High-Speed CO_2 Ramping Technique

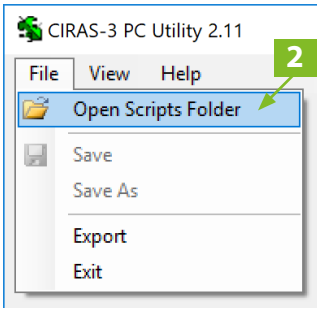
Rapid A/C_i Curves in Minutes

The CIRAS-3's ability to rapidly control CO_2 gas concentration while simultaneously and continuously recording data by utilizing the multi-level response scripts available in every CIRAS-3 has been available for some time. New interest in a linear ramp of CO_2 concentration prompted PP Systems to update the PC-based Script Editor–streamlining the process to create the response script file needed to create linear ramp response curves where the CO_2 reference level is changed at each recording interval.

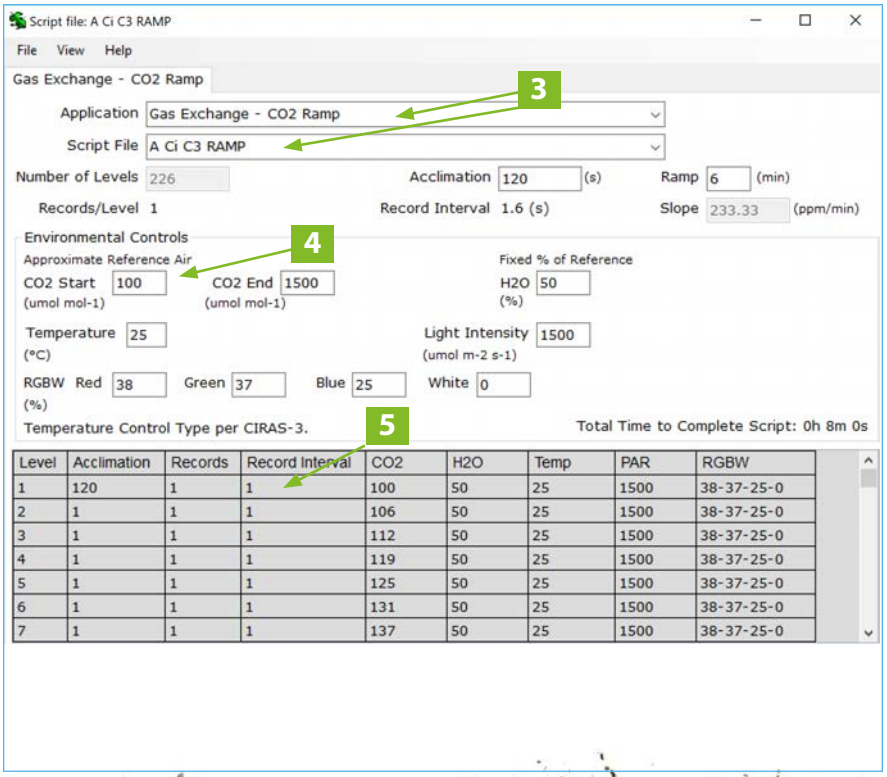
This application note describes the simple steps needed to create the script file, set up the CIRAS-3, run a linear ramp experiment, and record data. Data are plotted to illustrate the linear ramp capability, followed by a description of the post processing of the data to generate A vs. C_i curves from the ramped gas exchange data. **NOTE:** Version 2.00 or later of the CIRAS-3 PC Utility Program is required to utilize the ramp features described in this application note.

Script File Generation

1. *Start the CIRAS-3 PC Utility Program:* (**Start** > **All Programs** > **PP Systems** > **CIRAS-3 Utility**).
2. Choose **File** > **Open Scripts Folder.** Choose a location where the response scripts are saved. The default location is **User\MyDocuments\PP Systems\Ciras3_PC_Utility 2.XX\Response**.
3. When the script editor window opens, select **Gas Exchange - CO_2 Ramp** from the **Application** dropdown menu and choose your ramping script. **NOTE:** One ramping script for C_3 leaves and one for C_4 leaves are available from PP Systems. The two scripts differ primarily in the lower maximum CO_2 level reached for C_4 photosynthesis. Both may be edited by the user.



4. Edit **CO_2 Start**, **CO_2 End**, **Ramp (min)**, and other experimental parameters in any of the white text boxes. **NOTE:** Any changes will automatically update and the grid will be recomputed based on the new parameters.
5. **NOTE:** The "**1**" in the **Record Interval** column indicates that one data point is recorded for each recording interval, or one data point every 1.6 seconds.
6. Select **File** > **Save As** to save the new response script. **NOTE:** If using V2.00 the filename must include the word "ramp" to allow future editing in the Ramp Editor. It is not required in later versions of the program.



Level	Acclimation	Records	Record Interval	CO2	H2O	Temp	PAR	RGBW
1	120	1	1	100	50	25	1500	38-37-25-0
2	1	1	1	106	50	25	1500	38-37-25-0
3	1	1	1	112	50	25	1500	38-37-25-0
4	1	1	1	119	50	25	1500	38-37-25-0
5	1	1	1	125	50	25	1500	38-37-25-0
6	1	1	1	131	50	25	1500	38-37-25-0
7	1	1	1	137	50	25	1500	38-37-25-0

Set Up CIRAS-3 and Record Data

Once the script file is created in the PC Utility package, follow these steps to prepare the CIRAS-3 for a linear ramp process.

1. **NOTE:** Be sure the **\CIRAS3\Response** directory structure is in place on your USB drive and that you insert your drive in the **USB 2** port.

   To transfer the script file from the PC to the CIRAS-3: Navigate to **Operations** > **Rec Options** > **Edit Rsp Crv** > **Transfer**. Follow the prompts to import the script file into **Internal Memory Files**.

2. *Perform a **Stored Diff Bal Calibration** to allow the CIRAS-3 to have accurate offset information applied continuously throughout the linear ramp:* Navigate to **Operations** > **Calibration** > **Stored Diff Bal** and follow the prompts. Set the CO_2 min and CO_2 max to match the ramp min and max concentrations. The H_2O range can be left at the default of 0 to 100% or reduced to a smaller range closer to the H_2O operating point of the response script. Select **Start** and the CIRAS-3 will step through 6 levels between the min and max settings, 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 20-30 minutes and must be allowed to continue to completion. Under **Settings** make sure **Zero**, **Diff Bal Mode** is set to **Auto Zero**, **Stored Diff Bal**.

3. *Start the experiment:* The first ramp recording creates the baseline trace to characterize the time response of the system and stores the data for subsequent post processing. Set the appropriate operational settings similar to the start of the ramp on the *closed* and *empty (no leaf)* PLC3 Universal Leaf Cuvette. Navigate to **Operations** > **Rec Options**. Select **Response Curves** in the top line to enable the **Response Curve Scripts** dropdown menu. Select the correct response script for the linear ramp. Select either internal or external storage (USB flash drive) for the results data files. Select **Start** to begin the experiment. Select **Back** to return to the data or graph screen to watch the ramp script occur. Red triangles are shown on the graph at each recorded data point (at this recording interval, the red triangles overlap).

4. *Start the experiment with a leaf:* Repeat step 3, except with a leaf in the PLC3 Universal Cuvette chamber.

   NOTE: If the current environment of the leaf to be tested differs greatly from the first step in the ramping script, it is recommended to allow the leaf to equilibrate to those cuvette conditions prior to starting the ramping script with the leaf. 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 **End Recording**.

5. *Transfer data files to PC and begin post processing:* If data files were stored to the internal memory, use the **Operations** > **Rec Options** > **Transfer Data** screen to move the files to a USB flash drive.

6. *Post Processing:* The CIRAS-3's Stored Diff Balance capability makes post processing very simple – all done in Excel by adding just 3 new columns to the standard CIRAS-3 output file. To begin, open the file for the empty cuvette ramp in Excel. Copy the column of assimilation rates (A), which is column Y. 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, starting after a brief lag period (typically at about line 20).

7. *Compute C_i:* The sub-stomatal CO_2 concentration, C_i, is now recomputed 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.

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

Column Y from no-leaf data file copied here

Actual A = ramp A (Col Y) - empty A

C_i recomputed using actual A

ramp soy 2.xlsx - Excel

FILE HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW DEVELOPER TEAM Andy Lintz

AJ2 =Y2-AI2

	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL
1	VPD	A	E	WUE	rb	Adaxial	Tsensor	Tcontrol	Lcontrol	PLC	Status	empty A	actual A	actual Ci	
2	1.56	4.2	5.4	0.77	0.35	50	IR	LT	LED	U		-1.4	5.6	76.2532	
3	1.56	4.2	5.4	0.77	0.35	50	IR	LT	LED	U		-1.3	5.5	76.7112	
4	1.56	4.2	5.5	0.77	0.35	50	IR	LT	LED	U		-1.3	5.5	76.8269	
5	1.56	4.2	5.5	0.77	0.35	50	IR	LT	LED	U		-1.3	5.5	76.8269	
6	1.56	4.2	5.5	0.77	0.35	50	IR	LT	LED	U		-1.3	5.5	76.7692	
7	1.54	4.1	5.4	0.76	0.35	50	IR	LT	LED	U		-1.2	5.3	77.9491	
8	1.54	4.2	5.5	0.77	0.35	50	IR	LT	LED	U		-1.2	5.4	77.5618	
9	1.56	4.3	5.5	0.79	0.35	50	IR	LT	LED	U		-1	5.3	77.8491	
10	1.56	4.9	5.5	0.9	0.35	50	IR	LT	LED	U		-0.3	5.2	78.3028	
11	1.56	6.3	5.4	1.16	0.35	50	IR	LT	LED	U		1.3	5	79.1585	
12	1.56	7.9	5.4	1.45	0.35	50	IR	LT	LED	U		2.9	5	79.2104	
13	1.54	9.8	5.5	1.79	0.35	50	IR	LT	LED	U		4.6	5.2	78.6162	
14	1.54	12	5.5	2.22	0.35	50	IR	LT	LED	U		6.8	5.3	78.3267	
15	1.54	14	5.4	2.5	0.35	50	IR	LT	LED	U		8.7	4.9	79.9149	
16	1.54	16	5.4	2.92	0.35	50	IR	LT	LED	U		10.5	5.4	77.8618	
17	1.54	18	5.5	3.21	0.35	50	IR	LT	LED	U		12.3	5.2	79.2688	
18	1.54	19	5.5	3.5	0.35	50	IR	LT	LED	U		13.9	5.2	79.8632	
19	1.54	20	5.4	3.64	0.35	50	IR	LT	LED	U		14.7	5.1	81.7139	
20	1.54	21	5.4	3.84	0.35	50	IR	LT	LED	U		15.8	5.1	83.3139	
21	1.52	22	5.5	3.96	0.35	50	IR	LT	LED	U		16.3	5.3	84.3845	
22	1.52	22	5.5	4.07	0.35	50	IR	LT	LED	U		17.1	5.1	87.0215	
23	1.52	23	5.5	4.18	0.35	50	IR	LT	LED	U		17.2	5.6	86.7425	
24	1.54	23	5.4	4.21	0.35	50	IR	LT	LED	U		17.7	5.2	90.3688	

Sheet1 Sheet2 Sheet3

READY 130%

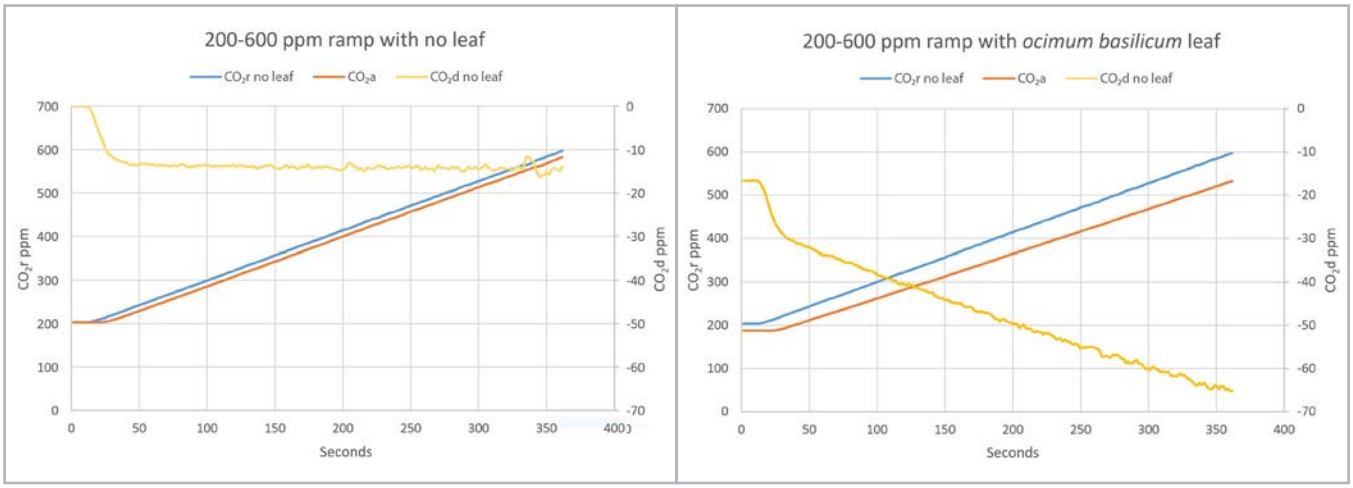
Plot actual A (row AJ) vs actual C_i (row AK) starting at row 20

Notes:

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 3] is stable for 4 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. The scripts provided allow curves up to saturating A to be completed in about 5 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.
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.

Sample Results

A linear ramp response curve from 200 ppm to 600 ppm over 6 minutes was created with the Script Editor with a 300 ml/min cuvette flow, a 100 ml/min analysis flow, and PAR set to1000 µmol mol^{-2} s^{-1}. The CIRAS-3 was warmed up and a Stored Diff Balance Calibration was performed. The response script was run once with an empty chamber, and again with an *ocimum basilicum* leaf in the PLC3 chamber.

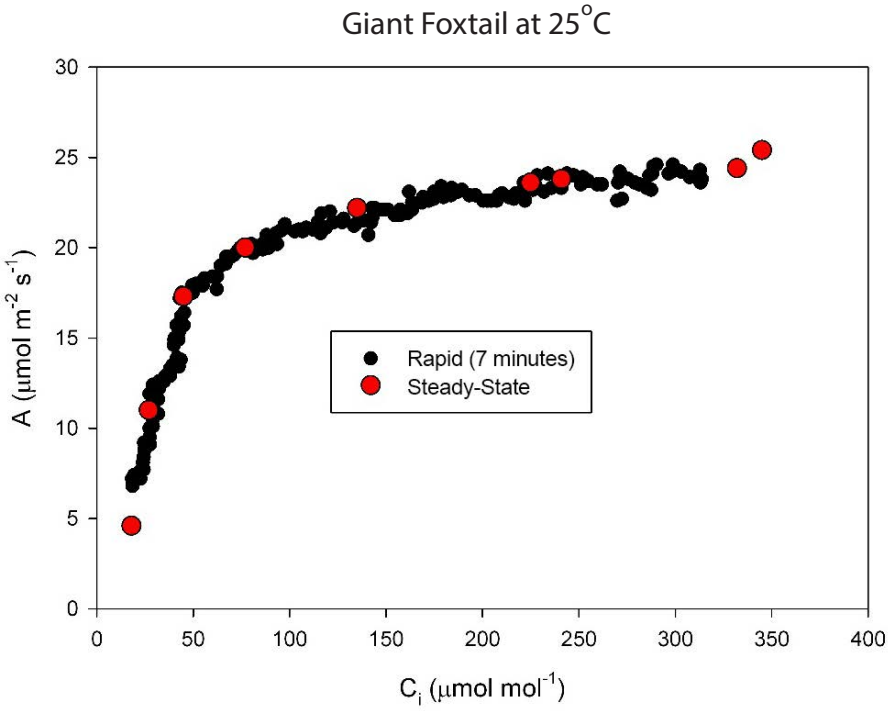


In the "no-leaf" case, the CO_2 differential is a relatively constant -14 ppm for most of the ramp, after starting out at 0 ppm during the 120 second acclimation time when the CO_2r is kept constant at 200 ppm. The -14 ppm CO_2d represents the response time of the system including cuvette mixing and gas transport back to the CIRAS-3 console, equivalent to 12.6 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-3'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 PLC3, the CO_2d begins at -17 ppm during the 120 sec acclimation (instead of 0 as in the 'no-leaf" case) because the leaf is actively assimilating. As the CO_2r increases from 200 to 600, the CO_2d increases from -16 ppm to -65 ppm at the end of the ramp.

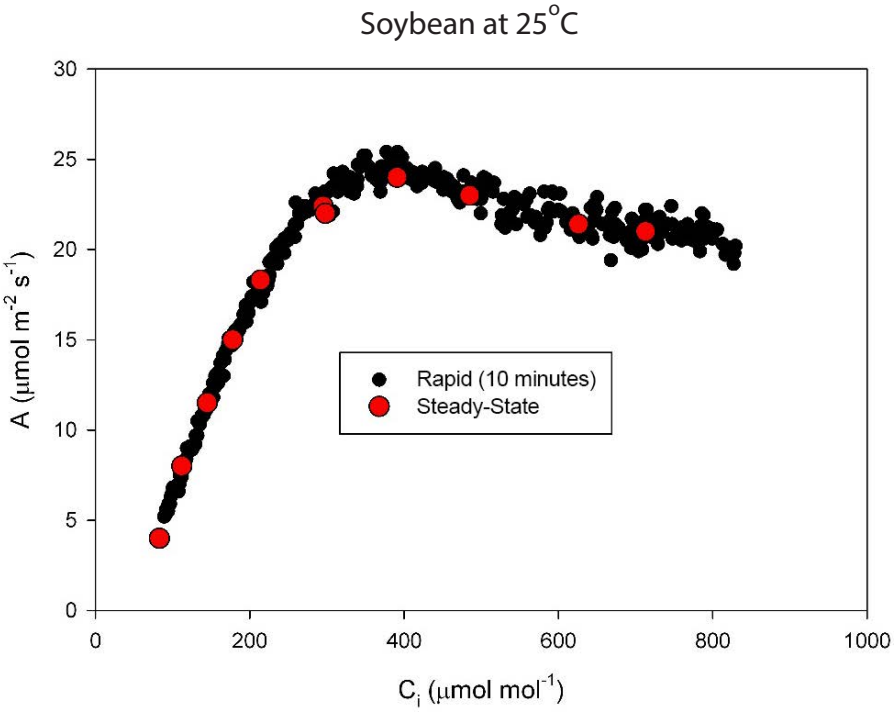
A vs C_i Comparisons

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.



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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