Multi-Flash - High Light Quantum Photosynthetic Yield Correction

Up to a - 41% error has been found in quantum photosynthetic yield measurements (ΔF/Fm’) and ETR measurements in high light environments (Loriaux S.D. 2006).

There has been increasing evidence since 1990 (Markgraf 1990) (Earl 2004) (Rosenqvist and vanKooten 2006), that at high actinic light levels, quantum photosynthetic yield, and ETR measurements did not correlate well with gas exchange carbon assimilation measurements. Even with very intense saturation pulses, chlorophyll fluorescent quantum photosynthetic yield and ETR measurements were found to underestimate values measured by gas exchange. It is believed to be caused by a form of non-photochemical quenching that prevents closure of all reaction centers in high light conditions even with the most intense saturation pulse (Earl 2004).

As a solution, Earl (2004) found that he was able to ramp saturation pulse intensities from lower to higher and use the maximum saturation fluorescence values from these various measurements to determine an infinite saturation intensity value. All test saturation values were set to be higher than should be necessary for complete reaction center saturation. Earl then uses these measured values and linear regression analysis to determine the fluorescence value for an infinitely intense saturation pulse. He applied this approach to both C_3 and C_4 plants and he found that the method restored the proper correlation of yield and ETR measurements to CO_2 assimilation.

More recent research, was done to corroborate the work done by Earl (2004) and Markgraf (1990) and verify the value of a similar solution called “multiphase single flash”. The research team that was used, included the man that developed quantum photosynthetic yield, Bernard Genty (Genty 1989, 1990). With Genty getting behind the issue and the research, the concept immediately increased in credibility.

A research team of team of Loriaux S.D., R.A Burns, Welles J.M., McDermitt D.K. and Genty B. found that in Z. mays, the standard saturation pulse method, when used under field conditions, that included high light, produced up to a -41% error in ETR measurements and in yield measurements. The research recommends the multiphase flash process for field measuring work. “Determination of Maximal Chlorophyll Fluorescence Using A multiphase Single Flash of Sub-Saturating Intensity” is the title of the poster.

Opti-Science has offered a similar approach based on Earl’s work for more than one year. Originally we hypothesized that such an approach might only be necessary if saturation pulse intensities were only in the 8,500 µmol range or less, but unpublished tests indicated that even at 15,000 µmols Earl’s findings were valid.

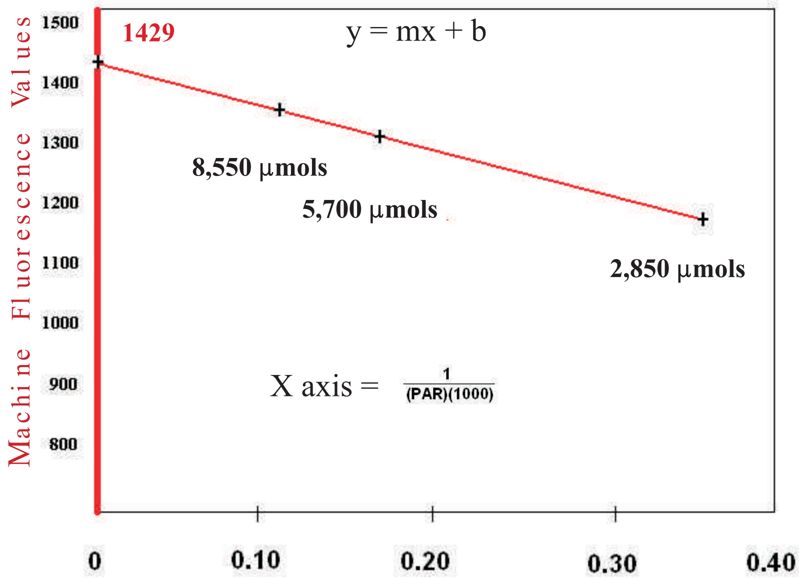
Opti-Sciences offers a “multi-flash protocol” for yield and ETR measurement that uses three stepped saturating light intensities to determine the Fm’ fluorescence intensity with an infinite saturation flash.

The protocol is to be used with a PAR Clip and provides the three saturation flashes in rapid succession. The first saturation flash has a duration of 0.3 seconds. It is followed by two higher intensity flashes, each with a duration of 0.34 seconds. The total duration for the three steps in the protocol is 0.98 seconds. These times were selected to provide the minimum time required for saturation of higher plant reaction centers at each step (Vredenburg 2004) (Loriaux 2006), and prevent or minimize any saturation flash NPQ. Optimal saturation duration times for higher plants has been found to be between 0.8 and 1.0 seconds. Longer times can create saturation pulse NPQ that can cause yield under estimation (Rosenqvist and van Kooten 2006).

These fluorescence intensity values are then used in the Earl formula to determine the fluorescence intensity with an infinite saturation pulse. The infinite value corresponds to the y-axis intercept using measured data in linear regression analysis. By inserting light PAR values on the X axis using 1/((PAR)(1000)), the y-axis intercept becomes the corrected infinite saturation pulse fluorescence value.

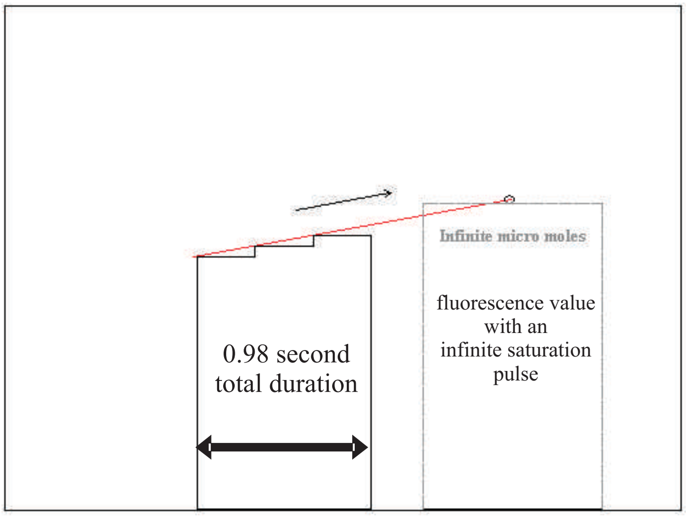
Infinite Saturation Pulse Fluorescence Determination Using Ramped Saturation Pulses & Linear Regression Analysis

The y-axis intercept is the infinite fluorescence value



Earl (2004) reports an R^2 value of better than 0.96 for both maize (C_4 plant) and cotton (C_3 plant) when relating corrected Fm' values to gas exchange measurements.

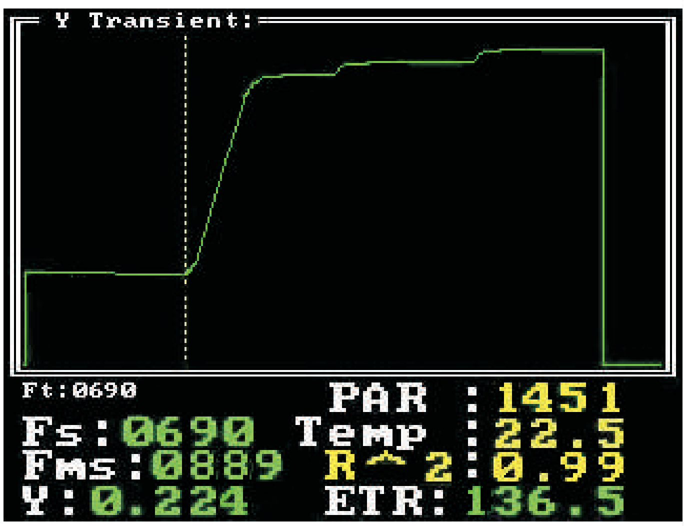
The process involves correction of fm' (or fms) values using linear regression analysis and fluorescence measurements of different saturation pulse intensities, above the expected saturation range.



0.3 seconds and 0.34 seconds were chosen to provide the minimum duration times required for higher plant saturation (Vredenburg 2004)(Loriaux 2006). The overall saturation pulse duration of 0.98 seconds was chosen to prevent saturation pulse NPQ from affecting Fm' measurements in higher plants (Rosenqvist and van Kooten 2006).

NPQ that appears at the back peak of a saturation pulse on longer duration flashes, can cause a small errors in yield and ETR measurements. The ideal saturation flash duration for higher plants ranges from 0.8 to 1.0 seconds to prevent NPQ (Rosenqvist and van Kooten 2006).

In the unlikely event that saturation pulse NPQ should occur, the back of the final, and highest step, would be curved downward on the graph.



Representative Multi-flash trace 0.98 second duration

This protocol, only offered to selected customers in the past, is offered in the current software release. The user may choose to use the new multi-flash protocol, or use the standard yield measuring saturation flash protocol. R^2 values are also included in the readout regarding measured fluorescence number correlation with infinity fluorescence determination.

$$ETR = (Yield)(PAR)(0.84)(0.5)$$

When measuring ETR, or electron transport rate, there are additional variables involved. While the average leaf absorption is estimated to be 0.84 or 84%, and the portion of energy that enters PSII is estimated to be 0.5 or 50%, the real values vary by plant species and plant type . Actual leaf absorption ranges from 0.7 to 0.9 (Eichelman H. 2004). PSII energy usage in land plants range between 0.4 and 0.6 (Edwards 1993) (Laisk A. 1996). Zea mays, for example, has been measured at 0.4 (Edwards 1993).

Available on the OS5p and the New OS1p

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