# High-Speed $CO_2$ Ramping Technique

## Rapid A/$C_i$ Curves in Minutes

The CIRAS-4 can change $CO_2$ gas concentration while simultaneously and continuously recording data. Linear ramps of $CO_2$ concentration are easier to program and record than in previous instruments. Now that there is no longer a need to define a response script for a ramp test in the CIRAS-4, the process is faster and more streamlined than ever.

This application note describes the simple steps needed to set up the CIRAS-4 to run a linear ramp experiment and record data. Data are plotted to illustrate the linear ramp capability, followed by a description of the post processing of the data to generate A vs. $C_i$ curves from the ramped gas exchange data.

## Set Up the CIRAS-4 and Record Data

The following steps illustrate the streamlined process to create A-$C_i$ curves with the CIRAS-4 in *minutes*.

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. 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 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 **$CO_2$r** tile. Set the starting $CO_2$ for the ramp to 100 µmol $mol^{-1}$. Next, navigate to **Control** > **Ramp**. Set the Direction to **UP**, the Step to 180 µmol $mol^{-1}$ per minute, 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 the main measurement screen **Measure** > **Photosynthesis**. Tap **Record Options**. Tap the **Data File Name** white box and enter a file name. Tap the **Enable Ramp** toggle so that a **1** is visible, indicating that it is enabled. The Interval will default to one second. Tap **Return.**
5. *Perform the Empty Leaf Ramp:* The first ramp recording creates the baseline trace to characterize the time response of the system and stores the data for subsequent post processing (*See Tip #2 on page 5*). With a closed cuvette, tap **Start** on the command menu (located to the right) to begin the ramp experiment. Use **Measure** > **Graph** or **Measure** > **Photosynthesis** to switch between graph and tile views while the ramp is ongoing. Once the linear ramp is complete, an additional 30 seconds of data are recorded and the $CO_2$ control will return to the initial Lower Limit.
6. *Perform the Ramp Experiment with a Leaf:* Tap **Record Options** on the command menu and enter a new file name as in step 4. Tap **Return**. 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**. You can also let it run until completion and an additional 30 seconds of data will be recorded. Either way, once the ramp is completed, the $CO_2$ control returns to the initial setting and is then ready for the next ramp experiment.

7. *Transfer data files to a PC to begin post processing:* Tap **Transfer Files** on the command menu. Insert a USB flash drive into either slot on the back of the CIRAS-4 console. Highlight the data files recorded in the previous steps and tap the ➔ button to move the files to the USB drive.

8. *Post Processing:* The CIRAS-4's Stored Diff Balance capability makes post processing very simple—all done in Excel simply by adding 3 new columns to the standard CIRAS-4 output file.

   To begin, open the file for the empty cuvette ramp in Excel. Copy the column of assimilation rates (**A**), which is column **AB**. Open the ramp file with the leaf in the cuvette, and paste the empty cuvette **A** rates into an empty column. Subtract the empty cuvette **A** rates from the rates obtained with the leaf in the cuvette. The differences are the actual assimilation rates, beginning after a brief lag period (typically at about line 20).

9. *Compute $C_i$:* The sub-stomatal $CO_2$ concentration, $C_i$, is now recomputed within the spreadsheet using the new actual Assimilation and the other values that have not changed ($CO_2$ analysis, and $g_s$ and E.):

$$C_i\,(\mu mol\,mol^{-1}) = \frac{\left[(g_c - \frac{E}{2}) \times C_{out}\right] - A}{(g_c + \frac{E}{2})}$$

   Where $g_c$ is the total conductance to $CO_2$ transfer:

$$g_c\,(mmol\,m^{-2}\,s^{-1}) = \left[\frac{1}{(1.585 \times r_s) + (1.37 \times r_b)}\right] \times 10^3$$

   [1.585 is the diffusion ratio of $CO_2$ and water in *air*, and 1.37 is the diffusion ratio of $CO_2$ and water in the *boundary layer*.]

   An Excel spreadsheet is available from PP Systems to use as a template for this calculation.

10. Plot A vs. $C_i$, starting at about line 20, after the linear ramp stabilizes.

Column **AB** from no-leaf data file copied here

Actual **A** = ramp **A** (Col **AB**) - empty **A**

**$C_i$** recomputed using actual **A**

Ramp Post Processing for App Note [Read-Only] - Excel

File Home Insert Page Layout Formulas Data Review View Help Acrobat TEAM Tell me what you want to do

AP2 =AB2-AO2

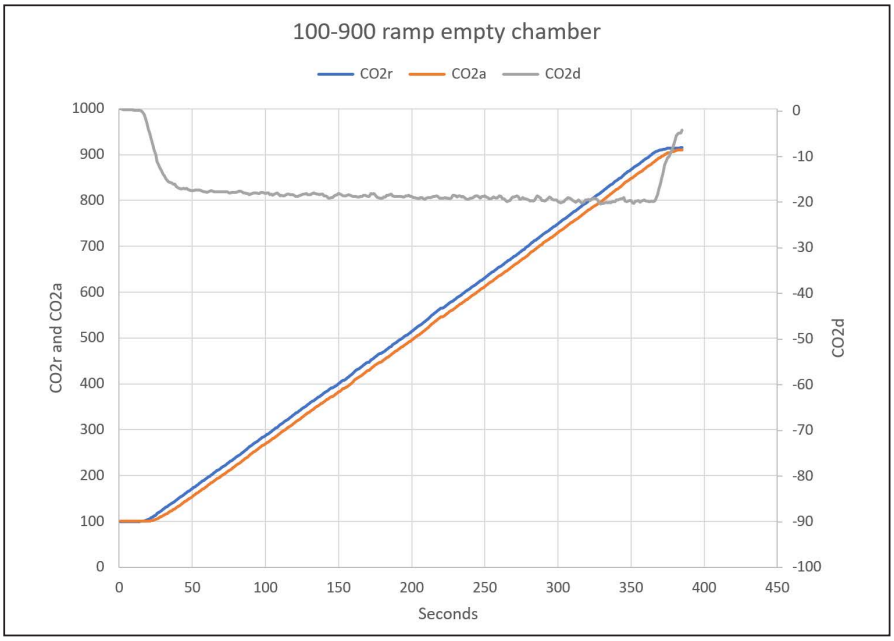
| | Y | Z | AA | AB | AC | AD | AE | AF | AG | AH | AI | AJ | AK | AL | AM | AN | AO | AP | AQ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Ci | gs | VPD | A | E | WUE | rb | StomataR | ZDiff | Ccontrol | Hcontrol | Tsensor | Tcontrol | Lcontrol | Accessory | Status | empty A | actual A | Ci |
| 2 | 88.1632 | 826.533 | 0.81187 | 1.94581 | 5.07638 | 0.38331 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.2143 | 2.1601 | 87.7053 |
| 3 | 88.1632 | 826.533 | 0.81187 | 1.94581 | 5.07638 | 0.38331 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.16965 | 2.11545 | 87.8147 |
| 4 | 88.3847 | 826.354 | 0.80894 | 1.88421 | 5.05704 | 0.37259 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.13647 | 2.02068 | 88.1173 |
| 5 | 88.4868 | 812.194 | 0.81666 | 1.84033 | 5.03937 | 0.36519 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.13038 | 1.97071 | 88.164 |
| 6 | 88.5127 | 815.089 | 0.81386 | 1.83704 | 5.03547 | 0.36482 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.09949 | 1.93653 | 88.3322 |
| 7 | 88.5685 | 803.093 | 0.82173 | 1.81536 | 5.0277 | 0.36107 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.10562 | 1.92098 | 88.37 |
| 8 | 88.6505 | 798.93 | 0.82426 | 1.80022 | 5.02329 | 0.35837 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.10896 | 1.90917 | 88.3775 |
| 9 | 88.6204 | 791.734 | 0.82657 | 1.79844 | 5.00274 | 0.35949 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.10621 | 1.90465 | 88.2912 |
| 10 | 88.4585 | 779.332 | 0.83544 | 1.81092 | 4.99617 | 0.36246 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.102 | 1.91291 | 88.1319 |
| 11 | 88.3076 | 774.346 | 0.83923 | 1.833 | 4.99428 | 0.36702 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.102 | 1.93499 | 88.0487 |
| 12 | 88.3008 | 770.971 | 0.83889 | 1.83103 | 4.9755 | 0.36801 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.07088 | 1.90191 | 88.1865 |
| 13 | 88.3733 | 761.071 | 0.84501 | 1.80767 | 4.96236 | 0.36428 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.04113 | 1.848 | |
| 14 | 88.6902 | 748.596 | 0.8528 | 1.73388 | 4.94502 | 0.35063 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.04234 | 1.7762 | |
| 15 | 88.6431 | 742.874 | 0.85504 | 1.74093 | 4.92868 | 0.35322 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.05032 | 1.7912 | |
| 16 | 88.404 | 728.91 | 0.86531 | 1.7747 | 4.91521 | 0.36106 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | -0.04365 | 1.8183 | |
| 17 | 88.5593 | 725.082 | 0.86636 | 1.75814 | 4.90112 | 0.35872 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 0.00523 | 1.7529 | |
| 18 | 88.4598 | 723.613 | 0.86741 | 1.81596 | 4.89924 | 0.37066 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 0.09421 | 1.7217 | |
| 19 | 87.7564 | 703.972 | 0.8829 | 1.99927 | 4.88124 | 0.40958 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 0.31881 | 1.6804 | |
| 20 | 87.3476 | 707.609 | 0.87863 | 2.22791 | 4.87693 | 0.45683 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 0.65164 | 1.5762 | |
| 21 | 86.5706 | 703.163 | 0.88256 | 2.5869 | 4.87482 | 0.53067 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 1.13546 | 1.4514 | |
| 22 | 85.7043 | 706.819 | 0.87705 | 3.03097 | 4.86384 | 0.62316 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 1.61891 | 1.4120 | |
| 23 | 84.5587 | 696.616 | 0.88607 | 3.54046 | 4.85849 | 0.72872 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 2.08032 | 1.4601 | |
| 24 | 83.3388 | 680.399 | 0.89892 | 4.0564 | 4.839 | 0.83827 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 2.53269 | 1.5237 | |
| 25 | 82.2797 | 674.573 | 0.90245 | 4.78035 | 4.82515 | 0.99072 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 3.08009 | 1.7002 | |
| 26 | 81.8839 | 666.801 | 0.9088 | 5.2308 | 4.81495 | 1.08637 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 3.52729 | 1.70351 | 93.0508 |
| 27 | 81.4851 | 664.13 | 0.90833 | 5.66711 | 4.79697 | 1.18139 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 3.95636 | 1.71074 | 94.0046 |
| 28 | 80.9036 | 647.482 | 0.92384 | 6.07185 | 4.78188 | 1.26976 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 4.55668 | 1.51517 | 95.5812 |
| 29 | 80.8395 | 646.286 | 0.91907 | 6.46346 | 4.74978 | 1.36079 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 4.90713 | 1.55633 | 96.7814 |
| 30 | 81.0793 | 636.965 | 0.92805 | 6.74176 | 4.74105 | 1.422 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 5.18082 | 1.56094 | 98.0977 |
| 31 | 81.342 | 628.291 | 0.93537 | 7.03579 | 4.7264 | 1.48861 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 5.43151 | 1.60427 | 99.2982 |
| 32 | 81.6165 | 625.237 | 0.93505 | 7.37476 | 4.70628 | 1.56701 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 5.68298 | 1.69179 | 100.544 |
| 33 | 81.952 | 616.238 | 0.94447 | 7.6693 | 4.69905 | 1.6321 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 5.86286 | 1.80644 | 101.789 |
| 34 | 82.7061 | 606.891 | 0.94769 | 7.86582 | 4.6574 | 1.68889 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 6.08198 | 1.78384 | 103.465 |
| 35 | 83.3151 | 597.179 | 0.95647 | 8.09492 | 4.63978 | 1.74468 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 6.30077 | 1.79415 | 105.163 |
| 36 | 84.4016 | 595.877 | 0.95613 | 8.31106 | 4.62978 | 1.79513 | 0.4 | 0.5 | AZSDB | LR | F% | IR | CT | LED | 2 | ML | 6.41071 | 1.90036 | 106.645 |

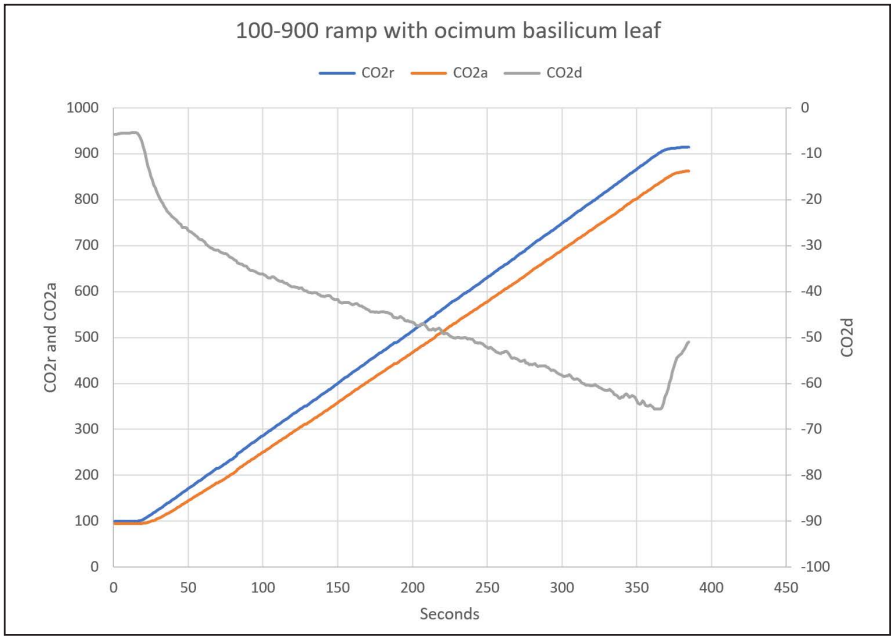
ramp2 w leaf

Plot actual A (row AP) vs actual $C_i$ (row AQ) starting at row 20

## Sample Results

A sample linear ramp experiment was recorded using a ramp from 100 to 900 µmol mol$^{-1}$ with a ramp step of 150 µmol mol$^{-1}$ min$^{-1}$ and cuvette flow of 250 ml/min. The CIRAS-4 was warmed up and a Stored Diff Balance Calibration was performed. Once the Stored Diff Bal was complete, a ramp data set was recorded with an empty chamber. Next, a second ramp data set was recorded with an *Ocimum basilcum* leaf and PAR set to 1000.



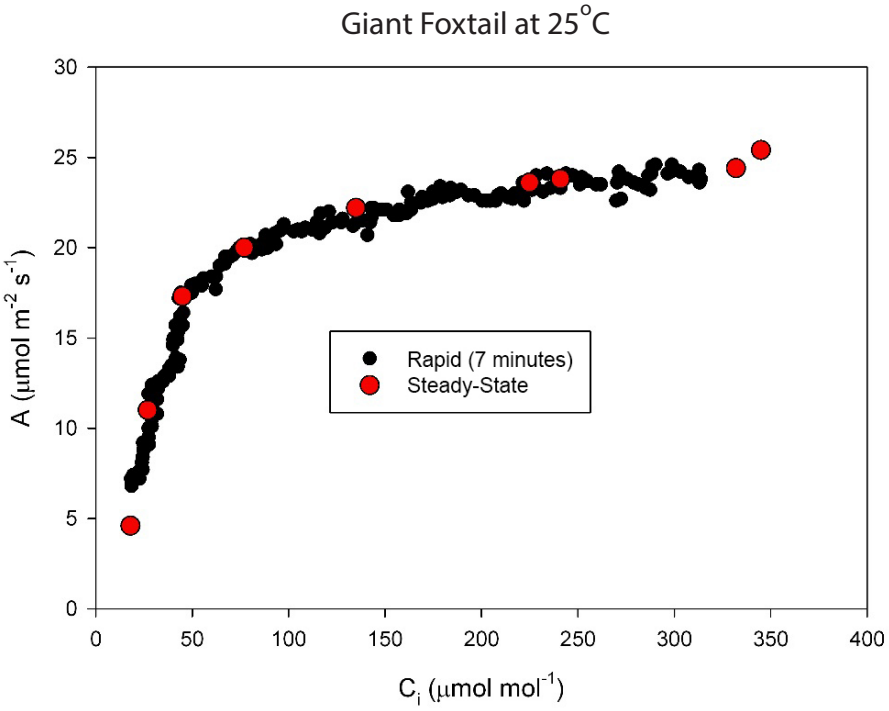




In the "no-leaf" case, the $CO_2$ differential is a relatively constant -20 µmol mol$^{-1}$ for most of the ramp, after starting out at 0 µmol mol$^{-1}$. The -20 µmol mol$^{-1}$ $CO_2$d represents the response time of the system including cuvette mixing and gas transport back to the CIRAS-4 console, equivalent to 13 sec with these particular settings. Faster response time can be obtained with higher cuvette flow rate, however the corresponding $CO_2$ differential will be lower. The CIRAS-4's ability to perform a Stored Diff Balance over the full range of the ramp prior to running the response script eliminates the need to correct the reference and analysis for accumulated channel difference.

With an active leaf in the PLC4, the $CO_2$d begins at -6 µmol mol$^{-1}$ (instead of 0 as in the 'no-leaf" case) because the leaf is actively assimilating. As the $CO_2$r increases from 100 to 900, the $CO_2$d increases from -6 µmol mol$^{-1}$ to -65 µmol mol$^{-1}$ at the end of the ramp.

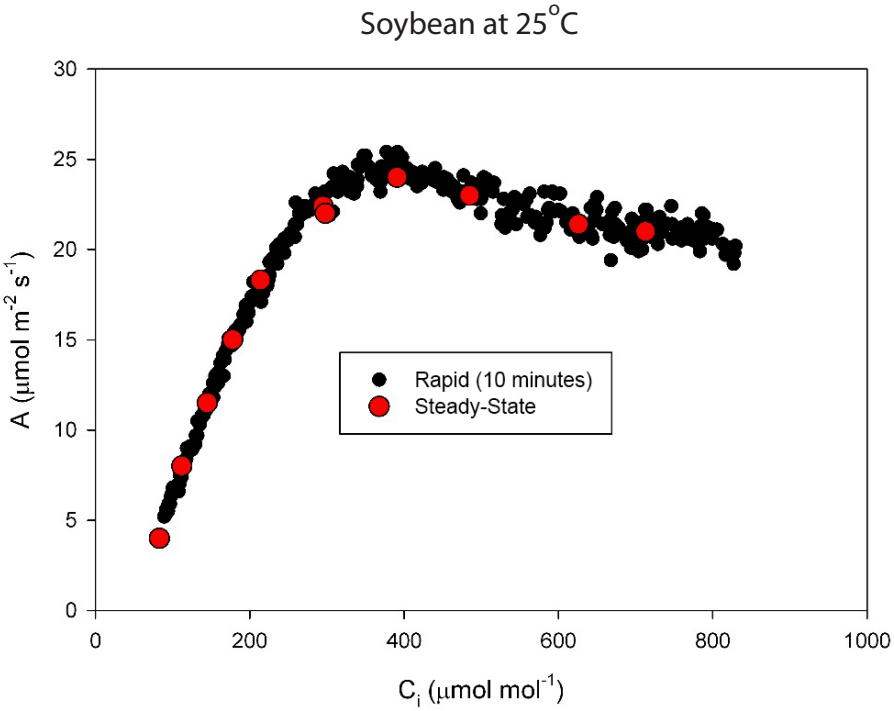
## A vs $C_i$ Comparisons

The Rapid A-$C_i$ curve technique and traditional point-by-point steady-state A-$C_i$ technique were compared on identical leaves a few minutes apart. Data on both $C_3$ (soybean) and a $C_4$ (giant foxtail) were made and show very good agreement between the two methods.




**Comparison of High-Speed A/$C_i$ Ramping (black points) to traditional point-by-point Steady-State (red points) for a typical $C_4$ Giant Foxtail leaf with PAR of 1500 µmol $m^{-2}$ $s^{-1}$ and Cuvette Flow of 300 ml/min. Reference $CO_2$ was ramped from 50 to 500 in 5 minutes (with one initial 2-minute acclimation). Each Steady-State point had a 2-minute acclimation time for total data recording time of 18 minutes.**

**Comparison of High-Speed A/$C_i$ Ramping (black points) to traditional point-by-point Steady-State (red points) for a typical $C_3$ Soybean leaf with PAR of 1500 µmol $m^{-2}$ $s^{-1}$ and Cuvette Flow of 300 ml/min. Reference $CO_2$ ramped from 100 to 1000 in 8 minutes (with one initial 2-minute acclimation). Each Steady-State point had a 2-minute acclimation time for total data recording time of 22 minutes.**



## Tips

1. The Stored Diff Balance Calibration should be performed after a 30-minute initial warmup prior to the beginning of 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. The *No Leaf (empty chamber)* ramp [see step 5] is stable for four hours or more and does not need to be redone unless experimental parameters are changed.
3. We have not encountered a situation where humidity values or $g_s$ values change rapidly enough during the ramp to cause substantial errors in $g_s$ 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 about five minutes, and $g_s$ changes with $C_i$ are typically minimal during that time.
4. Ramp speeds up to 233 ppm per minute are acceptable. Changing the speed of ramping in the range of 100 to 230 µmol $mol^{-1}$ per minute 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 $mol^{-1}$ per minute, starting at 100 µmol $mol^{-1}$ gives a complete A vs. $C_i$ curve in about five minutes, due to the fact that is has been our experience that saturating $CO_2$ is often 800-900 µmol $mol^{-1}$.
5. 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.
6. When performing ramps at a range of temperatures, we recommend setting the chamber temperature high enough to prevent condensation at 100% RH (i.e. above the current ambient temperature). We have also found that when performing ramps at a range of temperatures (common in field situations), the initial Stored Diff Bal should be performed with the cuvette temperature set high enough to prevent condensation at 100% RH (i.e. above the current ambient temperature). This will help to avoid warning messages associated with excessive humidity particularly outdoors where the humidity is generally much higher than indoors.
7. 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 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 will keep Rubisco activated and have stomata open. This is more efficient because 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 does ramping upward, if that information is of particular interest. However, the drawback to ramping downward is not knowing what high level of $CO_2$ is appropriate to start. If the starting $CO_2$ is too high, time is wasted. If it's too low, valuable information about the $CO_2$ level which saturates photosynthesis is lost. Both can be learned by trial and error, but that also takes time.

PP SYSTEMS

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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