MEASURING PHOTOSYNTHESIS BY GAS EXCHANGE SYSTEMS

(LI-6400)

/Petra Majer/

Goals of the practice:

- **Learn principles of gas exchange measurements and infra red gas analysis**
- **Get familiar with infrared gas exchange systems, in particular with the *LI- 6400***
- **Make photosynthesis measurements on different tobacco leaves**

PRINCIPLES

Gas exchange measurements

Measuring gas exchange is the most commonly utilized technique at present for commercial and research purposes in order to measure photosynthesis of individual leaves, whole plants or plant canopy. Gas exchange measurements provide direct measure of the net rate of photosynthetic carbon assimilation.

Main advantages of gas exchange measurements: instantaneous, non-destructive, direct.

CO_2 exchange systems use enclosure methods, where the leaf in closed in a transparent chamber. The rate of CO_2 fixed by the leaf enclosed is determined by measuring the change in the CO_2 concentration of the air flowing across the chamber. Because ambient atmospheric CO_2 concentration is only 0.04 % (400 ppm), it is difficult to measure photosynthetic CO_2 uptake and sensitive sensors are needed.

Infrared gas analysis

Heteroatomic gas molecules absorb radiation at specific infrared (IR) wavebands, each gas having a characteristic absorption spectrum. Infrared gas analyzers (IRGAs) measure the reduction in transmission of IR wavebands caused by the presence of CO_2 between the radiation source and a detector. The reduction in transmission is a function of the concentration of CO_2. The only gas normally present in the air with an absorption spectrum overlapping that of CO_2 is water vapour. Since water vapour is usually present in the air at much higher concentrations than CO_2, this interference is significant, but may be overcome simply by drying the air or measuring H_2O concentration by another IRGA.

PHOTOSYNTHESIS GAS EXCHANGE SYSTEMS

General parts of a gas exchange system:

- leaf chamber
- flow meter
- means of generating and controlling air flow over the leaf

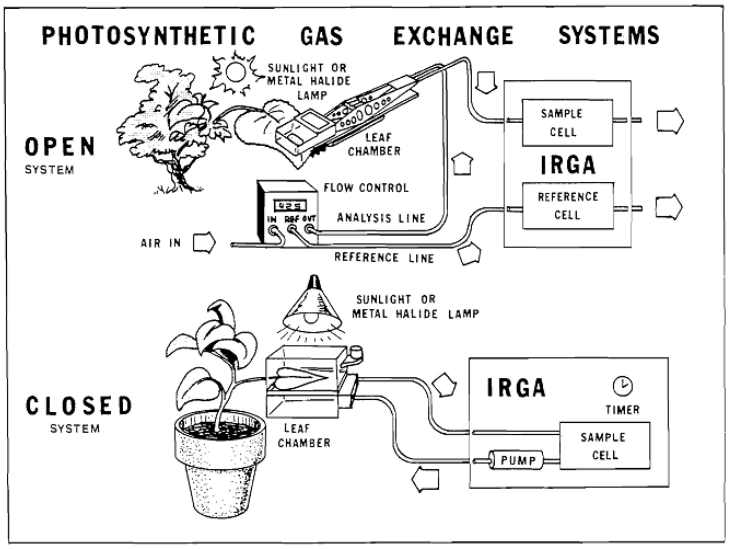
Open versus closed systems

In closed systems the signal from the sample cell is compared to the zero gas reference signal to provide an absolute measurement of CO_2 concentration. A leaf is enclosed in a chamber, sealed to avoid gas exchange with the atmosphere, and the rate at which the CO_2 and H_2O concentration changes in the chamber are monitored.

Major disadvantages of closed IRGA system:

- Photosynthesis measurements must be made within a few seconds after closing the leaf chamber (once the leaf is sealed in the chamber CO_2 concentration in the leaf chamber is continually decreases and water vapour increases).
- The operator has limited control over environmental conditions within the chamber.

Open systems are configured to allow air from a single source to enter both the analysis and reference lines. Air is continuously passed through the leaf chamber (to maintain CO_2 in at fixed concentration) and measurements of photosynthesis and transpiration are based on the differences in CO_2 and H_2O in an air stream that is flowing into the leaf cuvette (reference cell) compared to the air stream flowing out of it (sample cell). The rate of CO_2 uptake is used to assess the rate of photosynthetic carbon assimilation, while the rate of water loss is used to assess the rate of transpiration (counted on a leaf area basis).

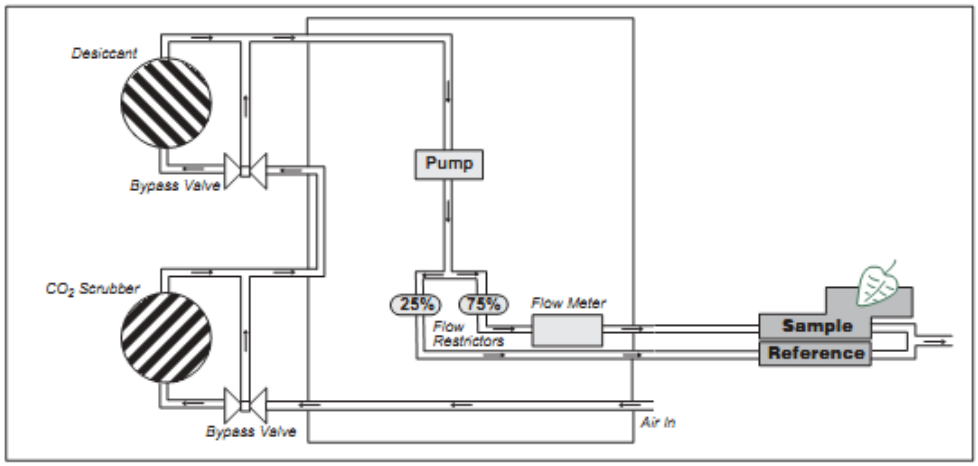


Schematic illustration of open and closed IRGA systems

(Illustration by Alan Rhodes, Mulkey & Smith 1998)

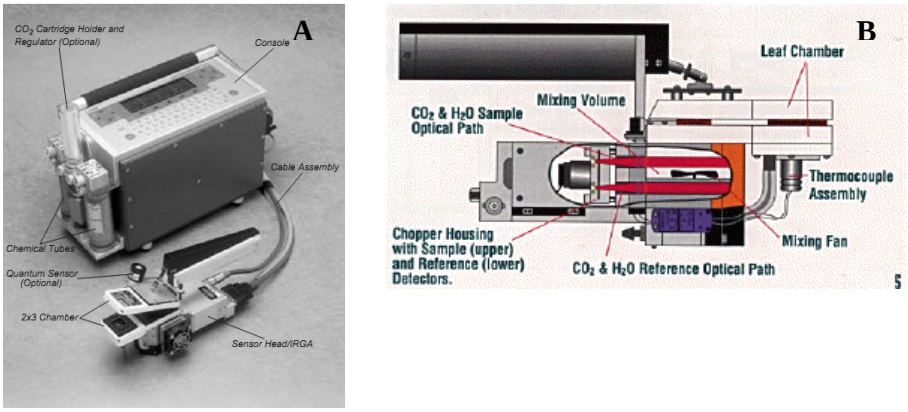
***LI-6400* Portable Photosynthesis System** (LI-COR, Lincoln, Nebraska, USA)

LI-6400 is an open system design photosynthetic gas exchange system. IRGAs (H_2O and CO_2) are in the sensor head, that gives a possibility for a tight control of conditions in the leaf chamber response to leaf changes. The incoming air stream can be conditioned for CO_2 concentration, humidity and temperature. 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, however, is best controlled by scrubbing all of it from the incoming air, and using CO_2 source to provide a stable concentration at the desired value (optional CO_2 injection system). The system is equipped with Peltier thermoelectric coolers for temperature control and light sensors. LI-COR offers a variety of interchangeable leaf chambers (coniferous, broadleaf, soil). The newest version, *LI-6400XT* is also capable of measuring fluorescence and gas exchange simultaneously over the same leaf area.



Schematic of *LI-6400* (Source: Using the LI-6400, Manual, version 5.3)

The reference analyzer measures incoming gas concentrations and is located directly below the sample analyzer. The sample and reference analyzers can be matched at any time, either manually or automatically, without altering conditions in the leaf chamber.



The *LI-6400* device (A) and the sensor head (B) (Source: Using the LI-6400, Manual, version 5.3)

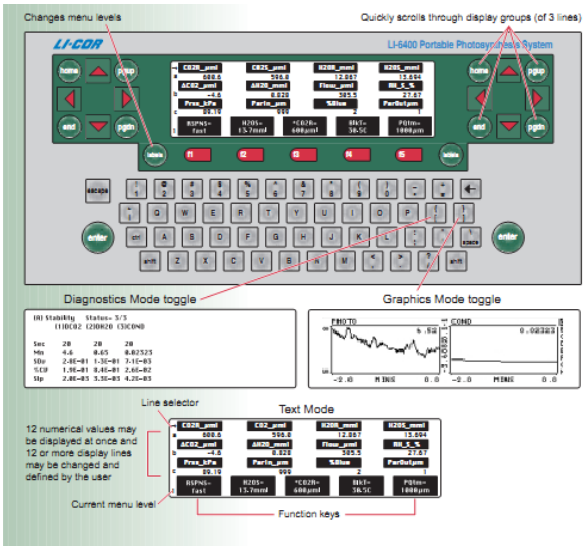
Main parts of the *LI-6400* device:

Console: a data logging computer with keypad and LCD screen

Sensor Head/IRGA: leaf chamber (for different leaf areas), spring-loaded latching handle, 2 Peltier thermoelectric coolers, sample and reference IRGAs, light sensor (external quantum sensor is optional)

Chemical Tubes: a tube containing soda lime (to remove CO_2 from incoming air stream) and a tube containing Drierite, a desiccant (to remove water vapour)

CO_2 source: cartridge + injector or tubing from a buffer volume



***LI-6400* console** (Source: Using the LI-6400, Manual, version 5.3)

What can we measure with *LI-6400*?

LI-6400 provides direct measurements of net photosynthetic rate and transpiration and indirect assessment of stomatal conductance and many other variabes.

- Instantaneous gas flux measurements (13 computed and 55 measured variables)
- Photosynthesis light response curves
- A/Ci curves: response of CO_2 uptake to intercellular mole fraction of CO_2
- Daily courses of gas exchange

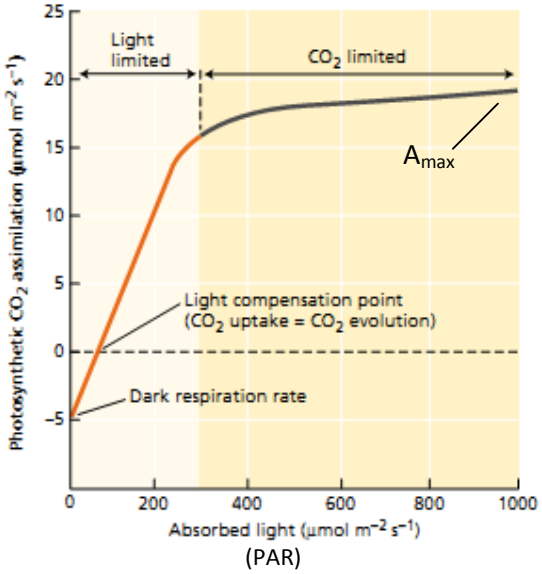
Main variables:	Abbreviations:		
	common	*LI-6400*	
Photosynthetic rate	A	*Phot*	µmol CO_2 m^{-2} s^{-1}
Transpiration rate	E	*Trmmol*	mmol H_2O m^{-2} s^{-1}
Stomatal conductance to H_2O	g_s	g_{sw}	mol H_2O m^{-2} s^{-1}
Intercellular CO_2 concentration	C_i	C_i	µmol CO_2 mol^{-1}

Photosynthesis light response curves (rapid/slow)

With increasing photosynthetically active radiation (*PAR*) chloroplast stroma becomes more alkaline that leads to the activation of Rubisco, and an increase in ATP and NADPH production, therefore to an increase in photosynthetic CO_2 assimilation (*A*). If we observe the relationship of photosynthetic CO_2 assimilation and light, we can see that initially *A* increasis linearly. It reaches the light compensation point at a certain *PAR* level, where CO_2 assimilation and respiration balances each other. The slope of the linear shows the maximal quantum efficiency of photosynthesis (0.04-0.06 mol CO_2 mol^{-1} photon). In this part of the response curve, photosynthesis is limited by the rate of electron transport, which is in turn limited by the amount of available light. With further increase in light photosynthesis becomes CO_2 limited, until where the curve reaches a light saturation point, where *A* is not responding to further increases in *PAR* level, and is limited by the carboxylation capacity of Rubisco or by triose phosphate metabolism.

Rapid light response curves: ca. 2 minutes at each PAR, not enough time for stomatal adjustment

Slow light response curves: 15-20 minutes at each PAR (till the stomatal adjustment happens)



Photosynthetic light response curve in a C3 plant (Source: Taiz & Zeiger 2010)

PRACTICAL

Samples:

Tobacco (*Nicotiana tabacum L. var Xanthi*), 6-week-old

1, ***greenhouse*** grown at ca. 200 µmol m^2 s^{-1} PAR

2, greenhouse grown at ca. 200 µmol m^2 s^{-1} PAR and placed ***outdoor*** under solar radiation for a few days

3, greenhouse grown at ca. 200 µmol m^2 s^{-1} PAR, water withdrawal for a few days (***drought stressed***)

Measurements:

1, Rapid light response curves (ca. 2 min per irradiation level)

a) greenhouse tobacco

b) outdoor tobacco

c) drought stressed tobacco

2, Effect of clouds

a) „fast cloud" (brief shade simulation)

b) „slow cloud" (long shade simulation)

DISCUSSION & QUESTIONS

Sources and further reading

Long SP, Farage PK, Garcia RL, 1996. Measurement of leaf and canopy photosynthetic CO_2 exchange in the field. Journal of Experimental Botany 47: 1629-1642.

Mulkey SS, Smith M, 1988. Measurement of photosynthesis by Infra Red Gas Analysis. IN: Bioinstrumentation, C.T. Lange, (ed). 79-84. Publication of the American Biology Teachers Association.

Taiz L, Zeiger E, 2010. Plant Physiology, Fifth Edition. Sinauer Associates, Inc. Sunderland, MA, USA.

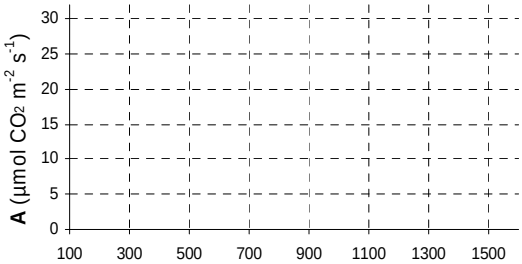
LI-COR, 2004. Using the LI-6400 Portable Photoszntheis System. Version 5 (2004) LI-COR Biosciences, Inc. Lincoln, NE, USA

http://www.licor.com/env/products/photosynthesis/measurements.html

DATA SHEET

Plant	PAR	A	E	g_s	C_i
	µmol m^{-2} s^{-1}	µmol CO_2 m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	µmol CO_2 mol^{-1}
greenhouse tobacco	100				
	300				
	500				
	700				
	900				
	1100				
	1300				
	1500				
outdoor tobacco	1500				
	(30 s) 300				
	1500				
	(2 min) 300				
	1300				
	1100				
	900				
	700				
	500				
	300				
	100				
drought stressed tobacco	100				
	300				
	500				
	700				
	900				
	1100				
	1300				
	1500				

Rapid Light Curve:



Rapid Light Curve: