Carbon Assimilation and Leaf Water Status in Sugar Beet Leaves during a Simulated Natural Light Regimen[1]

Donald R. Geiger*, Wen-Jang Shieh, Lynn Su Lu, and Jerome C. Servaites

Department of Biology, University of Dayton, Dayton, Ohio 45469-2320

ABSTRACT

Carbon assimilation and leaf water status were studied in sugar beet (*Beta vulgaris* L., Klein E-type multigerm) leaves during a light period in which illumination either increased rapidly to full irradiance or changed gradually in a sinusoidal manner as generally occurs during a natural day. A light regimen that simulated the light of a natural day was produced by adjusting irradiance with a neutral-density filter under the control of a computer. Under this light regimen, photosynthesis, transpiration, and stomatal conductance followed the irradiance pattern very closely and ribulose bisphosphate carboxylase was nearly fully activated. When illumination was increased rapidly at the beginning of a light period, transpiration also increased quickly, causing leaves to wilt to some extent. The activation state of ribulose bisphosphate carboxylase increased to only 52%, but ribulose bisphosphate level was nearly twice as high as during the simulated natural day. In spite of the differences in activation state and ribulose bisphosphate levels, photosynthesis rates were very similar under both regimens. Nevertheless, differences in parameters between leaves under the two irradiance regimens can affect how a plant responds to internal or external factors, and therefore, the rate at which irradiance increases at the beginning of a light period is an important consideration when interpreting data.

In the course of a light period, leaves are often subjected to a wide range of irradiance changes that can have significant physiological consequences. In nature, plants are exposed to a basic daily light regimen in which irradiance gradually increases to a maximum during a period of hours. Superimposed on this diurnal pattern may be changes in light level which happen during a fraction of a second to seconds as a result of events such as passing clouds, changing sun angle, and leaf motion. The physiological responses that enable plants to acclimate effectively to these various shifts in irradiance will have time constants that are consonant with the speed of the light changes.

To understand the role of irradiance in regulation of photosynthesis, the responses of leaves to each of these types of irradiance change should be characterized. To do this, it is important to use methods that enable one to measure the changes in leaf physiology that are associated with the light changes. For instance, because of recent technological advances, it is possible to study the response of photosynthesis during sunflecks and thereby understand the importance of this process for CO_2 assimilation (5).

To study regulation of photosynthesis in response to the basic diurnal light regimen, it is important that irradiance change gradually and reproducibly throughout the photoperiod. Responses to diurnal patterns of irradiance change can be studied outdoors or in a greenhouse, but the resulting measurements will be complicated by the effects of variable factors such as clouds. Studies of photosynthesis under artificial illumination generally are done under a light regimen in which irradiance at the start of the light period increases to full intensity within the first several minutes (RIL[2]). With this type of light regimen, irradiance changes very rapidly, making it unsuitable for studying the responses of photosynthesis or carbon metabolism to gradually changing irradiance characteristic of the basic diurnal light regimen.

The use of SND, a light regimen that simulates the changing light levels of a natural diurnal period, provides a convenient means for studying plant responses to gradually changing light. Measurements can be made during a reproducible photoperiod of specified duration, under controlled environmental conditions. Light regimens in which GIL is used have been used to advance our understanding of a number of topics including the onset of photoinhibition associated with midday depression of photosynthesis (7), diurnal metabolic control of starch and sucrose synthesis (10, 11), diurnal regulation of carbon allocation for export (1), and light regulation of the PCR cycle (12).

The present study was prompted by our discovery that the light-modulated enzymes of the PCR cycle are activated at different redox potentials in leaves, and therefore, regulation and operation of the cycle are sensitive to the rate at which irradiance changes throughout a day (12). It seems likely that, as a consequence, the rapid increase to full irradiance that occurs at the beginning of a light period under RIL will cause certain functions and responses of the plant to differ from those that occur under GIL. Plants have evolved under light

[2] Abbreviations: RIL, light regimen under which irradiance increases rapidly; glyphosate, *N*-(phosphonomethyl)glycine; NCE, net carbon exchange; PGA, phosphoglyceric acid; PRK, phosphoribulokinase; RuBP, ribulose bisphosphate; RWC, relative water content; GIL, light regimen that begins with a gradual increase in irradiance as occurs under natural daylight; PCR, photosynthetic carbon reduction.

regimens in which irradiance changes gradually, and therefore, their physiological and biochemical processes may respond differently when the day starts with an abrupt increase in irradiance. Homeostasis may be disturbed and oscillations may occur during the critical period of dark-light transition which may modify how the dark-light transition is accomplished. For example, sudden changes in irradiance are known to produce oscillations in gas exchange (17, 21), leaf water status (4), and metabolite levels and patterns of carbon metabolism (17, 19). Under GIL, there is more time for internal stability to be established following the dark to light transition, and therefore, the use of GIL may prevent certain persistent effects from occurring as a result of a rapid increase in light level to full irradiance. In fact, we have observed that the response of sugar beet leaves to glyphosate differs, depending on whether irradiance increases gradually or abruptly at the start of the photoperiod (16).

In the present study, we used a GIL light regimen to make a detailed comparison of the responses of photosynthesis, carbon metabolism, and leaf water status in sugar beet leaves under GIL with those under RIL. Differences in the time course and magnitude of enzyme activities, metabolite levels, and water status were observed between leaves of plants under RIL and those under GIL. Some of these differences appear to account for observed characteristics of PCR cycle regulation (12) and for differences in the response of photosynthesis and carbon metabolism to glyphosate under GIL and RIL (16).

MATERIALS AND METHODS

Plant Material

Sugar beet plants (*Beta vulgaris* L., Klein E-type multigerm) were grown in 6-L containers filled with a mixture of milled peat and vermiculite:sand (1:1, v/v) and watered three times daily with nutrient solution (18). Plants were maintained under a 14-h photoperiod at 25°C, 60% RH day and a 17°C, 75% RH night. Irradiance was increased in three steps to a maximum of 800 μmol photons m^{-2} s^{-1} PPFD at canopy level and then decreased in steps during the last half of the day. Incandescent lamps provided an initial 45-min period of photomorphogenic light at an irradiance of 25 μmol photons m^{-2} s^{-1}. Next, illumination was increased to 33% of the maximum intensity by high-pressure metal halide lamps and held at that level for 13.2 h. Irradiance was increased to the maximum level 3 h after the start of the photoperiod by high-pressure sodium vapor lamps and maintained at that level for 8 h. Photomorphogenic light was provided again during the final 7 min of the photoperiod to regulate the growth of petioles and leaf blade to produce plants whose appearance closely approximated that found in plants grown outdoors.

Illumination for Experimental Treatments

Illumination for experiments was provided by an equal number of 400-W high-pressure sodium vapor and metal halide lamps (Philips Lighting Co., Somerset, NJ), as was the case for the light source under which the plants were grown. At the start of a photoperiod under RIL, irradiance was found to increase to the maximum level within 10 min, with most

of the change occurring in the first 5 min. Under GIL, irradiance followed a sinusoidal time course that lasted for the duration of the photoperiod.

Apparatus for Simulating a Natural Diurnal Light regimen

Irradiance level for GIL was controlled by a motor-driven neutral-density filter interposed between the light source and the plant as shown in Figure 1. The filter consisted of a 0.6-m-wide, 12-m-long Mylar strip with a series of 50 0.18-m panels of decreasing optical density (Solar Tint of Ohio, Dayton). The filter panels were made by attaching to the Mylar film fields of evenly spaced opaque, reflective discs ranging from 5 to 17 mm in diameter or reflective window film which transmitted 30 or 50% of the incident light or both. At the beginning of the strip, there was a 0.8-m leader panel of reflective film that transmitted about 1% of the maximum irradiance and at the other end a clear panel. The discs and reflective film used to construct the filter panels were selected to provide a series of steps in irradiance that correspond to the levels found at intervals of 2% of the photoperiod during a natural day period. In addition to the moving filter strip, an IR-reflecting glass filter (Corning Pyrex Brand, Cincinnati Gasket and Packing Co., Cincinnati, OH) and a plastic prism diffuser were placed between the lamps and the plant to condition the light.

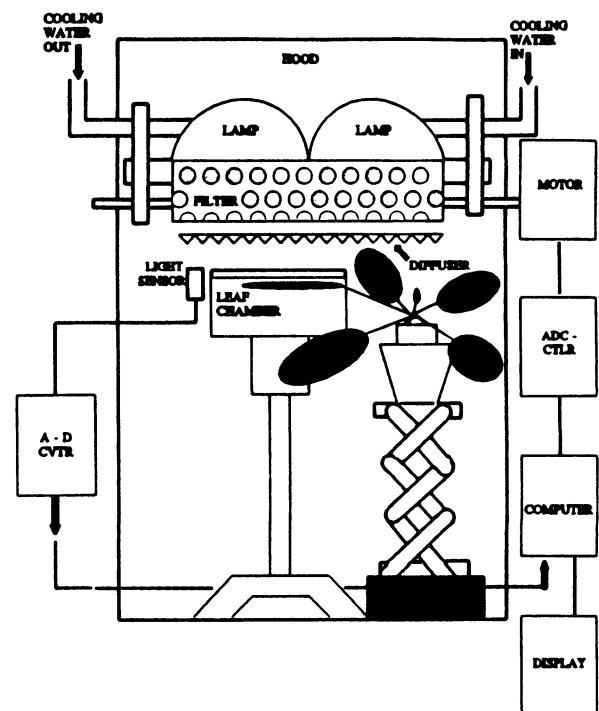The time course of irradiance was controlled by a computer

Figure 1. Apparatus for regulating light intensity to simulate the irradiance under a natural diurnal light regimen. The light level is sensed and converted to digital input (A-D CVTR) for processing by a computer. A signal is sent to a controller (ADC-CTLR) which then directs the motors to transport the neutral-density film to produce the required irradiance.
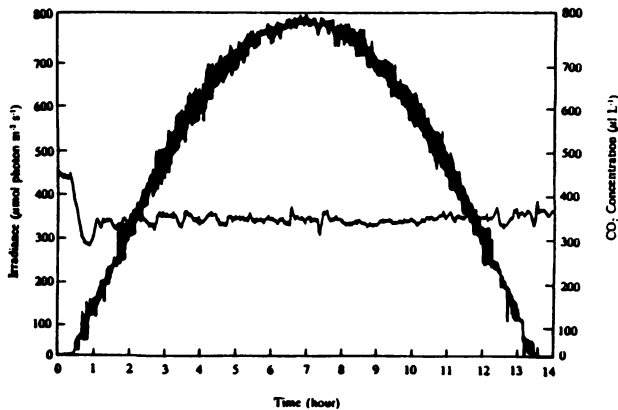
Figure 2. Recorder tracing of CO_2 concentration and sinusoidal time course of irradiance during a light period under GIL. Carbon dioxide level was controlled at approximately 350 μL L^{-1}.

(Commodore 64, Commodore Business Machines Inc., West Chester, PA) with an A/D converter interface which controlled movement of the filter to provide the sinusoidal irradiance regimen. The two transport motors operated continuously at a rate of 5 mm s^{-1}, moving the film to either increase or decrease irradiance. At 10-s intervals, the irradiance level was read and compared with the desired level, and a command was sent to continue or to reverse the travel of the film. This arrangement provided relatively smooth transitions in light level and varied irradiance as a sinusoidal function of time (Fig. 2).

Gas Exchange Measurements

Two recently mature sugar beet leaves on the same plant were enclosed in separate chambers which were connected in parallel to a closed apparatus for circulating the atmosphere and measuring the rates of CO_2 exchange and water loss by the leaves (1). The leaves were first used for measuring NCE with the plant under GIL, and then, on the following day, measurements were done on the same leaves under RIL. Results under this condition were were comparable to those obtained with plants from the growth room without a exposure to the GIL.

An IR gas analyzer (MSA model 3000, Pittsburgh, PA) was used to measure CO_2 concentration. NCE rate was determined by recording the output of a mass flow controller (Tylan model FC260, Carson, CA). Flow rate was regulated by a computer which used data from the IR gas analyzer to maintain the CO_2 in the atmosphere around the leaves at a set level (Fig. 2).

RH of the air entering and leaving the leaf chambers was measured with two humidity probes (Vaisala Humicap, Helsinki, Finland) to determine the rate at which water vapor needed to be removed by condensation to maintain a constant RH. Transpiration rate was calculated from the flow rate and the difference in RH of the air entering and leaving the leaf chambers. Stomatal conductivity was calculated using the equation of von Caemmerer and Farquhar (22).

Leaf Water Status Measurements

Leaf thickness was measured by a rotary transducer (Schaevitz, R30D). The RWC of discs taken from leaves was calculated as follows: RWC = ([fresh weight − dry weight]/[turgid weight − dry weight]) × 100. The fresh weight was measured immediately after the leaf discs were collected, turgid weight following equilibration of the discs in distilled H_2O for 1 h, and dry weight after discs were dried at 80°C for 12 h.

Enzyme and Metabolite Measurement

Leaf samples were taken from leaves outside the leaf chambers during gas exchange measurements. Leaf tissue was rapidly frozen in place by clamping tissue between brass cylinders cooled to liquid nitrogen temperature as described by Servaites et al. (11). Initial and total Rubisco activity and leaf levels of RuBP and PGA were determined in leaf extracts as described previously (10, 11). Ribulose-5-phosphate-dependent PRK activity was measured as in ref. 14.

RESULTS

Gas Exchange and Transpiration

The rate of NCE under RIL increased to a maximum during the first 2 h of the light period and then declined to 70% of maximum (Fig. 3A). Under GIL, the time course of NCE rate generally paralleled that of irradiance (Fig. 3B) except that NCE rates in the afternoon were lower than those at the same irradiance in the morning. The maximum NCE rate under
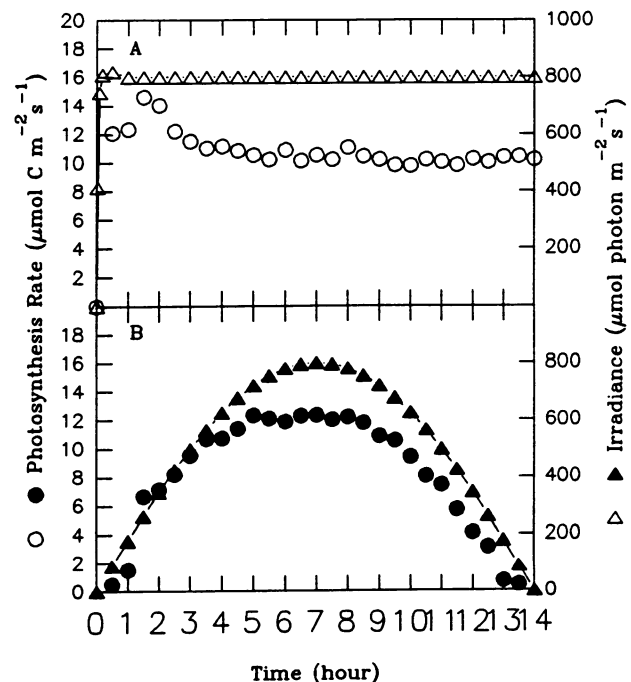

Figure 3. Time course of irradiance and of NCE rate in sugar beet leaves during a light period under RIL or GIL. NCE (O, ●) and irradiance (△, ▲) for plants under RIL (A, open symbols) or under GIL (B, solid symbols).
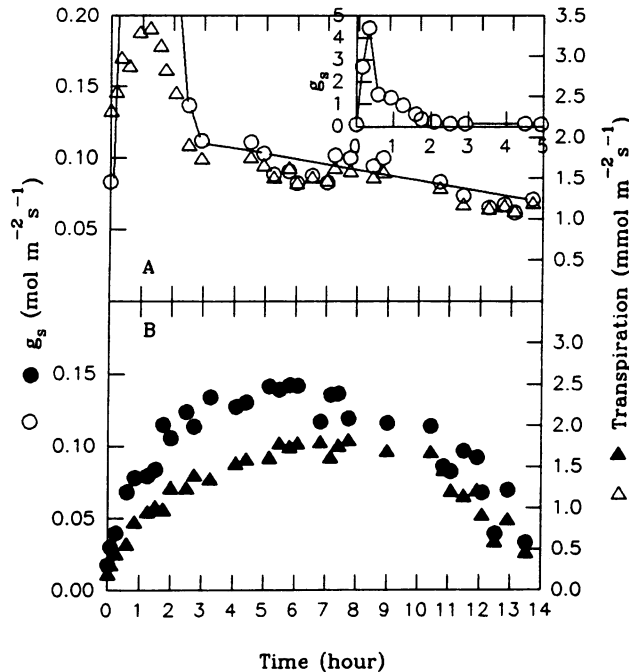
GEIGER *ET AL.*

Figure 4. Gas exchange by sugar beet leaves during a light period under RIL or GIL. Stomatal conductance (O, ●) and transpiration rate (Δ, ▲) for plants under RIL (A) or under GIL (B). Insert, full range of values for stomatal conductance for the first 5 h under RIL.

GIL, which occurred near midday, was sometimes slightly higher than the rates observed in leaves under RIL following the transient maximum.

During the initial 2-h period under RIL, g_s increased rapidly to a maximum that was many times the usual midday value and then decreased rapidly (Fig. 4A). At this time, transpiration rate increased and then decreased rapidly before entering a gradual decline which lasted the rest of the day. Under GIL, transpiration and g_s changed gradually along with the change in irradiance (Fig. 4B), g_s being slightly higher in the morning than in the afternoon. Whereas leaves remained turgid under GIL, they often became visibly wilted during the first part of the light period under RIL, even though the plants were well watered and their roots were not crowded.

Leaf Water Status

During the increase in transpiration at the start of the RIL, RWC decreased sharply to a minimum at about 0.5 h (Fig. 5A). As g_s decreased, transpiration rate decreased to the usual daytime range, and RWC rapidly returned to within about 3% of the value at the start of the light period. Leaf thickness, which generally followed RWC, reached a minimum approximately 0.9 h after the start of irradiance and then increased rapidly to a steady daytime level slightly below the initial thickness. In contrast, both RWC and leaf thickness were relatively steady under GIL after a small decrease during the first hour of the light period (Fig. 5B).

Activation of Enzymes of C₃ Cycle

Initial Rubisco activity generally followed the time course of irradiance, increasing rapidly under RIL and gradually under GIL (Fig. 6). In the latter case, there was a delay of about 2 h before the activation state began to increase. Total Rubisco activity was relatively steady throughout the day similarly under both RIL and GIL. Initial Rubisco activity reached a maximum near full activation under GIL, whereas under RIL initial activity was only 50% of the total activity.

The activity of PRK increased rapidly at the start of the light period under RIL and remained relatively constant for the remainder of the day (Fig. 6A). Under GIL, the activity increased relatively rapidly during the first 2 h and remained steady until it decreased during the final hour of the light period.

Levels of RuBP and PGA

Under RIL, the levels of RuBP and PGA increased steadily for about 1 h at the start of the photoperiod and thereafter remained constant (Fig. 7A). By contrast, under GIL, the time course of RuBP was dynamic, increasing slowly for the first 2 h, then declining during midday to a concentration only about half that found in leaves under RIL, and finally showing another transient increase later in the day as irradiance decreased (Fig. 7B). Under GIL, changes in PGA level generally were a mirror image of the changes in RuBP concentration.
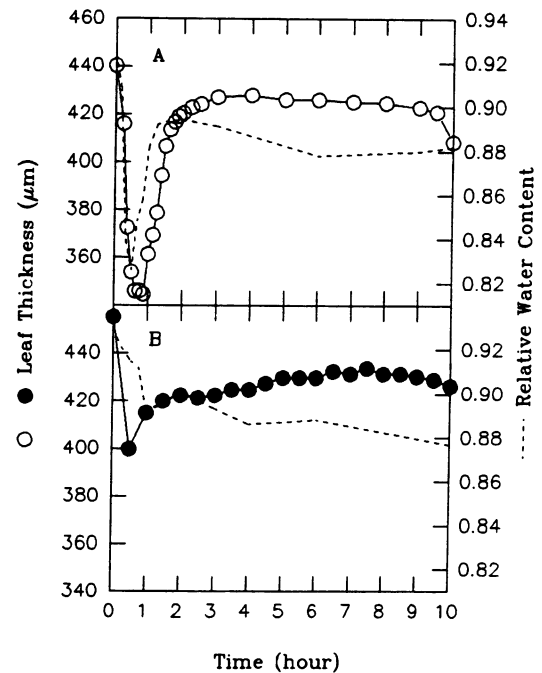

Figure 5. Water status and leaf thickness of sugar beet leaves during a light period under RIL or GIL. Leaf thickness (O,) and RWC (—--—) for plants under RIL (A) or under GIL (B).
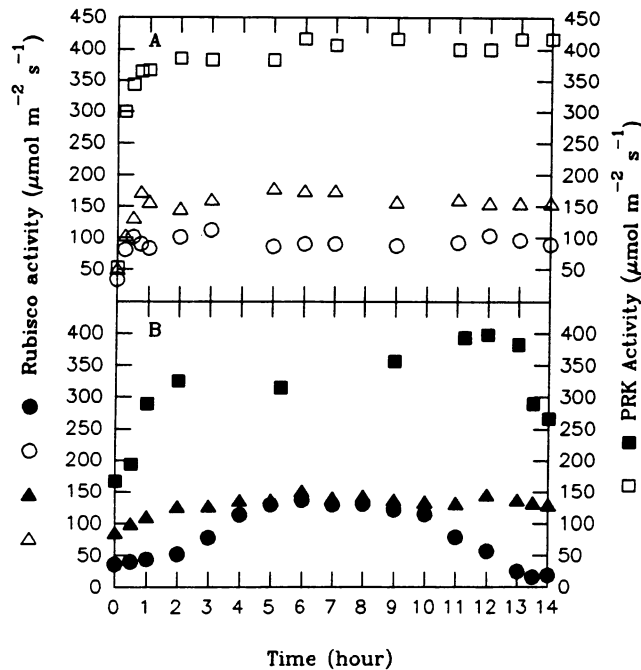
Figure 6. Activities of Rubisco and PRK in sugar beet leaves under RIL or GIL. Initial Rubisco activity (O, ●), total Rubisco activity (△, ▲), PRK (□, ■) for plants under RIL (A) or under GIL (B).
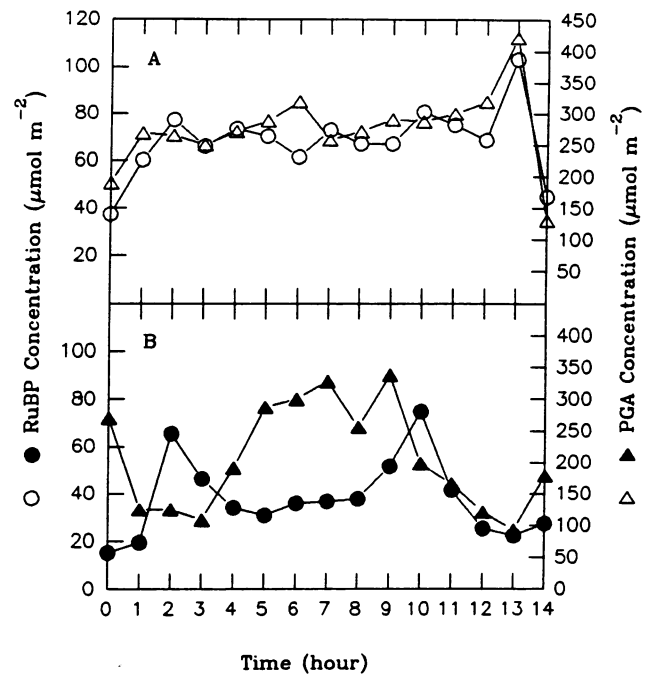

Figure 7. Levels of some RuBP and PGA in sugar beet leaves under RIL or GIL. RuBP level (O, ●) and PGA (△, ▲) levels for plants under RIL (A) or under GIL (B).

DISCUSSION

Photosynthesis, carbon metabolism, and leaf water relations show a variety of response patterns to changing irradiance when the day is begun with the gradually increasing light level characteristic of a natural diurnal light regimen. Changes in NCE, g_s, transpiration, and initial and total Rubisco activity under GIL generally followed the time course for irradiance level, demonstrating a direct response of these parameters to the gradual change in irradiance. The relatively steady time-course curves observed for leaf thickness and water content indicated that these parameters were able to adjust rather well to the gradual change in stomatal aperture and transpiration under GIL. The time courses for the levels of RuBP and PGA, by contrast, revealed a more complex, dynamic relation with irradiance level when the day started with a gradual increase in irradiance.

Starting the day with a rapid increase in irradiance resulted in transient changes in both carbon metabolism and leaf water status. The fact that NCE, transpiration, g_s, and leaf RWC returned to normal levels within the first 1 to 2 h demonstrates the ability of the plant to restore homeostasis relatively quickly under these conditions. During the remainder of the light period, the parameters associated with these processes did not change markedly in keeping with the steady irradiance under RIL. It seems likely that under the conditions used leaf water status did not markedly affect responses of photosynthesis under either GIL or RIL.

Although NCE rates from leaves on the same plant were within 10% of each other at midday under both RIL and GIL, Rubisco was close to being fully active under GIL but

only 50 to 60% active under RIL (Table I). Similar maximum levels of Rubisco activation, 50 to 70%, have been observed in chamber-grown plants (6, 13, 15). Full activation of Rubisco has been observed previously in leaves subjected to a natural diurnal light regimen (3, 14, 23). The differences in degree of activation of Rubisco observed here cannot be attributed to differences in irradiance level, because maximal irradiance was the same in both light regimens, but solely to starting the day under RIL and GIL irradiance patterns. The Rubisco activation process is able to proceed to near completion when irradiance level is increased slowly.

In a number of studies, the initial activity of Rubisco in leaves has been found to correlate with photosynthesis rates measured in those leaves (6, 8, 9, 20). In this study, plants under RIL had a lower initial Rubisco activity but the same NCE rate as leaves under GIL. The only factor that appears to be markedly different between the two is the midday level of RuBP, which is much higher in leaves under RIL (Table

Table I. *Midday Values for Parameters Associated with Carbon Assimilation under RIL or under GIL*

Parameter	GIL	RIL
NCE (μmol C m^{-2} s^{-1})	11.8 ± 0.6[a]	10.6 ± 0.4
Initial Rubisco (μmol C m^{-2} s^{-1})	131.5 ± 3.6	88.9 ± 1.9
Total Rubisco (μmol C m^{-2} s^{-1})	143.1 ± 6.3	171.7 ± 9.7
Rubisco activation	0.92 ± 0.02	0.52 ± 0.03
RuBP (μmol m^{-2} s^{-1})	40.5 ± 7.5	67.3 ± 4.7
PGA (μmol m^{-2} s^{-1})	305 ± 36	285 ± 27

[a] Mean ± SD.

I). At midday, the level of RuBP in the leaves under GIL was only half that in the leaves under RIL. Under GIL, the time course and levels of initial Rubisco activity and RuBP concentration appeared to change in a manner that was consistent with its role as a substrate for Rubisco. During the first 2 h of the light period, Rubisco activation state changed little, yet RuBP level increased rapidly. After 2 h, when Rubisco activation state is increasing, the RuBP level decreased rapidly. Clearly, the contrasting modes of adjusting NCE under GIL and RIL demonstrate that the rate of carbon assimilation can be established at moderate midday light levels in two ways: by establishing a high Rubisco activation state and a near-limiting level of RuBP or by establishing a large excess of RuBP and a modest level of Rubisco activation. This pattern of change in Rubisco activation and the levels of RuBP and PGA under GIL support the hypothesis that these metabolites interact with activation state to regulate Rubisco activity (12).

Although NCE and transpiration were maintained or restored to their normal midday state following their initial response to irradiance, some of the adjustments made during the dark-light transition period were found to produce changes in biochemical and physiological parameters which persisted for the remainder of the day. Persistent differences were observed in the levels of Rubisco activity and RuBP between leaves under the two light regimens, and these may explain differences observed in the subsequent responses of leaves to various treatments. For example, we found that photosynthesis rate in sugar beet leaves responded differently to the foliar application of the herbicide glyphosate, depending on whether the light regimen was GIL or RIL (2, 16). Glyphosate was found to slowly reduce the level of RuBP present in sugar beet leaves, and photosynthesis was reduced only when RuBP reached a critical minimum (15). Under RIL, because of the initial high level of RuBP present in leaves, inhibition of NCE occurred after about 4 h. Recently, we observed that in leaves under GIL photosynthesis rate and RuBP level decreased within 1 h following application of glyphosate (16).

LITERATURE CITED

1. **Fondy BR, Geiger DR, Servaites JC** (1989) Photosynthesis, carbohydrate metabolism and export in *Beta vulgaris* L. and *Phaseolus vulgaris* L. during a sinusoidal light regime. Plant Physiol **89**: 396–402
2. **Geiger DR, Tucci MA, Servaites JC** (1987) Glyphosate effects on carbon assimilation and gas exchange in sugar beet leaves. Plant Physiol **85**: 365–369
3. **Kobza J, Seemann JR** (1989) Regulation of ribulose-1,5-bisphosphate carboxylase activity in response to diurnal changes in irradiance. Plant Physiol **89**: 918–924
4. **Lang ARG, Klepper B, Cumming MJ** (1969) Leaf water balance during oscillation of stomatal aperture. Plant Physiol **44**: 826–830
5. **Pearcy RW** (1990) Sunflecks and photosynthesis in plant canopies. Annu Rev Plant Physiol Plant Mol Biol **41**: 421–453
6. **Perchorowicz JT, Raynes DA, Jensen RG** (1981) Light limitation of photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Proc Natl Acad Sci USA **78**: 2985–2989
7. **Raschke K, Resemann A** (1986) Midday depression of CO_2 assimilation in leaves of *Arbutus unedo* L.: diurnal changes in photosynthetic capacity related to changes in temperature and humidity. Planta **168**: 546–558
8. **Salvucci ME, Portis AR Jr, Ogren WL** (1986) Light and CO_2 response of ribulose-1,5-bisphosphate carboxylase/oxygenase activation in *Arabidopsis* leaves. Plant Physiol **80**: 655–659
9. **Seemann JR, Kobza J, Moore Bd** (1990) Metabolism of 2-carboxyarabinitol 1-phosphate and regulation of ribulose-1,5-bisphosphate carboxylase activity. Photosynth Res **23**: 119–130
10. **Servaites JC, Fondy BR, Li B, Geiger DR** (1989) Source of carbon for export from spinach leaves throughout the day. Plant Physiol **90**: 1168–1174
11. **Servaites JC, Geiger DR, Tucci MA, Fondy BR** (1989) Leaf carbon metabolism and metabolite levels during a period of sinusoidal light. Plant Physiol **89**: 403–408
12. **Servaites JC, Shieh W-J, Geiger DR** (1991) Regulation of photosynthetic carbon reduction cycle by ribulose bisphosphate and phosphoglyceric acid. Plant Physiol **97**: 1115–1121
13. **Servaites JC, Torisky R** (1984) Activation state of ribulose bisphosphate carboxylase in soybean leaves. Plant Physiol **74**: 681–686
14. **Servaites JC, Torisky RS, Chao SF** (1984) Diurnal changes in ribulose 1,5-bisphosphate carboxylase activity and activation state in leaves of field-grown soybean. Plant Sci Lett **35**: 115–121
15. **Servaites JC, Tucci MA, Geiger DR** (1987) Glyphosate effects on carbon assimilation, ribulose bisphosphate carboxylase activity, and metabolite levels in sugar beet leaves. Plant Physiol **85**: 370–374
16. **Shieh W-J, Geiger DR, Servaites JC** (1991) Effect of N-(phosphonomethyl)glycine on carbon assimilation and metabolism during a simulated natural day. Plant Physiol **97**: 1109–1114
17. **Sivak MN, Walker DA** (1987) Oscillations and other symptoms of limitation of *in vivo* photosynthesis by inadequate phosphate supply to the chloroplast. Plant Physiol Biochem **25**: 635–648
18. **Snyder FW, Carlson GE** (1978) Photosynthate partitioning in sugar beet. Crop Sci **18**: 657–661
19. **Stitt M** (1986) Limitation of photosynthesis by carbon metabolism. I. Evidence for excess electron transport capacity in leaves carrying out photosynthesis in saturating light and CO_2. Plant Physiol **81**: 1115–1122
20. **Torisky RS, Servaites JC** (1984) Effect of irradiance during growth of *Glycine max* on photosynthetic capacity and percent activation of ribulose 1,5-bisphosphate carboxylase. Photosynth Res **5**: 251–261
21. **van der Veen R** (1949) Induction phenomena in photosynthesis. II. Physiol Plant **2**: 287–296
22. **von Caemmerer S, Farquhar GD** (1981) Some relationships between biochemistry of photosynthesis and gas exchange of leaves. Planta **153**: 376–387
23. **Vu CV, Allen LH, Bowes G** (1983) Effects of light and elevated atmospheric CO_2 on the ribulose bisphosphate carboxylase activity and ribulose bisphosphate level of soybean leaves. Plant Physiol **73**: 729–734