The Single-Step CO_2 Response ($SSCO_2R^{™}$) Method

Rapid A/C_i Curves in Real Time Without Post Processing

The **Single-Step CO_2 Response ($SSCO_2R^{™}$) Method** is a new high-speed ramping technique that eliminates all post processing and generates the data for A vs. C_i directly on the CIRAS-4 console in real time.

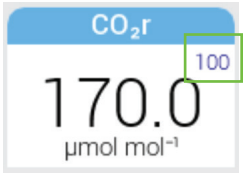
With The $SSCO_2R^{™}$ Method, reference and analysis channels have identical time responses to a CO_2 ramp and delta CO_2 would be zero during an empty chamber ramp, eliminating the need for any corrections to A or C_i.

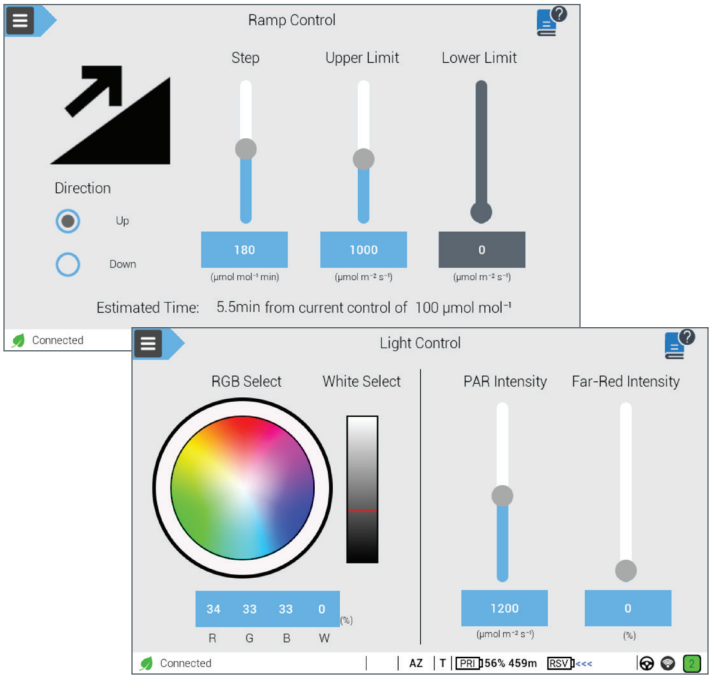
The $SSCO_2R^{™}$ Method is the fastest, most accurate, streamlined method available for the rapid measurement of A/C_i — *more measurements and data points in a much shorter period of time!*

This application note describes the simple steps needed to set up the CIRAS-4 to run a linear ramp experiment using the $SSCO_2R^{™}$ Method and record data. Data are plotted to illustrate the linear ramp capability.

Set Up the CIRAS-4 and Record Data

1. *Perform a **Stored Diff Bal Calibration** to allow the CIRAS-4 to have accurate offset information applied continuously throughout the linear ramp:* Install fresh desiccants and a new CO_2 cartridge in the CIRAS-4 and allow it to warm up for 30 minutes. Set the cuvette temperature to at least the current ambient temperature, to prevent condensation. Navigate to **Setup** > **Calibration** > **Stored Diff Bal** and tap **Start**. The CIRAS-4 will step through six CO_2 levels between 0 and 2000 µmol mol^{-1}, and six H_2O levels between 0 and 100% of ambient humidity, performing a Diff Balance at each level. It then computes a regression analysis of the resulting offsets that will be applied for every CO_2 concentration in real time. The Stored Diff Balance Calibration takes approximately 12 minutes and must be allowed to continue to completion. Tap the **Accept** button to complete the process.
2. *Set Zero Diff Bal Mode:* Navigate to **Control** > **General**. Tap the **Zero Diff Balance Mode** button, select **Zero/Stored Diff Bal** and **Save**.
3. *Set the Ramp Criteria:* Ramps in the CIRAS-4 always begin at the current control setting of CO_2 (as shown on the Measurement CO_2 tile) and return to that original CO_2 value when the ramp is complete. A typical ramp for a C_3 leaf might be an increasing ramp, starting at 100 µmol mol^{-1} and ending at 1100 µmol mol^{-1} with a ramp rate of 180 µmol mol^{-1} per minute for a total experiment time of 5.3 minutes. To set these ramp criteria, first navigate to **Measure** > **Photosynthesis** and tap the **CO2r** tile. Set the starting CO_2 for the ramp to 100 µmol mol^{-1}. Next, navigate to **Control** > **Ramp**. Set the **Direction** to UP and the **Step** to 180 µmol mol^{-1} per minute. Next navigate to **Control** > **Light** and set the **Upper Limit** to 1100 µmol mol^{-1} and **PARi** to 1200 µmol m^{-1} s^{-1}.



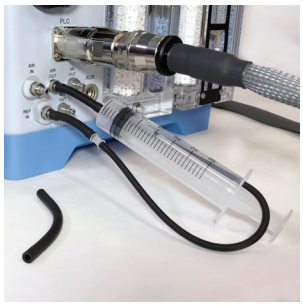


4. *Set the Recording Options for Ramp Processing:* Navigate to **Measure** > **Photosynthesis** > **Record Options**. Tap the **Data File Name** white box and enter a file name. Tap the **Enable Ramp** toggle to **1**, indicating that it is enabled. The Interval will default to one second. Tap **Return**.

5. *Test the volume/flow combination for zero CO_2 differential during a ramp:* Insert either tubing (see *Table 1* for approximate lengths required) or the Ramp Path Equalizer (STD581) between the **AIR OUT** and **REF IN** ports on the rear panel to increase the volume of the reference line. Close the chamber and begin a ramp. Adjust the cuvette flow rate (when using tubing to increase volume) or the syringe volume until the CO_2 differential is zero, then end the ramp.

   NOTE: This is only required once for a given cuvette flow rate.

$SSCO_2R$™ Method using long reference sampling tube



$SSCO_2R$™ Method using the STD581 Ramp Path Equalizer

Cuvette Flow Rate (cc/min)	1/8" Tubing Length (cm)
200	90
300	60
500	36

Table 1.

6. *Perform the Ramp Experiment with a Leaf:* Repeat Step 4. Now with a leaf in the PLC4 Universal Leaf Cuvette chamber, tap **Start**. If one watches the plot of A vs. Time for the ramp with the leaf, it becomes clear when CO_2 saturation occurs, at which time the ramp can be terminated by selecting **Stop**.

   NOTE: You can alternatively plot A vs. C_i directly on the console and stop recording when the curve has plateaued or let it run to completion.

7. *Transfer data files to a PC and use Excel® to plot A vs. C_i directly.* Usable data begins at about line 20, depending on flow rate, and ends slightly above zero CO_2r if ramping downward.

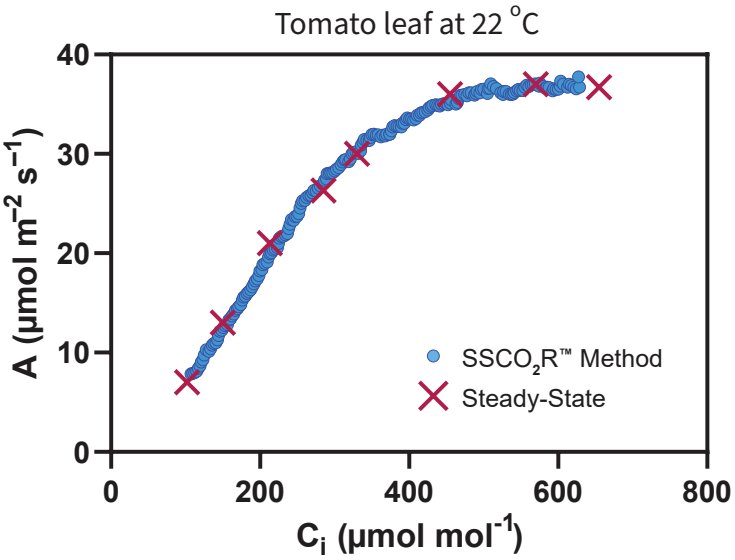


Figure 1.

Relationships between CO_2 assimilation rate (A) and internal CO_2 (C_i) for a tomato leaf measured at 22 °C and 1500 µmol m^{-2} s^{-1} PPFD near midday in the field—either under steady-state CO_2 condition—or during an upward ramping of CO_2 at a rate of 200 µmol mol^{-1} min^{-1} using the $SSCO_2R$™ Method.

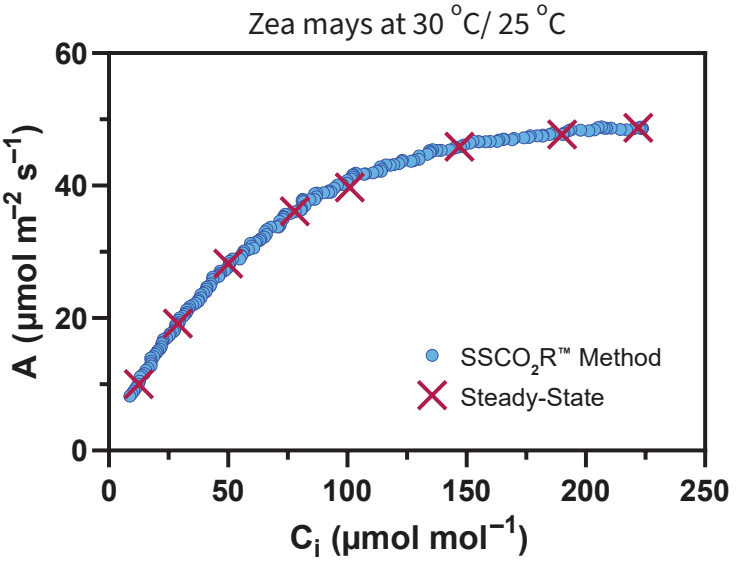
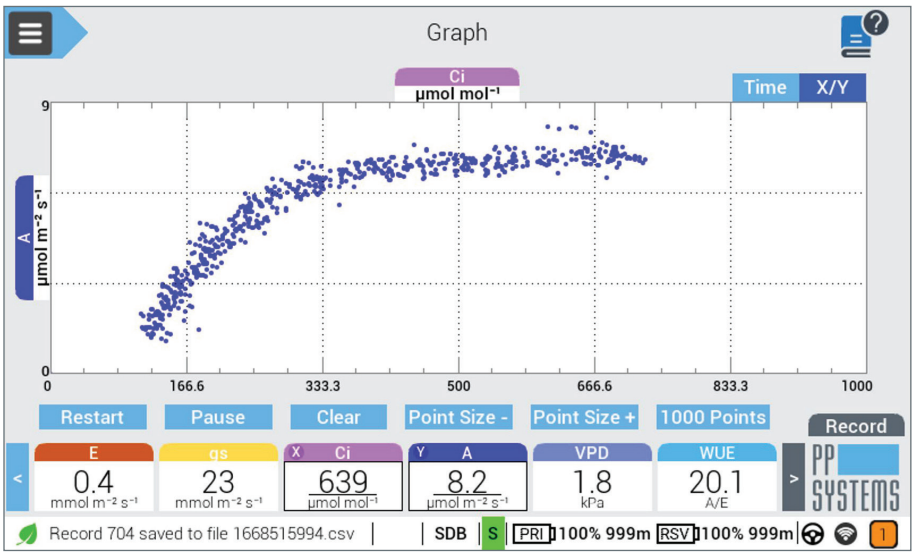


Figure 2.

Relationships between CO_2 assimilation rate (A) and internal CO_2 (C_i) for Zea mays grown at 30/25 °C day/night temperature, measured at 30 °C, 1800 µmol m^{-2} s^{-1} PPFD, and a VPD of 1.5 kPa. The CO_2 ramp was from 500 ppm down to zero at 150 ppm/min using the $SSCO_2R$™ Method.

Tips

1. Perform the Stored Diff Balance Calibration after a 30-minute initial warm up and before beginning a day of testing. The Stored Diff Balance will remain stable throughout a full 8-12 hour day of testing and will not be required again until the following day.
2. We have not encountered a situation where humidity values or gs values change rapidly enough during the ramp to cause substantial errors in gs and calculated C_i. A ramp speed of 100 to 230 µmol mol^{-1} per minute allows curves up to saturating A to be completed in approximately five minutes, and gs changes with C_i are typically minimal during that time.
3. Ramp speeds up to 233 ppm per minute are acceptable. Changing the speed of ramping in the range of 100 to 230 µmol mol^{-1}/min did not have a significant effect on the results in tests with Abutilon theophrasti (C_3). For typical C_3 leaves, a rate of 150 µmol mol^{-1}/min, starting at 100 µmol mol^{-1} provides a complete A vs. C_i curve in about five minutes, due to the fact that it has been our experience that saturating CO_2 is often 800 to 900 µmol mol^{-1}.
4. When executing multiple successive ramps, periodically perform a manual "zero" to keep the Auto-Zero function that takes place every 30 minutes from interrupting your CO_2 ramp.
5. When performing ramps at a range of temperatures, adjust Humidity values at each temperature to avoid condensation.
6. For most purposes, ramping up in CO_2 is a more efficient way to collect information on V_c, J, and TPU from A vs. C_i curves of C_3 plants compared to ramping down. When ramping CO_2 upwards, it is best to equilibrate the leaf to approximately 100 µmol mol^{-1} CO_2, using 100 µmol mol^{-1} as the starting CO_2 level of the ramp. This keeps Rubisco activated and stomata open. The upward ramp can be terminated once the apparent photosynthesis rate is no longer increasing as CO_2 goes up, as opposed to letting the ramp go to completion—saving considerable time on each ramp. Ramping down does provide more information at lower C_i values than ramping upward if that information is of particular interest. For example, CO_2 saturation is more predictable in C_4 species and ramping downward may be more efficient.



Example of A vs. C_i plotted in real time directly on the CIRAS-4 console.

If you would like to learn more about this application or speak with one of our experienced technical staff, please feel free to get in direct contact with us via any of the contact information listed below:

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