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Technical communication

Light-emitting diodes as a light source for photosynthesis research

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Abstract

Light-emitting diodes (LED) can provide large fluxes of red photons and so could be used to make lightweight, efficient lighting systems for photosynthetic research. We compared photosynthesis, stomatal conductance and isoprene emission (a sensitive indicator of ATP status) from leaves of kudzu (*Pueraria lobata* (Willd) Ohwi.) enclosed in a leaf chamber illuminated by LEDs versus by a xenon arc lamp. Stomatal conductance was measured to determine if red LED light could sufficiently open stomata. The LEDs produced an even field of red light (peak emission 656 ± 5 nm) over the range of 0–1500 μmol $m^{-2} s^{-1}$. Under ambient CO_2 the photosynthetic response to red light deviated slightly from the response measured in white light and stomatal conductance followed a similar pattern. Isoprene emission also increased with light similar to photosynthesis in white light and red light. The response of photosynthesis to CO_2 was similar under the LED and xenon arc lamps at equal photosynthetic irradiance of 1000 μmol $m^{-2} s^{-1}$. There was no statistical difference between the white light and red light measurements in high CO_2. Some leaves exhibited feedback inhibition of photosynthesis which was equally evident under irradiation of either lamp type. Photosynthesis research including electron transport, carbon metabolism and trace gas emission studies should benefit greatly from the increased reliability, repeatability and portability of a photosynthesis lamp based on light-emitting diodes.

Introduction

The light-emitting diode (LED) has been used as a light source for whole plant growth (Bula et al. 1991, Hoenecke et al. 1992) and for measuring in vivo chlorophyll fluorescence induction (Wickliff and Wickliff 1991). However, a thorough evaluation of a LED lamp for photosynthesis research has not been reported. Improvements in semiconductor technology has dramatically increased the light output of LEDs resulting in LED photon output levels useful for photobiological research. We evaluated a new type of red LED made of aluminum gallium arsenide (AlGaAs) and designed to minimize internal adsorption of photons resulting in increased electrical conversion efficiency (Steranka et al. 1988). These semiconductors can be fabricated to emit photons with peak wavelengths ranging from 630 to 940 nm (half band width of 20–25 nm). Electrical efficiency of these devices rivals the cool white fluorescent lamp and the spectral output overlaps with the photosynthetic action spectrum (Barta et al. 1992). Traditionally, the LED chip is encapsulated in an epoxy casing which focuses the emitted light into a viewing angle of 8° to 60°. An improvement on these devices has been made by installing the chips directly onto a ceramic heat sink resulting in virtually no infrared radiation and light emission over a 180° viewing angle (Ignatius and Martin 1992). An array of 250 LED chips creates an even field of red light with intensities equaling full sunlight yet remains cool to the touch, unlike many conventional photobiological lamps that require water jacketed cooling. Light intensity is regulated by varying the current through the LED array. This new light source is palm sized and portable.

The LEDs designed to emit longer wavelengths of light (AlGaAs, 660 nm) are more efficient than shorter wavelength emitters (SiC, 460 nm) and are therefore 1000 times brighter (Barta et al. 1992). It was important to select an LED type that is electrically efficient and which emits photons that are efficient for photosynthesis. We selected LEDs with a peak emission of 660 nm to provide light for photosynthesis. Providing all the photosynthetic light energy in such a narrow range of wavelengths may result in photosynthetic limitations for two reasons. First, the stomatal action spectrum is known to peak in the blue region of the light spectrum (Sharkey and Raschke 1981). Light which lacks blue photons may result in reduced stomatal conductance which in turn may affect leaf temperature, transpiration rate and $p(CO_2)$ in the leaf. Low stomatal conductance can limit the maximal rate of photosynthesis (Farquhar and Sharkey 1982). Measurement of leaf transpiration can be used to determine the stomatal conductance and its effect on $p(CO_2)$. Photosynthetic rates measured in saturating CO_2 will minimize the effect of stomatal aperture on photosynthetic rate.

The second reason that a narrow range of wavelengths may limit photosynthesis is that an imbalance of photons available to Photosystem I (P700) and Photosystem II (P680) could occur affecting the ratio of cyclic to whole chain electron transport, upsetting the balance between NADPH and ATP. An LED with peak emission of 660 nm (22 nm half band width) provides 80% of its photons below 680 nm. This means that 20% of the photons are not available to P680. The occurrence and magnitude of these potential effects can be assessed by measuring isoprene emission which is highly correlated with ATP status (Loreto and Sharkey 1993).

We measured photosynthetic rate and stomatal conductance as a function of CO_2 and light levels, using light from LEDs or a xenon arc lamp to assess perturbations in photosynthesis due to total photosynthetic irradiance in a narrow band of wavelengths versus white light. In some experiments, we also measured isoprene emission to asses any alteration of ATP status due to light quality.

Materials and methods

Plant material and growth conditions

Kudzu (*Pueraria lobata* (Willd) Ohwi.) plants were propagated by stem cuttings and grown to maturity in reach-in growth chambers (model E15; Conviron, Winnipeg, Manitoba, Canada) under 16 h photoperiod and day/night temperature 26 °/18 °C. Metal halide lamps, supplemented with incandescent lamps provided 1000 μmol photons $m^{-2} s^{-1}$ at the canopy. Each plant was grown in a 10 L plastic pot, filled with peatlite (Metro-Mix 360: Grace Sierra Co., Milpitas, CA, USA) and watered 4 times per day with half-strength Hoagland's solution (Hoagland and Arnon 1938).

Gas-exchange measurements

An aluminium leaf cuvette with a glass window (10 × 14 cm) enclosed a single mature terminal leaflet. Air entering the cuvette was mixed with mass-flow controllers (Datameterics type 825; Edwards High Vacuum, Wilmington, MA, USA) from cylinders of N_2, O_2 and 5% CO_2 in air. Air was passed through warm water for humidification and subsequently through a copper coil in a refrigerated water bath to established the desired dewpoint in the leaf cuvette. Leaf photosynthetic rates and conductance were calculated by measurement of CO_2 partial pressure and dewpoint of the air before entering and after exiting the leaf cuvette with an infrared gas analyzer (6262; LI-COR, Lincoln, NE, USA). Gas exchange equations used were as described by Von Caemmerer and Farquhar (1981). Temperature was maintained at 30.0 ± 0.5 °C using thermoelectric blocks and was measured by a copper-constantan thermocouple pressed along the abaxial surface of the leaf for 1 cm.

Isoprene measurements

Isoprene was measured by sampling the gas exiting the leaf chamber with a small pump supplied as part of the Scentoscreen portable gas chromatograph (Scentoscreen, Sentex Systems, Inc., Ridgefield, NJ, USA). The gas was pulled through a small preconcentrating column filled with Carbosieve S-III at a rate of 80 ml min^{-1} for 15 s. The gas path was then changed and a flow of argon (Grade 4.7) was passed through the

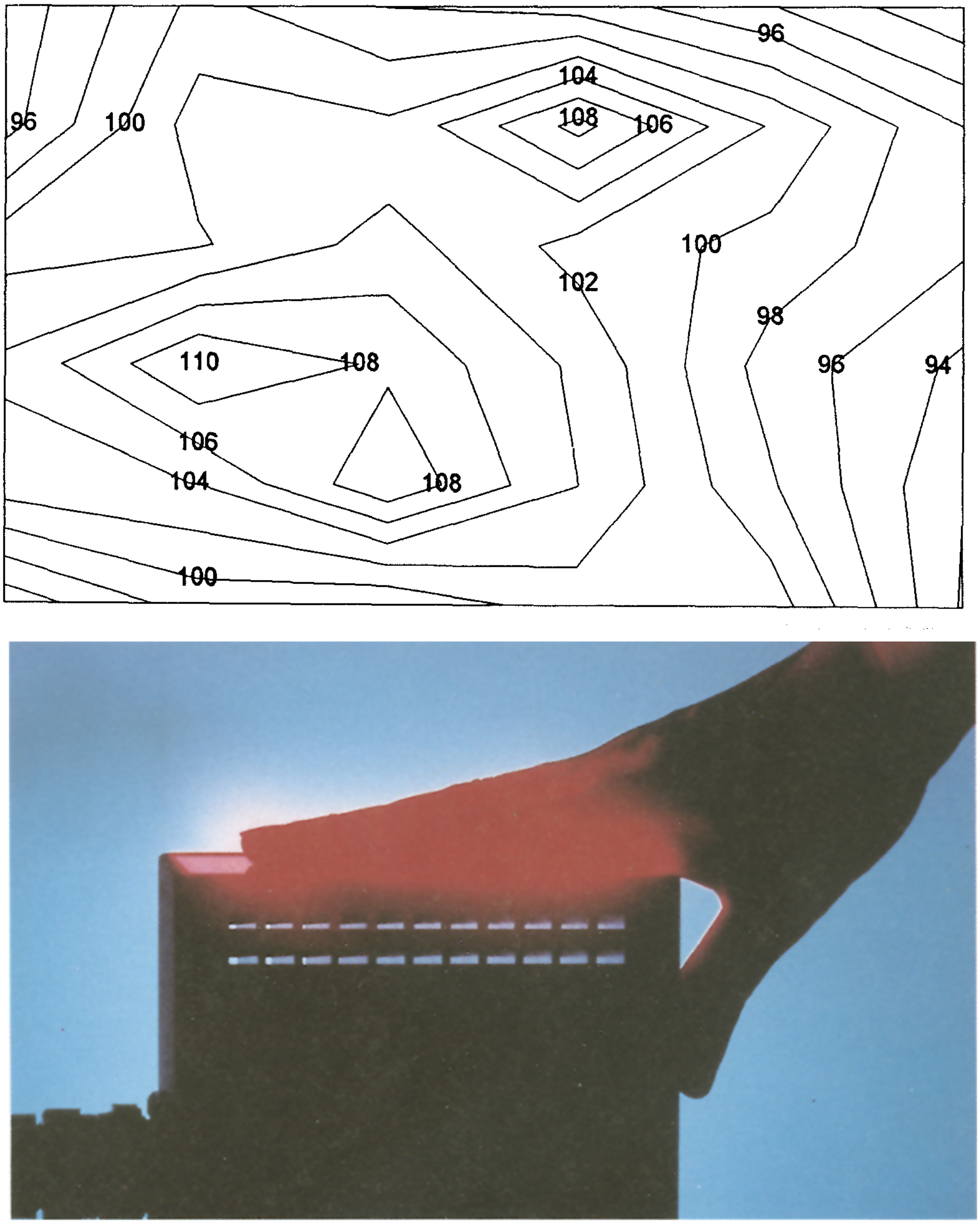

Fig. 1. (Top) Contour plot of the emitting surface of an LED lamp. Each line represents the percent of mean irradiance at half power for 36 light measurements dispersed evenly over the 6 × 10 cm emitting surface. (Bottom) The QBeam 2000 lamp head operating at full power emits light intensities equivalent to full sunlight yet remains cool to touch.

preconcentrator while it was rapidly heated using nichrome wire wrapped around the preconcentrator. The argon passed through a capillary column for volatile organic carbon (Vocol) supplied by Sentex and isoprene was measured by an argon ionization detector. Liquid isoprene (99.9% pure) from Fluka Chemical Co. (Milwaukee, WI, USA) was used to make gas standards of 250 ppb after twice diluting in glass flasks containing pure nitrogen.

Light sources and spectral measurements

A beam of white light from a xenon arc lamp was passed through a water filter to remove infrared radiation and reflected down onto the leaf cuvette for white light treatments. Intensity was modulated using neutral density filters. Red light was provided using a QBeam photosynthesis lamp (Quantum Devices, Inc., Barneveld, WI, USA) consisting of 250 LED chips mounted on a ceramic heat sink, backed by aluminum fins and a cooling fan. Red light intensities were modulated by a 10 turn potentiometer which controlled current to the LED array. Light intensity was measured using a quantum sensor (LI-185, LI-COR, Lincoln, NE, USA) and spectral characteristics were measured using a spectroradiometer (LI-COR). Quantum sensor irradiance values were 2% lower than spectroradiometer values (400–700 nm).

Results

Lamp characteristics

When operated at half power, the emitting surface average irradiance was 963 ± 12 μmol $m^{-2} s^{-1}$ (average ±SE) measured by 36 independent quantum sensor readings placed 5 mm from the emitting surface. A contour plot shows changes in light intensity as percent of the mean (Fig. 1). At full power, the emitting surface average irradiance was 1912 ± 15 μmol $m^{-2} s^{-1}$ (average ± SE) yet the surface of the lamp was cool to touch (Fig. 1). A power versus light output curve was made using the remote probe of a spectroradiometer placed inside the gas exchange cuvette. The average photon output

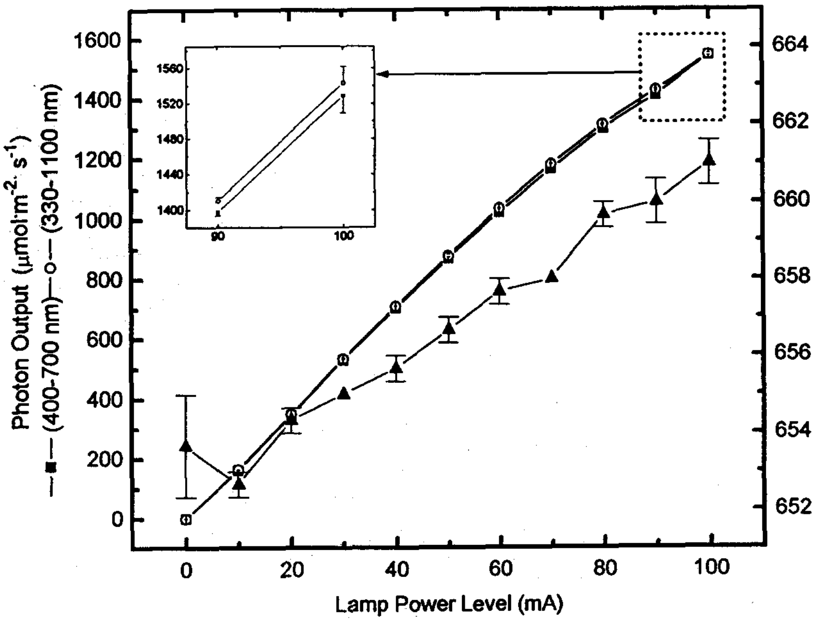

Fig. 2. Photon output and spectral quality of light from light emitting diodes. Photosynthetic light intensity (■), total light intensity (O) and peak photon emission (▲) based on measurements from a spectroradiometer and remote probe in a glass covered leaf cuvette. Each data point represents 3 sets of measurements. Error bars indicate standard error of the mean and were smaller than the symbol size for the photon output but are resolved in the inset. A set of data includes a range of 10 measurements taken from low to high power and from high to low power.

was nearly linear over the range of 0 to 1500 μmol $m^{-2} s^{-1}$ (Fig. 2). Spectral quality of the LED light varied from 652 nm peak irradiance to 661 nm peak irradiance at low and high power, respectively. This shift in wavelength could be suppressed by controlling the LED temperature (data not shown).

Photosynthetic rate and isoprene response to light

Under ambient CO_2, the photosynthetic response to red light deviated slightly from the response measured in white light (Fig. 3). At low light intensities (175 μmol $m^{-2} s^{-1}$) the quantum yield of photosynthesis was 0.027 ± 0.002 mol CO_2 mol photons^{-1} and 0.022 ± 0.002 mol CO_2 mol photons^{-1} from incident irradiance of LED and xenon are light. Stomatal conductance followed a similar pattern as photosynthesis in response to light intensity (Fig. 3). At low intensities, stomatal conductance was greater in red light than in white light. In 175 Pa CO_2, the photosynthetic response curve to light was virtually identical between the two light treatments (Fig. 4). The response of isoprene emission to light was similar in white light and red light (Fig. 5). Although there was a slight deviation in the mean isoprene emission rate at 500 μmol $m^{-2} s^{-1}$ between the two light treatments, the difference was not statistically significant ($p = 0.05$).

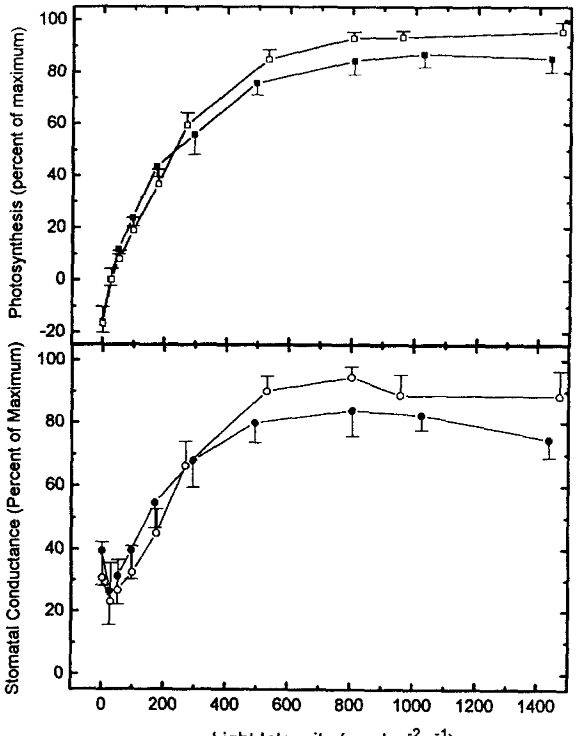

Fig. 3. Net photosynthesis and stomatal conductance, as percent of maximum in white light as a response to light intensity from xenon arc (open symbols) and LED (closed symbols) lamps. All values represent the average of 5 different kudzu leaves. The mean value for maximum photosynthesis was 9.7 μmol $m^{-2} s^{-1}$ and the mean value for maximum stomatal conductance was 0.12 mol $m^{-2} s^{-1}$. Leaf temperature was 30 °C and CO_2 partial pressure was maintained at 35 Pa.

Photosynthetic response to CO_2 level

The response of photosynthesis to CO_2 partial pressure was similar under the LED and xenon arc lamps which provided equal photosynthetic irradiance of 1000 μmol $m^{-2} s^{-1}$ (Fig. 6). There was no statistical difference between the white light and red light measurements at each paired set of data. Some leaves exhibited feedback limitation of photosynthesis as a decline in photosynthesis with increasing $p(CO_2)$ and this unusual behavior was independent of lamp type. However, photosynthesis of leaves which had been water-stressed was reduced in red light relative to white light levels (data not shown).

Discussion

The LED lamp is an efficient, durable, light weight, solid state lamp which can provide light intensities equalling full sunlight at the emitting surface. Each chip emits light in 180°, resulting in even light distribution close to the emitting surface (Fig. 1). In the laboratory and field, the LED lamp offers greater control of light intensity (Fig. 2) which enables easy repetition of light treatments relative to natural lighting and use of neutral density filters. With adequate control of power input, LED technology will be an excellent choice for quantum requirement studies because it provides repeatable low light intensities and no spectral shift when lamp temperature is held constant. However, large changes in

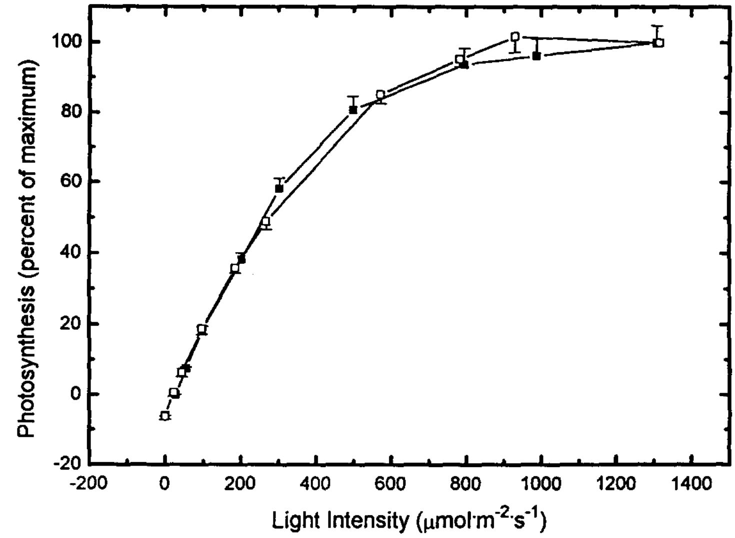

Fig. 4. Net photosynthesis, as in Fig. 3, except that 175 Pa CO_2 was maintained.

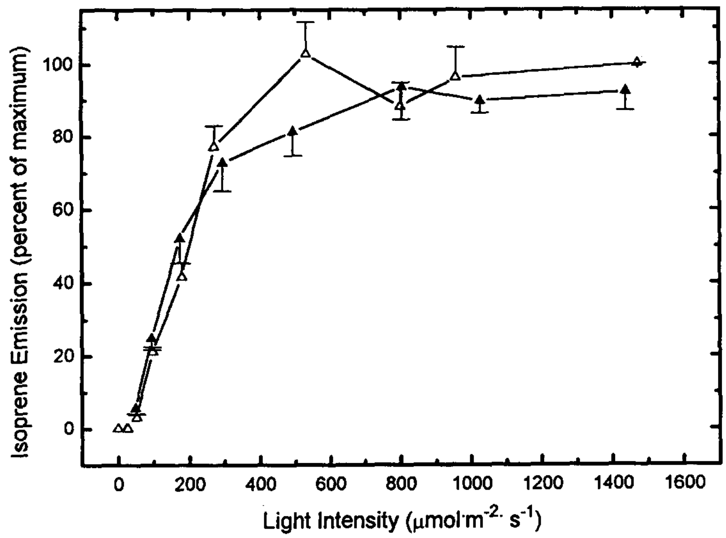

Fig. 5. Isoprene emission from kudzu leaves irradiated with xenon arc (△) or LED (▲) light. Leaf temperature was 30 °C and the partial pressure of CO_2 was maintained at 35 Pa. All values represent the average of 5 different leaves. The mean value for maximum was 76 nmol m^{-2} s^{-1} isoprene.

LED photon output result in up to a 9 nm spectral shift (Fig. 2). This effect is largely due to heat buildup at the LED chip. If the emitting surface temperature is maintained at a constant temperature this effect can be minimized (unpublished data). As a solid state device, the LED has a rise and fall time of approximately 80 ns (Barta et al. 1992) and therefore may be beneficial for intermittent, pulsed, or sunfleck photosynthesis research.

The photosynthetic response to light was similar for the LED lamp and the xenon arc lamp treatments. Photosynthesis was greater in red light at lower light intensities and slightly lower in high light. Although the differences were not significant at the 5% level in a pairwise t-test of

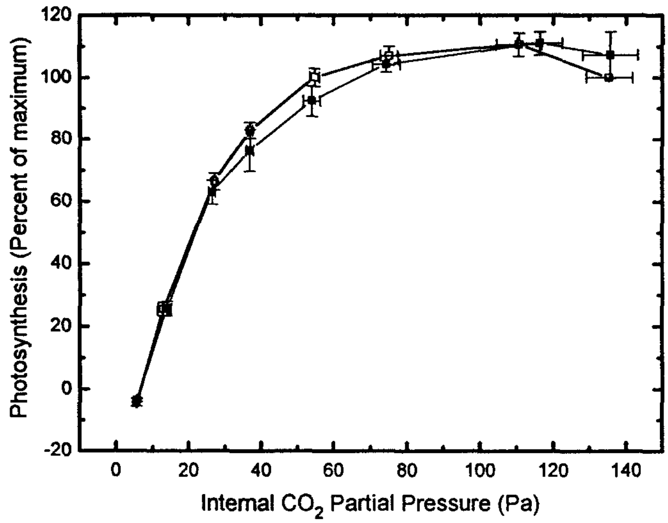

Fig. 6. Photosynthesis as percent of maximum from kudzu leaves in response to varying levels of internal CO_2 partial pressure and 1000 μmol $m^{-2} s^{-1}$ of light from xenon arc (□) and LED (■) lamps. Leaf temperature was 30 °C. All values represent the average of 5 different leaves. The mean value for maximum was 14 μmol $m^{-2} s^{-1}$ CO_2.

each measurement, the incident quantum yield of photosynthesis was 0.027 and 0.022 in red and white light, respectively, and these were significantly different (P = 0.01) (Fig. 3). The greater quantum yield in LED light may, in part, be due to greater photosynthetic action from red photons verse blue or green photons found in the xenon arc light. However, stomatal conductance followed a similar pattern, crossing at the same point in the light curve as photosynthesis (Fig. 3). Although stomata are most responsive to blue photons, red photons provide sufficient signal for stomata to open (Sharkey and Raschke 1981). Addition of blue photons to the red LED light may increase stomatal conductance sufficiently to match that in white light.

The deviation in photosynthetic rates between the two lights is likely to be caused by the difference in stomatal conductance. Lower stomatal conductance is more significant at high photosynthetic rates due to reduced pCO_2 in the leaf (Farquhar and Sharkey 1982). When saturating CO_2 levels were used, stomatal limitations were minimized and the photosynthetic response to red light overlapped that of white light (Fig. 4). Under those conditions, the incident quantum yield was 0.047 and 0.043 under LED and xenon arc light, a difference that was not significantly different (P = 0.11). A thorough evaluation of quantum yield from different peak wavelength emitting LEDs and white light is required to discern the difference between these light sources.

Measurements of isoprene emission rates are not different between the white light and red light under any irradiance level. Because isoprene emission is correlated (r = 0.8 to 0.95) with ATP status (Loreto and Sharkey 1993) we believe that the LED light does not upset the ATP balance, relative to white light from a xenon arc lamp, under any irradiance level. Another indicator of ATP status is the decline in photosynthesis as $p(CO_2)$ increases (Sharkey 1990). This behavior was unaffected by lamp type, again indicating that the ATP status was not upset by the red light.

Photosynthetic response to increasing CO_2 was independent of lamp type (Fig. 6). A thorough evaluation of quantum requirement may resolve slight differences in photosynthetic action between LEDs that differ in peak wavelength emission and between a white light source. The red LED light does not affect the magnitude of feedback inhibited photosynthesis or the frequency of occurrence. Preliminary data of photosynthesis in water stressed leaves shows patchiness may cause a greater reduction on photosynthesis in red versus white light (unpublished data).

Conclusion

The LED has many advantages over conventional light sources for photosynthesis research. Although care should be taken when interpreting photosynthetic measurements where stomatal patchiness has been induced, other research such as electron transport, carbon metabolism and trace gas emission should benefit greatly from the increased reliability, repeatability and portability of a photosynthesis lamp based on light emitting diodes.

Acknowledgments

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