

## State Transitions Affect Fv/Fm & Yield Measurements

“The photosynthetic state transitions are a process of changing the balance of energy flows into the photosystem I and photosystem II reaction centers” (Ruban, Johnson 2009). “...state transitions are an essential strategy for photosynthetic organisms helping them utilize every photon” (Ruban, Johnson 2000). All oxygenic photosynthetic plants have both photosystem I & II reaction centers, but the percentage of one type to another varies with different plants. Laisk (1996) found that the ratio of PSII to PSI centers varied from 0.42 to 0.6 in different terrestrial plants. In cyanobacteria and red algae about 85% of chlorophyll is associated with PSI (Allen, Mullineau 2004). ***Fluorescence measurements made without taking these transitions into account can lead to erroneous results.***

### Basic Theory:

State transitions were discovered in the late 1960s by Bonaventura and Myers (1969) and Murata (1969 a and b). Current state transition models have their beginnings in the 1980s with Allen 1981, Benett 1983, and Horton 1983, and continue to be refined (Rubin, Johnson 2009). Triggered by changes in the spectral quality of light, it is a process that takes *ten to twenty minutes* to complete (Ruban, Johnson 2009), (Lichtenthaler 1999). State transitions are also triggered by light intensity rate limitations of one of the two photosystems to allow balanced distribution of light. (Allen, Mullineau 2004). At low light levels  $q_T$ , the fluorescence measuring parameter related to state transitions and a component of NPQ &  $q_N$ , can account for up to 67% of total non-photochemical quenching and about 5% of *non-photochemical quenching* at high light levels in higher plants (Lichtenthaler 1999).

### The effects of state transitions at different light levels in Soybean samples at steady state photosynthesis.

Light radiation in $\mu\text{mols}$	$q_T$ as a % of Total $q_N$ (or The Effects of LHCI <sub>II</sub> Antenna Size Reduction Due to State Transition as a Percentage of Total Non-photochemical Quenching)
10 $\mu\text{mols}$	67%
50 $\mu\text{mols}$	62%
100 $\mu\text{mols}$	57%
200 $\mu\text{mols}$	39%
300 $\mu\text{mols}$	30%
500 $\mu\text{mols}$	22%
1000 $\mu\text{mols}$	10%
2000 $\mu\text{mols}$	5%

(Approximate values from a graph in Lichtenthaler 1999 on page 10)

**Non-photochemical quenching as measured by  $q_N$  includes mechanisms that are involved in photoprotection, light balancing (state transitions), and other heat dissipation schemes involved in photoinhibition and photodamage. These mechanisms take time to reach equilibrium at specific light levels. If they have not reached equilibrium, errors in Yield measurement can occur. These same mechanisms also require similar times to relax when dark-adapting for Fv/Fm measurements.**

### How do state transitions work?

Energy imbalances between photosystem I and photosystem II are reflected in the oxidation-reduction state of the plastoquinone pool. The state of this pool regulates the operation of two enzymes that phosphorylate and dephosphorylate some of the LHCI<sub>II</sub>b antenna polypeptides (Light Harvesting Complex for photosystem II enriched with mostly chlorophyll b). Photophosphorylation changes the affinity of some of these LHCI<sub>II</sub> antenna from photosystem II to photosystem I. This causes some of these complexes to *detach and migrate* to PSI and serve as antennae for PSI. When the plastoquinone pool is more oxidized, it activates a second

enzyme to dephosphorylate these transient LHCII antennae and cause migration to PSII reaction centers. Kinase is the enzyme that causes phosphorylation and phosphatase is the enzyme that causes dephosphorylation. (Ruban, Johnson 2009).

In addition, Papageorgiou reports that when dark-adapting for fluorescence measurement, higher plants and algae shift toward state 1 conditions and cyanobacteria shifts to state 2 conditions. (Papageorgiou G.C. Tismilli-Michael M. Stamatakis K. 2007)

### **How do state transitions affect fluorescence measurements?**

Chlorophyll fluorescence measurements from Fv/Fm and quenching parameters to Yield and ETR can all be affected by state transitions if proper care is not taken. State transitions affect Fm and Fo if dark adaptation is not long enough. The time required for complete state transition adjustment when moving from light adapted to dark-adapted states is ten to twenty minutes (Ruban, Johnson 2009).

The same time scales are involved when going from a dark-adapted state to a light adapted state *or when changing light levels*. Light adapted Fms (or Fm') and Fs (or F) will also be effected until steady state photosynthesis has been reached (Lichtenthaler 1999), (Ruban, Johnson 2009). Allen and Mullineau (2004) report that the range of light balancing by state transitions can cause a change in yield measurements by 10% to 30% depending on the organism. This shows one must be aware of state transitions and determine when steady state occurs for Yield and ETR as well as dark adapting for Fv/Fm, and quenching measurements. Maxwell and Johnson (2000) claim that *steady state photosynthesis is reached in 15-20 min.* in 22 different British terrestrial plants.

While some authors have reported that state transition quenching is negligible in higher plants, the results of Lichtenthaler (1999), Krause (2004), and Allen, Mullineau (2004) indicate that at low and medium light levels, state transitions can be a greater factor in non-photochemical quenching. It is only at high light levels that state transitions may become negligible (Krause 2004), (Walters and Horton 1991,1993), (Schansker and van Rensen 1999). Fv/Fm, Yield, and ETR measurements are affected by non-photochemical quenching.

Researchers may want to test for measurement variations in new species. For dark adapted tests, one can evaluate different dark adaptation times to find the maximum Fv/Fm value. For light adapted measurements, one can make yield measurements until significant variation stops. When taking yield measurements, allow enough time for the fluorescence signal to return to steady state after the saturation pulse. While the time span between saturation pulses depends on what is being measured, it has been suggested by Rosenqvist and van Kooten (2006) that saturation pulses from 1-2 min. apart allow for full relaxation of the fluorescence level to steady state photosynthesis.

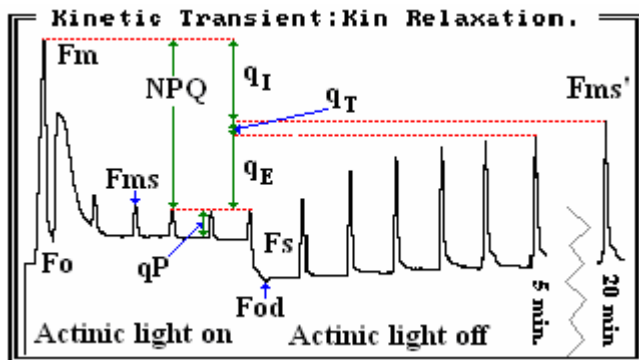
**The Opti-Science OS5p offers the necessary kinetic and quenching relaxation protocols to work with state transition research. Both *puddle model* and *lake model* parameters are available on the OS5p**

### **Parameters used to measure state transitions:**

**qT** is called state transition quenching but it is not a true quenching, because it reflects a decrease in LHCII antenna size (Ruban, Johnson 2009). qT can be near 67% of total non-photochemical quenching at low light levels in terrestrial plants and as low as 5% of total non-photochemical quenching at high light levels. (Lichtenthaler 1999).

To measure state transitions various methods can be used. For greater detail on the process and a discussion of current theories and research, refer to Ruban, Johnson (2009) and Allen, Mullineau (2004).

One way to measure the affects of state transitions is to use a quenching relaxation analysis protocol. In this case, a predetermined programmable measuring routine is set in motion. The sample is dark-adapted and  $F_m$  is determined. Next, an internal actinic light is turned on at a predetermined intensity value. The actinic light remains on until the plant sample reaches steady state photosynthesis. Saturation pulses are triggered at predetermined intervals while the actinic light is on to allow measurement of  $F_{ms}$  (or  $F_m'$ ) at steady state. After steady state has been reached the actinic light is turned off, and a far red light is turned on for about ten seconds to rapidly re-oxidize PSII. Saturation pulses continue to be triggered at predetermined times without actinic illumination to allow the observation and measurement of quenching mechanisms.



$q_T$  can be described as  $q_T = F_{ms}' - F_{ms} / F_m$  at twenty minutes –  $q_E$  (or  $F_{ms}' - F_{ms} / F_m$  at five minutes) (Muller Niogi 2004), or alternatively using  $q_N$  it may be described as  $q_T = F_{ms}' - F_{ms} / F_m - F_{od}$  at twenty minutes –  $q_E$  (or  $F_{ms}' - F_{ms} / F_m - F_{od}$  at five minutes) (Lichtenthaler 1999). Both Parameters definitions use a dark-adapted  $F_m$  value and quenching relaxation measurements after steady-state photosynthesis has been reached. The ability to completely separate  $q_T$  from  $q_E$  has been brought in question by some because there may be some overlap of events Maxwell and Johnson (2000).

There is no vanKooten and Snell standard definition for  $q_T$ . One definition is  $NPQ = q_E + q_T + q_I$  (Muller Niogi 2004). An alternative expression is  $q_N = q_E + q_T + q_I$  (Krause 2004). Both parameters for determining  $q_T$  are used by different researchers. The newer lake model quenching parameter designations do not offer a parameter for state transition measurement. In lake model nomenclature it is buried in with other mechanisms. For this reason,  $q_T$  is still used by those studying state transitions.

### Other parameters used.

**IB** or imbalance =  $F_{sI}'$  (The highest fluorescence level immediately after the far red light is turned off.) –  $F_{sI}$  (The fluorescence steady state level with far red light turned on) /  $F_0$  (The dark adapted minimal fluorescence value) or **IB** =  $F_{sI}' - F_{sI} / F_0$ . It allows the assessment of the effects of antenna composition on spectral differences of the two photosystems. It can also be used to determine light source wavelength choice for experimentation. (Ruban, Johnson 2009).

**qS** is a measure of the efficiency in rebalancing electron transport after a spectral change in light quality.  $qS = F_{sI}'$  (or fluorescence with the far red light turned off in state I) –  $F_{sII}$  ( or fluorescence in state II with the far red light on /  $F_{sI}' - F_{sII}$  (or fluorescence with the far red light turned on while in state II). **qS** =  $F_{sI}' - F_{sII} / F_{sI}' - F_{sII}$  (Ruban, Johnson 2009).

$1/t_{1/2}$  is the time it take fluorescence to decline by half of the total amount during transition. (Ruban, Johnson 2009).

## Definitions:

**F<sub>m</sub>** - is maximal fluorescence measured during the first saturation pulse after dark adaption.

**F<sub>s</sub>** – also known as F is the fluorescence level related to the actinic light and is a reflection of the level of photosynthetic activity (F<sub>s</sub> is also known as steady state fluorescence signal).

**F<sub>ms</sub>** – also known as F<sub>m</sub>' is the saturation pulse value that is not dark adapted. They are at a lowered values due to NPQ or non-photochemical quenching. When this parameter has reached steady state, it is used to calculate photosynthetic Yield along with F<sub>s</sub>. F<sub>ms</sub> at steady state is also used to calculate q<sub>N</sub>, NPQ, q<sub>P</sub>, q<sub>L</sub>, Y(NPQ), Y(NO), q<sub>E</sub>, q<sub>T</sub>, and q<sub>I</sub>.

**F<sub>o</sub>** - is the dark adapted initial fluorescence

**F<sub>od</sub>** – also known as F<sub>o</sub>', is the minimal value after the actinic light has been turned off and after a far red light is turned on for several seconds. It represents F<sub>o</sub> with non-photochemical quenching.

**F<sub>v</sub>/F<sub>m</sub>** = (F<sub>m</sub> – F<sub>o</sub>) / F<sub>m</sub> This is a dark adapted test used to determine Maximum quantum yield. This ratio is an estimate of the maximum portion of absorbed quanta used in PSII reaction centers (Kitajima and Butler, 1975). Another way to look at F<sub>v</sub>/F<sub>m</sub> is a measurement ratio that represents the maximum potential quantum efficiency of Photosystem II if all capable reaction centers were open. 0.83 is the approximate optimal value for most plant species with lowered values indicating plant stress. It is important to properly dark adapt samples for this test. F<sub>o</sub> will be raise and F<sub>m</sub> will be lowered if dark adaption is inadequate. Since dark adaption requirement can vary with species and light history, testing should be done to ensure proper dark adaption, (See the “Stress Guide” for guidance on dark adaptation).

**Yield** = (F<sub>ms</sub> – F<sub>s</sub>) / F<sub>ms</sub> -This test is also known as ΔF/F<sub>m</sub>'. Yield of PSII is a fast light adapted test normally taken at steady-state photosynthesis levels. It provides a measure of actual or effective quantum yield. This ratio is an estimate of the effective portion of absorbed quanta used in PSII reaction centers. (Genty, 1989) It is affected by closure of reaction centers and heat dissipation caused by non-photochemical quenching.

**NPQ** is non-photochemical quenching. It is a measure of heat dissipation and a combined total for the combination of photo-protective mechanisms, state 1 and state 2 transitions, and photo-inhibition and photo-damage. NPQ = q<sub>E</sub> + q<sub>T</sub> + q<sub>I</sub>. NPQ is an alternate expression of non-photochemical quenching. It provides an estimate of quenching without knowledge of F<sub>od</sub> (or F<sub>o</sub>'). The advantage of NPQ over q<sub>N</sub> depends on the specific application. NPQ is more heavily affected by non-photochemical quenching that reflects heat-dissipation of excitation energy in the antenna system. So it may be thought of as an indicator of 'excess excitation energy'. Alternatively, NPQ is relatively less sensitive to the part of non-photochemical quenching associated with q<sub>N</sub> values lower than 0.6. This range of q<sub>N</sub> is affected by Δp<sub>h</sub> of the thylakoid lumen and the xanthophyll cycle, which are an important aspect of photosynthetic regulation. (Bilger & Björkman, 1990)

**q<sub>N</sub>** is similar to NPQ but requires F<sub>od</sub> or F<sub>o</sub>' in the calculation. q<sub>N</sub> is defined as the coefficient of non-photochemical fluorescence quenching. The original definition of this term implied that fluorescence quenching affects primarily the 'variable fluorescence' (F<sub>v</sub>) and not the minimal fluorescence (F<sub>o</sub>). In cases where q<sub>N</sub> is greater than 0.4 this may not be a good assumption. This will affect the calculated q<sub>N</sub> value. By using the Far-Red source after actinic illumination, the PSII acceptors re-oxidized and PSI is reduced. A new F<sub>od</sub> value is measured and used for corrections to the quenching coefficients. (puddle model) (van Kooten & Snel, 1990) (Stern –Volmer 1919)

**q<sub>E</sub>** is the quenching parameter that represents the photo-protective mechanisms in the leaf that allow rapid compensation for changes in light levels due to cloud cover and increased light intensity. It is directly related to Δp<sub>h</sub> of the thylakoid lumen and the xanthophyll cycle. (puddle model) (Muller P., Xiao-Ping L., Niyogi K. 2001)

**q<sub>T</sub>** is the parameter that represents state 1 and state 2 transitions. (puddle model) (Muller P., Xiao-Ping L., Niyogi K. 2001), (Lichtenthaler 1999), (Ruban, Johnson 2009).

**q<sub>I</sub>** is the quenching parameter that represents photo-inhibition and photo-damage. (puddle model) (Muller P., Xiao-Ping L., Niyogi K. 2001)

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