Quantifying and manipulating the angles of light in experimental measurements of plant gas exchange

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Abstract

Diffuse light has been shown to alter plant leaf photosynthesis, transpiration, and water-use efficiency. Despite this, the angular distribution of light for the artificial light sources used with common gas exchange systems is unknown. Here we quantify the angular distribution of light from common gas exchange systems and demonstrate the use of an integrating sphere for manipulating those light distributions. Among three different systems, light from a 90° angle perpendicular to the leaf surface (± 5.75°) was <25% of the total light reaching the leaf surface. The integrating sphere resulted in a greater range of possible distributions from predominantly direct light (i.e., > 40% of light from a 90 ± 5.75° angle perpendicular to the leaf surface) to almost entirely diffuse (i.e., light from an even distribution drawn from a nearly 0° horizontal angle to a perpendicular 90° angle). The integrating sphere can thus create light environments that more closely mimic the variation in sunlight under both clear and cloudy conditions. In turn, different proportions of diffuse light increased, decreased, or did not change photosynthetic rates depending on the plant species observed. This new tool should allow the scientific community to explore new and creative questions about plant function within the context of global climate change.

Introduction

Experimental measurements of leaf gas exchange are a cornerstone of plant physiology in both basic and applied settings. The portable infrared gas analyzers used to conduct these measurements can control different environmental factors (e.g., humidity, CO_2, light) known to affect plant carbon and water exchange. The ability to manipulate light conditions is of particular interest, and portable infrared gas analyzers typically utilize artificial light sources composed of a mix of different colored light emitting diodes (at least red and blue, but often more) that allow for the precise control of both the quantity and spectral distribution of light (*e.g.,* LI-COR Biosciences, 2021). However, the light experienced by plants can vary not only in quantity and spectral quality, but also with respect to the angle at which that light strikes the leaf surface (often referred to as angle of incidence).

The plant physiology community generally and implicitly assumes that the angle of incidence of light emanating from artificial light sources is perpendicular to the leaf surface. This is logical insomuch as the light source sits directly over the leaf chamber similar to the sun generally occurring above a plant canopy on a clear midsummer day. Both solar zenith angle and atmospheric scattering affect the angle of incidence of sunlight, leading to a wide range of light environments experienced by plants. Despite this, information on the angle of incidence emanating from artificial light sources for gas exchange is not readily available. One of the primary companies that manufactures gas exchange equipment (LI-COR Biosciences) has also confirmed that they have not quantified the angle of incidence for light in their systems (M. Johnson, pers. comm.). As a variable known to affect leaf gas exchange, it is important that users know the angle of incidence in their experimental setups.

Moreover, plants are frequently subject to light that is not direct (collimated), but rather a mix of direct and diffuse (scattered) light (Steven 1977). Diffuse light occurs when aerosols (e.g., pollution or clouds) scatter the light from the sun, changing both the quantity of light reaching the leaf surface and the angle of incidence. Due to atmospheric scattering and aerosols, even the clearest day will have some proportion of light that is diffuse; these values are often at least 15% of total light and can reach as high as 30 or 40 % under midday conditions (Steven 1977, Spitters et al. 1986). There is also evidence that the annual diffuse fraction of light was 44% in 2000 (the most recent year with data), an increase of approximately 15 % since 1900 (Mercado et al. 2009). Finally, plants (and leaves) occurring below the canopy, under shade houses, or in greenhouses with diffusive glazing consistently experience diffuse light.

Diffuse light can have a significant effect on leaf optics and gas exchange (Vogelmann 1993, Vogelmann & Martin 1993, Brodersen *et al.* 2008, Berry & Goldsmith 2020), with implications for ecosystem level carbon and water fluxes (Misson *et al.* 2005, Mercado*et al.* 2009, Baguskas *et al* . 2021). Therefore, we must also consider the distributions of the angles of incidence of light as a critical variable to control when conducting gas exchange measurements. The light source for gas exchange systems would ideally reflect generalized environmental conditions, as well as allow, for example, the manipulation of the fraction of direct compared to diffuse light to the range of values that plants experience.

Knowing this, we see a major methodological gap: a variable (angle of incidence of light) known to affect leaf gas exchange (1) has neither been quantified in the most commonly used gas exchange systems, (2) nor is there a way to manipulate this variable in these systems. Here, we describe the results of a series of experiments in which we characterize the distribution of the angles of light from common artificial light sources used on portable infrared gas analyzers. We then demonstrate the use of an integrating sphere to effectively control the distribution of the angle of light reaching the leaf surface, creating a range of possible distributions from almost entirely direct light (light from a 90° angle perpendicular to the leaf surface) to almost entirely diffuse (light from an even distribution drawn from a nearly 0° horizontal angle to a perpendicular 90° angle). Finally, we demonstrate that manipulating the distribution of angles of light has different impacts on leaf photosynthesis depending on the plant species. Our objective is to advance our understanding of, and our ability to measure, how differences in the distribution of light angles reaching leaves affects plant gas exchange.

Methods

We quantified the angular distribution of light using the small (6800-02) and large (6800-03) light sources from the LI-6800 portable infrared gas analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA) and the small light source (6400-02B) from the LI-6400XT portable infrared gas analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA). The general workflow for our experiments is summarized in Figure 1.

Quantification of Light Angle

To assess the angular distribution of light sources that accompany different portable infrared gas analyzers used for plant gas exchange, we measured the amount of light reaching a fiber optic cable mounted at different angles (horizontal to vertical) below the light source. The fiber optic cable was mounted to a rotational stage that allowed for it to be tilted at precise angles across a 180° field of view. The cable had a 105 µm core diameter and a 0.1 numerical aperture, such that the angle of acceptance was 11.5° (M96L02; ThorLabs, Inc., Newton, New Jersey). The fiber optic cable was mounted just below the top of the leaf chamber (where a leaf would be located) with the light source mounted on top. The fiber optic was then rotated at 11.5° increments across the field of view (horizontal-vertical-horizontal) to collect spectra at 17 discrete angles (a total of 184°). The fiber optic cable was connected to a CCS100 compact spectrometer (350-700 nm; ThorLabs, Inc., Newton, New Jersey) and data were recorded using the ThorLabs software associated with the spectrometer.

Spectra were collected across the leaf chamber area at random positions (n =10 to 25 positions per experimental setup). Because the rotational stage only moves along one axis, we collected spectra in two cardinal directions (i.e., front-to-back and left-to-right) to integrate the angular distribution of light in both directions. Light quantity was held constant at 1390 µmol m^{-2} $s^{-1}$and the distribution of wavelengths was held constant using the same proportions of light emitting diodes (LED) in the light source (65% red, 20% blue, 10% green, and 5% white).

We also assessed if changes to the ratios of LEDs used, the total intensity of light, or the method of controlling light affected the angular distribution of light. All additional tests were done using the LI-6800 large light source. To test the effects of changing the ratio of the LEDs, we compared an LED distribution of 90% red and 10% blue to our previously conducted measurements using 65% red, 20% blue, 10% green, and 5% white. The 90/10 ratio of red to blue light is referenced on the LI-COR Biosciences website as a commonly used LED ratio (LI-COR Biosciences 2021). To test changes to intensity, we did additional measurements with the total PAR at 1000 µmol $m^{-2}s^{-1}$ and compared that to our previous measurements conducted at 1390 µmol m^{-2} s^{-1}. Finally, we measured the angular distribution of light using the "Percent" control mode on the light-control tab of the LI-6800 operating system and compared this to measurements using the "Setpoint" control. This was done to ensure that the percentage of each LED remained constant through our experiment. When using the "Percent" control, the user is able to hold the percentage of each LED color constant despite small fluctuations in total intensity or feedback in the system.

Integrating Sphere

Integrating spheres have a rich history in plant physiology and have been used to measure leaf absorptance, total radiation, and leaf area (Ehleringer *et al* . 1976, Idle & Proctor 1983, Serrano *et al.* 1997). Here, we developed an integrating sphere to provide control over the distribution of angle of light reaching the leaf surface. Specifically, we built an integrating sphere with an adjustable mount for the large light source (6800-03) typically mounted to the large chamber (6800-13) on the LI-6800 (Figure 2). The sphere was 19.2 cm in internal diameter. Along one side of the chamber was an opening with rails on either side; the light source is mounted to a guide that slides along the rails between the top and the bottom, with a screw to hold it in place at the desired position. Small inserts of different sizes are slid into the rails to close that part of the sphere not covered by the light source. The bottom of the sphere has a slot that connects to the top of the large plant chamber. The inside of the sphere is uniform and smooth with no internal structures. The integrating sphere was printed in white polylactic acid (PLA; MH Build; Matterhackers Inc., Lake Forest, California, USA) on a desktop 3D printer (Ultimaker S5, Utrecht Netherlands). The inside of the sphere and the inserts were primed by sanding with 120 grit sandpaper and then covered with ultrawhite barium sulfate coating (Avian-B Coating, Avian Technologies New London, New Hampshire, USA) purchased through Edmund Optics (Barrington, New Jersey, USA). This coating is intended to create a highly reflective surface ($>$ 97%) that effectively scatters light. The ultrawhite coating was applied as directed, which required a dilution with an alcohol solution (95% ethanol, 5% methanol) of approximately 1:1. The solution was mixed using a stir bar for 15-30 min with additional alcohol added periodically until homogenous. The solution was then evenly sprayed onto each piece with an aerosol sprayer delivered at 70 psi (Preval Sprayer, Nakoma Products, Bridgeview, Illinois). Each layer was allowed to dry until it was generally dry to the touch, typically about 5 – 10 minutes at ambient room temperature. A complete sphere, including insert and mounts, would typically require 20 layers and 200 – 300 mL of total solution. The design for this sphere is patent pending (Docket 1959206.00015).

We quantified the angular distribution of light with the integrating sphere interfaced on top of the LI-6800 large leaf chamber. Then, using the integrating sphere, we quantified the angular distribution of light for four distinct experimental setups: (1) the light source at 90° directly on top of our integrating sphere (i.e., light presumably emanating perpendicular to the leaf surface), (2) the light source at a 67.5° on the integrating sphere, (3) the light source at a 45° on the integrating sphere, and (4) the light source at a 0° on the side of the integrating sphere (i.e., light presumably emanating parallel to the leaf surface). In each of these setups, the quantity of light and the proportion of LEDs in the light source were held at the same values that were used for measurements without the integrating sphere.

Plant photosynthesis from direct to diffuse light

To assess the effects of different distributions of angles of light on leaf photosynthesis, we collected experimental leaf gas exchange data from three different plant species. Data were collected on sun-exposed leaves of mature trees of *Heteromeles arbutifolia* (Lindl.) M. Roem.

and *Citrus sinensis* (L.) Osbeck (5 individuals each) at the Fullerton Arboretum (California State University at Fullerton) during February and March 2020. Data on *Persea americana* Mill. (8 individuals) were collected on sun-exposed leaves of potted trees (approximately 2 m tall) on the campus of Chapman University (Orange, California) from June to September 2020. Instantaneous gas exchange was quantified on leaves in five different distributions of light using the integrating sphere. The sphere was mounted to the LI-6800 as described above. A fully expanded, mature leaf was placed in the chamber and allowed to stabilize under the following chamber conditions: total PAR of 1295 µmol m^{-2} s^{-1}, temperature of 27 °C, a CO_2 of 410 µmol mol^{-1}, a relative humidity of 50%, fan speed of 10,000 rpm, and a flow rate ranging from 500 to 1000 µmol s^{-1}. Each leaf was first measured with the light source at one of the endpoints of the integrating sphere (0° or 90°, alternating which was first). Once the measurement was recorded, the light source was moved to the next position on the sphere and again allowed to stabilize. This process was repeated at all five light positions. Relationships between photosynthesis

and the angle of the integrating sphere were analyzed using linear regression.

Data processing

To determine the total quantity of light for each spectrum, we integrated the area under the curve from 400-700 nm, the wavelengths commonly referred to as photosynthetically active radiation (PAR). We did this using numerical integration via the trapezoidal rule, where a series of trapezoidal areas are created under the curve and then summed to determine the total area. A trapezoid was computed between each data point resulting in 3647 distinct trapezoids for our data set. The numerical integration was computed using a Python script and processed in JupyterLab v2.2.6 (Project Jupyter, Worldwide). The total integrated area was summed from each of the 17 distinct curves created for each replicate. The percent of light arriving at each angle was determined as the amount of light at that angle divided by the total summed value. The 10 to 25 replicates for each experimental setup were averaged to create figures that demonstrate the percentage of PAR arriving as a function of angle of light. Using the percentage of PAR instead of raw intensity values allows us to more easily compare across curves from different experimental settings. At specific angles of interest, two sample t-tests were run to compare differences in spectra. All analyses and figures were made in R Studio v1.4.110 (R Studio, Boston, USA) using R v4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Angular light distribution of traditional gas exchange setups

We tested the angular distribution of light with the LI-6800 small and large leaf chambers and the LI-6400 small leaf chamber (Figure 3; Table 1). For all results, we refer to 90° as the position where the light is positioned directly perpendicular to the leaf surface The LI-6800 small and large leaf chambers produced similar light angle distributions (Figure 3a and 3b) with 23.5 ± 5.2 % and 23.9 ± 7.0 % of light coming from 90 ± 5.75° (t = 0.21, d.f. = 20.5, P =0.84). Both chambers had dramatic declines in the quantity of light beginning around 28.75° from perpendicular in either direction and very little light (< 9% total) came from other angles. The LI-6400 small chamber had a similar, albeit slightly broader, distribution with much greater variation at each position (Figure 3c, Table 1). This chamber had 15.7 ± 12.2 % of light coming from 90 ± 5.75° which, while broader, was not significantly different from either LI-6800 chamber (with LI-6800 large chamber: t = 2.02, d.f. = 11.05, P =0.07; with LI-6800 small chamber: t = 1.85, d.f. = 12.13, P =0.09). Light was much more evenly distributed through 90 ± 40.25°, ranging from 10 to 20 % at each position. At each angle, the standard deviation reflects the variation in the angular distribution of light across different locations within the leaf chamber. The particularly high variance for the LI-6400 small chamber was due to greater spatial variability (at different positions in the chamber) in light angle distribution.

We also tested if changes to the ratios of LEDs used, the total intensity of light, or the method of controlling light affected angular distribution of light. None of these changes affected the angular distribution of light (Figures S1 and S2). Specifically, when we changed the ratio of LEDs from 65% red, 20% blue, 10% green, and 5% white to 90% red and 10% blue, the percentage of light arriving at 90 ± 17.25° was a non-significant change from 65.9 ± 9.9 % to 60.7 ± 5.9 % (t = 1.11, d.f. = 26.83, P =0.28). When we then tested a change in intensity by lowering the PAR from 1390 µmol $m^{-2}s^{-1}$ to 1000 µmol $m^{-2}s^{-1}$, the percentage of light arriving at 90 ± 17.25° was 61.8 ± 9.0 % (Figure S1; t = 0.20, d.f. = 14.57, P =0.85). Lastly, we tested two different methods to control light intensity and LED ratios on the LI-6800 and again found no change to the angular distribution of light. The percentage of light arriving at 90 ± 17.25° was 65.5 ± 9.9 % when run by controlling light as a "Setpoint" and 63.7 ± 9.9 % when controlling light as a "Percentage" (Figure S2; t = -0.27, d.f. = 12.53, P =0.79).

Angular light distribution with the integrating sphere

We found that the integrating sphere alters the distribution of light from the LI-6800 light source by both making light more direct when mounted directly on top of the sphere (90°) and more diffuse when the light is mounted on the side of the sphere (0°) (Figure 4). For the control (no integrating sphere on the LI-6800 large leaf chamber), the percent of light arriving at 90 ± 5.75° was 23.9 ± 7.0 % and the percent of light arriving at 90 ± 17.25° was 65.9 ± 9.9 %. With the light mounted directly on top of the sphere, these percentages increased significantly to 43.3 ± 5.4 % and 77.0 ± 15.4 %, respectively (t = 12.00, d.f. = 44.90, P < 0.001). Conversely, when the light is mounted on the side of the sphere (0° or 180°), these percentages were reduced to only 9.9 ± 0.5 % and 29.9 ± 0.8 %, respectively (compared to the control: t = -10.85, d.f. = 24.56, P < 0.001). The intermediate positions on the sphere provide intermediate percentages of predominantly direct light, allowing the user to manipulate the amount of diffuse light reaching the leaf surface by rotating the light source around the outside of the integrating sphere.

Photosynthetic response to different distributions of angles of light

To assess the effects of different distributions of angles of light on leaf photosynthesis, we collected experimental leaf gas exchange data using the integrating sphere on three different plant species (Figure 5). We observed distinct responses in each species, including a significant increase in photosynthetic rates with increasing proportion of diffuse light in *C. sinensis* (slope = -0.024, p < 0.0001), a significant decrease in photosynthetic rates with increasing proportion of diffuse light in *H. arbutifolia* (slope = 0.023, p < 0.0001), and no significant change in photosynthetic rates with change in the proportion of diffuse light in *P. americana*(slope = -0.0155, p = 0.20).

Discussion

Our advances in understanding how carbon and water move across leaf surfaces has relied heavily on leaf-level gas exchange systems for more than 50 years. These gas exchange systems have led to revolutionary advances in our understanding of plant function in both basic (e.g., terrestrial carbon cycling) and applied contexts (e.g., crop productivity). These same instruments also rely heavily on light sources that are assumed to approximate characteristics of light from the sun traveling through our atmosphere and arriving at the leaf surface. However, practically all of these measurements have been taken while ignoring a prevailing component of solar radiation: the angle of light arriving at the leaf. Recent work has demonstrated that the distribution of light angles directly impacts leaf physiology (Figure 5; Brodersen et al. 2008, Berry & Goldsmith 2020, Baguskas et al. 2021). However, until now, we were unable to control this characteristic of light. We quantified the angular distribution of light coming out of common gas exchange analyzers and demonstrated how adding an integrating sphere to the setup can allow for reliable manipulation of the angles of light hitting the leaf surface. Our vision is that this resource, combined with clear evidence that diffuse light alters plant function, will inspire new research directions for plant researchers.

Assessing angular distributions of light

The integrating sphere in this study was able to alter the angular distribution of light from light that was more direct (Figure 4A) to more diffuse (Figure 4D) than that available with the standard light source alone. This range facilitates measurements that more closely mimic the angular distribution of light from the sun. Under clear skies conditions, the proportion of diffuse light varies as a function of scattering by particles or aerosols in the atmosphere and ranges from 15 – 40 % of total light under midday conditions (Steven 1977, Spitters et al. 1986). This was further supported by our own measurements where 14 - 20 % of midday light under clear skies arrived as diffuse light in Winston-Salem, North Carolina during the summer of 2021(Figure S3). The 90° position on the integrating sphere provided a similar fraction of diffuse light. Moreover, the angular distribution of light from the sun acts as a more leptokurtic distribution where data are more concentrated around the mean with sharp reductions as you move away from the mean (Perez et al. 1993). The integrating sphere also creates this leptokurtic curve shape, whereas the light sources we tested all create a more normal distribution.

Similarly, the integrating sphere creates a more realistic light environment for diffuse light conditions, which commonly occur with cloud cover or high atmospheric pollution. Our data demonstrated an equal percentage of light arriving at each angle in the middle 80.5° (9.5 – 10.4 % at each angle) followed by a gradual reduction at other angles. In overcast or cloudy conditions, nearly all light is arriving as diffuse under the conditions described above (Steven 1977, Spitters et al. 1986). But the dynamic movement of clouds, combined with the changing zenith angle of the sun, leads to angular distributions of light that are constantly changing and somewhere in between the endpoints. The integrating sphere allows for more realistic representations of this dynamic light environment when carrying out experiments.

Implications for plant biology

There is a growing body of research that suggests that plant leaf gas exchange varies as a function of diffuse light (Vogelmann 1993, Vogelmann & Martin 1993, Brodersen et al. 2008, Urban et al. 2014, Hughes et al. 2015, Earles et al. 2017, Berry & Goldsmith 2020). The directionality and magnitude of these changes vary from a 20 % reduction to a 100 % increase in photosynthesis under diffuse light, although the reason for such variation among different species remains unresolved. In this study we have added data (Figure 5) demonstrating (1) three distinct responses among three different species and (2) that intermediate proportions of diffuse light lead to intermediate levels of photosynthesis. The integrating sphere combined with these results open opportunities for developing relationships between photosynthesis and different proportions of diffuse light.

By quantifying the angular distribution of light of gas exchange systems and providing a new tool that allows for manipulation of this variable, we provide the opportunity to explore these observations and to open other new directions for research. Most fundamentally, we need to understand how plant gas exchange varies in response to diffuse light across species and given different environmental contexts. Uncovering the mechanism that explains these varied responses across species will be critical to our understanding of plant functional biology. In addition, no studies have yet explored how changes to diffuse light gas exchange changes as a function of changes to other environmental variables such as CO_2 concentration, moisture availability (soil or atmospheric), temperatures, or even the spectral distribution of light. These interactions will become critical to our understanding of plant function as global climate continues to change. This includes the effects of new cloud regimes, changing aerosol patterns, and the potential for atmospheric geoengineering to alter the fraction of diffuse light and have concomitant impacts on plant function. Lastly, leaf-level observations will need to be reconciled with ecosystem measurements demonstrating changes in primary productivity under diffuse light conditions (e.g., Roderick et al. 2001, Gu et al. 2002, Alton et al. 2007, Mercado et al. 2009, Williams et al. 2014, Cheng et al. 2015).

Conclusions

Measurements of plant gas exchange are ubiquitous and fundamental to our understanding of plant function; the community has made tens of thousands of observations of plant leaf gas exchange since portable infrared gas analyzers became commonly available. In turn, it has also developed the ability to carefully control environmental parameters to which gas exchange may vary, including the amount and spectral distribution of light. We have quantified the angular distribution of light in common portable infrared gas analyzers and developed a complementary tool that will allow for more reliable control of this variable. Determining the extent and explanations of the response to the angular distribution of light has implications for all measurements that rely on our understanding plant gas exchange.

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Table 1 . The percentage of light arriving at the leaf chamber as predominantly direct light (90° - perpendicular to leaf surface). Data are shown as the percentage of total light arriving at 90 ± 5.75° (11.5° angle of acceptance) and arriving at 90 ± 17.25° (34.5° angle of acceptance). The percentages shown represent how much of total light (from all angles 0° to 180°) was arriving from the 90° position. Data represent means and one standard deviation.

Light setup	**Percent arriving at 90 ± 5.75°**	**Percent arriving at 90 ± 17.25°**
LI-6800 3x3 cm chamber	23.5 ± 5.2	71.4 ± 8.6
LI-6800 6x6 cm chamber	23.9 ± 7.0	65.9 ± 9.9
LI-6400 2x3 cm chamber	15.7 ± 12.2	49.8 ± 18.8
Sphere 90°	43.3 ± 5.4	77.0 ± 15.4
Sphere 67.5°	7.8 ± 3.2	37.4 ± 18.8
Sphere 45°	6.7 ± 3.2	23.0 ± 5.5
Sphere 0°	9.9 ± 0.5	29.9 ± 0.8

Figure 1.

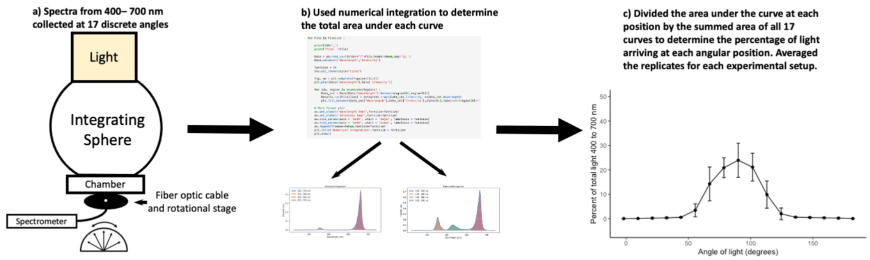


Figure 1. Workflow diagram from data collection of spectra to figures that quantify the angular distribution of light for different experimental setups of common portable plant leaf infrared gas analyzers. First (a), spectra were collected with a fiber optic connected to a rotational stage and a spectrometer. The relative intensity was collected at 17 discrete angles to capture the total light of the light source. For each spectrum (b), we used numerical integration through a programming script to calculate the total area under the curve from 400 to 700 nm. Finally (c), the total area under the curve at each angular position was divided by the summed area under the curve for all 17 spectra collected for each experimental setup. For each experimental setup, 10 to 25 replicates were conducted and averaged.

Figure 2.

Figure 2. Image of the LI-6800 large leaf chamber and light source attached to the integrating sphere. As seen, the light source is mounted at approximately 45° along the track. Photo credit: Gregory R. Goldsmith.

Figure 3

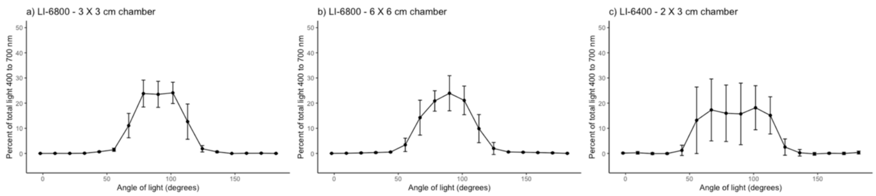


Figure 3 . The percentage of light (400 to 700 nm) arriving at the leaf chamber as a function of the angular distribution of light. Data are shown for three commonly used gas exchange chambers; the LI-6800 3 x 3 cm chamber (a; "small leaf chamber"), the LI-6800 6 x 6 cm chamber (b; "large leaf chamber") and the LI-6400XT 2 x 3 cm chamber (c). Each panel had the light source in its traditional position mounted directly above the leaf chamber. Data represent means and one standard deviation.

Figure 4

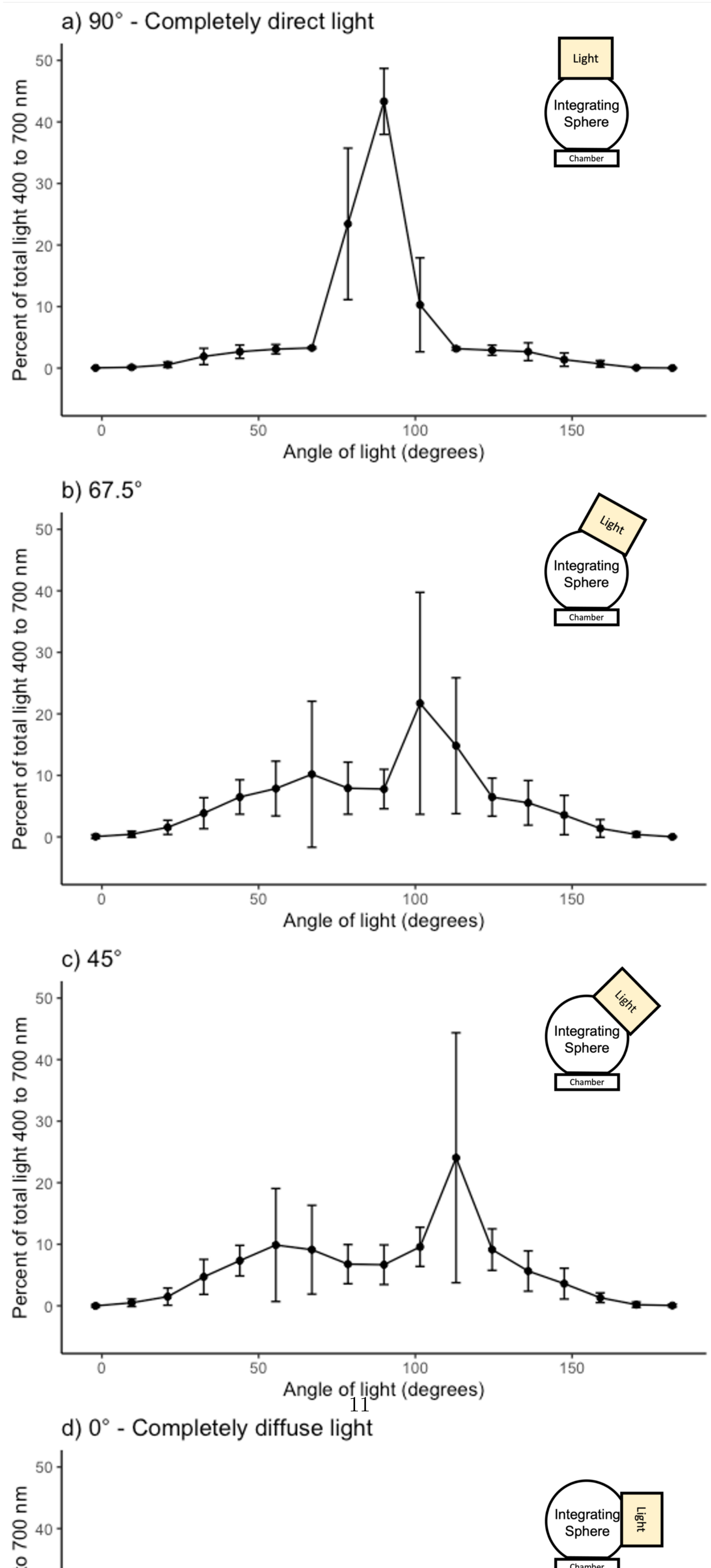

a) 90° - Completely direct light
Light
Integrating Sphere
Chamber
Percent of total light 400 to 700 nm
Angle of light (degrees)
b) 67.5°
Light
Integrating Sphere
Chamber
Percent of total light 400 to 700 nm
Angle of light (degrees)
c) 45°
Light
Integrating Sphere
Chamber
Percent of total light 400 to 700 nm
Angle of light (degrees)
d) 0° - Completely diffuse light
Integrating Sphere
Light
Chamber

Figure 4 . The percentage of light (400 to 700 nm) arriving at the leaf chamber as a function of the angular distribution of light with an integrating sphere mounted to the top of the chamber. Each panel represents the light source mounted at a different position along the track on the side including at 90° (a), 67.5° (b), 45° (c), and 0° (d). The diagram in the corner of each figure is a visual representation of the experimental setup. Data represent means and one standard deviation.

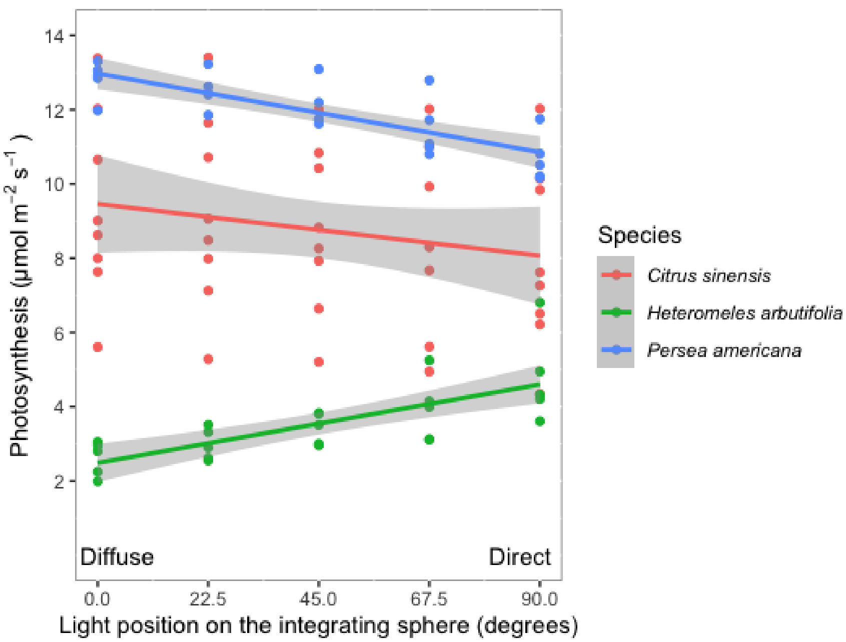


Figure 5 . Relationship between photosynthesis and the position of the light source on the integrating sphere for three plant species. Each light position corresponds to a distinct light environment ranging from predominantly direct (0°) to predominantly diffuse (90°). Lines represent linear regressions and 95% confidence intervals (n = 5 individuals each for *C. sinensis* and *H. arbutifolia* , 8 individuals of *P. americana*).

Supplementary Material

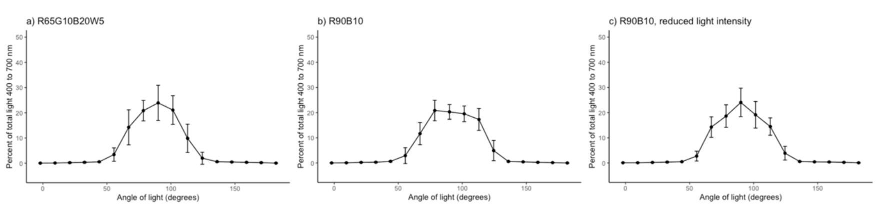


Figure S1. The percentage of light (400 to 700 nm) arriving at the leaf chamber as a function of the angular distribution of light with the LI-6800 large leaf chamber. These panels show tests where we changed the ratio of LEDs in the light head and the light intensity. Panel (a) shows the LED ratio of 65 % red, 20 % blue, 10 % green, and 5 % white at 1390 μmol m^{-2} s^{-1} PAR. Panel (b) shows the same PAR level but with a ratio of 90 % red, 10 % blue. This ratio of LEDs is a common ratio used for gas exchange measurements. Panel (c) shows the sale LED ratio as panel (b) but with the PAR level at 1000 μmol m^{-2} s^{-1}. Data represent means and one standard deviation.

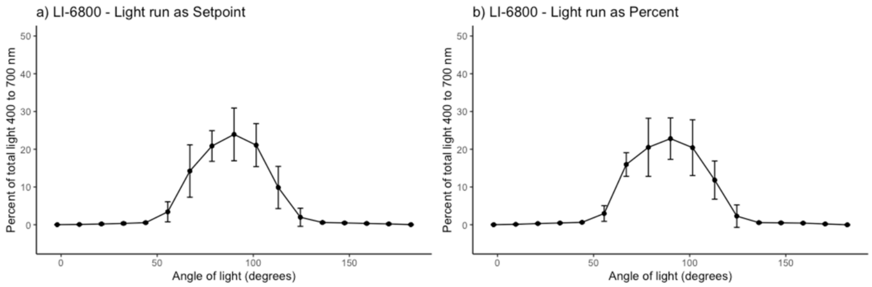


Figure S2. The percentage of light (400 to 700 nm) arriving at the leaf chamber as a function of the angular distribution of light. Panel (a) represents controlling the intensity and ratio of LEDs by using the "Setpoint" feature on the LI-6800. Panel (b) uses the "Percentage" feature. This test was done after conversations with colleagues at LI-COR Biosciences indicating that the two different methods could affect the quality and quantity of light (M. Johnson and D. Lynch, pers. comm.). Both experimental setups held the LED ratio of 65 % red, 20 % blue, 10 % green, and 5 % white and the light intensity at 1390 µmol m^{-2} s^{-1} PAR. Data represent means and one standard deviation.

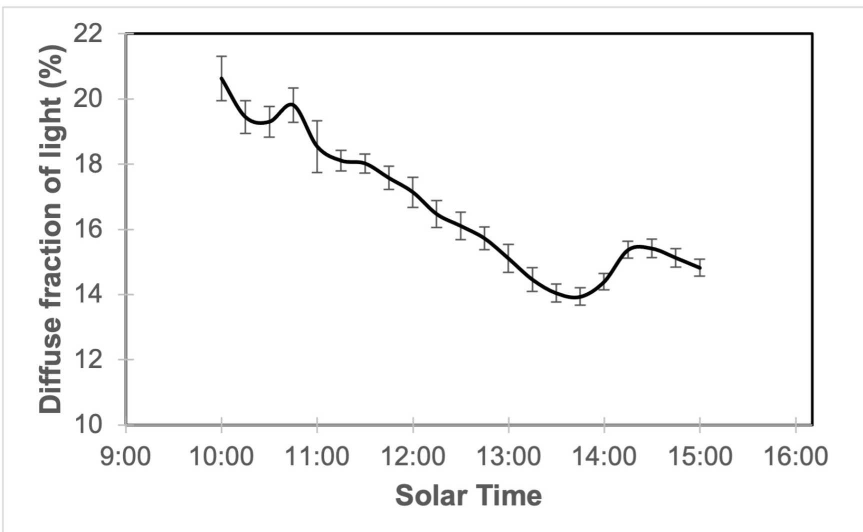
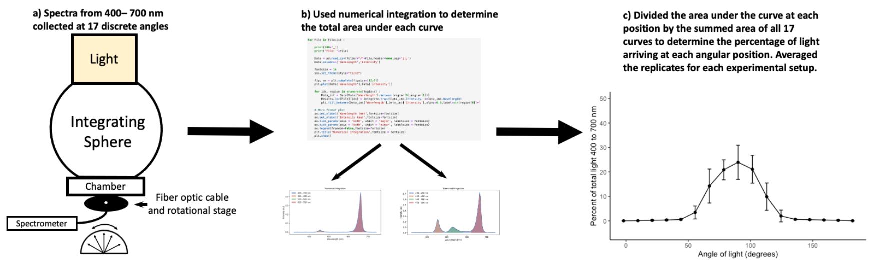
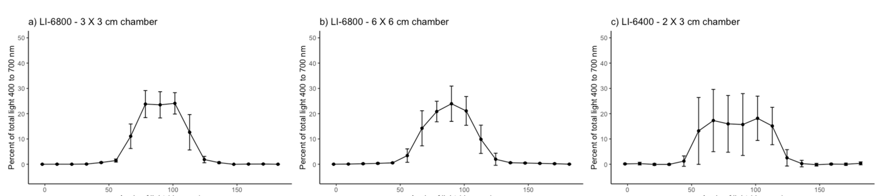
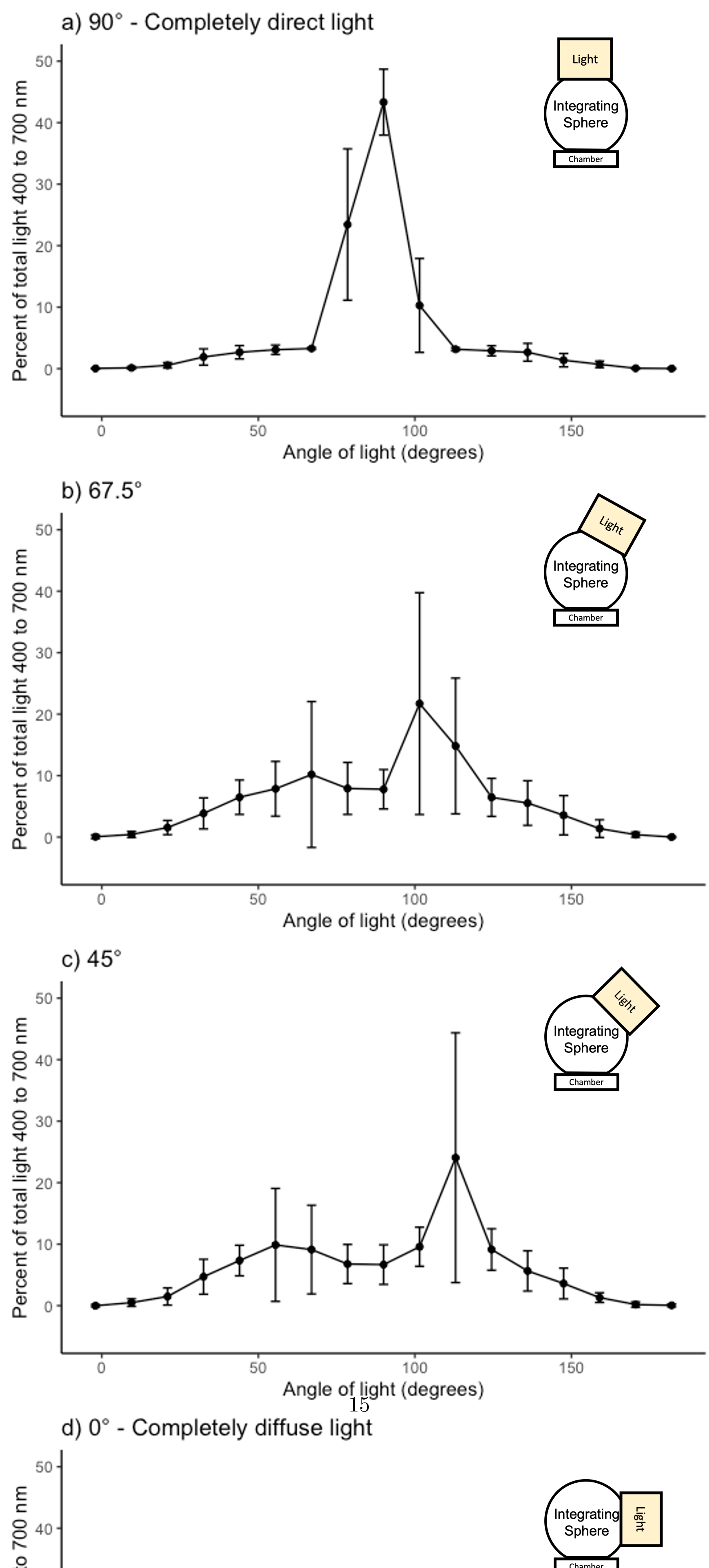


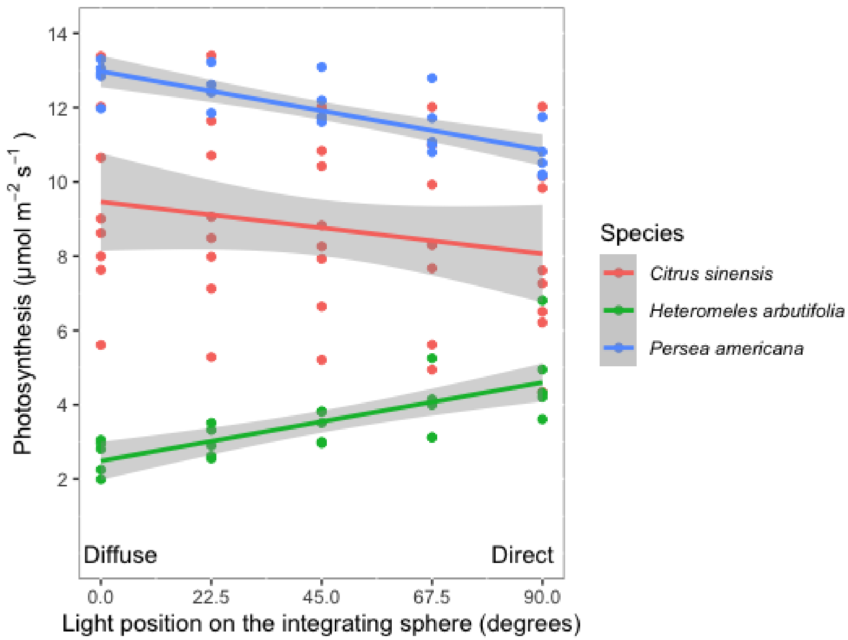
Figure S3. The diffuse fraction of light in Winston-Salem, North Carolina during seven consecutive clear days in the summer of 2021 (July 25 – 31, 2021). Midday (10:00 – 15:00) photosynthetically active radiation (PAR) was measured using a BF5 sunshine sensor (Delta-T Devices, Cambridge, United Kingdom), which determines the fraction of PAR arriving as direct and diffuse. These values were used to determine the percent fraction of PAR that was diffuse at each time point. The values over this period range from 14 % to 20 %, similar to other measurements of diffuse fraction of light during clear sky conditions.


a) Spectra from 400– 700 nm collected at 17 discrete angles
Light
Integrating Sphere
Chamber
Fiber optic cable and rotational stage
Spectrometer
b) Used numerical integration to determine the total area under each curve
c) Divided the area under the curve at each position by the summed area of all 17 curves to determine the percentage of light arriving at each angular position. Averaged the replicates for each experimental setup.
Percent of total light 400 to 700 nm
Angle of light (degrees)


0°
90°
LI-COR


a) LI-6800 - 3 X 3 cm chamber
b) LI-6800 - 6 X 6 cm chamber
c) LI-6400 - 2 X 3 cm chamber
Percent of total light 400 to 700 nm
Angle of light (degrees)


a) 90° - Completely direct light
Light
Integrating Sphere
Chamber
Percent of total light 400 to 700 nm
Angle of light (degrees)
0
10
20
30
40
50
0
50
100
150
b) 67.5°
Light
Integrating Sphere
Chamber
Percent of total light 400 to 700 nm
Angle of light (degrees)
c) 45°
Light
Integrating Sphere
Chamber
Percent of total light 400 to 700 nm
Angle of light (degrees)
d) 0° - Completely diffuse light
Integrating Sphere
Light
40
50



Photosynthesis (µmol m^{-2} s^{-1})
14
12
10
8
6
4
2
Diffuse
Direct
0.0
22.5
45.0
67.5
90.0
Light position on the integrating sphere (degrees)
Species
Citrus sinensis
Heteromeles arbutifolia
Persea americana