LI-6400; Determination of photosynthetic parameters by the Gasometry measurements

May 2019

You are going to be introduced to gasometric methods allowing us to measure photosynthetically activity of plants. Precisely you will work with the device LI-6400XT, widely used gasometric system used in plant physiology and ecophysiology. Principles of measurement and data evaluation are going to be demonstrated on plants with C3 and C4 metabolism. Refresh your knowledge about differences between those photosynthetic pathways, Plant Physiology, Taiz and Zeiger; or illustration video on youtube:

https://www.youtube.com/watch?v=HbLg4lMpUa8; aj.).

Introduction

In the process of photosynthesis the leaf assimilates CO2 coming from the ambient atmosphere. At the same time there is CO2 released during the respiration. In the case of unstressed green leaf with the supply of irradiance, the photosynthesis dominates over the respiration. Here, we measure the balance of both processes called „net photosynthesis“ (A_n). It is a flux of assimilated CO2 per leaf area unit per time ($\mu mol(CO_2)m^{-2}(leaf)s^{-1}$). With the gasometric system we measure the exchange of gases between the leaf and the ambient atmosphere. The change in CO2 concentration is measured by the Infrared gas analyser (IRGA). A_n is strongly influenced by:

i) the Intensity of Radiation (PAR; Photosyntheticly active radiation, $\mu mol_{(photons)}*m^{-2}*s^{-1}$). The relationship is shown at the „Light curve“

ii) the CO2 concentration in the atmosphere or substomatal cavity, respectively. This relationship is shown at the A/Ci curve.

From those two curves, we can determine photosynthetic parameters characteristic for the plant (or leaf).

1 Main principle of the measurement

1.1 Gas Analysis by Infrared Radiation

Heteroatomic molecules have characteristic absorption spectrum in the infrared region. Homonuclear gases (f.e. O2, N2) have zero Electric dipole moment and do not absorb in this irradiance. The major absorption peak wavelengths of CO2 is 4.25 μm with secondary peak at 2.66, 2.77 and 14.99 μm, respectively. IR radiation in the 2.7 μm range is absorbed as well by water vapour. The absorption of radiation by a specific heteroatomic molecule is directly proportional to its concentration in an air sample.

The IRGA system is composed of four main parts: source of infrared (IR) radiation, assimilation chamber, optical filter and detector. Molecules of CO2 in the cuvette cause a decline in the radiation and reduce the signal on the detector that is transferred to the concentration.

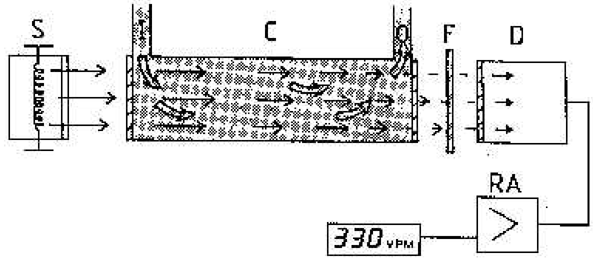

Fig. 1: The schema of simple InfraRed Gas Analyser (IRGA): InfraRed irradiance. The infra-red irradiance from the source (S) goes through the cuvette (C). The air circulate in (I) and out (O). The infrared radiation goes through the wide-band filter (F) and is analysed by a detector (D). The signal from the detector is amplified (RA) and recorded as the concentration.

1.2 The set up of the Open gasometric system LI-6400

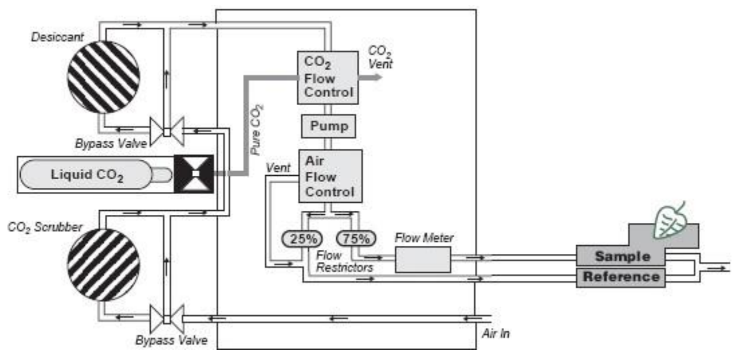

Fig. 2: The Flow Schematic: The atmospheric air is sucked in by a membrane pump. There are chemical tubes for scrubbing CO_2 and H_2O, and air can be diverted through these tubes in any proportion desired. CO_2 is best controlled by scrubbing all of it from the incoming air and injecting just enough CO_2 from an external source. The air is controlled by a flow control before entering the chamber

2 A/C_i and Light curves

The output of the gasometry measurement are photosynthetic curves. **A/Ci curve** shows the rate of net assimilation (A_n) plotted against CO2 concentration in the substomatal cavity (C_i) with constant radiation and temperature. Similarly, the **Light curve** shows the rate of net assimilation (A_n) plotted against the irradiance (PAR) with constant CO2 concentration and temperature.

Both curves have shape of unbalanced hyperbole.

2.1 *A*/C_i curve

A/C_i curve (net assimilation rate plotted against intercellular CO2 concentration) can provide a number of insights into the biochemistry of a leaf or plant. The curve start at the point of **day respiration rate** (R_{day}), with increasing CO2 concentration we reach the **CO2 compensation point** (Γ_c, CO2 concentration for which photosynthesis and respiration are balanced and A_n=0), the carboxylation efficiency (V_{cmax}, the initial slope), and **the maximum photosynthetic rate** (A_{nmax}).

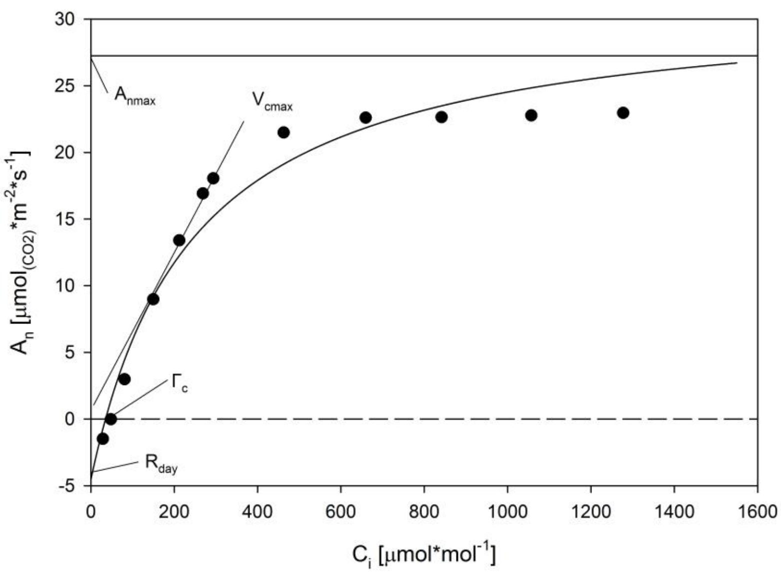

Fig. 3: The relation of the net assimilation (An) on the CO2 concentration in the substomatal cavity (C_i).

The CO2 compensation point is crucial for determining the carbon metabolism. C3 plants have higher Γ_c in comparison with C4 plants.

2.2 Light curves

The Light curve shows a relationship between the rate of assimilation (A_n) and the Irradiance (PAR, $\mu mol_{(photon)}.m^{-2}.s^{-1}$). The curve has again shape of hyperbola.

Starting from total darkness, in which there can be no photosynthesis, the first few photons to be absorbed by the leaf will be used with greatest efficiency. As light increases, the efficiency drops, and eventually subsequent increases in light yield little or no increase in photosynthesis. Thus, a light response curve can provide measures of **dark respiration rate** (R_{dark}), **the light compensation point** (Γ_I, absorbed quantum flux for which photosynthesis and respiration are balanced; A_n=0), **the quantum efficiency** (*AQE*, the initial slope), and **the maximum photosynthetic rate** (A_{nmax}).

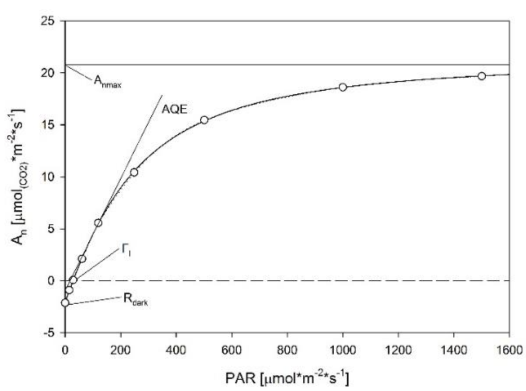

Fig. 4: The relation of the net assimilation (An) on the photosynthetically active radiation (PAR).

2.3 Mathematical models of photosynthetic curves

The shape of unbalanced hyperbole of A/Ci and light curves indicate the enzymatic reaction of both relations. For mathematical modeling we use equations based on Michaelis-Menten kinetics:

$$v = \frac{V_{max} * [S]}{[S] + K_m} \tag{5}$$

where v is the rate of formation of product, V_{max} is the maximum rate achieved by the system, [S] is a concentration of the substrate (in our case of CO_2 or PAR, respectively), K_m is Michaelis-Menten constant (the substrate concentration at which the reaction rate is half of V_{max}).

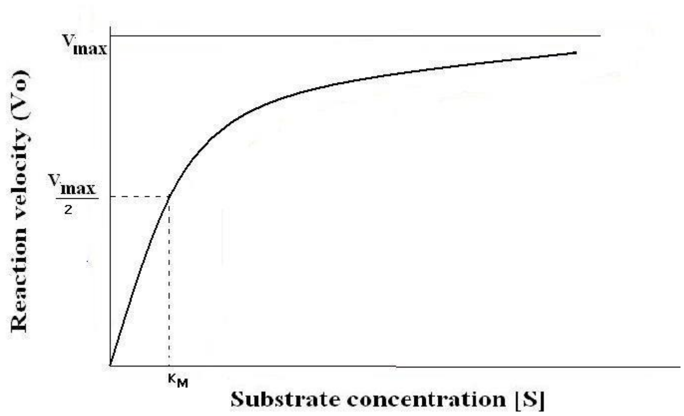

Fig. 5: Michaelis-Menten saturation curve for an enzyme reaction showing the relation between the substrate concentration and reaction rate.

For modeling *A/C_i curves* we can use model derived from Michaelis-Menten saturation curve:

$$A_n = \frac{A_{max} * (c_i - \Gamma_c)}{c_i + K} - R_{day} \tag{6}$$

where A_{max} is the maximal rate of net assimilation, c_i is concentration of CO2 in the substomatal cavity, Γ_c is the compensation concentration of CO2, K is value of C_i, when An=Amax/2 and R_{day} is the respiration (together with photorespiration).

For modelling the *Light curve* we can use the following equation:

$$A_n = \frac{AQE * PAR * A_{max}}{AQE * PAR + A_{max}} - R_{dark} \tag{7}$$

where AQE is the apparent quantum efficiency, *PAR* (photosynthetically active radiation) is intensity of the irradiance, A_{max} is the maximal rate of photosynthesis, R_{dark} is dark respiration.

The compensation irradiance (Γ_I) can be than calculated as:

$$\Gamma_I = \frac{R_{dark} * A_{max}}{AQE * (A_{max} - R_{dark})} \tag{8}$$