*, 1997; Osborne *et al.*, 1997; Curtis & Wang, 1998; Theobald *et al.*, 1998; Würth *et al.*, 1998). Many of these effects are related; for example the reduced expression of photosynthesis-related genes is signalled by a build-up of carbohydrates resulting from increased photosynthesis rates at elevated CO₂ (Goldschmidt & Huber, 1992; Van Oosten *et al.*, 1994; Van Oosten & Besford, 1996; Büssis *et al.*, 1997; Würth *et al.*, 1998). It has been suggested that a similar mechanism can influence the diurnal pattern of photosynthesis through sink limitations of photosynthesis when carbohydrates build up in the mesophyll cells (Sinha *et al.*, 1997; Greer, 1998).

Our objective was to determine what endogenous and exogenous factors control photosynthesis in understory hardwood trees. We used saplings grown outdoors in the forest-atmosphere-carbon-transfer-and-storage 1 (FACTS-1) research site, where an intact pine forest is fumigated with CO₂ using free-air-CO₂-enrichment (FACE) technology to elevate the atmospheric CO₂ concentration by 20 Pa. We expected plants grown at elevated CO₂ to accumulate more carbohydrates throughout the day than those at ambient CO₂ (Rey & Jarvis, 1998; Würth *et al.*, 1998). Thus the CO₂ treatment served as a manipulation of the carbohydrate status of the leaves, which allowed us to investigate endogenous control via photosynthetic end-product build-up. To examine the endogenous and exogenous controls of photosynthesis, we measured leaf gas-exchange, chlorophyll fluorescence and leaf water potential, and leaf carbohydrate levels repeatedly throughout the day.

MATERIALS AND METHODS

Study site

Measurements were made on understory saplings at the FACTS-I research site in Chapel Hill, NC,

USA (35E 97' N, 79E 09' W). At this site, three 30-m-diameter plots were continuously fumigated to raise the atmospheric CO₂ concentration to 20 Pa above current ambient levels (Hendrey & Kimball, 1994; Lewin *et al.*, 1994; Hendrey *et al.*, 1999). Three additional fully-instrumented rings were fumigated with compressed air as controls. Fumigation was initiated in August 1996, and all measurements were made in June and August 1998.

The plots were established in a loblolly pine plantation which has been unmanaged since its planting in 1983. We selected three deciduous species that had established naturally in the understory: sweetgum (*Liquidambar styraciflua* L., 811 stems ha⁻¹), eastern redbud (*Cercis canadensis* L., 160 stems ha⁻¹), and red maple (*Acer rubrum* L., 170 stems ha⁻¹). Subject trees were <3 m tall.

Gas-exchange

Leaves of five individuals of each species were tagged at the beginning of each day, and the photosynthetic light-response of each leaf was measured five times during the day (every 2 h beginning at 08.00 hours). Each light-response-curve took <15 min to complete, and there was no evidence of previous measurements affecting the light-response of a leaf (E. H. DeLucia, unpublished). Measurements were made on six consecutive days in August 1998 which had similar weather conditions and were uninterrupted by rain. Photosynthetic CO₂ response (A–C_i curves) were measured on sweetgum trees three times daily (08.00, 13.00 and 16.00 hours).

Photosynthetic measurements were made using an open gas-exchange system (model LI-6400; LICOR Inc., Lincoln, NE, USA). Leaves were clamped in the standard 6 cm² cuvette. Light was provided by a red-blue light emitting diode (LED) array, and ambient CO₂ partial pressure (C_a) was maintained during measurement at 37 Pa for leaves measured in control plots and 57 Pa for leaves measured in treatment plots. Data were logged at 14 light levels between 800 and 0 μmol m⁻² s⁻¹ PFD, and the leaf was given between 45 and 300 s to stabilize. Leaves usually stabilized within 60 s. Leaf temperature was maintained at 25°C and the dew-point in the gas-exchange cuvette was maintained between 16 and 20°C which maintained a constant leaf-air VPD during measurements. Low-oxygen measurements were made by providing a mixture of 98% N₂ + 2% O₂ to the inlet port of the system, and controlling C_a to 60 Pa.

The maximum quantum yield of CO₂ fixation was calculated by regression of the linear region of the light-response curves (3–6 points; usually at PFD <60 μmol m⁻² s⁻¹) on an incident-light basis. The maximum rate of RuBP regeneration (J_{max}) and of Rubisco carboxylation (V_{c,max}) were calculated by fitting A–C_i curve data (eight points per curve) to the

equations of Farquhar *et al.* (1980) by non-linear least-squares regression as in Harley & Tenhunen (1991). Model parameters were from Harley & Baldocchi (1995).

Chlorophyll fluorescence

Chlorophyll fluorescence was measured following each gas-exchange measurement using a portable pulse-modulated fluorometer (model PAM-2000, Heinz Walz GmbH, Effeltrich, Germany). Maximum photochemical efficiency of PSII (F_v/F_m) was measured in leaves that had been dark adapted for 30 min.

Xylem pressure potential

Pressure potential (Ψ) was measured with a pressure bomb (model 3005, Soilmoisture Equipment Corp., Santa Barbara, CA, USA) from five trees of each species in each ring. Measurements coincided with gas-exchange and fluorescence measurements.

Leaf carbohydrate analysis

Ten 1.05-cm² punches (14–20 mg d. wt) were collected from leaves adjacent to those used for gas-exchange and chlorophyll fluorescence measurements. Samples were dried at 70°C for 3 d, and analyzed for non-structural carbohydrates as described in Tissue & Wright (1995).

Micrometeorology

The temporal variability of meteorological parameters was measured at a single site near the center of a treatment and a control plot. PFD, CO₂, and water vapor concentration were sampled at 1-min intervals. Total PFD is defined as the sum of diffuse and direct PFD and was measured with a gallium-arsenide photodiode (Hamamatsu Corp., Middlesex, NJ, USA), which was cosine-corrected with a small teflon diffusion-disc and calibrated under sunlight against a quantum sensor (model LI-185, LI-COR, Inc.). Diffuse PFD was measured with a photodiode as already described with a shadow band mounted 6 cm from the surface. To measure CO₂ (data not reported) and water vapor concentrations, ambient air was drawn by a small pump through a 1.5 m piece of tubing to an IRGA (model 6262, LI-COR, Inc.). All sensors and gas inlets were mounted 1 m above the forest floor. The 5 V outputs from the IRGA and the bridged photodiode output were recorded on a data logger (model CR21x, Campbell Scientific Inc., Logan, UT, USA). Ambient air temperature was logged by the FACTS-I site control system.

Statistical analysis

Diurnal data were analyzed using repeated-measures ANOVA (CO₂ × time of day; Potvin & Lechowicz,

1990). Treatment comparisons of light-saturated photosynthesis rates and quantum yields were made using nested ANOVA where plots are nested within a treatment (elevated CO₂ or control). Linear trends were compared using ANCOVA as described in Sokal & Rohlf (1995). Statistics were calculated using SYSTAT (SPSS Inc., Chicago, IL, USA). Probability values ≤ 0.05 were considered significant.

Individual saplings in the same plot were considered statistically independent because the spatial variation in CO₂ within each plot was greater than the variation between plots (K. F. Lewin, unpublished). Since *C. canadensis* and *A. rubrum* saplings did not occur in all plots, five individuals were sampled in each of two plots (one at 36 Pa and one at 56 Pa).

RESULTS

Micrometeorology

The environmental conditions in both plots were similar, so data from a representative day in an elevated CO₂ plot are shown (Fig. 1). PFD averaged 121 μmol m⁻² s⁻¹ with a median of 68 μmol m⁻² s⁻¹ between 08.00 and 17.00 hours, and ranged from 18 to 1470 μmol m⁻² s⁻¹. One quarter of the readings were > 60 μmol m⁻² s⁻¹ but these provided 75 % of the total daily photon flux. Diffuse PFD during the middle of the day was more normally distributed with a mean of 28.7 and a median of 29.6, ranging from 4.8 to 61.1 μmol m⁻² s⁻¹. Vapor pressure deficit (VPD) exhibited a strong diurnal variation, climbing from near zero in the morning to 1.7 kPa during the middle of the day. The peak in VPD occurred after 16.00 hours which coincided with the maximum daily air temperature.

CO₂ effects on light-limited and light-saturated photosynthesis

To assess whether growth under CO₂ enrichment affected the photosynthetic response to PFD, we measured the light-limited quantum yield ($\Phi_{CO_2,app}$) and the light-saturated rate of CO₂ assimilation on *L. styraciflua* trees between 10.00 and 15.00 hours (Table 1). When measured at the same C_a, $\Phi_{CO_2,app}$ did not differ between trees grown at elevated and ambient CO₂ ($P = 0.654$, $n = 18$). However, at their respective growth CO₂ concentration, plants at 56 Pa CO₂ had 20 % greater quantum yields (0.066 vs 0.055; $P < 0.001$). Light-saturated rates of photosynthesis were 30 % higher for plants grown at elevated CO₂ when measured at the same CO₂ conditions as control plants ($P = 0.043$, $n = 18$). Comparisons of plants at their respective growth CO₂ shows a 100 % increase in light-saturated

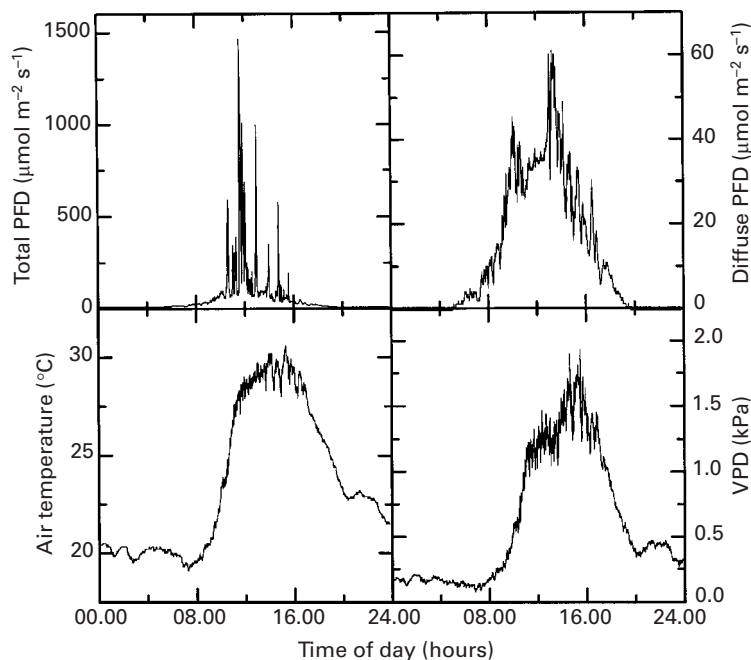


Fig. 1. Diurnal variations in diffuse and total (diffuse + direct) irradiance (PFD), air temperature, and vapor pressure deficit (VPD) in the understory at the FACTS-I research site. Data points were collected once per minute on 11 June 1998 and were not averaged or smoothed.

Table 1. Apparent quantum yield ($\Phi_{CO_2,app}$) and light-saturated photosynthesis rates (A_{sat}) of understory Liquidambar styraciflua trees grown at ambient (36 Pa) and elevated (56 Pa) CO_2

Measurement conditions	$\Phi_{CO_2,app}$		A_{sat}	
	Grown at ambient CO_2	Grown at elevated CO_2	Grown at ambient CO_2	Grown at elevated CO_2
36 Pa CO_2	0.055 ± 0.001	0.057 ± 0.002	3.9 ± 0.43	5.1 ± 0.42
56 Pa CO_2	0.066 ± 0.002	0.066 ± 0.002	6.0 ± 0.62	7.8 ± 0.61

Measurements were made at 21 kPa O_2 and either 36 or 56 Pa CO_2 . Means \pm SE are presented.

Table 2. Quantum yield of understory Liquidambar styraciflua, Cercis canadensis and Acer rubrum saplings at the FACTS-I research site

Species	21 kPa O_2		2 kPa O_2	
	36 Pa CO_2	56 Pa CO_2	36 Pa CO_2	56 Pa CO_2
<i>Liquidambar styraciflua</i>	0.060 ± 0.002	0.067 ± 0.002	0.090 ± 0.003	0.092 ± 0.003
<i>Cercis canadensis</i>	0.063 ± 0.001	0.072 ± 0.002	0.093 ± 0.002	0.098 ± 0.003
<i>Acer rubrum</i>	0.055 ± 0.002	0.066 ± 0.002	0.088 ± 0.003	0.090 ± 0.005

Measurements were made at ambient CO_2 and O_2 partial pressures (ambient) or at 2 kPa O_2 and 60 Pa CO_2 (low O_2). All measurements were made in June 1998. Data are reported as means \pm SE.

photosynthesis (7.8 vs $3.9 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$; $P < 0.001$) for elevated CO_2 grown plants.

To assess whether acclimation to elevated CO_2 had occurred in all three species under investigation, we measured the light-limited quantum yield on all species at their growth CO_2 with 21 kPa O_2 and at 60 Pa CO_2 with 2 kPa O_2 (Table 2). When measured at their ambient CO_2 and O_2 , quantum yields were

between 10 and 20% higher at elevated CO_2 for all three species ($P < 0.001$, $n = 36$). When we suppressed photorespiration by making measurements at 2 kPa O_2 , we detected no difference in light-limited quantum yield between plants grown at ambient and elevated CO_2 ($P = 0.136$, $n = 36$). Therefore, we found no evidence for acclimation of light-limited quantum yield to elevated CO_2 .

Diurnal variance in photosynthesis

We measured the photosynthetic PFD-response repeatedly to investigate the diurnal patterns in photosynthesis (Fig. 2). There was no change in light response in the light-limited region (i.e. $< 100 \mu\text{mol m}^{-2} \text{s}^{-1}$). All five lines diverged in the light-saturated region of photosynthesis. The light-saturated rate of photosynthesis was low at 08.00 hours, and increased until 12.00 hours, at which point it decreased throughout the afternoon. This pattern is similar to that for stomatal conductance. Photosynthesis did not, however, appear to be limited by C_i since the low rate of photosynthesis at 08.00 hours occurred at a C_i similar to the midday values. The decline in C_i in these data is atypical as there was no significant time of day effect on C_i (see below). The coefficient of variation in light-saturated photosynthesis was between 27 and 44%. We detected no differences

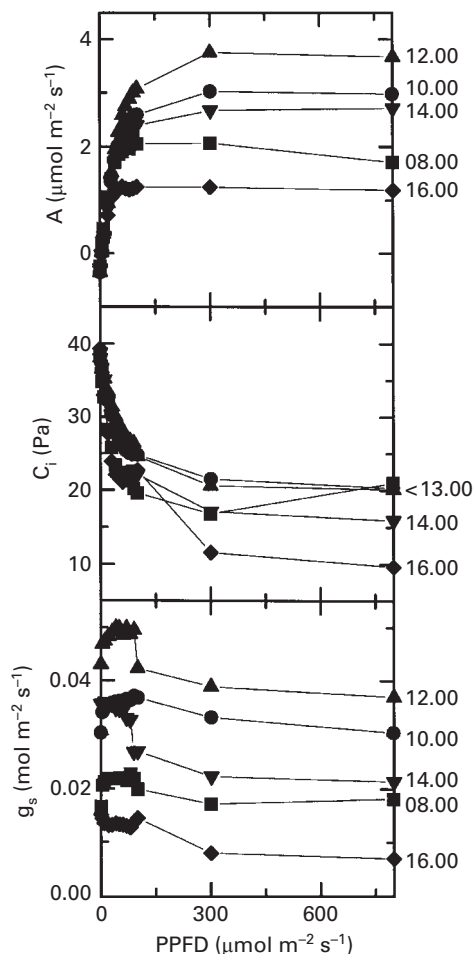


Fig. 2. Light-response of photosynthesis (A), leaf-airspace CO_2 partial pressure (C_i) and stomatal conductance (g_s) at five times during the day. Values were calculated from gas-exchange measurements made on a single *Liquidambar styraciflua* leaf at five times during the day. Data were collected on 18 June 1998. Symbols represent curves measured at different times of day and numbers to the right specify the time at which each curve was measured.

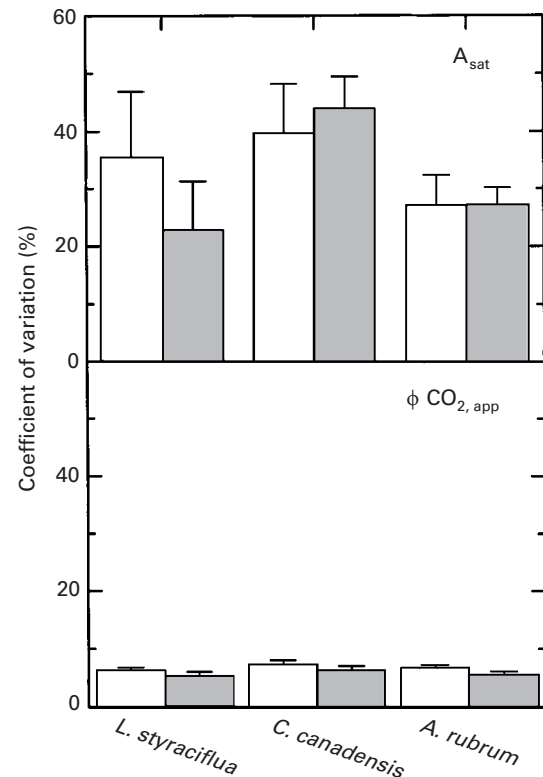


Fig. 3. Coefficient of variation in light-saturated- and light-limited- photosynthesis. Gas-exchange measurements were made on three species grown in two CO_2 treatments (open bars, 36 Pa; shaded bars, 56 Pa CO_2). The light-saturated rate of photosynthesis (A_{sat}) was measured at $800 \text{ mol m}^{-2} \text{ s}^{-1}$ PFD, and quantum yield (ΦCO_2) was calculated as described in the Materials and Methods section. All measurements were made from 10–20 June, 1998. Bars represent means \pm SE ($n = 5$).

between the CO_2 treatments (Fig. 3; $P = 0.62$, $n = 24$). The coefficient of variation in quantum yield averaged 7%, and there was no detectable CO_2 effect ($P = 0.11$, $n = 24$).

Diurnal patterns in light-saturated photosynthesis

Photosynthesis varied diurnally in all three species (Fig. 4; $P \leq 0.001$ for all three species). In most cases, the highest rates occurred at noon and declined thereafter. Photosynthesis was higher at 08.00 hours, relative to the rest of the day, for plants in elevated rather than ambient CO_2 . Photosynthesis rates did not correspond with the diurnal changes in leaf water potential. Water potential was generally highest at 08.00 hours and declined through the day, but the decline was large only in *C. canadensis*. There was no CO_2 effect on leaf water potentials during most of the day, except that in *L. styraciflua* and *C. canadensis* leaf water potentials were slightly lower in plants grown at ambient CO_2 during the afternoon (time of day by treatment interaction; $P = 0.007$).

Diurnal variation in stomatal conductance (Fig. 5) corresponded to some extent with photosynthesis

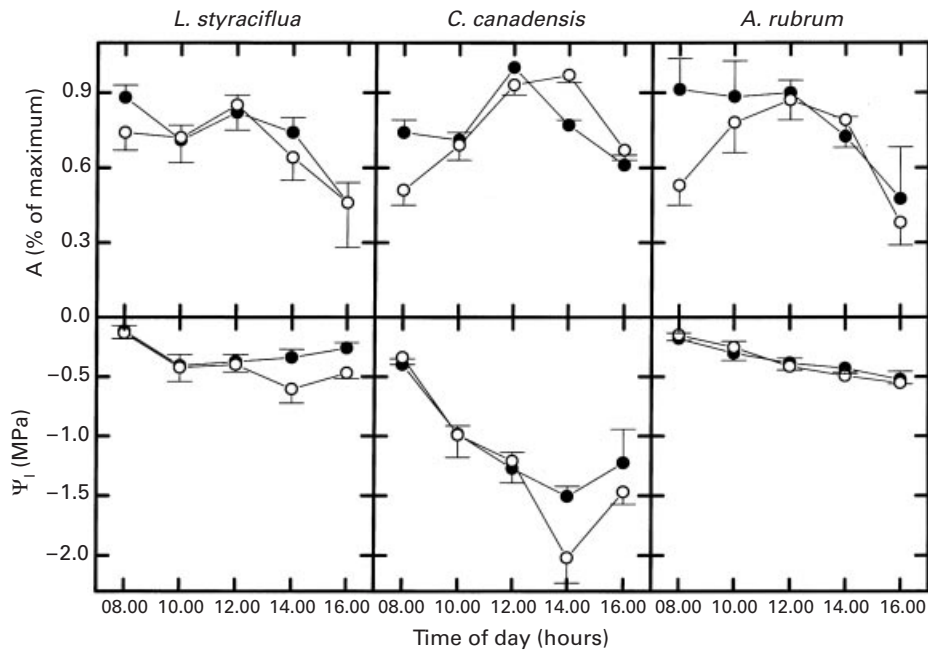


Fig. 4. Diurnal patterns of photosynthesis (A) and leaf water potential (Ψ) in three species of understory seedlings. Gas-exchange measurements were made repeatedly on each leaf as described in the Materials and Methods section. Water potential measurements were measured from the same trees, but different leaves were used each time. Measurements were made on trees grown at 36 Pa (open circles) and 56 Pa (closed circles) CO_2 . Data points represent means \pm SE ($n = 10$ for *Liquidambar styraciflua*, $n = 5$ for *Cercis canadensis* and *Acer rubrum*).

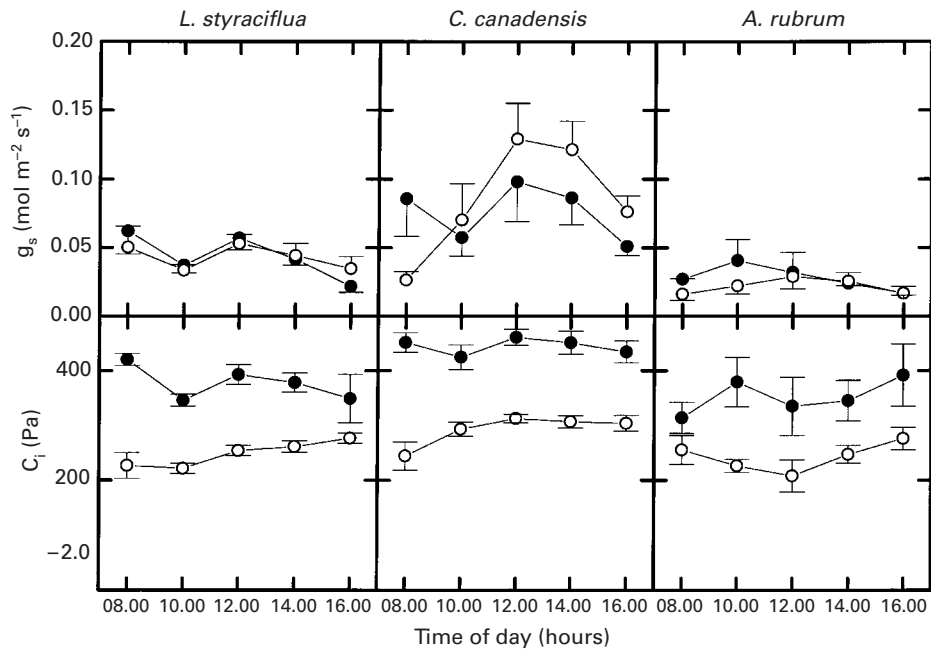


Fig. 5. Diurnal patterns of stomatal conductance (g_s) and leaf-airspace CO_2 partial pressure (C_i). Gas-exchange measurements were made repeatedly on each leaf as described in the Materials and Methods section. Measurements were made on trees grown at 36 Pa (open circles) and 56 Pa (closed circles) CO_2 . Data points represent means \pm SE ($n = 10$ for *Liquidambar styraciflua*, $n = 5$ for *Cercis canadensis* and *Acer rubrum*).

(Fig. 4). The variation was significant for *L. styraciflua* and *C. canadensis* ($P = 0.016$, $n = 18$; $P < 0.001$, $n = 10$) but not for *A. rubrum* ($P = 0.584$, $n = 7$). The co-varying of photosynthesis and stomatal conductance resulted in C_i remaining relatively constant throughout the day for all three

species (*L. styraciflua*: $P = 0.06$, $n = 18$; *C. canadensis*: $P = 0.498$, $n = 10$; *A. rubrum*: $P = 0.380$, $n = 7$). Because of its higher stomatal conductance, C_i was higher in *C. canadensis* than in the other two species.

To investigate the potential involvement of photo-

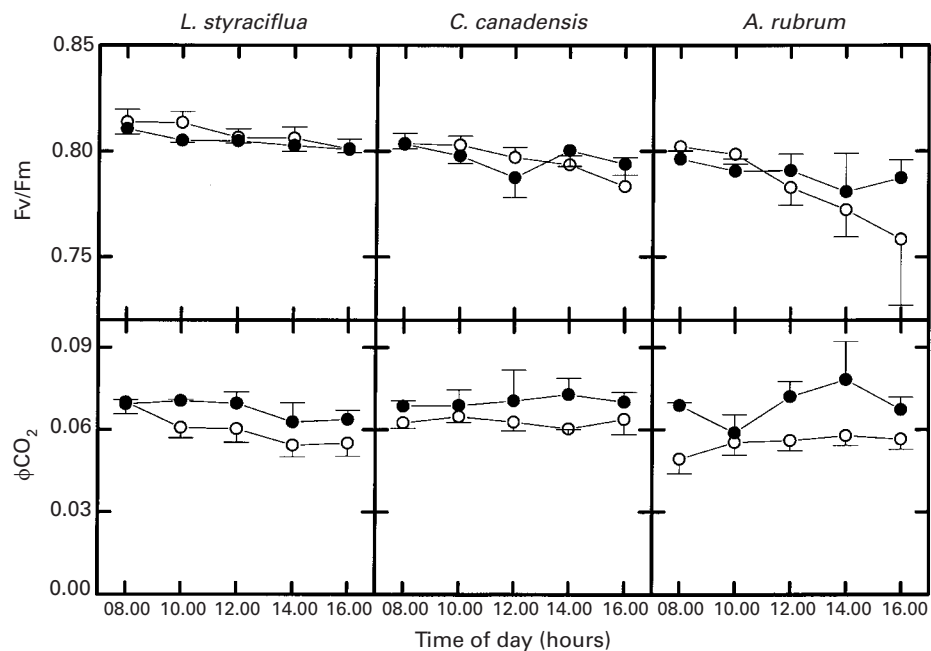


Fig. 6. Diurnal patterns in maximum photochemical yield (F_v/F_m) and the maximum light-limited quantum yield of CO_2 fixation (ΦCO_2). F_v/F_m was measured repeatedly on leaves after a 30 min dark acclimation. ΦCO_2 was calculated from the initial slope of photosynthesis vs PFD response curves measured by gas exchange. Measurements were made on trees grown at 36 Pa (open circles) and 56 Pa (closed circles) CO_2 . Data points represent means \pm SE ($n = 4$ for all species).

inhibition in the diurnal pattern of photosynthesis, we measured the maximum photochemical efficiency of PSII, F_v/F_m , and the maximum light-limited quantum yield of CO_2 fixation, ΦCO_2 (Fig. 6). Although F_v/F_m declined steadily throughout the day in two of the three species (*L. styraciflua*: $P < 0.001$, $n = 7$; *C. canadensis*: $P = 0.014$, $n = 8$), the changes were relatively small. There was no detectable decline in *A. rubrum* ($P = 0.098$, $n = 7$). Light limited quantum yield did not vary diurnally in *A. rubrum* or *C. canadensis* (*C. canadensis*: $P = 0.404$, $n = 8$; *A. rubrum*: $P = 0.215$, $n = 7$). The statistically significant decline in ΦCO_2 in *L. styraciflua* ($P < 0.001$, $n = 7$) was $< 10\%$ at elevated CO_2 and $< 20\%$ at ambient CO_2 . In all cases, declines were linear throughout the day.

To investigate the relationship between photosynthesis and Rubisco kinetics throughout the day, we calculated $V_{c_{\max}}$, which estimates the total activity of Rubisco, and J_{\max} , which estimates the maximum electron transport rate, using A- C_i curves collected from *L. styraciflua*. $V_{c_{\max}}$ decreased throughout the day (Fig. 7; $P = 0.01$, $n = 8$), and there was a significant afternoon decline in J_{\max} ($P = 0.002$, $n = 8$).

Diurnal patterns in leaf carbohydrate content

To determine whether the build-up of photosynthetic end products in the mesophyll can influence the patterns in photosynthesis rates, we measured soluble and insoluble leaf carbohydrates

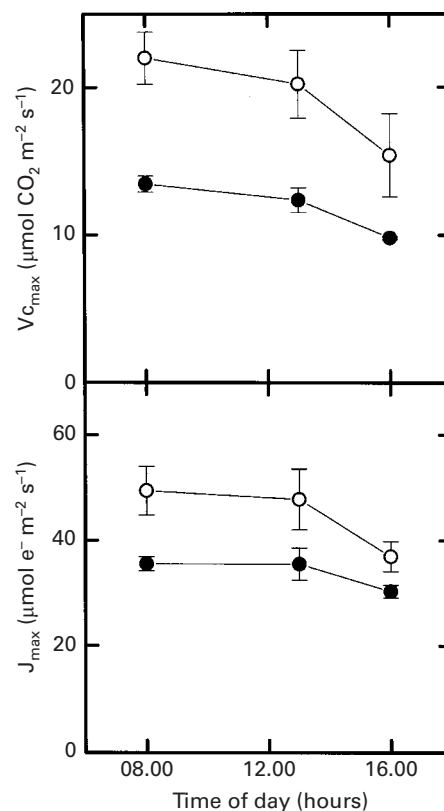


Fig. 7. Diurnal patterns in maximum carboxylation rate ($V_{c_{\max}}$) and maximum electron transport rate (J_{\max}) estimated from CO_2 -response (A- C_i) curves. Gas-exchange measurements were made repeatedly on leaves of *Liquidambar styraciflua* growing at 36 Pa (open circles) and 56 Pa (closed circles) CO_2 . Parameters were estimated from gas-exchange measurements as described in the Materials and Methods section. Data points represent means \pm SE ($n = 5$).

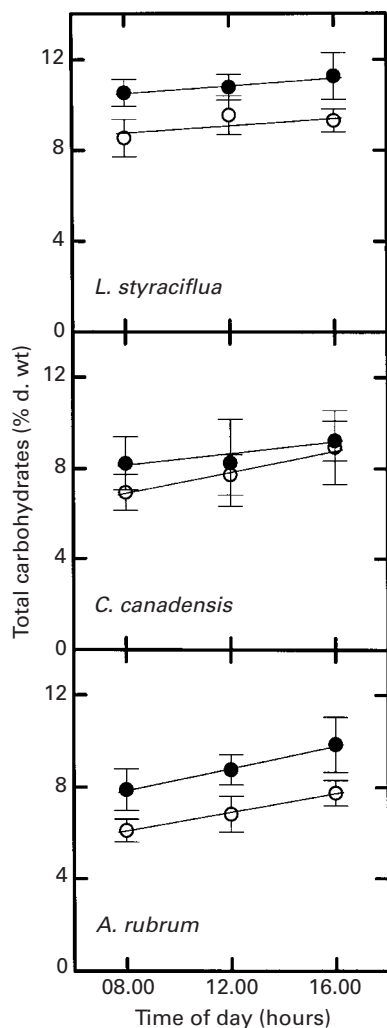


Fig. 8. Diurnal accumulation of nonstructural carbohydrates in leaves of saplings growing at 36 Pa (open circles) and 56 Pa (closed circles) CO₂. Leaf punches were collected three times per day and analyzed for carbohydrates. Data points represent means \pm SE. Linear regression was used to fit lines.

(Fig. 8). Leaves grown at elevated CO₂ contained more total carbohydrates than controls in *L. styraciflua* ($P = 0.007$, $n = 24$) and *A. rubrum* ($P = 0.004$, $n = 24$), but not *C. canadensis* ($P = 0.482$, $n = 24$). Although there was significant diurnal accumulation of carbohydrates in *A. rubrum* leaves ($P = 0.024$), we detected no difference in the slopes of the lines ($P > 0.6$), indicating that there was no difference in accumulation rate between elevated CO₂ and control plants.

DISCUSSION

Light-saturated photosynthesis rates in understory saplings varied by as much as 40% throughout the day. This percentage change is similar to plants grown in full sunlight (Correia *et al.*, 1990; Wise *et al.*, 1991; Barton & Gleeson, 1996; Sinha *et al.*, 1997; Greer, 1998; Pathre *et al.*, 1998). However, the diurnal pattern of photosynthesis (low in the

morning and afternoon, with maximum rates at noon) was only seen in one other study to our knowledge (Barton & Gleeson, 1996), with most showing a decline between 12.00 and 14.00 hours. In this study, low photosynthetic rates coincided with decreasing Rubisco carboxylation efficiency and electron transport rates (Fig. 7), both of which are associated with the afternoon decline in photosynthesis (Raschke & Resemann, 1986; Wise *et al.*, 1991; Geiger & Servaites, 1994; Greer, 1998; Tenhunen *et al.*, 1984).

These results indicate that care must be taken when interpreting photosynthesis measurements made in the field. Light-saturated photosynthesis rates can vary as much as 40–60% during the day. This is substantially larger than the 30% variation in light-saturated photosynthesis attributable to the CO₂ treatment (e.g. Table 1). This variation can be caused by several factors; including stomatal conductance, mesophyll carbon reduction capacity, and electron transport capacity, all of which can be influenced by environmental factors such as light and VPD. Gas-exchange measurements by themselves do not provide enough information to deduce which, if any, of these mechanisms is controlling photosynthesis. Some or all of these factors may explain the apparent discrepancy between the apparent upregulation of light-saturated photosynthesis at elevated CO₂ (Table 1) and the apparent decrease in $V_{c_{max}}$ and J_{max} (Fig. 7). The low A_{max} in the ambient CO₂ rings is associated with low stomatal conductance which corresponds with low mesophyll capacity for photosynthesis. Thus the apparent CO₂ effect on A_{max} may be attributed to diurnal regulation rather than acclimation to elevated CO₂. According to our results, comparisons between treatments will be most accurate when data are collected during the middle hours of the day (between approx. 10.00 and 15.00 hours), when light-saturated photosynthesis rates are relatively constant. However, it is likely that this period is different for each field site, time of year and species, and must be evaluated on a case-by-case basis.

While the overall pattern of photosynthesis throughout the day was not significantly affected by the CO₂ treatment, there was a stimulation of early morning photosynthetic rates at elevated CO₂ in all three species (Fig. 4). In *C. canadensis*, this pattern appears to be associated with elevated stomatal conductance and slightly elevated C_i (Fig. 5), but this is not evident in the other two species. Likewise, our fluorescence data and $A-C_i$ curves do not provide any insight into this phenomenon. This phenomenon may be potentially important in understanding what controls daily carbon gain in an elevated CO₂ environment and further investigations in this area are warranted.

While there was significant diurnal variation in light-saturated photosynthesis, there was little vari-

ation in light-limited photosynthesis. The coefficient of variation in light-limited ΦCO_2 was *c.* 7% in all species. The slight diurnal decline in Fv/Fm suggests that increases throughout the day, as has been suggested in willow canopies (Ögren & Sjöstrom, 1990). However, the declines were small compared with those in the willow studies or in studies demonstrating photoinhibition in rainforest understory species (Watling *et al.*, 1997). We conclude that the changes in Fv/Fm and $\Phi\text{CO}_{2,\text{app}}$ contributed only marginally to the diurnal patterns in photosynthesis.

We calculated the light-limited quantum yield of photosynthesis ($\Phi\text{CO}_{2,\text{app}}$) in the different CO_2 treatments. The only difference we found was caused by suppression of photorespiration during the measurement. This is demonstrated by the lack of detectable differences in $\Phi\text{CO}_{2,\text{app}}$ between control and treatment plants when measured under the same CO_2 (Table 1) or low O_2 (Table 2) conditions. This is consistent with expectations that elevated CO_2 reduces photorespiration during the measurements without long-term acclimation of ΦCO_2 (Osborne *et al.*, 1997). Our measurements indicate that $\Phi\text{CO}_{2,\text{app}}$ is quite high for these plants. Assuming the absorptance (α) of the leaves is near 0.85, increasing ΦCO_2 by *c.* 20% brings ΦCO_2 near the maximum theoretical value of 0.125. Quantum yields this high have been reported in many species in different studies (Demmig & Björkman, 1987; Long *et al.*, 1993). While growth at elevated CO_2 is expected to elicit changes in N investment between carbon reduction and electron transport reactions of photosynthesis (Medlyn, 1996), these would not be detected with measurements of ΦCO_2 because it is, by definition, measured under light-limiting conditions. Since $\Phi\text{CO}_{2,\text{app}}$ is the product of ΦCO_2 and α , its constancy indicates no change in either parameter which is consistent with previous reports (Osborne *et al.*, 1997). Greater investment in electron transport components could increase the range of PFD over which photosynthesis increases linearly. This effect, however, would be masked by the diurnal patterns in stomatal conductance and mesophyll photosynthetic capacity (Figs 2, 5 and 7) making determination of electron transport acclimation difficult.

To test for endogenous control of photosynthesis by carbohydrates, we used the CO_2 enrichment experiment at the FACTS-I site to manipulate the carbohydrate content in leaves of plants growing in a forest. Build-up of carbohydrates can induce changes in the photosynthetic capacity of the mesophyll. Most notably, excess carbohydrates can suppress the expression of photosynthesis-related genes, including Rubisco small subunit which causes a reduction in Rubisco protein (Van Oosten *et al.*, 1994; Büssis *et al.*, 1997). Although this is not likely to play a significant role in the diurnal variation in photo-

synthesis, it is partially responsible for the decreased $V_{c,\text{max}}$ of plants grown at elevated CO_2 (Büsis *et al.*, 1997). Furthermore, excess carbohydrates can influence photosynthesis directly by increasing the probability of feedback on photosynthesis by reducing the rates of sucrose and starch synthesis (Pammeter *et al.*, 1993). Two of the species we studied, *L. styraciflua* and *A. rubrum*, had significantly higher leaf carbohydrate contents at elevated than ambient CO_2 . Because the diurnal patterns in photosynthesis and stomatal conductance are similar between all three species, it is unlikely that leaf carbohydrate content influenced photosynthesis patterns. The expected effect of growth at elevated CO_2 , a decrease in $V_{c,\text{max}}$, occurred in *L. styraciflua* leaves. Thus, photosynthesis was influenced by an endogenous factor, but there was no evidence of this endogenous influence on the diurnal pattern of photosynthesis.

The coincident decline in photosynthesis with the highest VPD suggests that this may be an important signal of the afternoon decline of photosynthesis. Raschke & Resemann (1986) found that increases in VPD throughout the day depressed photosynthetic rates in a Mediterranean shrub. Responses to VPDs may be mediated either by mesophyll sensitivity to the rate of transpiration (Sharkey, 1984) or stomatal closure in response to leaf-to air vapor pressure difference (Yong *et al.*, 1997). In both cases, the VPD response included reductions in the carboxylation efficiency of the mesophyll, an effect commonly associated with the midday decline in photosynthesis (Tenhunen *et al.*, 1984; Wise *et al.*, 1991; Geiger & Servaites, 1994). This explanation is most consistent with our observations. The maximum VPD we observed occurred later in the day and coincided with the afternoon decline in photosynthesis.

Most of the time, light in the understory is diffuse and non-saturating for photosynthesis, the constancy of light-limited photosynthesis indicates that plants assimilate CO_2 slowly and continuously throughout the day. However, sunflecks allowed leaves to be exposed to as much as $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. This could potentially influence the diurnal pattern of photosynthesis. Plants grown in deep understory shade can become photoinhibited if they receive prolonged full PFD, which would be in excess of what they can use (Watling *et al.*, 1997). For example, light stress has been implicated in the afternoon decline in photosynthesis because of an observed drop in light-limited quantum yield during the afternoon (Correia *et al.*, 1990) and a correlation between daily photon interception and photoinhibition (Ögren & Sjöstrom, 1990). Our data indicate that photoinhibition did not play a strong role in determining the diurnal pattern of photosynthesis in our system. The efficiency of photosynthesis, determined by the light-limited quantum

yield of CO₂ assimilation, were high in all three species and remained relatively constant throughout the day. Likewise, Fv/Fm did not decrease substantially, and light-saturated photosynthesis rates were highest at midday when sunflecks were most common in the understory.

On the other hand, sunflecks provided 75 % of the daily PFD, so diurnal changes in sunfleck use efficiency could also influence daily carbon gain (Sims & Pearcy, 1993; Pearcy & Yang, 1998). This is controlled primarily by the induction state of carbon-reduction enzymes such as Rubisco (Sassenrath Cole & Pearcy, 1994; Krall *et al.*, 1995), and stomatal conductance of the leaf just before the sunfleck (Lei & Lechowicz, 1997; Valladares *et al.*, 1997). We observed diurnal patterns in both of these traits. Furthermore, both traits were highest during the middle part of the day, when sunflecks occurred most frequently. During the afternoon, photosynthesis and stomatal conductance declined together, which allowed C_i to remain constant. The maintenance of a high C_i is also thought to improve sunfleck-use-efficiency by maintaining photosynthetic carbon reduction enzymes in their induced states (Krall *et al.*, 1995; Lei & Lechowicz, 1997). The diurnal patterns in photosynthesis observed in these understory trees maximized their sunfleck-use efficiency, a trait that correlates with seedling survival in the understory.

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