

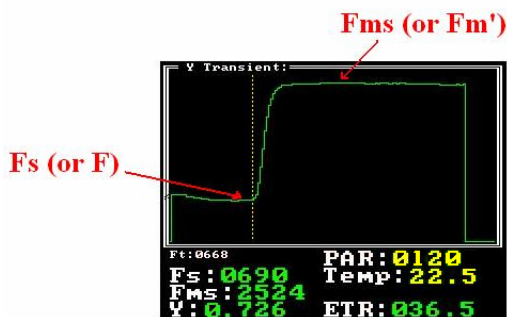
## “Yield” (quantum photochemical yield) - *Value and Limitations*

Yield (or  $\Delta F/F_m'$  or  $F_m - F_s / F_m$ ) is a time tested *light adapted* parameter that is more sensitive to more types of plant stress than  $F_v/F_m$ . A survey of existing research shows this to be true. While  $F_v/F_m$  is an excellent way to test for many kinds of plant stress and the health of Photosystem II, ***Quantum Photochemical Yield is a test that allows the measurement of the efficiency of PSII under actual environmental and physiological conditions.***

Quantum Photochemical Yield is a measurement ratio that represents achieved efficiency of photosystem II under current steady-state photosynthetic lighting conditions. (Genty 1989, 1990), (Maxwell K., Johnson G. N. 2000), (Rascher 2000) It is affected by closure of reaction centers and heat dissipation caused by non-photochemical quenching (Schreiber 2004).

As ambient light irradiates a leaf, about 84% is absorbed by the leaf, and approximately 50% of that light is absorbed by the antennae associated with PSII and transferred to PSII (Photosystem II) reaction centers. Under normal non-stressed conditions, most light energy is channeled into photochemistry with smaller amounts of energy channeled into heat and fluorescence. In photosystem II, this process is competitive so that as plant stress occurs, mechanisms that dissipate heat, photo-protect the leaf, and balance light between photosystem II and photosystem I, change the output of fluorescence and heat. In other words, conditions that maximize photochemistry minimize fluorescence and heat dissipation and conditions that maximize fluorescence minimize photochemistry and heat dissipation.

Once these mechanisms have achieved an equilibrium at a specific light level and temperature, steady state photosynthesis has been achieved. At this point, a modulated fluorometer can be used to expose a plant to a very intense short light pulse called a saturation pulse. It is designed to momentarily close or chemically reduce all capable PSII reaction centers while at steady state photosynthesis. Apart from the known exceptions listed under “*Correlation to Carbon Assimilation*” later in this discussion, quantum photochemical yield will reflect changes in the function levels of PSII antennae, PSII reaction centers, electron transport, carbon assimilation, and regulatory feedback mechanisms.



Graphic display of a single Yield measurement taken with a PAR Clip.

Yield measurements may also be taken with an Open Body Clip (without PAR or temperature measurement).

Yield is  $(F_m - F_s) / F_m$  or  $\Delta F / F_m'$

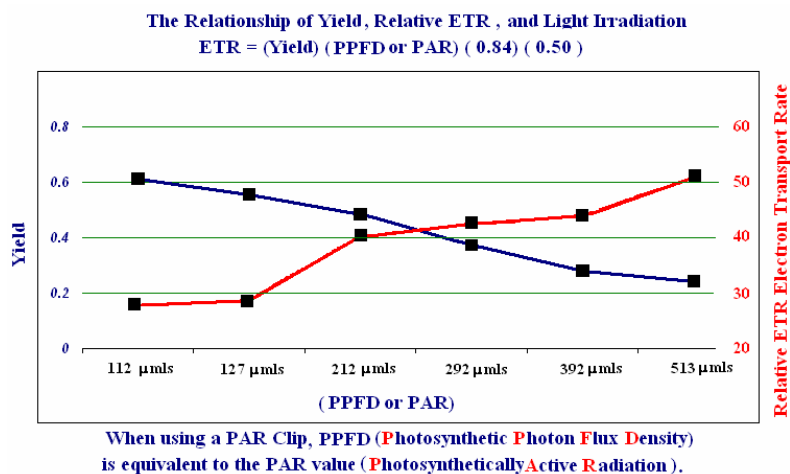
Light adapted measurement taken at steady state photosynthesis

***Yield will change at different light levels and temperatures*** so it can be of great value to use a heavily recommended accessory called a PAR Clip that measures yield relative to light intensity or irradiation level and temperature. **It is possible to misinterpret results if PAR and temperature changes are not taken into account. One leaf may appear to be stressed compared to another when the only difference is light irradiation level.**

PAR Clips measure Photosynthetically Active Radiation between the wavelengths of 400 nm and 700nm. When the dimensions per square meter per second in micro-mols or micro-einsteins are added, this parameter becomes Photosynthetic Photon Flux Density (or PPFD) (micromols and microeinsteins are equivalent, and when using a PAR Clip, PAR and PPFD are equivalent). *If irradiation and temperature are not controlled it is possible to falsely interpret data.* PPFD or PAR must be measured very close to the sample or errors can result. In addition, it is important not to change the orientation of a leaf and to avoid shading the sample measuring area with the PAR clip or by other means. Extraneous reflections and breathing on the sample should also be avoided (Rosenqvist and van Kooten 2006).

PAR Clips also allow measurement of relative ETR or relative Electron Transport Rate. ETR is a parameter designed to measure the electron transport of PSII. It has also been found to correlate well with CO<sub>2</sub> assimilation (with a few exceptions listed below). More advanced fluorometers provide built-in illuminators for greater experimental control of light irradiation intensity. This allows pre-illumination with a controlled predetermined intensity value for sample comparison. *(A future Application Note will deal more fully with relative ETR and PAR or PPFD measurement).*

For reliable Yield and ETR measurements, photosynthesis must be at steady state and with illumination on the same side of the leaf that is being measured (see number eight under correlation to carbon assimilation). Steady state photosynthesis is an equilibrium condition reached after a several minutes of exposure to existing light radiation conditions. Maxwell and Johnson (2000) tested 22 different species of British plant and found that steady state occurred in fifteen to twenty minutes in the plants measured. Measurements taken under variable lighting conditions may not provide reliable Yield results (Rascher 2000). No dark adaptation is required for Yield measurements.



### Correlation to Carbon assimilation:

In 1989, Genty developed the yield measurement and provided strong evidence of a linear correlation between Yield measurements, Electron Transport Rate, and CO<sub>2</sub> assimilation for C<sub>4</sub> plants (Baker and Oxborough 2004) and many others have confirmed the relationship (Edwards and Baker 1993), (Krall and Edwards 1990, 1991), (Siebke 1997). It was found that a curve-linear correlation between Yield and CO<sub>2</sub> assimilation exists for C<sub>3</sub> species where photorespiration can also use significant products of electron transport (Genty 1990), (Harbinson 1990), (Baker and Oxborough 2004).

*The strong relationship between Yield and CO<sub>2</sub> assimilation correlation 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 QA and the reduction of plastoquinone to plastoquinol. This raises F<sub>m</sub> (or F<sub>m</sub>’) 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 yield in higher plants.
3. Cold stress can produce a non-linear correlation with CO<sub>2</sub> assimilation. Electron transport of PSII in cold stressed corn far exceeds the requirements for CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> assimilation can be diagnostic for water stress in C<sub>3</sub> plants. C<sub>3</sub> plants exhibit strong electron transport rates for early and moderate levels of water stress even when CO<sub>2</sub> 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 than three times that of CO<sub>2</sub> 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 very high light stress levels, the correlation between ETR and CO<sub>2</sub> 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<sub>2</sub> 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).
9. In CAM plants, gas exchange measurements are not possible during daylight hours so Yield measurements can provide insights into daytime light reactions (Rosenqvist and van Kooten 2006).

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). For this reason, it is not uncommon for authors to differentiate between work done under non-photorespiratory conditions and under photorespiratory conditions (e.g. Earl 2004), (e.g. Genty B, Harbinson J., Baker N.R. 1990).

**Comparison of Yield and Fv/Fm for stress measurement**  
**For more detailed information request the Opti-Sciences Desktop Stress Guide.**

<b>Stress</b>	<b>Yield</b>	<b>Fv/Fm</b>
<b>Water Stress</b>	Yield <i>with heat</i> applied is sensitive to <i>very early water stress</i> in C3 plants. (Burke 2007)	Not sensitive to early or moderate water stress in most plants (Bukhov & Carpentier 2004) (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008)
<b>Water Stress</b>	In C4 plants ETR can be used for water stress. The ratio of ETR to carbon assimilation is consistent. <b>It does not work in C3 plants.</b> $ETR = (Yield) (PAR) (0.84)(0.50)$ (Cavender-Bares & Bazzaz 2004) Yield is sensitive to water stress in C4 plants (da Silva J. A. & Arrabaca M.C. 2008).	Not sensitive to early or moderate water stress in most plants (Bukhov & Carpentier 2004) (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008).  Fv/Fm is not sensitive to water stress in C4 plants, grasses are tested (da Silva J. A. & Arrabaca M.C. 2008).
<b>Water Stress</b>	Fs a component of Yield is sensitive to moderate water stress at saturation light levels. Fs/Fo is a normalized ratio that uses predawn dark adaptation and steady state fluorescence measurement at high light levels for moderate water stress. (Flexas 1999),( Flexas 2000), ( Flexas 2002)	Not sensitive to early or moderate water stress in most plants (Bukhov & Carpentier 2004) (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008)
<b>Nitrogen Stress</b>	Yield can be used for <i>early</i> nitrogen stress by adding <i>intense light</i> (Cheng 2001)	Fv/Fm is not sensitive to nitrogen stress until very low levels are reached. (Baker 2004)
<b>Light stress</b>	Yield can also be used for light stress in steady state sensitive to light stress. (Cavender-Bares & Bazzaz 2004)	Fv/Fm can be used to detect light stress (Adams & Demming-Adams 2004)
<b>Heat stress</b>	Yield can also be used for <u>moderate heat stress</u> from 35 degrees centigrade and higher in oak. (Haldimann P, & Feller U. 2004)	Fv/Fm can be used to detect severe heat stress in cotton above 45 degrees centigrade. (Crafts-Brander and Law 2000)
<b>Sulfur Stress</b>	<b>Yield is not sensitive to sulfur stress until starvation levels are reached. (Baker 2004)</b>	Fv/Fm is not sensitive to sulfur stress until starvation levels are reached. (Baker 2004)
<b>CO2 Stress</b>	<b>Not sensitive to early or moderate CO2 stress. (Siffel &amp; Braunova 1999)</b>	Fv/Fm is sensitive to early or moderate CO2 stress. (Siffel & Braunova 1999)
<b>Salt Stress</b>	Not sensitive to NaCl stress in Rice, but it is sensitive to NaCl stress in sorghum and chickpea. (Moradi & Ismail 2007) (Netondo 2004) (Eyidogan 2007)	Not sensitive to NaCl stress in Rice, but it is sensitive to NaCl stress in sorghum and chickpea. (Moradi & Ismail 2007) (Netondo 2004) (Eyidogan 2007)
<b>Nickel</b>	ETR, a parameter derived from Yield at a known light level, is sensitive to nickel stress (Joshi & Mohanty 2004), (Tripathy 1981) (PAR Clip required)	Not sensitive to nickel stress. (Joshi & Mohanty2004)
<b>Zinc</b>	Fs in Yield is a good indicator of zinc stress. (Joshi & Mohanty 2004) (Tripathy & Mohanty 1980) (Krupa 1993)	Not sensitive to zinc stress. (Joshi & Mohanty2004) (Tripathy & Mohanty 1980) (Krupa 1993)
<b>Cold</b>	Yield is sensitive to Cold stress(Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams1994), (Adams1995), (Ball 1995).	Fv/Fm is sensitive to Cold stress(Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams1994), (Adams1995), (Ball 1995).
<b>Herbicide</b>	Sensitive to most types of herbicides. See the Opti-Sciences Stress guide for specific information.	Sensitive to most types of herbicides <b>Not sensitive to DCMU (Nedbal &amp; Whitmarsh 2004).</b> See the Opti-Sciences Stress guide for specific information
<b>Pesticides</b>	Is sensitive to tested pesticide stress <i>including Trimax</i> . See the Opti-Sciences Stress guide for specific information	Is sensitive to tested pesticide stress but <b>not Trimax</b> . See the Opti-Sciences Stress guide for specific information
<b>Chemical Stress</b>	See the Opti-Sciences Stress guide for specific information	See the Opti-Sciences Stress guide for specific information
<b>Other Stress</b>	See the Opti-Sciences Stress guide for specific information	See the Opti-Sciences Stress guide for specific information.

**The Opti-Sciences OS5p and the OS1-FL can be used to make Yield measurements. Both units may be used with optional digital PAR Clips.**

**Yield is the more versatile fluorescence measuring parameter, but it is best to use a system that offers multiple test parameters for diverse stress applications. While systems that provide true yield measurements tend to cost more than ones that provide just Fv/Fm measurements, they offer greater capability.**

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