Novel Red, Green, Blue LED Light Source and Whole Plant Chamber Make Photosynthetic Assessment of Small Plants Possible

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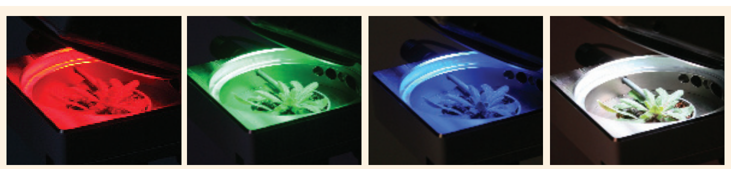
Introduction

- Difficulty measuring photosynthesis on small, low-stature plants is overcome in the 6400-17 Whole Plant Arabidopsis (WPA) Chamber.
- The 6400-18 RGB (red, green, blue) Light Source, in conjunction with this novel chamber, facilitates rapid assessments of photosynthetic capacity.
- Independently controlled red, green and blue LEDs in the RGB Light Source can be used to measure photosynthetic efficiency at different wavebands and intensities.
- Various blocking techniques used to isolate above ground carbon fluxes in the WPA Chamber are demonstrated.
- Photosynthetic response of wild-type *Arabidopsis thalina* (Col-0) and soybean (*Glycine max* cv. U98-311442) to light intensity at different wavebands are presented.

Light Source and Chamber Design

- The 6400-18 RGB Light Source illuminates a 7 cm diameter area with a spatial uniformity of ± 10% over 90% of the area.
- Intensities of white light can be achieved from 0 to 2000 µmol m^{-2} s^{-1} by providing equal quanta of red, green and blue light.
- Independently controlled red, green and blue (wavelength peaks at 460, 522 and 635 ± 5 nm, respectively) LEDs allow for color selection (Figure 1).
- The 6400-17 WPA Chamber encloses entire plants in a 7 cm diameter chamber (Figure 2).
- The chamber bottom seals to the growth container, either 38 mm Cone-tainers™ or 65 mm round pots, with either an o-ring or flange seal.



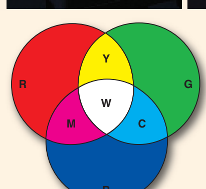


Figure 1. The RGB Light Source has independently driven red, green and blue LEDs that provide illumination singly and in concert. By varying the output from each type of LED, different colors of light can be produced, allowing exploration of plant response to light quality.

Figure 2. The RGB Light Source and WPA Chamber interface with the LI-6400XT Portable Photosynthesis System. Plant and container are sealed inside the WPA with an o-ring or flange to facilitate rapid assessment of individual plants. The top of the WPA seals to the chamber bottom with an airtight o-ring.

Blocking Soil CO_2 Flux

- When no blocking technique was employed, enclosing the growth container within the WPA Chamber resulted in measurement errors. Errors were the result of CO_2 fluxes from soil and roots inside the containers.
- Different methods of blocking soil CO_2 flux were tested by injecting pure CO_2 through a hole in the side of the container 1 – 1.5 cm below the soil surface.
  - Exhaust restrictions forced mass flow through the soil and were able to partially suppress CO_2 flux (Figure 3).
  - Suppression increased when an alternate exhaust path was provided by punching small vent holes in the containers.
  - A 3 - 5 mm thick pottery clay cap decreased CO_2 flux into the chamber by 99.8% (Table 1).

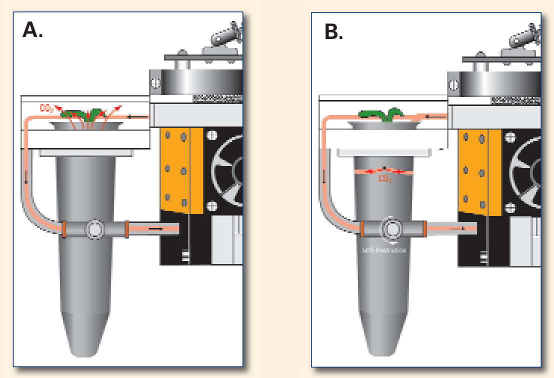


Figure 3. The exhaust path of the Whole Plant Arabidopsis chamber is shown with the optional Adjustable Exhaust Valve Assembly installed. **A.** An unrestricted exhaust path allows CO_2 to flow freely into the chamber from the roots, soil and outside the container. **B.** Influx was decreased by restricting the needle valve 25 – 50 %, diverting 100 – 150 µmol s^{-1} of flow through the soil and out a small vent hole (~0.5 mm) in the container. This caused a small overpressure in the chamber of about 0.15 kPa, which is slightly higher than the normal chamber over pressure of about 0.02 kPa. Matching the sample and reference IRGAs in the LI-6400XT removed the effect of the overpressure on the CO_2 concentration measurement.

Table 1. Pottery clay formed an effective cap to block CO_2 flux from the soil. The pottery clay cap consisted of a 3 – 5 mm thick layer of clay with a 4 mm hole in the center for a plant to grow through. Covering the soil with pottery clay altered the ΔCO_2 (maximum CO_2 concentration during spike – average CO_2 concentration prior to injection) following injection of 250 ml CO_2 through the side of the container just below the soil surface. The exhaust valve restriction had little effect on increasing the effectiveness of the clay cap. The values are the average ΔCO_2 ± standard error of the measurement for the number of samples indicated (n).

Injected gas	Hole Closed ΔCO_2	n	Partially Open ΔCO_2	n	Hole Open ΔCO_2	n
Pure CO_2	195.3 ± 21.8	5	60.9 ± 22.1	3	0.14 ± 0.06	5
CO_2-free Air	-0.07 ± 0.29	2			0.11 ± 0.05	2

Arabidopsis Rosette Photosynthesis

- Plants were germinated on soil and transplanted 5 to 10 days post-germination to individual containers with pottery clay caps.
- Plants were grown under short days at 175 µmol m^{-2} s^{-1} fluorescent light for 6 to 8 weeks prior to measurements.
- Plants with about 10 cm^2 leaf area typically provided the best results due to minimal self shading (data not shown).
- Arabidopsis rosette photosynthetic responses to different wavebands of light were similar despite differences in absorbance (Figure 4).

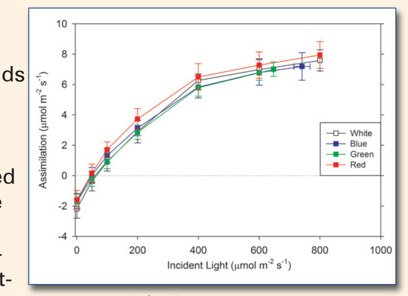


Figure 4. Photosynthetic responses of Arabidopsis rosettes to different wavebands of light were similar despite differences in absorbance. Photosynthetic response to increasing light was measured in the WPA Chamber with the RGB Light Source. To isolate above ground fluxes both exhaust tube restriction and pottery clay caps were used to suppress soil CO_2 flux. The light intensity given is the intensity incident on the plane of the majority of leaves of the rosette. Total plant leaf area was determined photographically with ImageJ (NIH, http://rsbweb.nih.gov/ij/). Values are the average photosynthetic assimilation rate ± standard error.

Leaf Photosynthesis for Soybean and Arabidopsis

- Arabidopsis absorption in the blue and red wavelengths was 30% higher than in green wavelengths (Figure 5).
- Soybean absorption across all wavelengths was high without a large dip in the green wavelengths.
- Arabidopsis and soybean weighted absorption spectra between red and green light varied by 9% and 6% respectively (Table 2).
- Similar to intact Arabidopsis rosettes, there were no significant differences in the photosynthetic response to different wavebands for individual Arabidopsis and soybean leaves (Figures 6A & 7).
- Quantum yield (µmol CO_2 / µmol photons) was very similar for all wavebands in Arabidopsis (Figure 6B).

Figure 5. Maximum light absorption by Arabidopsis and Soybean leaves is near the peak wavelengths of the red and blue LEDs in the RGB Light Source. The broader output spectrum of the green LEDs likely accounts for the similar photosynthetic responses, despite the differences in absorbance. Arabidopsis and soybean leaf absorption spectra were measured from 400 to 700 nm using a spectroradiometer (LI-1800 with integrating sphere) on the adaxial side of the leaf.

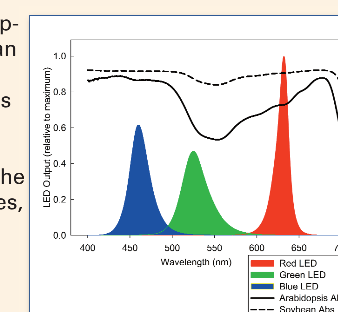


Table 2. The weighted absorption (αw) across the wavebands for the three LED types showed little variation for Arabidopsis and soybean. The calculated weighted absorption ($\alpha\omega$) is the leaf absorption (α) multiplied by the LED output (Q) integrated for the wavelengths between (λ) 400 to 700 nm, and is normalized to the light intensity (Q).

$$\alpha_w = \frac{\int_{400}^{700} \alpha(\lambda)Q(\lambda)d\lambda}{\int_{400}^{700} Q(\lambda)d\lambda}$$

LED Color	Arabidopsis	Soybean
White	0.74	0.90
Red	0.72	0.90
Green	0.63	0.87
Blue	0.86	0.93

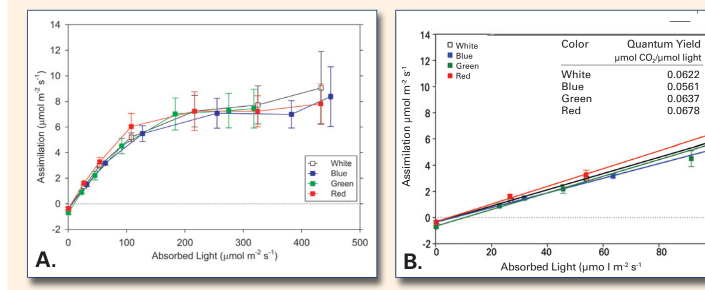
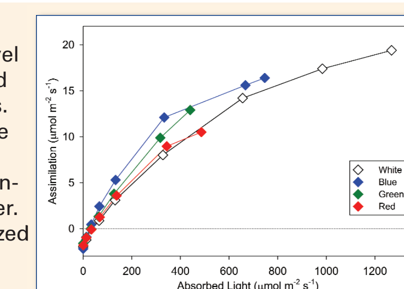


Figure 6. Leaf-level photosynthesis was measured with the RGB mounted above the LI-6400XT's standard clear-top 2x3 chamber. **A.** Arabidopsis leaf level photosynthesis responded similarly to all wavebands. The light intensity is the absorbed light normalized to each LED's output. **B.** The quantum yield from the light response curves was calculated from the linear relationship of absorbed light to assimilated CO_2 ($R^2 > 0.95$). The quantum yield for each waveband is reported in the upper right. Values are the average photosynthetic assimilation normalized to leaf area ± standard error of four samples.

Figure 7. Soybean leaf level photosynthesis responded similarly to all wavebands. Measurements were made with the RGB mounted above the LI-6400XT's standard clear-top 2x3 chamber. Absorbed light is normalized to each LED's output.



Conclusions

- The WPA Chamber and RGB Light Source make possible photosynthetic measurements of small or low stature plants.
- The RGB Light Source has independently controllable red, green and blue LEDs which facilitate the exploration of photosynthetic response to light quality.
- Despite differences in absorption, whole plant and leaf-level photosynthetic response at different wavebands were similar in Arabidopsis.
- Soybean showed little variation in absorption across the photosynthetic region of the spectrum and as a result had similar response to light in different wavebands.

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