http://www.carboschools.org

PHOTOSYNTHESIS, I MEASURE IT!

Introduction to the project

This activity is suitable for the curriculum in biology since it focuses on the process of photosynthesis measuring the real flux of carbon dioxide at leaf level. It is supported by professional instruments provided by the research institute and strictly dependent on the weather, so we suggest running the activity on a sunny day in spring or summer to get the highest values of photosynthesis. The frequency of the survey should be at least fortnightly.

Photosynthesis is the most important biological process occurring within the carbon cycle. Terrestrial and aquatic vegetation and some bacteria contribute to the removal of CO_2 from atmosphere through this process. In this way carbon is stored in organic matter while H_2O is consumed and carbohydrates and oxygen are produced. The basic carbohydrates produced by the process at night represent the first step of the gross primary production.
Even if some of the CO_2 is released again during night respiration, the vegetation, at global scale, represents a sink of carbon, due to the positive balance between the carbon uptake (photosynthesis) and carbon release (by respiration).

Within this project, students approach the concepts of plant physiology and have a direct and concrete experience of the gas exchanges occurring in the leaf during photosynthesis.

Preparation time: about 30 minutes to prepare materials (warming and setting up the device) and to select species and leaves.
And 10 minutes to explain the principle how the measuring device works and 1 hour for measurements.
Duration of activity: one year for seasonal variability.

- One to two hours for the introduction seminar. Topics: CO_2 in atmosphere; the carbon cycle; the role of vegetation within the carbon cycle; the photosynthesis and gas exchanges, introduction to the instrument
- One hour for each data collection campaign. Topics: use of the instrument, methodology, data acquisition.
  Frequency depends upon the set schedule for the project; it can be once or twice a month or once a season.
- three hours (depending on the informatics skills of the students) for data analysis and discussions
- two hours for the presentation of results (preparation of posters)

Application: biology curriculum; the activity is strictly connected to plant physiology.

Student Age: 16-18

Time for data analysis and discussion: data elaboration and discussion phase could take some time for the relevant number of parameters. We suggest observation of the variability over the year and in different species or to look at the position of the leaves in the canopy (sun/shade leaves, young/old leaves).

Previous knowledge required:
A prerequisite for this activity is that students should already have a basic knowledge of photosynthesis. The carbon cycle and the role of vegetation and gas exchanges at leaf level should be explained.
Informatics skills and general knowledge of Excel.

Cost: CIRAS or LICOR devices are the common equipment of Plant Physiology Research Centers. Their cost is high and the research partner might be able to lend it to the teachers involved, preferably handled by the technician.

Materials:
- **CIRAS-1** is an Infrared Gas Analyzer by PPSystem (Hitchin, UK) that instantaneously measures the photosynthesis (i.e. the µmol of CO_2 absorbed by a surface unit of leaf in a unit of time) and other parameters: absorbed CO_2 concentration, water vapor and transpiration rate, leaf temperature, cuvette temperature, Photosynthetically Active Radiation (PAR).
- Program for downloading data, and PC with worksheets;
- Green leaves: an easily accessible shrub or a small tree is required. Evergreen species allow to run measurements during the whole year.
- Plastic bags, pen and note book;
- Planimeter or graph paper to measure the leaf area.

Procedure:

1) Go to the garden and select a tree (or shrub) to observe. Select some sun exposed leaves. You can record observations on different conditions:
   - Position in the canopy: higher-middle-lower;
   - Location of the leaves: shade leaves (located in the inner part of the canopy) and sun leaves (located in the external part of the canopy);
   - different species

2) Run the measurements when the sun is high (11:00-14:00 hrs) if the instrument does not have an additional lamp. CIRAS has a **PAR** sensor for measuring the **P**hotosynthetically **A**ctive **R**adiation used by the pigments. A known flux of CO_2 (ppm) enters inside the cuvette and the consequent outlet flux is analyzed. The display gives the value of absorbed (-) or produced (+) CO_2 if photosynthesis or respiration occurred respectively.

3) During the measurement be careful to keep the leaves attached to the branch. They should be measured in their normal state, in as much as possible natural conditions.

4) Set the CIRAS following its manual for use or let the technician do it; when it has finished its calibration the measurements can start;

5) The following data should be noted: date, time, sky conditions, description of the site, species, number of leaf , position of the leaf in the canopy, number of plot and start-end records in CIRAS and any note useful for the elaboration. 6) Seal the leaf inside the cuvette, expose the head of CIRAS to the direct solar radiation(look at the PAR sensor) and take records (manually is preferable to make the students familiar with the data

collection);
7)Collect data: 5 values for each leaf. The sealing can influence the leaf activity increasing the leaf temperature with possible consequent stomatal closing.
8) Remove the leaf from the cuvette and repeat the measurement.
9) Detach the leaf and put it in a plastic bag, labeling it with a code (species, number of plot, records) and refrigerate it for later leaf-surface measurements.
10) Choose another leaf and repeat the procedure.
11) When all the leaves are measured and records are taken, the data can be downloaded from the CIRAS and imported in Excel. The proper program and cable to dump the data to the PC are needed. Several parameters are taken by the instrument, the presence of the assistant or the technician is helpful to understand the most important parameters for the student analysis.
12) Particular attention is given to:
- CO_2 ppm absorption
- Photosynthesis & Respiration
- Stomatal Conductance
- Transpiration (water loss)

13) Elaboration:
- Observation of the variability of the parameters during the measurements campaign or during the time the leaf has been sealed.
- Basic statistical analysis of the parameters (mean values, standard deviation)
- Observation of the values for different leaves according to their positions, or leaves of different species
- The parameters obtained by the instrument are referred to a reference value of 2,5 cm^2 (cuvette surface)
- If the leaf surface is measured (by planimeter or graph paper) it is possible to calculate the parameters referred to the leaf surface.

Presentation of Results:
Example of results obtainable (data collection 2009):
In table 1, mean values of the parameters for different species taken on the 19th of February at 14:00, have been calculated:
The values are referred to 2,5 cm^2 surface and they can be relative to the whole leaf surface if measured. The "sun leaves" of *N. Oleander* shown lowest values of CO_2 assimilation while the leaves of *P. laurocerasus* located in the lower part canopy had highest values.

	Diff CO_2 ppm '-' absorbed '+' released (ppm)	PAR (μmol /m^2s)	TRANSPIRATION water lost (mol/m^2s)	GS stomatal conductance to water vapor (mol m^{-2} sec^{-1})	LEAF Temper. (°C)	PN '+'Photosynt. '-'Respiration (μmol CO_2/m^2s)
	Prunus laurocerasus, lower part of the canopy, lighted leaf					
mean	**-35.3**	**1706**	**0.12**	**6.7**	**18.4**	**14.3**
st dev	11.83	8.08	0.10	5.51	0.60	4.90
	Prunus laurocerasus, higher part of the canopy, lighted leaf					
mean	**-17.4**	**1558**	**0.103**	**5.667**	**18.900**	**7.033**
st dev	5.543	18.009	0.031	1.528	0.000	2.194
	Nerium oleander, higher part of the canopy, lighted leaf					
mean	**-10.7**	**1390**	**0.070**	**3.333**	**19.433**	**4.300**
st dev	1.457	71.042	0.030	1.528	0.058	0.656

These values mean that we expect that at those environmental conditions (the meteorological station is useful for the site description), for instance *P. laurocerasus* leaves, on average absorb 14,3 μmol CO_2 $m^{-2}sec^{-1}$ in the lower part of the canopy and 7 μmol CO_2 $m^{-2}sec^{-1}$ in the higher part of the canopy, removing 35,3 ppm and 17,4 ppm of CO_2 from the atmosphere, respectively ,

while the other species look less active.
The higher assimilation rates observed in the lower part of the canopy could presumably be due to the lower air turbulence at leaf level that ensures a higher content of CO_2 in the air layer just above the leaf and makes its assimilation easier.
The *P. laurocerasus* leaves also show higher values of stomatal conductance with consequent higher water loss than other species.
Photosynthesis should be measured during spring and summer, which are favorable seasons for the physiological activity even if limitation by some external factors like soil humidity, air temperature can occur.

Discussion of results:
Have you noticed differences among different species?

Do you suppose a CO_2 enriched atmosphere to be limiting or positive for plants?

Can the photosynthesis rate be increased by the green house effect?

When is the CO_2 molecule fixed? And when are carbohydrates synthesized?
A second part of the experiments' description may be added as a guide or an aid to other teachers who would want to do the experiments in their schools. This can include the following:

1. **Suggested further experiments:**
   Extraction of pigments
   Observing Carbon dioxide uptake of different species of Phytoplankton and oxygen production by an aquatic plant.

2. **Suggested reading (literature or links):**

1993. Ecofisiologia vegetale, W.Larcher. Edagricole, Bologna. Original title: Ökologie der Pflanzen.
2003. CIRAS-1 PORTABLE PHOTOSYNTHESIS SYSTEM TUTORIAL Version 1.10. A Guide For Beginners. PPSystem.
2003. CIRAS-1 Portable Photosynthesis System Operator’s Manual Version 1.20
© 2000 PP Systems.

3. **Some tips or notes on things that one should pay attention to when preparing the experiment:**
   The project is possible when the sun is at zenith (day time around 11-14 hr), depending on the latitude.
   Evergreen species can be useful if the project is run during winter-autumn seasons.

Developed for CarboSchools by Francesca Ugolini, CNR, Firenze, Italy. Mail: f.ugolini@ibimet.cnr.it
(last change: 19-Oct-09)

This publication has received funding from the European Community's Seventh Framework programme under grant agreement number 217751. It is licensed under Creative Commons Attribution-Noncommercial-Share Alike 3.0 License. For details see http://creativecommons.org./licenses/by-nc-sa/3.0/