OPTI-SCIENCES, INC.

Application Note #1209

# A Comparison of Photosynthesis $CO_2$ and $H_2O$ Gas Exchange Measurement and Chlorophyll Fluorescence Measurement

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## Announcement:

***Opti-Sciences is now the exclusive importer, distributor, and repair center for ADC BioScientific products in the US.***

***ADC has been making $CO_2$ and $H_2O$ gas exchange systems for photosynthesis since 1970. Its innovative designs make ADC the world leader in field portable gas exchange photosynthesis systems. ADC also manufactures excellent solutions for Soil $CO_2$ flux, leaf area measurement, and atmospheric $CO_2$ measurement. Visit our website for more information. www.optisci.com***

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Photosynthesis Gas exchange measuring systems use Infra red gas analyzers (IRGAs) to measure $CO_2$ and sometimes $H_2O$. They also have sensors for barometric pressure, temperature, light PAR values, flow rate meters, and sometimes humidity sensors to measure photosynthesis.

The sample leaf to be measured is enclosed in a measuring chamber and the leaf is held gently in place by springs and neoprene seals. Measuring begins by allowing the leaf to reach $CO_2$ equilibrium in the measuring chamber. The time required to reach equilibrium is species dependant, and it has nothing to do with brand of instrument. This time can range from forty five seconds to one hundred and twenty seconds.

Once equilibrium has been reached the actual measurement takes a few seconds. This measurement can then be run many times as necessary. The instrument measures $CO_2$ in the air before it goes into the leaf chamber and compares it to air after it passes through the measuring chamber.

The parameters that are measured include: $CO_2$ level before and after the measuring chamber, $H_2O$ before and after the measuring chamber, barometric pressure, chamber temperature, leaf temperature, ambient temperature, air flow rate, and PAR light level.

From these direct measurements, a number of mathematically derived parameters become available including, net $CO_2$ assimilation, and Transpiration. There are also a number of derived parameters that require some additional assumptions. These parameters include: Stomatal Conductance, Stomatal resistance to $H_2O$, boundary resistance to $H_2O$, mesophyll conductance, and Intercellular $CO_2$ concentration.

In addition, measuring light curves can provide results at varying light levels and A/Ci curves ($CO_2$ net assimilation/ intercelluar $CO_2$ concentration) are an excellent way to study plant stress.

*So why should gas exchange be used to measure plant stress?*

Gas exchange will directly measure all types of plant stress regardless of the type, and it will detect stress very early. Fluorometers on the other hand will measure plant stress that affects PSII. In some cases gas exchange will detect plant stress before fluorometer techniques.

Measuring times are significantly slower with gas exchange systems due to the leaf equilibrium requirement. As As result, this technique is better suited to smaller populations of plants.

**Examples:**

Heat stress.

One study found that Gas exchange detected heat stress in Quercus at about 30°C. Photosynthetic yield and NPQ tests used by fluorometers first detected heat stress at 35°C. Fv/Fm, also used by fluorometers, first detected heat stress at 45°C (Haldiman P, & Feller U. 2004).

Nitrogen Stress.

Nitrogen stress can be detected early by gas exchange. With fluorometers, one needs to use specialized ratio fluorescence such as the FRFex360/FRFex440 test created by Sampson(2000), or use a special assay that combines both light stress and nitrogen stress created by Cheng (2001). Without one of these methods it is difficult to measure nitrogen stress at a useful level. ( Chlorophyll Content meters do offer a workable solution and a more cost effective solution for nitrogen stress. -see the OSI CCM-200)

Water Stress.

Both gas exchange and fluorescence can be used to detect water stress in C4 plants. However, in C3 plants Gas exchange will measure water stress at very early stages while standard fluorometer methods will only detect moderate to severe water stress. A fluorescence assay does exist for the measurement of very early water but involves using a leaf punch, and combining heat stress and water stress (Burke 2007)

Sulfur Stress

Sulphur stress can be detected by gas exchange, but it can only be detected by fluorometers at starvation levels (Baker and Rosenqvist 2004) ( Chlorophyll Content meters do offer a workable solution and a more cost effective solution for sulfur stress. -see the OSI CCM-200)

***LCpro+* field portable**
**$CO_2$ gas exchange system**
**with micro climate control**

***LCi* field portable**
**$CO_2$ gas exchange system**
**for ambient measurements**

## Chlorophyll Fluorescence

Chlorophyll fluorometers, or special fluorometer assays may be used to detect most types of plant stress at usable levels.

Fluorometer are used to measure variable fluorescence of the PSII system. It has been found that stresses that affect PSII change the amount of PSII fluorescence that is measured. PSII is sensitive to plant stresses of most types. (For detailed information please refer to the Opti-Sciences Stress Guide available on line or by phone.

Fv/Fm is the most used fluorescence parameter for detecting the health of PSII and for measuring plant stress. The sample must be dark adapted for reliable measurement (see the application note on dark adaptation from OSI)

Photosynthetic Yield and Electron transport rate (or ETR) are tests taken while the leaf is in sunlight with no dark adaptation. They allow measurement of PSII while photosynthesis is happening. Systems that measure Photosynthetic Yield and ETR are more expensive than units that measure just Fv/Fm, and require the measurement of light level(or PAR) and leaf temperature for reliable results. These parameters will detect more types of stress or detect stress earlier than Fv/Fm in some cases ( See the OSI Stress Guide).

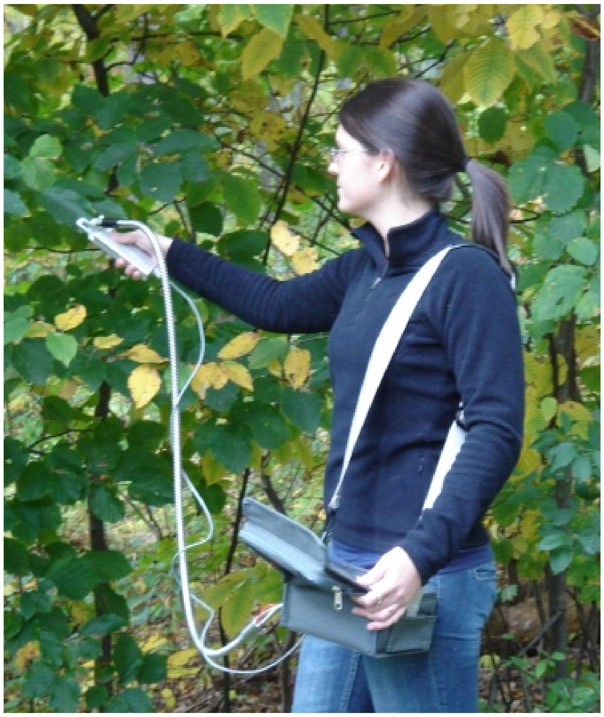
Fluorometers are also very valuable in studying the light reaction of photosynthesis.
Photoprotective mechanisms, state transitions, and photoinhibiotion can be studied with a fluorometer.

### *So why should chlorophyll fluorometer be used to measure plant stress?*

In general, fluorometers are great for most types of plant stress measurements. They offer a cost effective way to make measurements in the field, with large numbers of samples, very quickly. Most fluorescence measurements take only a few seconds. In addition, portable fluorometers also offer light weight field portable solutions with long battery life.

**OS5p Fluorometer with PAR clip and all-day shoulder harness**

**OS1p Fluorometer with PAR clip and shoulder strap**



## Correlation of Fluorescence Measurements to Gas Exchange Carbon Assimilation:

In 1989, Genty developed the fluorescence photosynthetic yield measurement and provided strong evidence of a linear correlation between Yield measurements, Electron Transport Rate, and $CO_2$ assimilation for C4 plants (Baker and Oxborough 2004). Many others have confirmed the relationship (Edwards and Baker 1993), (Krall and Edwards 1990, 1991). It was found that a curve-linear correlation between Yield and $CO_2$ assimilation exists for C3 species where photorespiration can also use significant products of electron transport (Genty 1990), (Harbinson 1990), (Baker and Oxborough 2004).

***The strong correlation between Yield and $CO_2$ assimilation has been reaffirmed repeatedly by many researchers with the following caveats:***

1. There is small percentage of chlorophyll fluorescence that comes from photosystem I that does not change with light intensity (PPFD). Therefore, the error is greatest at very high light levels when yield is minimized and PSI fluorescence remaining constant. This error is not large (Baker Oxborough 2004).

2. "Super-saturating flash" error is produced by using a very intense saturation light source that is longer than 2 ms causing multiple turnovers of primary PSII receptor $Q_A$ and the reduction of plasotoquinone to plastoquinol. This raises Fms (or Fm') and can cause an overestimate of Yield by less than 10% (Baker and Oxborough 2004), (Schreiber 2004). Use of a super-saturation flash is by far the most common method of measuring photosynthetic yield in higher plants.

3. Cold stress can produce a non-linear correlation with $CO_2$ assimilation. Electron transport of PSII in cold stressed corn far exceeds the requirements for $CO_2$ assimilation by more than three to one, indicating that under these conditions other electron sinks are at work. The ratio of ETR (a product of Yield, PAR, leaf absorption ratio, and PSII absorption ratio) to $CO_2$ assimilation under cold stress can be diagnostic for cold stress. (Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. 1998)

4. The ratio of ETR to $CO_2$ assimilation can be diagnostic for water stress in C3 plants. C3 plants exhibit strong electron transport rates for early and moderate levels of water stress even when $CO_2$ assimilation has decreased due to water stress. This indicates that there are other electron sinks for electron transport. (Ohashi 2005). This problem of early water stress measurement and detection may be overcome by using heat with Yield to measure very early water stress (Burke 2007).

5. Mangrove leaves growing in the tropics. Here again electron transport rate is more that three times that of $CO_2$ assimilation. It is believed that this is mostly due to reactive oxygen species as an electron sink. (Baker Oxborough 2004), (Cheeseman 1997)

6. Measurements not taken at steady state photosynthesis can lead to non-linearity caused by state transitions. This error can be in the range of 10% to 30% depending on the organism (Allen and Mullineau 2004). The error can be avoided by allowing plant samples to reach steady state photosynthesis, a process that takes between fifteen and twenty minutes (Maxwell and Johnson 2000).

7. At high light stress levels, the correlation between ETR and $CO_2$ assimilation breaks down. It is thought by some to be caused by the inability of the most intense saturation light sources to completely close all PSII reaction centers under high light stress conditions. To compensate for this issue, Earl (2004) uses saturation pulses at various levels and extrapolates the results of a saturation pulse at infinity using linear regression analysis. This method restores the correlation of ETR and $CO_2$ assimilation and it is an option that is offered On the Opti-Sciences OS5p.

8. While linear correlation and curvilinear correlation are possible (Genty 1989), (Genty 1990), (Baker Oxborough 2004), *exact* correlation between fluorescence ETR and gas exchange ETR is not possible due to the fact that fluorescence comes from only the upper most layers of the leaf while gas exchange measurements measure lower layers as well (Schreiber 2004).

As illustrated by the exceptions listed above, *in some cases* …" the relationship between light reactions and dark reactions is not straightforward"… The energy molecules ATP and NADPH can be used for carbon fixation and for photorespiration (Rosenqvist and van Kooten 2006), or light reaction electrons may flow to other electron sinks (Ohashi 2005), (Baker Oxborough 2004), (Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. 1998).

## Deciding on the best solution

| Category | Chlorophyll Fluorescence | $CO_2$ Gas Exchange |
|---|---|---|
| Cost | Chlorophyll fluorometers range in price from the low thousands of dollars to the mid teens. | Gas exchange equipment is generally more expensive. Prices start at about $17,500 and go to the mid-twenty thousand-dollar range for ADC products. |
| Time to measure | Most fluorescence measurements are made in two to three seconds. Several measurements can be made on a large population of samples very quickly. For dark adaption Fv/Fm, dark adaptation clips can be attached to hundreds of leaves. In this case, the researcher only waits once for dark-adapting.<br>Light curves and quenching analysis take much longer | Leaf equilibrium takes forty five seconds to two minutes before a measurement can be made on all brands of equipment. The actual measurement takes only a few seconds. Light curves and A/Ci curves take much longer.<br>Flow rates can affect measuring times. |
| Weight | Highly field portable. Weights of OSI systems range from 3 lbs. to 5 lbs. pounds | Highly field portable - ADC consoles weigh from 5 lbs., to 9.7 lbs. Other manufactures are not as field portable - weights are in the 16 lbs and 23 lbs. range. |
| Battery life | The OS5p has a battery life of 16 hours. The OS1p has a battery life of 10 hours. | The LCpro+ has a battery life of 16 hours. The LCi has a battery life of 10 hours. Competitors offer about four hours of battery life or less with a single battery. |
| Preventive maintenance and calibration | Calibration of a PAR sensor is recommended every two years. No preventive maintenance. | Calibration is recommended twice a year with preventive maintenance once a year. |
| Stress capability | Most types of stress can be measured with a chlorophyll fluorometer. Some types of stress require special assays (See OSI Stress Guide for more details. | All types of stress can be measured early. |
| Area of research focus | Fluorometers are very helpful in the study of the light reaction, photoprotective mechanisms, state transitions, and photoinhibition. | Gas exchange measures photosynthesis in the dark reaction. |

## Combining Fluorescence an gas exchange:

Fluorescence and $CO_2$ gas exchange have been used for combined measurement for some time.  These configurations are more expensive, and heavier, but they can offer advantages for some types of research.

## When does the combination of a fluorometer with CO2 gas exchange equipment offer advantages?

1. **Cold stress** can produce a non-linear correlation with $CO_2$ assimilation. Electron transport of PSII in cold stressed corn far exceeds the requirements for $CO_2$ assimilation by more than three to one, indicating that under these conditions other electron sinks are at work. The ratio of ETR (a product of Yield, PAR, leaf absorption ratio, and PSII absorption ratio) to $CO_2$ assimilation under cold stress can be diagnostic for cold stress. (Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. 1998)
2. **The ratio of ETR to $CO_2$ assimilation** can be diagnostic for **water stress** in C3 plants. C3 plants exhibit strong electron transport rates for early and moderate levels of water stress even when $CO_2$ assimilation has decreased due to water stress. This indicates that there are other electron sinks for electron transport. (Ohashi 2005). This problem of early water stress measurement and detection may be overcome by using heat with Yield to measure very early water stress (Burke 2007).
3. **Mangrove leaves growing in the tropics**. Here again electron transport rate is more that three times that of $CO_2$ assimilation. It is believed that this is mostly due to reactive oxygen species as an electron sink. (Baker Oxborough 2004), (Cheeseman 1997).
4. In **heat stress studies**, gas exchange equipment can detect stress at about 30 degrees C. and fluorescence photochemical yield does not detect heat stress until about 35 degrees C. Electron transport in relation to reversible Rubisco inhibition can be studied (Haldimann 2004).
5. The study of **light stress.** Under high light conditions PSII reaction centers are not fully saturated by very high intensity saturation pulses used in modulated fluorometers. This can be measured with combined instrumentation (Earl 2004) (The multiflash cabability of the OS5p fluorometer uses regression analysis to correct for this issue using only a fluorometer. (For more information contact OSI)
6. **$R_1$** photo-respiration  work. The rate of $CO_2$ release in photorespiration  or $R_1$ in determined by combined gas exchange and fluorescence ETR measurment. For the first time this can be measured with the combination of fluorescence and gas exchange. This is an important function for studies of C3 plants where a curvilinear correlation exists with carbon assimilation due to photorespiration...
(Long and Bernacchi 2003)
7. **$g_m$** = mesophyll conductance can now be measured directly with chlorophyll fluorescence and gas exchange. The difference between Electron transport of fluorescence and the J or ETR of gas exchange is a function of $g_m$. In the past, this had to be estimated; now it can be directly measured (Long and Bernacchi 2003).

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