

Direct Real-Time Monitoring and Assessment of Single-Leaf Carbon Fixation and Respiration Rates for *Arabidopsis thaliana* by Mass Spectrometry



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Overview

- An atmospheric monitoring mass spectrometer designed for real-time data acquisition
- Plant metabolic pathway switching characterized via light cycling
- Demonstration of method for determining single-plant-leaf CO₂ fixation and respiration rates

Methods

Gas sampling system

A Shimadzu QP2010 quadrupole mass spectrometer using electron impact ionization was fitted with a novel patented atmospheric inlet technology that allows instrument inlet and sample throughput flow metering (Figure 2). Atmospheric sample stream-filtering capabilities are built into the design to trap particulates for further downstream analysis, such as ICP or biological agent identification.

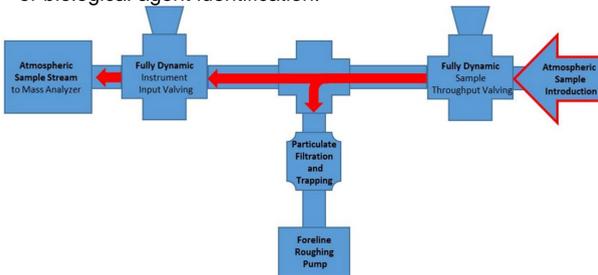


Figure 2. Design of the atmospheric inlet technology

Single-plant-leaf monitoring

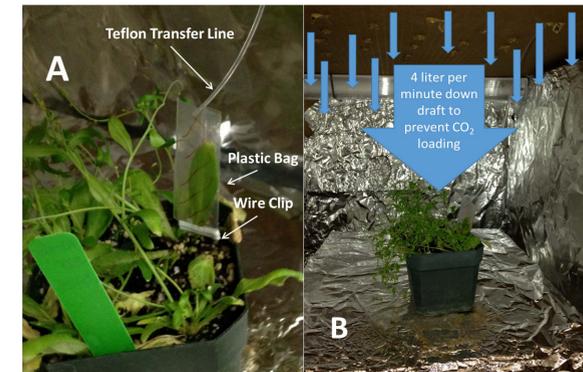


Figure 3. A) Isolated single leaf and B) plant orientation in chamber with downdraft flow diagram

Plants were placed in a 4-L/min downdraft environmental chamber developed in-house (Figure 3B). Individual plant leaves were isolated in plastic bags and sampled through a press-fitted Teflon transfer line (Figure 3A). A wire clasp kept the bag collapsed around the leaf surface to reduce dilution effects without obstructing flow. All plants were acclimated in darkness and room air for 24 hours with a constant transfer line flow of 2.0 mL/min prior to experiments.

The darkness-acclimated state served to 1) deplete the plant of all starch reserves, and 2) establish an initial baseline concentration of CO₂ (ppm) by combining the atmospheric CO₂ value (background) with that produced by the subject leaf via photorespiration, respiration, or other CO₂-producing reactions. A sampling rate of 2.0 mL/min was used to obtain highly resolved (in time) metabolic processes. A General Electric F15T8, 510 lumen, mercury vapor light source was used for its spectral characteristics (Figure 4). Each plant was then excised at the base of the wire clip, dried, and weighed for ppm/mg calculations.

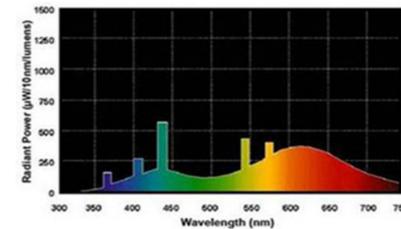


Figure 4. Exposure light spectral power distribution graph

Results

Instrument Calibration

Instrument calibration standards were volumetrically prepared in 3-L Tedlar gas bags at five concentrations using room air scrubbed of CO₂. Approximately 100 scans were collected and averaged at each concentration.

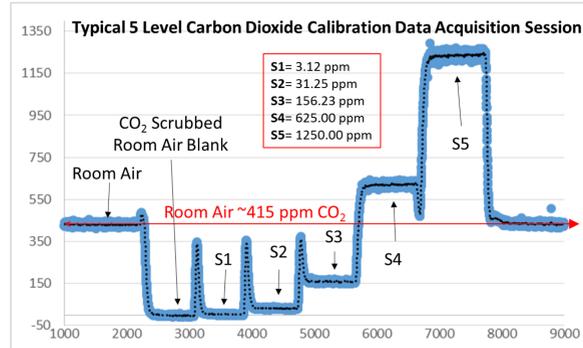


Figure 5. The novel inlet design allowed Tedlar gas bag standards to be continuously changed during a single monitoring session without flow or signal disruption

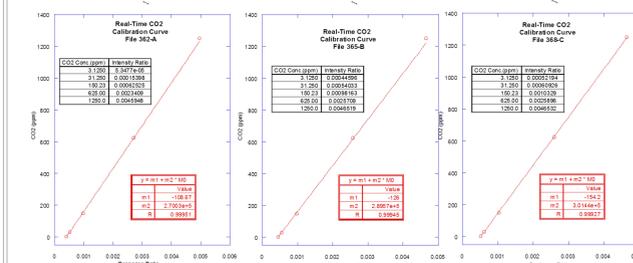


Figure 6. CO₂ calibration graphs for each single-leaf exposure sessions used in this experiment

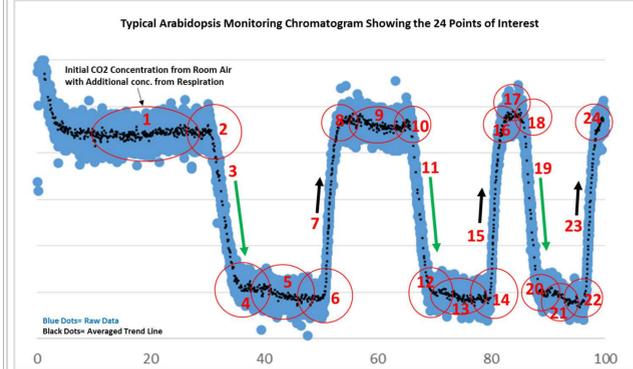


Figure 7. For each 100-minute single-leaf light-exposure-monitoring session, 12,000 scans were acquired

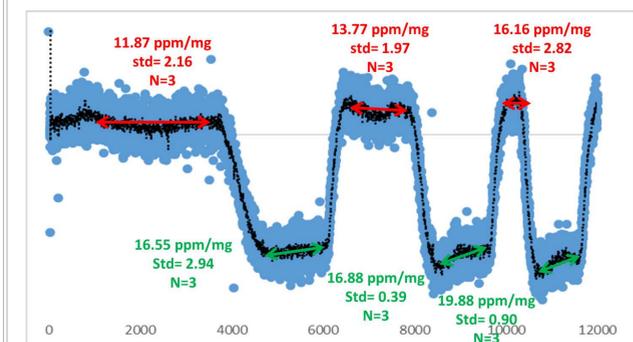


Figure 8. Averaged triplicate single-leaf rate data Included is total CO₂ fixed/mg of leaf, CO₂ released during increased respiration during dark reaction cycles.

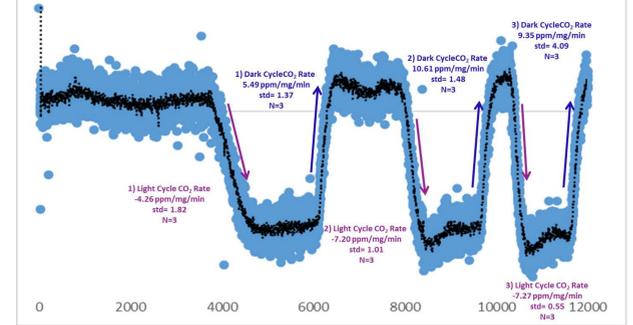


Figure 9. Light-to-dark and dark-to-light reaction CO₂ rates.

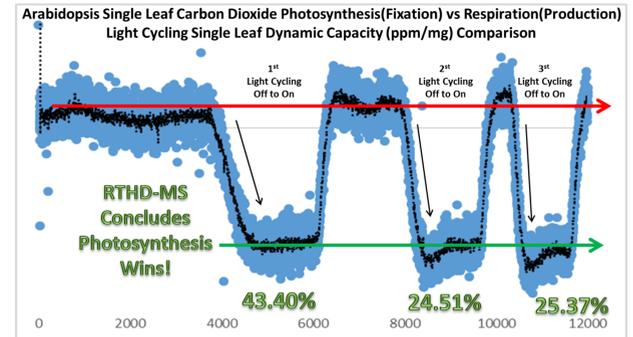


Figure 10. Data showing a single leaf of *Arabidopsis* consistently consumes more atmospheric CO₂ than it produces. Difference can be used to calculate total CO₂ fixed by the leaf over a given time.

Conclusions

- RTHD-MS provides highly time-resolved data for monitoring CO₂ photosynthesis/ carbon fixation and respiration in plants
- Single-leaf monitoring provides detailed, real-time data for CO₂ uptake during light-dark cycling
- Carbon fixation rate and total amount were demonstrated directly through gas measurements
- RTHD-MS showed excellent resolving power to reveal metabolic activity on the single-mg per plant leaf scale.

Acknowledgments

Samples were analyzed using capabilities developed under the support of NIH National Institute of General Medical Sciences (8 P41 GM103493-10). This project was supported by the U.S. Department of Energy (DOE) Office of Biological and Environmental Research (BER) Pan-omics program at Pacific Northwest National Laboratory (PNNL) and performed in the Environmental Molecular Sciences Laboratory, a DOE-BER national scientific user facility on the PNNL campus. PNNL is a multiprogram national laboratory operated by Battelle for the DOE under contract DE-AC05-76RL01830.



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