

Infra-red Gas Analyzer with Custom LED Module Attachment for Multi-spectral Photosynthetic Analysis

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Infra-red gas analyzer (IRGA)

Plants get energy from the sun. In photosynthesis, carbon dioxide (CO_2) and water (H_2O) from the air is converted to oxygen (O_2) and in addition the plant yields sugar (the complete equation is: $6\text{CO}_2 + 6\text{H}_2\text{O} = \text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2$). Inside the leaf, a portion of H_2O is in the gas phase, and during transpiration some of the water (and other gases) are released through the stomatal pores, whereas CO_2 is absorbed from the atmosphere to fuel the photosynthetic process. The amount of CO_2 taken up by the plant can be used as a measure of the photosynthetic rate.

Certain wavelengths of infra-red (IR) light are absorbed by trace gases common to the atmosphere. The specific frequency of light absorbed is characteristic of the gas. The IR gas analyzer (IRGA) is used to detect target gases with high precision and this has allowed plant physiologists to measure plant surface photosynthetic rates with high accuracy.

At the Lighting Enabled Systems and Applications center (LESA), we use the Combined Infra-Red Analysis System (CIRAS-3) instrument from PP Systems (Boston, MA). This instrument has four IRGAs: one each for CO_2 reference, CO_2 analysis, H_2O reference and H_2O analysis. Using a sealed chamber or cuvette (Figure 1), a leaf sample is exposed to an actinic light (light used to induce photosynthesis) while IR gas absorption is measured to quantify the sample's photosynthetic rate. IR light is applied to the target gas as it fills the sample cell, and the reduction in IR light due to absorption by H_2O or CO_2



Figure 1. Leaf cuvette of the CIRAS-3

CO_2 is then quantified instantaneously by a detector.

Custom multi-LED module attachment for multi-spectral photosynthetic analyzes

The LED attachment available as a light option for the CIRAS-3 instrument (PLC3 Universal LED Light Unit) has red (620-630 nm), green (520-535 nm), blue (465-485 nm) and full spectrum white LEDs that can be turned on/off independently to test plant photosynthetic responses to different spectral distributions of light. Unfortunately, this light is missing a far-red light channel.

The Tunable Irradiance Growth Efficacy Research (TIGER) LED module developed at LESA as a horticultural research fixture (GLASE Technical article [Issue 3](#)), was adapted to fit the CIRAS mini chamber. The TIGER Cub is equipped with 6 different LED channels: 3 blue light channels (400 nm, 420 nm, 450 nm), 1 green (530 nm), 1 red (660 nm), and 1

and 1 far-red), which can be individually calibrated and adjusted.



Figure 2. Different lights on in the TIGER Cub. The TIGER Cub is designed to mount directly to the leaf cuvette and consists of a single TIGER module with a re-configured LED array to accommodate the small window of the CIRAS-3 cuvette.

450 nm), 1 green (530 nm), 1 red (660 nm), and 1 far-red (735 nm), which all can be individually calibrated and adjusted. The TIGER Cub is designed to be placed directly on top of the CIRAS-3 leaf cuvette and consists of a single TIGER module with a re-configured LED array to accommodate the small window of the leaf cuvette (Figure 2).

The TIGER Cub LED light was built to allow the plants to undergo IRGA measurements using the same six discrete light wavelengths as in the growth chamber where light is provided by the TIGER LED modules, which are used by GLASE researchers for indoors crop cultivation under supplemental lighting.

Lighting control is done through the same control software as on a full TIGER research system to keep the TIGER Cub as similar as possible to the grow lights. While the user interface for the TIGER research system is focused on scheduled events, the user interface for the TIGER Cub is designed to easily apply immediate actions for taking measurements with the IRGA. With this set-up, it is easy to perform IRGA measurements under any combination of the 6 wavelengths, either in continuous lighting mode or in flexible pulsed lighting mode for more advanced photosynthetic studies.

Applications

By using the exact same light during photosynthetic measurements as in the growth chamber, we can accurately get a measure of the photosynthetic rate under different light spectra (Figure 3). Importantly, TIGER Cub includes different wavelengths of blue light (400 nm, 420 nm, 450 nm) that are important for fine-tuning pigment production (e.g. anthocyanins). Also, the effect of far-red light can be analyzed with the IRGA. Far-red light has recently been argued to be important for photosynthesis (Zhen & Bugbee, 2020).

At LESA, we are using the IRGA with TIGER Cub to test horticultural lighting in different ways:

1) Photosynthetic light response curves under narrow band LEDs.

We have performed single wavelength light response curves on lettuce, tomato and strawberry using each of the 6 LED channels (under the GLASE research program). In this work we could confirm that red light is best for photosynthesis compared to either blue or green light (measured as μmol of CO_2 fixed/ μmol of light) (Figure 3).

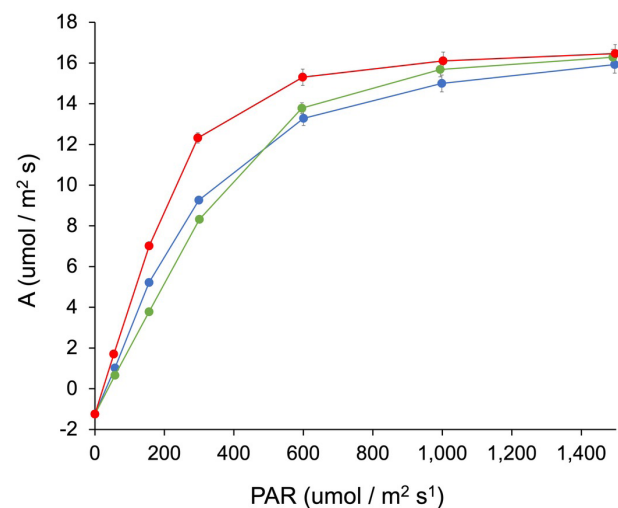


Figure 3. Lettuce mono-chromatic light response curves. Green 'Rex' lettuce was tested under blue (450 nm), green (530 nm) and red light (660 nm).

2) Selection of light spectrum for crop growth experiments.

Before starting a growth trial, we compare the photosynthetic rates of plants exposed to different new spectra for the selection of new light recipes.

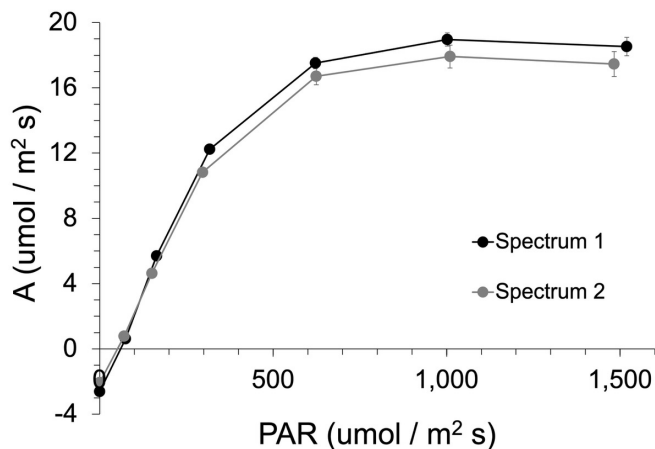


Figure 4. Lettuce multi-spectral light response curves. Different white light spectra were tested on green ‘Rex’ lettuce. Spectrum 1: 8% blue (450 nm), 2% green (530 nm), 84% red (660 nm) and 6% far-red (735 nm) light. Spectrum 2: 14% blue, 24% green, 61% red and 1% far-red light.

Performing light response curves, we for example compared different “white” spectra (Figure 4).

3) High frequency pulsing analysis

The TIGER Cub attachment was key to our analysis of photosynthesis of baby leaf lettuce under pulsed light conditions (Miliauskiene et al. 2021).

In addition to being able to match the LED lights used during growth, the TIGER Cub enables high frequency pulsing (<1.5 kHz) of the light and

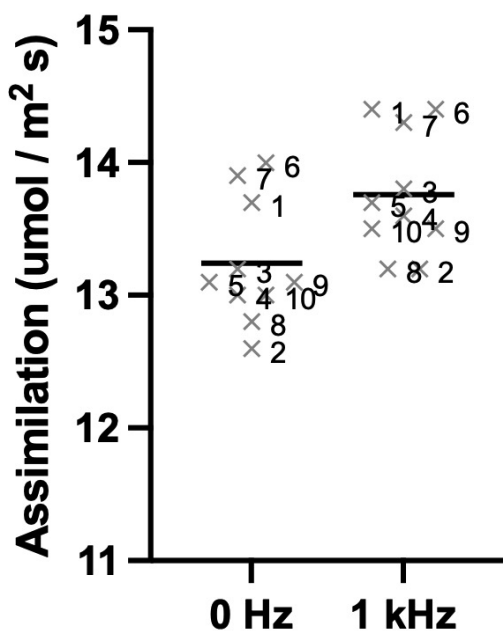


Figure 5. Comparison of CO₂ assimilation in baby leaf lettuce ‘Defender’ under constant (0 Hz) vs. 1 kHz pulsed light. 10 paired measurements were taken per leaf as indicated at the data points (Miliauskiene et al. 2021).

facilitates a direct comparison of different frequencies of pulsed light on the same leaf area (Figure 5).

References:

Zhen S, Bugbee B. Far-red photons have equivalent efficiency to traditional photosynthetic photons: implications for re-defining photosynthetically active radiation. *Plant, Cell & Environment*. 2020;43:1259-1272. <https://doi.org/10.1111/pce.13730>

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