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Efficiency of photosynthesis in continuous and pulsed light emitting diode irradiation

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Abstract

The light utilization efficiency and relative photon requirement of photosynthesis in pulsed and continuous light from light emitting diodes (LEDs) has been measured. First, we chacterized the photon requirement of photosynthesis from light of LEDs that differ in spectral quality. A photon requirement of 10.3 ± 0.4 was measured using light from a 658 nm peak wavelength (22 nm half band width) LED over the range of 0–50 μmol photons m^{-2} s^{-1} in 2 kPa O_2 in leaves of tomato (*Lycopersicon esculentum* Mill., cv. VF36). Because the conversion of electrical power to photons increased with wavelength, LED lamps with peak photon output of 668 nm were most efficient for converting electricity to photosynthetically fixed carbon. The effect of pulsed irradiation on photosynthesis was then measured. When all of the light to make the equivalent of 50 μmol photons m^{-2} s^{-1} was provided during 1.5 μs pulses of 5000 μmol photons m^{-2} s^{-1} followed by 148.5 μs dark periods, photosynthesis was the same as in continuous 50 μmol photons m^{-2} s^{-1}. When the pulse light and dark periods were lengthened to 200 μs and 19.8 ms, respectively, photosynthesis was reduced, although the averaged photon flux density was unchanged. Under these conditions, the light pulses delivered 10^{17} photons m^{-2}, which we calculate to be equivalent to the capacitance of PS I or PS II. Data support the theory that photons in pulses of 100 μs or shorter are absorbed and stored in the reaction centers to be used in electron transport during the dark period. When light/dark pulses were lengthened to 2 ms light and 198 ms dark, net photosynthesis was reduced to half of that measured in continuous light. Pigments of the xanthophyll cycle were not affected by any of these pulsed light treatments even though zeaxanthin formation occurred when leaves were forced to dissipate an equal amount of continuous light.

Abbreviations: CWF – cool white fluorescent; EPS – xanthophyll epoxidation state; LED – light emitting diode; LUE – light utilization efficiency; PFD – photon flux density; PR – photon requirement (for CO_2 fixation); PS II – primary donor in Photosystem II; RPR – relative photon requirement

Introduction

Advances in light emitting diode (LED) technology have made them an excellent light source for photosynthesis research (Tennessen et al. 1994) and plant growth (Barta et al. 1992; Ignatius et al. 1992; Bula et al. 1994). The LED can make photon fluxes well in excess of 2000 μmol m^{-2} s^{-1} yet can be turned fully on and fully off extremely rapidly (200 ns).

Pulsed lighting has been used to study details of photosynthetic electron transport (Emerson and Arnold 1932; Kok 1956; Jursinic and Pearcy 1988; Chow et al. 1989) and carbon metabolism (Sharkey et al. 1986; Stitt 1986; Kirschbaum and Pearcy 1988; Sassenrath-

Cole et al. 1994). Time constants for photosynthetic processes can be divided into three ranges: (1) primary photochemistry, (2) electron shuttling and (3) carbon metabolism. Primary photochemistry, from light harvesting through charge separation in the reaction centers, occurs in picosecond to nanosecond speeds (Diner 1986). Slightly slower reactions involving microseconds (μs) to milliseconds (ms) occur in electron shuttling between the photosystems (Whitmarsh and Cramer 1979; Harbinson and Hedley 1988; Whitmarsh 1992). Finally, carbon metabolism within the chloroplast occurs in seconds, while sucrose metabolism and enzyme activation takes minutes (Kirschbaum and Pearcy 1988; Sassenrath-Cole and Pearcy 1992). These three photosynthetic processes can be uncoupled by providing pulses of light within the appropriate range for each process. At high frequencies, pulsing light treatments can be used to separate the light reactions (light harvesting and charge separation) of photosynthetic electron transport from the dark reactions (electron shuttling) of photosynthetic electron transport.

Photosynthetic responses to pulsed light is ecologically relevant because the light environment experienced by leaves is often highly variable (Norman and Tanner 1969; Desjardins et al. 1973). Much of the light used for photosynthesis by leaves within canopies is from sunflecks (Pfitsch and Pearcy 1989; Pearcy 1990). These sunflecks range from milliseconds to minutes in duration and their photon flux densities can be as bright as full sunlight.

We have used LEDs to study the effects of μs to ms light pulses on photosynthesis of intact leaves. We measured the efficiency of the LEDs at converting electrical power to light used in photosynthesis over a range of wavelengths. The light-response of photosynthesis was measured in continuous light and compared to the situation where the same total photon flux was delivered in intense pulses lasting just 1% of the time. We used up to 5000 μmol m^{-2} s^{-1} pulses and frequencies from 5000 to 0.5 Hz. The capacitance of Photosystem II was assessed by lengthening the pulse on and off times (maintaining the same integrated light intensity) until photosynthesis became less efficient in the pulsed light than in the continuous light. Under conditions of excess light, leaves can induce dissipation mechanisms that correlate with de-epoxidation of the pigment violaxanthin (Demmig Adams et al. 1990), or experience a sustained decrease in photosynthetic efficiency, described as photoinhibition (Björkman 1981). Therefore, photoinhibition and de-epoxidation of violaxanthin was tested for in leaves dissipating pulses of light and compared to leaves dissipating the same light energy provided continuously.

Materials and methods

Plant material and growth conditions

Tomato plants (*Lycopersicon esculentum* Mill., cv. VF36) were grown in reach-in growth chambers (model E15; Conviron, Winnipeg, Manitoba, Canada) under a 16 h photoperiod, 70% relative humidity, and day/night temperatures of 26°/18 °C. Metal halide lamps, supplemented with incandescent lamps, provided 1000 μmol m^{-2} s^{-1} photons at the canopy top. Each plant was grown in a 10 L plastic pot, filled with peatlite (Metro-Mix 360: Grace Sierra Co., Milpitas, CA, USA) and watered to excess 4 times per day with half strength Hoagland's solution B (Hoagland and Amon 1938).

Gas exchange measurements and calculations

An aluminum leaf cuvette with a glass window (4.9 cm^2) was clamped onto single terminal leaflets of mature leaves. Fully expanded leaves of 30 day old tomato plants were used. Leaf photosynthesis and conductance were calculated by measurement of CO_2 partial pressure and dewpoint of the airstream entering and exiting the leaf cuvette with an infrared gas analyzer operated in differential mode (model 6262; LI-COR, Lincoln, NE, USA). Gas exchange equations used were as described by von Caemmerer and Farquhar (1981). Air entering the cuvette was mixed with mass-flow controllers (Edwards High Vacuum, Wilmington, MA, USA) from cylinders of N_2, O_2 and 5% CO_2 in air. This mixture was passed through warm water for humidifcation and subsequently through a copper coil in a refrigerated water bath to establish the desired dewpoint in the leaf cuvette. Reference air was cooled to 0 °C in order to obtain a reference dewpoint for differential measurements. Leaf temperature was maintained at 20 ± 0.5 °C using a water bath and was measured by a copper-constantan thermocouple pressed along the adaxial surface of the leaf for 1 cm. After measurements were taken, the PR (Eq. (1)) was calculated (photons absorbed CO_2^{-1} absorbed) over a range of 0 to 50 μmol photons m^{-2} s^{-1}.

$$\text{Photon Requirement} = \frac{\int_0^t \text{Light}(\mu\text{mol photons m}^{-2}\,\text{s}^{-1})}{\int_0^t \text{Assimilation}(\mu\text{mol CO}_2\,\text{m}^{-2}\,\text{s}^{-1})} \quad (1)$$

The time constant of seconds was used to integrate light absorption and CO_2 uptake over the microsecond light and dark durations. The PR is sometimes called quantum requirement and its inverse is known as quantum yield. By integrating absorbed photons and carbon assimilation over time, the PR of photosynthesis from lightflecks can be directly compared to that of continuous light (Eq. (2)).

$$\text{Relative Photon Requirement} = \frac{\text{PR}_{\text{continuous light}}}{\text{PR}_{\text{lightfleck}}} \quad (2)$$

In this way, the photosynthetic efficiency (quantum efficiency) in a light pulse or in fluctuating light can be directly compared to that of constant light. We also calculated a second measure, termed light utilization efficiency (LUE) of photosynthesis, based on calculations of Chazdon and Pearcy (1986b) (Eq. (3)).

$$\text{LUE} = \frac{\text{measured assimilation}_{\text{lightfleck}}}{\text{expected assimilation}_{\text{continuous}}} \quad (3)$$

Its intended use was to relate total carbon uptake from a *single* lightfleck to carbon assimilation in continuous light of the lightfleck intensity and duration. For measured assimilation in a lightfleck, we determined carbon assimilation from μs light pulse based on steady state carbon assimilation per second divided by the number of light pulses per second. Expected assimilation was extrapolated from mean photosynthesis of six leaves in response to photon flux density.

Light sources and spectral measurements

Light was provided using lamps made from LEDs with peak photon emission of 658, 661, 668, 676, 690, and 698 nm (22 nm half band width) custom manufactured by Quantum Devices, Inc. (Bameveld, WI). Each LED lamp consisted of 20 individual diodes evenly distributed over the area of the leaf chamber (4.9 cm^2). The LEDs were mounted on a ceramic heat sink as described by Ignatius and Martin (1992). Each lamp was pressed against the frame of the leaf cuvette that was maintained at 20 °C, eliminating the thermally-induced spectral shift noted in earlier work (Tennessen et al. 1994). A cool white fluorescent (CWF) lamp (2 - F48-T12) and neutral density filters were used to provide white light to the leaf cuvette. Light quality and intensity were measured using a spectroradiometer (LI-1800, LICOR, Lincoln, NE, USA) connected fiberoptically to a remote probe placed inside the leaf cuvette. The photon flux density (PFD) was measured for photons in the range of 330–1100 nm. Leaf absorptivity was measured using an integrating sphere (LI-1800-12S, LI-COR) and spectroradiometer by measuring reflectance and transmittance from each leaf sample after photosynthesis was measured. Absorptivity was calculated as described by Ehleringer (1981). Values for absorptivity were calculated as percent of total incident photons for each nanometer of light (330–1100 nm).

Pulsed photon fluxes

Photon pulses were attained with forty 661 or 668 nm LEDs mounted on a ceramic plate that covered the leaf cuvette window. For integrated PFD of 100 μmol m^{-2} s^{-1} or less, 661 nm LEDs were used. To produce higher integrated photon fluxes, the more efficient 668 nm diodes were used. The LED lamp was switched on and off with an overall frequency of 6.7 kHz (150 μs total cycle time). The lamp had a rise time of 200 ns to reach full intensity. We measured photosynthesis under light/dark times of 15/135, 7.5/142.5 and 1.5/148.5 μs which required instantaneous pulse PFD of 500, 1000, and 5000 μmol m^{-2} s^{-1}, respectively, to achieve an integrated PFD of 50 μmol m^{-2} s^{-1}. The duration of pulse and subsequent dark times were lengthened proportionately to maintain equal integrated PFD. As the pulse/darkness cycle was lengthened, turnover of an unknown electron transport carrier became rate limiting and a capacitance was calculated from the number of photons per pulse. The longest pulse time was 3 ms, separated by 297 dark. Pulses were generated using an electronic switching circuit. Pulse light intensity and duration was measured using a UDT photodiode and UDT filter #2169 (10D, United Detector Technologies, Hawthome, CA, USA) calibrated against values from the spectroradiometer. Photodiode output was linear from 0 to 12 000 μmol photons m^{-2} s^{-1} when calibrated using a red LED lamp as the photon source. In pulsed light, the photodiode exhibited a rise time of 80 ns using photons from a single LED driven by a sig-

nal generator (Model 3020, Dynascan Corp. Chicago, IL, USA). An analog oscilloscope (Tectronix, Model 465B, Beaverton, OR, USA) was used to measure circuit voltage, pulse frequency, pulse width, and the LED response time; while a digital oscilloscope (Model 206, Nicolet Instrument Corp., Madison, WI, USA) was used to measure photodiode response for light measurements. Values from both oscilloscopes were used to determine the PFD for each pulse and on/off cycle and to calculate integrated PFD as described by Sager and Giger (1980).

Due to the importance of accurate integration of the intense, short light pulses for this work we tested our photodiode calibration against an Epply pyranometer (Epply Laboratory, Inc., Newport, RI, USA), which integrated the light energy in these submillisecond pulses. The photodiode and pyranometer measurements were within 5% when used to determine the rate of photon arrival per second from pulses of less than 25 μs duration and within 10% for pulses of longer duration. The photodiode always measured lower. All results are reported based on photodiode measurements.

Xanthophyll induction and analysis

After reaching steady-state photosynthesis (30 min) under continuous PFD, some leaves were subjected to 2 ms pulses of 5000 μmol photons m^{-2} s^{-1} separated by 198 ms darkness treatment for one hour. This provided an integrated PFD of 50 μmol m^{-2} s^{-1}. Treatments of continuous red and continuous white light, with and without CO_2 as an acceptor, were used as positive and negative controls. Pigment was extracted in a cold room (4 °C) in low light (<15 μmol photons m^{-2} s^{-1}). Frozen tissue was ground with a glass pestle in Eppendorf tubes using 1 ml 85% acetone followed by centrifugation for 10 min. The pellet was twice resuspended in 1 ml acetone, ground, and again centrifuged. The three supemants were combined, diluted with acetone and water to 4 ml and filtered through a 0.20 μm syringe filter, resulting in an 82% acetone extract. Acetone extracts were stored at −20 °C under nitrogen until analyzed.

A 20 μl aliquot of the acetone extract was chromatographed on a C18 reverse phase HPLC column (Zorbax ODS non-endcapped 4.6 × 250 mm, Dupont Co.; supplied by MACMOD Analytical, Inc., Chadds Ford, PA, USA). A two solvent system, as described by Thayer and Björkman (1989), was used for a 35 min separation. A flow rate of 1 cm^3 min^{-1} and 30 °C

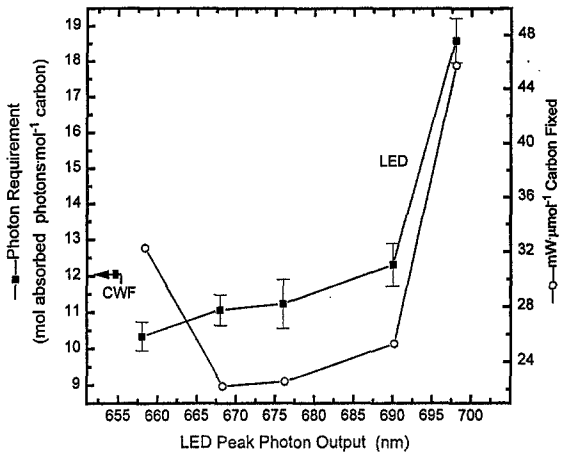

Fig. 1. Photon requirement of photosynthesis (■) and LED electrical conversion efficiency of carbon fixation (○) as affected by spectral quality of light provided by LEDs. As a control, the photon requirement of photosynthesis in cool white fluorescent light (CWF) was measured. Photosynthesis was measured in 2 kPa O_2 and 35 Pa CO_2 at 20 °C. Data represent mean and standard error for five tomato leaves. Photon requirement was calculated over a photon flux density, ranging from 10 to 50 μmol photon m^{-2} s^{-1}, in 10 μmol photon m^{-2} s^{-1} increments. Photon flux density was based on absorbed photons (330–1100 nm). Electrical conversion efficiency (○) is given in mW μmol $carbon^{-1}$ based on the product of mW μmol $photons^{-1}$ and PR (μmol photons μmol carbon $fixed^{-1}$).

temperature were maintained throughout the run. Each run was followed by a 10 min isocratic elution to clean the column and re-establish the first solvent. Pigments were detected by absorbance at 445 nm and peaks were integrated with a peak integrator (Spectra Physics model SP4400, San Jose, CA, USA). The peak integrator was calibrated by injecting lutein, α-carotene and β-carotene of which molar concentrations were determined using a spectrophotometer as described by Davies (1976). Lutein, α-carotene, and β-carotene standards were obtained from Sigma (St. Louis, MO, USA). Conversion factors from lutein to antheraxanthin (A), violaxanthin (V) and zeaxanthin (Z), at 445 nm, and the epoxidation state calculation [EPS = (Z + 0.5 A) / (Z + A + V)] were taken from Thayer and Björkman (1989).

Results

LEDs with a peak output wavelength of 658 nm resulted in the lowest photon requirement (PR) for photosynthesis (Fig. 1). However, LEDs convert electricity to light more efficiently at longer wavelengths. When the conversion of electricity to photons by LEDs was mul-

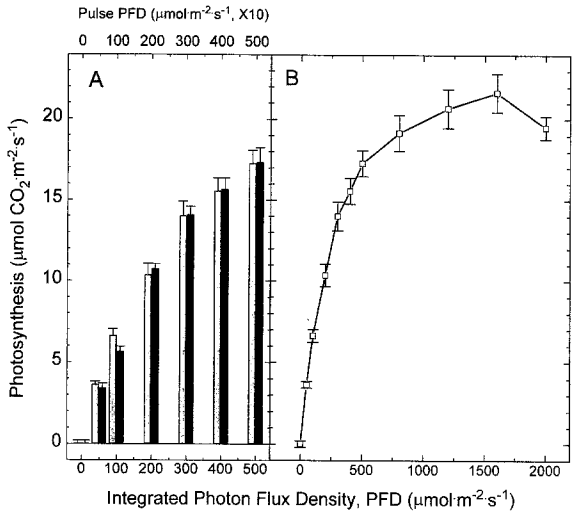

Fig. 2. Response of tomato leaf photosynthesis to photon flux density in 2 kPa O_2 and 35 Pa CO_2 at 20 °C. Data represent mean and standard error for photosynthesis of 5 different tomato leaves. (**A**) Leaf photosynthesis in pulsed (grey bars) and continuous (black bars) light. Photon fluxes were provided using a 668 nm LED lamp and on/off times of 20/180 μs. Pulses provided 10 times the photon flux density of continuous light, but integrated photon flux density of continuous and pulse treatments were equal. (**B**) Tomato leaf light response of photosynthesis.

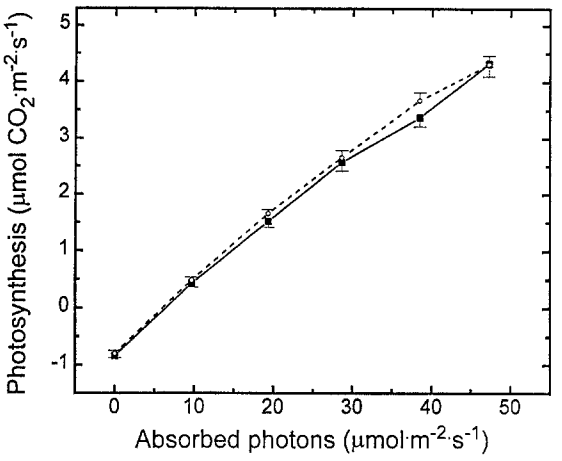

Fig. 3. Photosynthetic response to increasing absorbed photons of equal integrated photon flux densities provided in pulses (○) or continuous (■) light. Pulses from a 661 nm LED lamp provided 1.5 μs, 5000 μmol m^{-2} s^{-1} intermittent PFD during a cycle time of 150 μs. Leaves were at 20 °C and exposed to 2 kPa $O_2$2 and 35 Pa CO_2. Each data point represents a mean and SE for photosynthesis of 5 different tomato leaves.

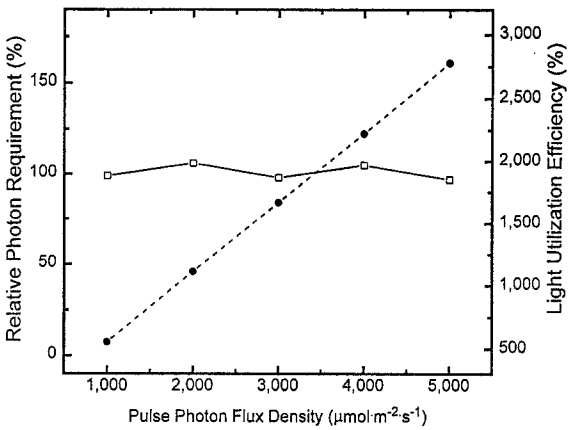

Fig. 4. Calculated light utilization efficiency (●) (Eq. (3)) and relative photon requirement (□) (Eq. (2)). Calculations are based on measurements of leaf photosynthesis under microsecond light pulses of 1, 2, 3, 4 and 5 × 10^3 μmol photons m^{-2} s^{-1} (Fig. 3) as a percent of photosynthesis extrapolated from continuous light of the same pulse light intensity (Fig. 2).

tiplied by the PR, an electrical requirement for LED light used by photosynthesis was determined. The electrical requirement is shown in Fig. 1 for five LED arrays with varying peak emission wavelengths. This calculation includes wavelength specificity of 1) the photon requirement of photosynthesis and 2) the electrical efficiency of the LED lamp. The LED lamp with the highest efficiency for converting electrical power to photosynthetic carbon fixation had a peak emission wavelength of 668 nm (Fig. 1). This array was used in many of the pulsed light experiments, but some of the low light experiments (PFD < 100 μmol photons m^{-2} s^{-1}) were done with an array with a peak output of 661 nm. The PR of CWF light is shown in Fig. 1 for comparison.

The response of photosynthesis to PFD was measured in continuous light and compared to the response when the light was provided 10% of the time in pulses 10 fold higher than the continuous PFD (Fig. 2). The frequency used was 5 kHz. We were limited to pulses of 5000 μmol photons m^{-2} s^{-1} since more intense pulses caused failure of the LEDs, even though the most efficient wavelength-LEDs had been selected. Over the range of 0 to 500 μmol photons m^{-2} s^{-1}, photosynthesis in the pulsed light was the same as photosynthesis in continuous light. This indicates that the photosynthetic apparatus of leaves can use all of the photons in 5000 m^{-2} s^{-1} pulses, providing the frequency is 5 kHz.

We next focused on low integrated PFD in which photosynthesis responds essentially linearly with light. For these measurements, we provided all of the photons in just 1% of the time in pulses of up to 5000 μmol photons m^{-2} s^{-1}. The frequency was 6.7 kHz and the LEDs had a peak emission wavelength of 661 nm. We could find no difference in leaf carbon assimila-

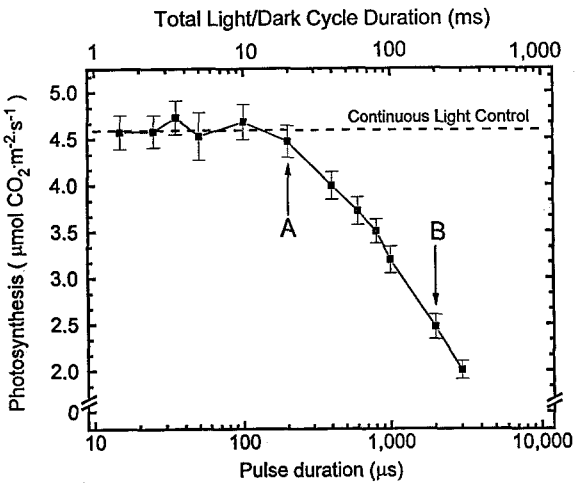

Fig. 5. Reduction in photosynthesis with increasing light pulse duration (increasing photons pulse^{-1}). The duration of light on time and relative off time was increased while integrated photon flux remained constant (50 μmol m^{-2} s^{-1}). Photosynthesis decreased (→ A) when the light pulse duration was 200 μs or longer (10^{17} photons m^{-2} pulse^{-1} or greater) and was reduced to half (→ B) when 2 ms light pulses were used. Control leaf photosynthesis in 50 μmol photons m^{-2} s^{-1} continuous light was 4.6 μmol CO_2 m^{-2} s^{-1}. Each data point represents a mean and SE for photosynthesis of 5 different tomato leaves. The leaf temperature was maintained at 20.0 ± 0.5 °C.

tion between continuous and pulsed PFD under these conditions (Fig. 3). We also used 5% and 10% duty cycles and saw no effects (data not shown). The photon requirement in continuous light was the same as in pulsed light as shown by the relative photon requirement (Fig. 4). However, the light utilization efficiency (LUE) at the highest pulse PFD tested was 27 times that of continuous light (Fig. 4) using our measured value of 9 photons per carbon in 5000 μmol m^{-2} s^{-1} pulsed PFD (Fig. 3) and a predicted value of 250 photons per carbon in 5000 μmol m^{-2} s^{-1} continuous light (Fig. 2).

The capacitance of the leaves was tested by decreasing the pulse frequency while holding the integrated PFD constant. Both the dark time and pulse duration were made longer so that the pulse remained at 1% of the total cycle time and 5000 μmol photons m^{-2} s^{-1} to give an averaged PFD of 50 μmol m^{-2} s^{-1}. When the pulse duration was 100 μs or shorter, photosynthesis was unaffected by the pulsing regime (Fig. 5). Photosynthesis declined with longer light pulses. With a 2 ms pulse duration, photosynthesis was about one-half of that in continuous PFD (Fig. 5).

When photons incident on leaves cannot be used for photosynthesis they can cause photoinhibition (sustained reduction in photosynthetic efficiency), or they can induce dissipation mechanisms that include de-epoxidation of violaxanthin to zeaxanthin. We tested for photoinhibition by holding leaves in a 1% duty cycle of 2 ms 5000 μmol photons m^{-2} s^{-1} light pulses followed by 198 ms darkness. Under these conditions, carbon assimilation was nearly half the control rate of 4.6 μmol m^{-2} s^{-1} in an equal average PFD of 50 μmol photons m^{-2} s^{-1} (Fig. 5). Control leaves in continuous light and leaves treated with 2 ms pulses were irradiated for 1 h, during which time photosynthesis was steady. Following this treatment, leaves were placed into continuous PFD (50 μmol photons m^{-2} s^{-1}) and in all leaves, photosynthesis equalled pre-treatment rates. Because the reduction in photosynthesis in fluctuating light was transient, and not sustained in continuous light, we conclude that the excess light (unaccounted for in photosynthesis) did not result in photoinhibition of these leaves.

The xanthophyll epoxidation state (EPS = [Z + 0.5A] / [Z + A + V]) was also measured for leaves exposed to a 1% duty cycle of 2 ms, 5000 μmol photons m^{-2} s^{-1} light pulses for 1 h. There was essentially no de-epoxidation (EPS = 1.2 ± 0.3%) caused by this pulse light treatment relative to the continuous light control (EPS = 2.1 ± 0.4%) (Fig. 6). Since the averaged PFD was 50 μmol m^{-2} s^{-1} and photosynthesis was one-half in our treatment, we compared the epoxidation state with that of a leaf exposed to 25 μmol m^{-2} s^{-1} PFD in the absence of O_2 and CO_2. This forced the leaf to dissipate the same PFD as was unaccounted for by photosynthesis in leaves irradiated with 2 ms light pulses. Leaves treated this way exhibited substantial de-epoxidation. To compare positive controls with previously reported values, we treated leaves with 2500 μ m^{-2} s^{-1} white PFD in the absence of electron acceptors and found an epoxidation state of 45%. In a final positive control of 1500 μmol m^{-2} s^{-1} red PFD from LEDs in the absence of O_2 and CO_2 we found an epoxidation state of 62%. This shows that red light was more effective at causing de-epoxidation than white light and that any de-epoxidation that occurred would have been detected by our methods.

Discussion

The LEDs with a peak emission wavelength of 668 nm exhibited the greatest efficiency of converting electricity to photosynthetically useful light (Fig. 1). For this reason, we recommend the 668 nm wavelength LED

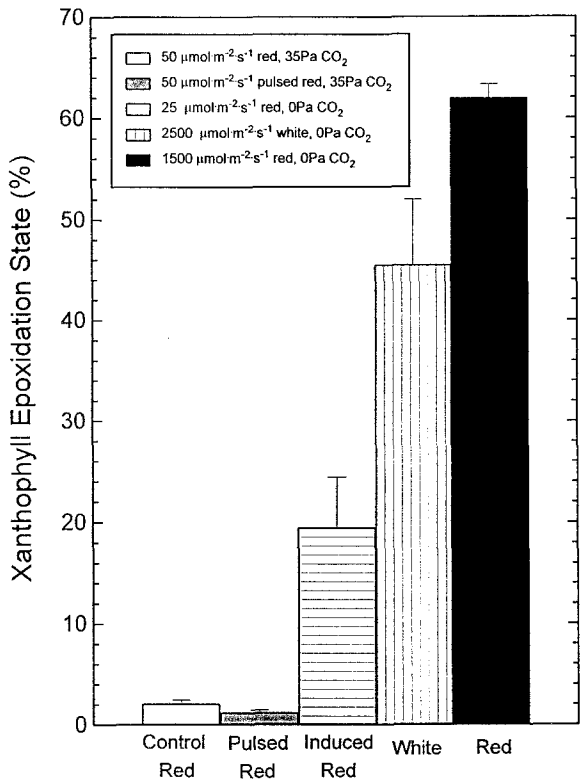

Fig. 6. Chloroplast xanthophyll pigment de-epoxidation in tissue irradiated for one h under light treatments. 'Control red' represents xanthophyll epoxidation state (EPS) from leaves irradiated with 50 μmol m^{-2} s^{-1} of continuous LED irradiation while 'pulsed red' represent that of tissue irradiated with 2 ms pulses of 5000 μmol photons m^{-2} s^{-1} separated by 198 ms dark time. 'Induced red' represents leaves treated with 25 μmol photons m^{-2} s^{-1} in CO_2 free and 2% O_2 air. Red and White represent violaxanthin de-epoxidation in red (1500 μmol photons m^{-2} s^{-1}) and white light (2500 μmol photons m^{-2} s^{-1}).

when high photon fluxes are desired. The response of photosynthesis to photon flux density (PFD) was the same when the light was delivered in pulses as when it was delivered continuously, provided the frequency was in the range of kHz (Fig. 2). In detailed measurements at low PFD, no effects of pulsing on photosynthesis were seen, even though pulses of up to $2\frac{1}{2}$ times sunlight were used (Fig. 3). The photosynthetic apparatus integrates the pulsed light and uses it as efficiently as continuous light.

As the pulses were lengthened, photosynthesis in the pulsed light fell below that in continuous light (Fig. 5). This may result from the light pulses delivering more photons than can be turned over by or stored in electron transport components. We found that pulses with more than 6×10^{17} mol photons m^{-2} could not be used as efficiently as pulses with less than that amount. We believe that this is a measure of the number of PS II centers in the leaf as Chow et al. (1989) have shown using a similar flashing light technique. The reason that this technique measures PS II is because of the time constants of the various processes of photosynthesis and the pool sizes of intermediates involved. Movement through the cytochrome complex can take ms (Whitmarsh and Kramer 1979; Harbinson and Hedley 1988), so very little whole-chain electron transport could occur during the short pulses we used. The pool size of PS II is likely to be close to what we measured with flashing light based on our measured value of 700 mmol Chl m^{-2} and 650 mol Chl mol^{-1} PS I (Whitmarsh and Cramer 1979). There could be more PS II than PS I but some of the PS II could be inactive (Chylla and Whitmarsh 1989, 1990; Ort and Whitmarsh 1990; Nedbal et al. 1991). From this information we estimate the PS II pool size to be 6×10^{17} mol m^{-2}. We estimate that plastoquinone provides a capacitance of 10^{19} mol photons m^{-2} (Whitmarsh and Cramer 1979) and that the triose phosphate pool provides about 10^{20} mol photons m^{-2} (Badger et al. 1984). The measured capacitance (10^{17} photons m^{-2}) is much lower than either of these downstream intermediates and support our theory that PS II is limiting.

While this capacitance is remarkable on the time scale used in these measurements, it is of little consequence for postillumination CO_2 fixation. This is because carbon metabolism intermediates have much greater capacitance than electron transport intermediates. In sun leaves, the pool of RuBP alone provides a post illumination CO_2 fixation of 5.3 μmol CO_2 m^{-2} in *Phaseouls* and 14.5 μmol CO_2 m^{-2} in *Alocasia* leaves (Sharkey et al. 1986). Here the $6{\cdot}10^{17}$ e^- m^{-2} stored in PS I and PS II could provide for the fixation of 0.25 μmol CO_2 m^{-2} assuming 4 e^- per CO_2. The ATP required for this amount of fixation could come from the movement of the electrons stored in PS II through plastoquinone to PS I.

Conversion of violaxanthin to zeaxanthin is known to occur when excess light energy is provided to leaves. No conversion of violaxanthin to zeaxanthin was evident when the pulse duration was 2 ms, even though the rate of photosynthesis was reduced by half its original amount (Fig. 6). Since zeaxanthin formation is associated with a light dependent decrease in thylakoid lumen pH (Hager 1969), the lack of zeaxanthin formation in our study is consistent with a slow transport of electrons to the plastoquinone shuttle.

Finally, we address whether plants use intermittent light (in kHz frequencies) as effectively as they use continuous light. Despite analyses showing that intermittent light is at best, only as good as continuous light and that it can be used much less efficiently than continuous light (as was the case for the data presented here and as reviewed by Sager and Giger (1980)), the idea that plants use intermittent light better than continuous light is still being considered (Barta et al. 1992). This idea stems in part from a misinterpretation of the Light Utilization Efficiency (LUE) measure of Chazdon and Pearcy (1986). They show that the photosynthesis that can occur in response to a light fleck can be greater than the photosynthesis expected in continuous light at that PFD. With our pulses at 5000 mmol m^{-2} s^{-1} we calculate a LUE of 2700%. However, in both the sunfleck work of Pearcy and colleagues and the work reported here, in no case were equal numbers of photons used more efficiently when provided in pulses than when provided continuously. The relative photon requirement calculation shows that the photon requirement in pulsed light is 100% of that in continuous light under the conditions given here (Fig. 4). In the light fleck work, the greater than 100% LUE comes about because of capacitance provided by carbon metabolism intermediates (Sharkey et al. 1986) and in this work the greater than 100% LUE comes about because of the capacitance of electron transport which we conclude is predominantly PS II.

Leaves can integrate and use μs pulses of light as efficiently as continuous light, providing pulse PFD do not exceed the capacitance of electron transport carriers. The technique we describe could be adapted to portable gas exchange systems using LED lamps. Addition of background far-red light from diodes could improve the method by effectively closing PS I (Chow et. al. 1989). Thus, pulsed LEDs could provide a field portable method for measuring functional PS II in intact leaves. The 668 nm LED provides the greatest potential for converting electricity to photosynthetic carbon assimilation. Although there is no advantage to providing photons in pulses relative to a continuous stream, the frequency and peak PFD of light pulses attainable with LED lighting may be desirable for further research on capacitance and limitations of photosynthetic electron transport.

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