Measurement of Photosynthesis by Infra-red Gas Analysis

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INTRODUCTION

Because CO_2 is only 0.03 percent of our atmosphere, measurement of photosynthetic CO_2 uptake is problematic. Early investigators relied on large volumes of gas exchanged between a plant and the surrounding environment in order to obtain reasonable estimates of photosynthesis. Such techniques employed the absorbtion of CO_2 into an alkaline solution, or the reaction of CO_2 with an alkali metal oxide such as CaO. Manometric methods were based on the change in volume of the enclosed air space around a plant or group of plants, whereas gravimetric methods simply used the change in weight of the chemical reactant. More modern studies have employed radioactively labelled $^{14}CO_2$, and measured the rate at which this substrate is removed from the air surrounding a plant. Presently photosynthesis is commonly measured by infra-red gas analysis, which allows the measurement of parts-per-million fluxes of CO_2 in an airstream moving through a transparent chamber enclosing a leaf. In addition to permitting the investigator to quickly assay small CO_2 fluxes, this technique avoids the logistic problems associated with radioactive substances and can be employed in the field.

PRINCIPLE OF INFRA-RED GAS ANALYSIS

Heteroatomic gases absorb radiation in specific wavebands. CO_2 strongly absorbs in the intermediate infra-red wavebands, and this is expected to cause the global "greenhouse effect" as levels of atmospheric CO_2 increase. Infra-red gas analyzers measure the reduction in transmission of infra-red wavebands caused by the presence of a gas between the radiation source and a detector. The reduction in transmission is a function of the concentration of the gas. Dispersive infra-red analyzers employ sequentially applied monochromatic radiation to determine the concentration of various species in complex mixtures of gases. In contrast, photosynthesis systems employ non-dispersive analyzers which assay the concentration of a particular species of gas. These use broad spectrum infra-red radiation which is made selective for CO_2 by the use of filters in the optical path. Typically, detectors designed for CO_2 exhibit cross-sensitivity to the absorption spectrum of water vapor. Although filters minimize this interference, it is necessary to correct the apparent CO_2 concentration if there is significant water vapor in the airstream. Alternatively, water vapor may be condensed or chemically removed just before the airstream enters the analyzer.

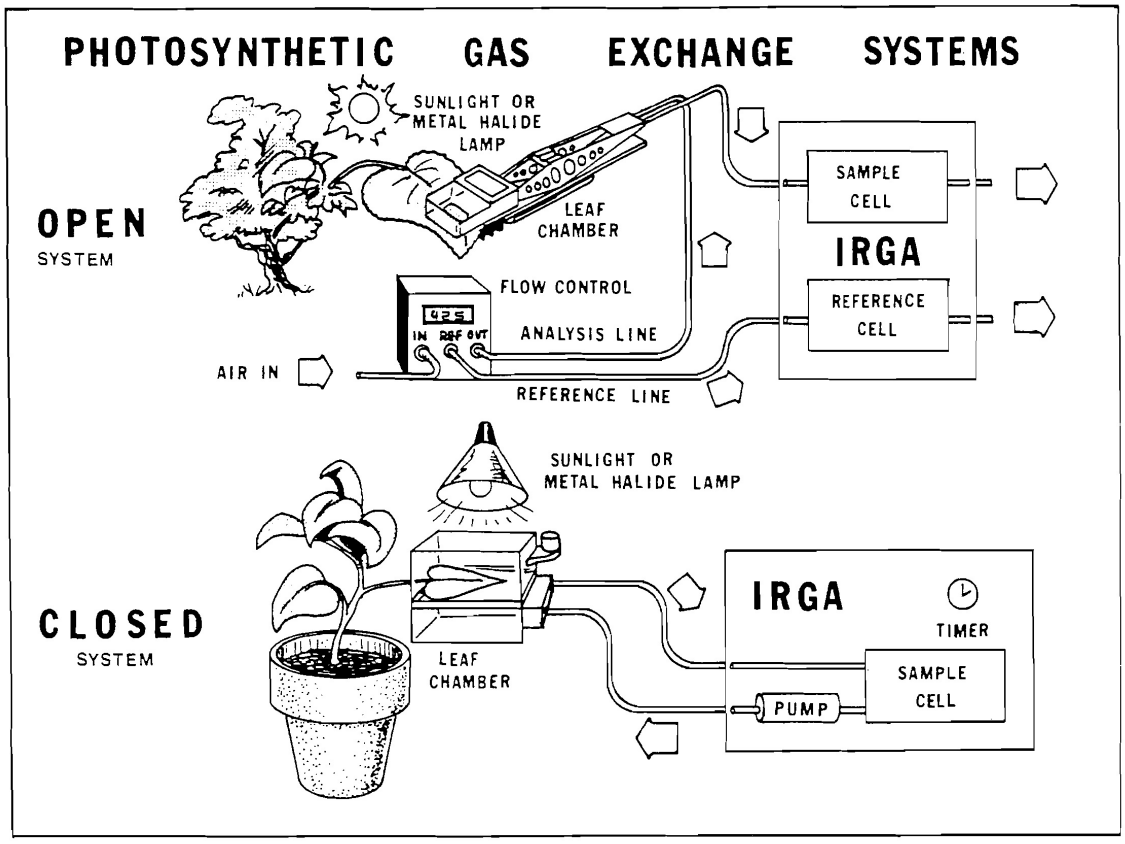
PHOTOSYNTHETIC GAS-EXCHANGE SYSTEMS

Accurate photosynthetic measurements require the concerted operation of each component of the photosynthesis system. In addition to the infra-red gas analyzer (IRGA), all gas-exchange systems consist of a leaf chamber, flow meter, and some means of generating and controlling the air flow over the leaf (Figure 1). In some systems, flow is precisely maintained with a mass flow controller, which generates a specified velocity by heating or cooling a small portion of the airstream. Leaf chambers may have a humidity sensor for measuring transpiration, a light sensor for measuring irradiance, and a fan to ensure adequate mixing of air in the leaf chamber. Operation in the laboratory requires an artificial light source with a spectral composition similar to that of sunlight, but with a minimum of heat producing infra-red wavebands (e.g., metal halide). Although photosynthesis may be measured in any terrestrial plant, cacti and other plants which have extremely modified leaves my require special leaf chambers. In addition, leaf chambers may include filters and heat-exchangers for reducing infra-red heating of the leaf.

Photosynthesis systems may be configured in either an open or closed fashion (Figure 1.) Open systems (also called differential systems) measure the concentration of CO_2 in the airstream passed over a leaf relative to air which has not been exposed to the leaf. Closed systems (depletion systems) continually

circulate air through the leaf chamber and measure the amount of CO_2 taken from a fixed volume of air during the time that the leaf is inclosed. Either configuration can be used to obtain acceptable photosynthetic measurements, but the circumstances where each should be used are dictated by differences in portability, speed of measurement, accuracy, and level of environmental control in the leaf chamber. The characteristics of each system are described below.

Open System: Open systems are configured to allow air from a single source to enter both the analysis and reference lines. After passing through the leaf chamber, the analysis air is pumped to the IRGA, while the reference air is pumped directly from the source to the IRGA. Most IRGA's use two cells to assay the reference and analysis gas simultaneously, whereas some assay the analysis and reference air sequentially with a single cell by switching the airstreams with a solenoid. Absolute concentrations of CO_2 in the analysis and reference lines are determined by comparison with air in an internal loop in which CO_2 has been chemically removed. Air may come from a pressurized tank or from the atmosphere so long as the CO_2 concentration is stable. For most studies, the CO_2 concentration should be close to the global mean of about ~~340 pm~~ . Leaf chambers used with open systems enclose small volumes. usually only a portion of a leaf, so the small CO_2 fluxes will significantly alter that concentration of the analysis air. Accordingly. a single measurement may require only a few seconds.



Technical Illustrations by Alan Rhodes

Closed Svstems: A closed gas-exchange system is operated with two closed gas flow loops. A reference cell is placed in series with a chemical CO_2 scrubber to maintain a zero CO_2 concentration in the reference loop. The sample cell is in a closed loop which includes the leaf chamber and a desiccant circuit through which all of a portion of the leaf chamber air sample may be passed. This diversion through a desiccant allows some control over the humidity in the closed chamber. which continues to increase as the plant transpires. The signal from the sample cell is compared to the zero gas reference signal to provide an absolute measurement of CO_2 concentration. In a closed IRGA system. an attached

leaf is enclosed in a chamber, sealed to avoid gas exchange with the atmosphere, and rate at which the CO_2 concentration in the chamber changes is monitored, typically for 10 - 20 seconds. Net photosynthesis is then calculated using this rate of change of CO_2 concentration.

There are two major disadvantages to using a closed IRGA system: photosynthesis measurements must be made within a few seconds after closing the leaf chamber: and the operator has limited control over environmental conditions within the chamber. Once the leaf is sealed in the chamber, CO_2 concentration in the leaf chamber- is continually decreasing. Consequently. if the leaf has a high photosynthetic rate, resulting in a rapid reduction of the chamber CO_2 concentration, measurements must be made quickly to avoid the possibility of a direct effect of low CO_2 concentration on photosynthesis. This limits the amount of time one may allow for a leaf to adjust to a particular experimental condition (light level. temperature, etc.). This problem may be partially overcome in some closed systems by using an external flow switch which allows the operator to open the system and draw outside air into the chamber while the leaf acclimates prior to beginning measurements.

The second limitation concerns the control of temperature and relative humidity within the chamber during measurement. Because the closed system was designed to be portable. it typically does not include heat-exchange devices for maintenance of constant air temperatures within the chamber. In addition, the air stream cannot be consistently humidified to a desired level. whereas steady state humidity control is commonly a part of open systems.

There are however. at least three advantages to the closed IRGA system: it is compact and light-weight, comparatively low-priced, and relatively simple to calibrate and operate. This makes it an appropriate instrument to use in secondary and undergraduate field courses.

Note: Modified and updated by J.E. Silvius, February, 2008.

About the Authors:
Stephen Mulkey (Ph.D. University of Pennsylvania, 1986) studies gas-exchange and drought tolerance of understory species in tropical forests of Central America. See http://snre.ufl.edu/people/mulkey.html for BIO and recent publications.

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Pre-Lab Preparation Questions – Based on your reading of the Mulkey-Smith article, please answer the following questions and bring your answers to the meeting in which they will be discussed:

1. Explain how an infrared gas analyzer (IRGA) measures CO_2 concentration?
2. Distinguish "closed systems" from "open systems" that use the IRGA to measure photosynthesis.
3. Recalling your experience with indirect calorimetry and manometric measurement of animal respiration, what is there about plants and photosynthesis that require more sophisticated systems?
4. How are the problems noted in Question #3 addressed by a "closed system?" What problems remain?
5. Describe how you would use an "open system" to quantify the rate of photosynthesis as rate of CO_2 uptake and assimilation by a plant leaf.
6. List environmental factors that affect photosynthesis and transpiration in leaves and consider how you would design a means of determining the effects of each on leaf gas exchange.

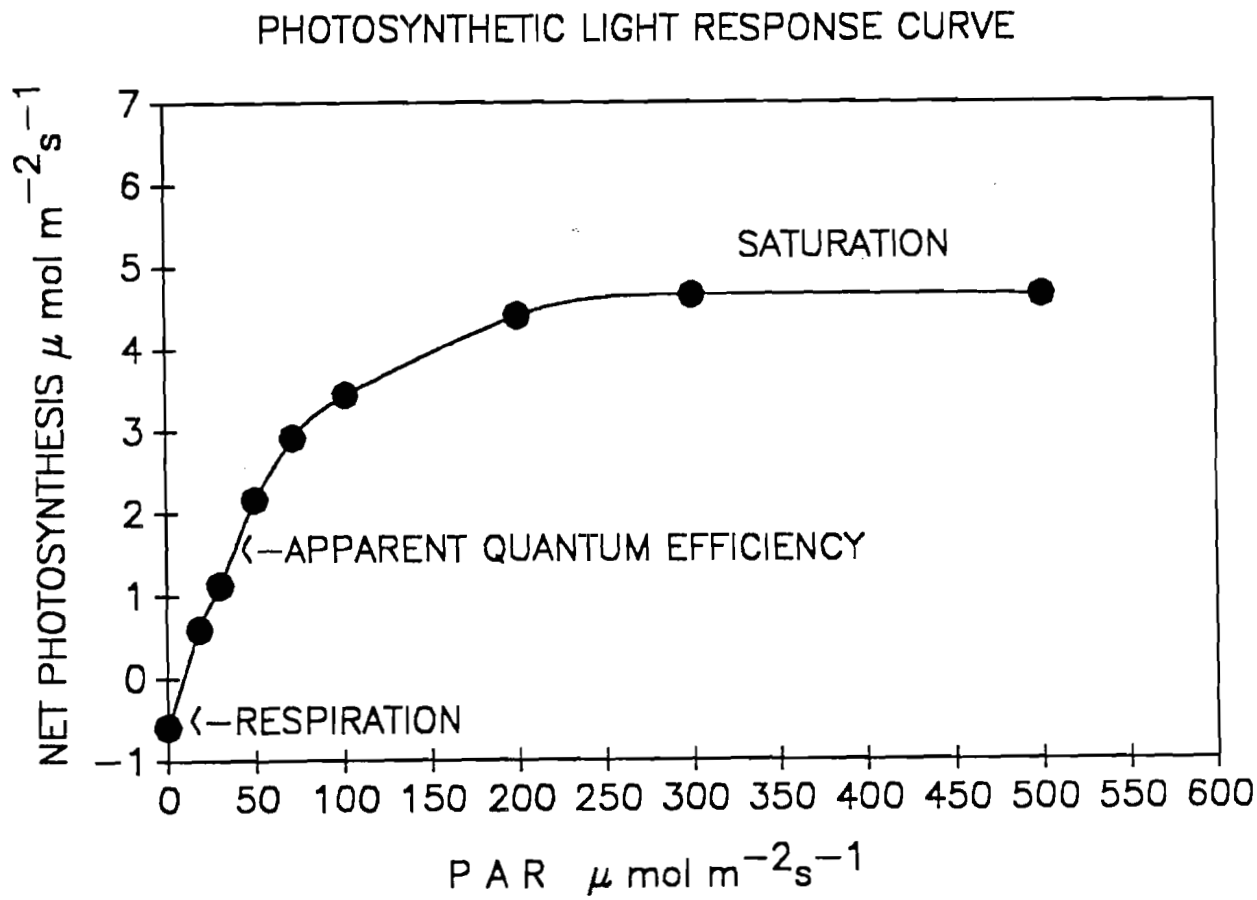

Figure 2. Example of a photosynthetic light response curve. See text for an explanation of the various parts of the curve.