OPTI-SCIENCES
8 Winn Avenue • Hudson, NH 03051 • USA
Phone: 603-883-4400 Fax: 603-883-4410
Email: sales@optisci.com Website: www.optisci.com

Basic Desktop Plant Stress Guide:

The following pages represent a compilation of research done using chlorophyll fluorescence for plant stress detection, and measurement. It is organized by plant stress type, with important introductory notes listed first.

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Note: Recent chloroplast migration work has created game changing research that should make researchers reconsider, dark adaptation times, the times to reach steady state photosynthesis under light adapted conditions, the types of actinic light sources that should be used for chlorophyll fluorescence measurements, and photosynthesis measurements (Cazzaniga 2013, Dall'Osta 2014). See the application note on chloroplast migration for details.

This is a *basic version* of the plant stress guide a more *advanced version* is available on our website along with other important application notes at: www.optisci.com . This guide is intended as a starting point for research. Results may sometimes vary by species, plant type, or special interest.

Results were compiled from world wide published research, *independent of fluorometer brand name*. While chlorophyll fluorescence is sensitive to most types of plant stress, in some cases, this is not true. In those cases, quality special fluorescence assays are listed, or other quality alternative solutions are suggested.

The best tests for different types of plant stress are listed on the following pages. Tests are listed by plant stress type and in order, with the best tests listed first. For more information, contact Opti-Sciences at 603-883-4400, or **www.optisci.com**

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Cook book checklists for reliable chlorophyll fluorescence measurements

Specific Plant Stress Types

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Cookbook checklists

To ensure reliable measurements, follow the "cookbook checklists" on the following pages as a recipe for success.

First –F_V/F_M

F_V/F_M allows comparison of samples in a known dark-adapted state.

To get an accurate measurement, one has to follow tested guidelines.

1. **Dark-adapt properly** knowing the plant's light history. It takes only a few minutes for the xanthophyll cycle and the Δ ph of the thylakoid lumen to return to a dark-adapted state. (State transitions (where they exist), however, take between fifteen to twenty minutes (Ruban 2009) (Lichtenthaler 1999) . These times can vary somewhat in field plants, and can take slightly longer. Deactivation of Rubisco in the dark, takes between 12 -18 minutes in vascular plants and from 9 minutes to 28 minutes in some photoplankton (MacIntyre 1997). In plants that have a high actinic light history, chloroplast migration occurs and relaxes during a twenty to *thirty five minute* window (Cazzaniga S. 2013). In addition, field plants and other plants that have been exposed to photoinhibition conditions for a number of hours, will retain a certain amount of NPQ for up to 60 hours (Lichtenthaler 2004). This means that even if dark adaptation is overnight, there will almost always be some residual NPQ built into most summer field sun grown leaf measurements of F_V/F_M after a sunny day. This is all right as long as one is comparing samples with a similar light history. But it will cause problems when samples have a different light history unless one is measuring "light stress" and comparing results. It is common for researchers to choose dark adaptation times anywhere from twenty minutes to thirty five minutes, or overnight, using pre-dawn values. If the leaf has been exposed to high light conditions, *thirty-five minutes would be a safe dark adaptation time* to account for chloroplast migrations (Cazzaniga S. 2013) (Dall'Osta 2014). Shorter times may be used to study the effects of plant protective mechanisms. For more information contact OSI for the Dark adaptation application note. (These guidelines are different for quenching measurements and for Rapid Light Curves.) . *Some Journal research reviewers have their own ideas regarding reliable dark adaptation times. If you plan to publish, it may make sense to check with reviewer. The equivalent of overnight dark adaptation is acceptable for all known reviewers.*
2. **Modulation light intensity setting** $F_V/F_M = (F_M-F_O)/F_M$. Minimum fluorescence , is a measurement of a fully oxidized photo system II before any Q_A, or quinone "A" has been reduced. It is a dark adapted value, measured by exposing the leaf antennae to a very low intensity modulated light. The intensity must be set high enough to properly allow minimum detection of chlorophyll fluorescence, but not high enough to drive photochemical reduction of any Q_A, the primary quinone in the light reaction. If it is set too high, it will drive photochemical reduction of any Q_A and provide an F_O value that is too high. *When setting the modulating light intensity, the Ft value or fluorescence signal should not rise over a 30 second period* when a leaf is exposed to the modulated light. If it does, the intensity must be lowered. *OSI now offers an automated modulated light set up routine for its OS5p+,OS1p,OS30p+ , iFL , Y(II) meter, and F_V/F_M meter chlorophyll fluorometers.*
3. **Shade leaves vs. Sun leaves**. – The F_V/F_M ratio will be slightly higher on sun leaves than on shade leaves (Lichtenthaler 2004).
4. **F_V/F_M will be higher with a white saturation pulse than a red saturation pulse.** Some fluorometers use a red saturation pulse. This is not an issue for comparative measurements of plant stress with similar instruments, but values measured on a fluorometer with a white saturation pulse should not be directly compared to measurements of a fluorometer with a red saturation pulse. There is evidence to show that systems with a red saturation pulse correlate but measure consistently slightly lower than systems with white light saturation lights. (Cessna 2010)

5. **Maximum F_V/F_M values vary with species**. The average maximum F_V/F_M value is between 0.79 - 0.83 (Maxwell and Johnson 2000).
6. **Field plants should only be compared to field plants and green house plants should be compared to green houseplants** due to light history. (Lichtenthaler 2004)
7. **It is common to use the youngest fully mature leaf blade for diagnosis of deficiencies in plants** (Reuter and Robinson 1997)
8. **The duration of the saturation pulse** should be between 0.5 seconds and 1.5 seconds for higher plants, and 25 to 50 milliseconds for Phytoplankton and cyanobacteria. (Schreiber 1995). Times outside these ranges increase the error in F_V/F_M measurements. Shorter durations prevent complete saturation of PSII regardless of the light intensity. Longer durations create a form of saturation pulse NPQ that rounds the tail end of the pulse maximum value, and reduces the average maximum saturation pulse value. A figure of 0.8 is sometimes set at the factory for some fluorometers, because it works for all higher plants tested. Some fluorometers have automated routines that ensure the correct saturation pulse duration. *This is not as important in the latest chlorophyll fluorometers from Opti-Sciences. They now use an eight point 25 millisecond rolling average to determine F_M at its highest point, regardless of saturation pulse duration, as long as the saturation pulse is wide enough to saturate the sample. Therefore, saturation pulse NPQ will not affect measurements with modern Opti-Sciences chlorophyll fluorometers. It is available on the OS5p+,OS1p,OS30p+ , iFL , Y(II) meter, and F_V/F_M meter chlorophyll fluorometers.*
9. **Saturation pulse intensity.** Dark adapted leaves saturate easily with lower saturation pulse intensities. It may take a few hundred µmols to saturate shade leaves and sun leaves will saturate below 1,500 µmols. Lower values may not fully saturate PSII, and provide an error. Higher values always work with dark adapted samples. (Ralph 2005) (Requirements are different for Y(II).) Very intense saturation pulses will only damaged dark adapted plants if they are too frequent at the same location. Research has shown that once per hour is safe in the dark (Albert Porcar-Castell 2008).
10. **Some F_V/F_M fluorometers have the ability to pre-illuminate dark adapted leaves with far-red light.** When this feature is used for five to ten seconds before an F_V/F_M measurement takes place, it activates PSI, and ensures that all electrons have been drained from PSII before the measurement of F_O. While this feature ensures that PSII is completely re-oxidized, it does not relax the xanthophyll cycle, state transitions, chloroplast migration, and photoinhibition. *Time is still required in a darkened environment to relax all forms of NPQ and to obtain a reliable F_V/F_M measurement.* (Maxwell and Johnson 2000)
11. **Part of the minimum fluorescence, F_O, and maximum fluorescence, F_M, in $F_V/F_M = (F_M-F_O)/F_M$ contains Photosystem I fluorescence** as well as PSII fluorescence. With F_V/F_M, one is trying to measure the maximum variable fluorescence of PSII in a dark-adapted state. PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap with PSII. This produces an error. In C_3 plants, about 30% of F_O fluorescence is due to PSI, and in C_4 plants about 50% of F_O fluorescence is due to PSI fluorescence. PSI produces about 6% of the fluorescence found in F_M in C_3 plants, and about 12% of Fm in C_4 plants (Pfundle 1998). This not a problem when comparing F_V/F_M measurements for plant stress because PSI fluorescence does not change. It remains constant.
16. **Chlorophyll fluorescence heterogeneity** is measurement variation over the surface of a single leaf. While for most applications, it is not of concern, it can create problems when measuring some types of plant stress and under certain conditions. According to Baker (2008), plants under **drought stress**, **cold stress**, **CO_2 stress, or biotic stress** show significant patchy chlorophyll fluorescence heterogeneity. Fv/Fm does not work for drought stress until it is severe, but it may be used for cold stress. This means that if measurements are taken with a standard chlorophyll fluorometer, on different parts of the same leaf, there may be significant variation. The problem may be overcome by developing a sampling pattern, and making multiple measurements on a single leaf, and averaging the results. See the Opti-Sciences application note on chlorophyll fluorescence heterogeneity for more information. (Correspondence with Claus Buschmann). The iFL averages chlorophyll fluorescence measurements over the large chamber area, eliminating heterogeneity as an issue
17. **Light history –** Compare samples with similar light history only. Field plants and other plants that

have been exposed to photoinhibition conditions for a several hours, can retain a certain amount of NPQ for up to 60 hours (Lichtenthaler 2004). This means that even if dark adaptation is overnight, there will almost always be some residual NPQ built into most summer field measurements of F_V/F_M plants that have been exposed to photoinhibitory light conditions for several hours. Measurements should not be compared to plants that have been exposed to overcast conditions for this reason unless some form of light stress is the focus of the experiment. If photoinhibition measurement is the focus, it may make sense to partially shade the samples from photoinhibitory conditions for at least 60 hours.

The best experiments are ones that take these issues into account. PSI fluorescence is involved in all measurements. It does not vary with light level or plant stress. (Schreiber 2004). With this in mind, comparing samples with similar light histories allows comparison of many types of plant stress. The Plant Stress guide provided by Opti-Sciences references papers that deal with specific types of plant stress and limitations of different chlorophyll fluorescence parameters for measuring plant stress.

There are fluorescence solutions and assays available that are sensitive to most types of plant stress. F_V/F_M is not as sensitive as Y(II) for many types of plant stress. However, It does have the advantage that all samples, with similar light histories, can all be dark adapted to the same known state. Light level does not need to be controlled.

F_V/F_M is not a sensitive test for drought stress, heat stress, nitrogen stress, nickel stress, sulfur stress, zinc stress, some herbicides and salt stress in some types of plants (Opti-Sciences Plant Stress Guide 2014). It can be used effectively in most other types of plant stress. *For specific research results on specific types of plant stress, see the specific type of plant stress of interest.*

Cookbook checklist OJIP

OJIP - has an additional requirement when compared tor F_V/F_M

"Strasser OJIP" is the OJIP protocol most used for measuring plant stress. Measurements require a fixed and calibrated actinic light intensity to get reliable measurements. It was found by Vredenburg (2011) that some of the OJIP peaks and timing for the peaks change at different light intensities. Early Strasser work was done at 3,000 µmols. Later Strasser work was done at 3,500 µmols. (The OS30p+ automatically calibrates it's red actinic light source to 3,500 µmoles when the instrument is turned on.

Follow the F_V/F_M checklist for all other OJIP requirements for reliable measurement.

Cookbook checklist before making light adapted Y(II) or $\Delta F/F_M$' measurements.

Y(II) or $\Delta F/F_M$' is the quantum yield of PSII. It is a normalized measurement ratio that is an indication of the amount of energy used in photochemistry by PSII under steady-state photosynthetic lighting conditions (Genty 1989), (Maxwell K., Johnson G. N. 2000). Y(II) is affected by closure of reaction centers and heat dissipation caused by non-photochemical quenching. (Schreiber 2004) Photochemistry, heat dissipation, and chlorophyll fluorescence compete for light energy absorbed by the leaf. First reported by Bernard Genty in 1989, this light adapted test became possible with the advent of modulated fluorometers. It is the most versatile plant stress measuring parameter, because it has been shown to detect more types of plant stress, earlier, than any other chlorophyll fluorescence protocol. Measurement requires built in IR filtering.

PAR is photosynthetically active radiation. Radiation on the leaf is measured between the wavelengths of 400nm to 700 nm. PAR sensors and thermisters for measuring temperature are calibrated to other instruments that are traceable to the NIST. It is recommended that recalibration should occur every two years. Most modern sensors are solid state, so drift is minimal. **Y(II) is sensitive to most types of plant stress.** We have listed some important notes below.

Checklist before making Y(II) measurements:

F_M' = maximum fluorescence in a light adapted environment at steady state photosynthesis. F_S' = the fluorescence signal in a light adapted environment at steady state photosynthesis.
Y(II) is = $(F_M' - F_S') / F_M' = \Delta F/F_M'$

1. **Leaves must be at steady state photosynthesis.** Above canopy leaves on a clear day, in the field, are considered to be at steady state photosynthesis. (Maxwell and Johnson 2000). *In the past*, it was thought that this process took between 15 to 20 minutes on a sunny day. (Maxwell and Johnson 2000). However, it can take from twenty to thirty five minutes at higher actinic light levels (Cazziniga 2013) (Dall'Osta 2014). New evidence requires that chloroplast migration time be included in the time to reach steady state photosynthesis. At higher actinic light levels, chloroplasts migrate from the top of cells in the dark, and at lower light levels, to the sides of cells at high light levels. This process takes between *20 to 35 minutes*. The fluorescence changes previously thought to be caused by state transitions, and by "acute" photoinhibition, are actually caused by chloroplast migration. It is a mechanism that increased leaf transmittance and decreases leaf absorptance. Chloroplast migration can account for up to 30% of non-photochemical quenching at higher light levels. Chloroplast migration changes both F_S and F_M'. (Cazzaniga S. 2013) (Dall'Osta 2014).
2. **It is dangerous to make Y(II) measurements on below canopy leaves in the field**. The shade from higher leaves and wind can interrupt a plant's adjustment to steady state under ambient conditions. The xanthophylls cycle, and Δph of the thylakoid lumen adjust in several seconds to several minutes. It can take longer in field plants, up to seven minutes. (Baker 2008) (Lichtenthaler 1999). State Transitions take between fifteen and twenty minutes to completely adjust (where they exist). It has been found that state transitions were a big factor at lower light intensities where they existed, but they were not as much of a factor at high light intensities. Chloroplast migrations take between 20 and 35 minutes at high actinic light levels. Rapid light curves and F_V/F_M may be better solutions for below canopy work where appropriate. Rapid light curves are designed for measuring the affects of rapid light changes. *The alternative, is to use an internal fluorometer actinic light source, under a shroud, expose the leaf sample to light for up to thirty five minutes, to reach steady state, and then make a measurement.* If necessary, use a shroud, a tripod, and a PAR clip. Use the internal actinic illuminator to pre-illuminate samples to steady state photosynthesis to get reliable Y(II) measurements.
3. **Y(II) values vary with light level and with temperature**. The higher the light level, the lower the YII) value. When measuring Y(II) in the field, it is extremely important to measure leaf irradiation or light level, at the leaf level, and at the same angle of as the leaf orientation. Y(II) also varies with leaf temperature. Comparing Y(II) values taken at different light levels, and different temperature levels, can introduce significant errors, unless it is the change, at different light levels and heat levels, that is of interest. This is commonly done with a PAR Clip. (Genty 1989), (Genty 1990) *PAR clips are essential for most field and laboratory Y(II) applications.* Light intensity varies with the square of the distance from the light source. In the field, small changes in distance make little difference because the sun is so far away. In the laboratory or in growth chambers, small distance changes can make a significant difference in PAR light reaching the leaf surface.
4. **Shade leaves vs. Sun leaves**. – The Y(II) ratio will be higher on Sun leaves than on shade leaves for the same light irradiation level (Lichtenthaler 2004). Light level will affect each differently.
5. **Field plants should only be compared to field plants** and green houseplants should be compared to green houseplants due to light history. (Lichtenthaler 2004)
6. **Leaf orientation.** When making a Y(II) measurement, it is important not to change the orientation of the leaf. The leaf is at steady state photosynthesis in its current orientation. Changing the orientation

changes the amount of light falling on the leaf, and the leaf will no longer be at steady state photosynthesis.

7. **It is common to use the youngest fully mature leaf blade for diagnosis of deficiencies in plants** (Reuter and Robinson 1997)
8. **The duration of the saturation pulse** should be between 0.5 seconds and 1.5 seconds for higher plants, and 25 to 50 milliseconds for algae and cyanobacteria (Schreiber 1995). Times outside these ranges increase the error in Y(II) measurements. Shorter durations prevent complete saturation of PSII regardless of the light intensity (Roseqvist & van Kooten 2006). Longer durations create a form of saturation pulse NPQ that rounds the top trailing edge of the pulse maximum value, and reduces the average maximum saturation pulse value (Roseqvist & van Kooten 2006). Some fluorometer allow adjustment of this parameter, and others are preset at the factory at either. 0.8 seconds, or 1.0 seconds for higher plants.. *This is not as important in the latest chlorophyll fluorometers from Opti-Sciences. They now use an eight point rolling average to determine F_M' at its highest point, regardless of saturation pulse duration, as long as the saturation pulse is wide enough to saturate the sample. The eight point rolling average prevents saturation pulse NPQ from being a problem. This feature exists on the OS5p, the OS5P+, the OS1p, the iFL the OS30p+, the Y(II) meter & the F_V/F_M meter*
9. **Saturation pulse intensity**. Saturation pulse intensity is more of an issue with Y(II) than with F_V/F_M. When dark adapting, shade leaves will saturate at a few hundred µmols, and sun leaves will usually saturate below 1,500µmols (Ralph 2004). However, a problem has been found when measuring Y(II) at high light levels. It has been discovered that at high actinic, or sun light levels, leaves resist the complete closure of all PSII reaction centers that is expected when using the most intense saturation pulse. Even with a 20,000 µmol saturation pulse, some reaction centers remain open. As a result up to a 41% error was found in Y(II) measurements using standard square saturation flash techniques at high actinic light levels (Loriaux 2006) (Loriaux 2013) when compared to gas exchange measurements. To correct for this issue, a method was developed using a multiple phased single saturation flash was used. The fluorescence intensity output was measured for each phase. The initial maximum saturation flash of 7,000 µmols for 0.3 seconds was made and then, a 20% down ramp in light intensity was created at a rate of 0.01 mol photons $m^{-2}s^{-2}$. Finally, a second 0.3 second flash at 7000 µmols was used to detect any saturation pulse NPQ. The measured fluorescence results were then subjected to east squares linear regression using PAR values of PAR/10,000. The Y axis intercept represented a fluorescence value with an infinitely intense saturation flash. The Loriaux 2013 paper was co-Authored by Bernard Genty, the creator of Y(II). The Loriaux 2013 method is an included option on the OS5p+, the iFL, the OS1p and the Y(II) meter chlorophyll fluorometers. One can still use the standard square topped flash if desired.
10. **PSI fluorescence -** Part of the fluorescence signal contains PSI fluorescence as well as PSII fluorescence. With Y(II), one is trying to measure variable fluorescence of PSII in a light adapted state. PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap with PSII. This produces a small error but it is not a problem for comparing similar samples, because PSI fluorescence does not change with light intensity, temperature, or plant stress. (Baker, Oxborough 2004)
11. **"Super-saturating flash" error** is produced by using a very intense saturation light source that is longer that 2ms, causing multiple turnovers of primary PSII receptor Q_A and the reduction of plasotoquinone to plastoquinol. This raises F_M' (or F_{MS}') and can cause an overestimate of Y(II) 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 of PSII in higher plants. *As long as one is interested in plant stress and not exact correlation to CO_2 carbon assimilation, this is not an issue.*
12. **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 Y(II), 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)

13. **The ratio of ETR to CO_2 assimilation can be diagnostic for drought stress in C_3 plants**. C_3 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 2006). *This problem of early water stress measurement and detection may be overcome by using a special assay discussed in Burke 2007 and Burke 2010. A discussion of the Burke assay can be found at* www.optisci.com. Request the water stress application notes. The iFL is ideal for comparing ETR to CO_2 assimilation.
14. **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)
15. While linear correlation occurs between Y(II) and ETR with CO_2 assimilation in C_4 plants and curvilinear correlation between Y(II) and ETR with CO_2 assimilation in C_3 plants, (Genty 1989), (Genty 1990), (Baker Oxborough 2004), *exact* correlation between fluorescence ETR and gas exchange carbon assimilation is not possible due to the fact that most fluorescence comes from only the upper most layers of the leaf, while gas exchange measurements measure lower layers as well (Schreiber 2004).
16. **Chlorophyll fluorescence heterogeneity** is measurement variation over the surface of a single leaf. While for most applications, it is not of concern, it can create problems when measuring some types of plant stress and under certain conditions. According to Baker (2008), plants under **drought stress**, **cold stress,** and **CO_2 stress** show significant patchy chlorophyll fluorescence heterogeneity. This means that if measurements are taken with a standard chlorophyll fluorometer, on different parts of the same leaf, there may be significant variation. The problem may be overcome by developing a sampling pattern, making multiple measurements on a single leaf and averaging the results. Gas exchange systems with integrated fluorometers, such as the iFL-LCpro-SD, measure over a large area and eliminate this issue as a problem. See the Opti-Sciences application note on chlorophyll fluorescence heterogeneity for more information.
17. **Light history** - Since chronic photoinhibition takes up sixty hours to relax, there can be un-relaxed photoinhibition built into all light adapted measurements made on samples that have a "high" light history from the previous day or two. It is also likely that a few overcast days will allow complete relaxation of photoinhibition. For this reason, it is important to take this variable into account when comparing samples measured on different days and under different conditions. Light history should be considered when designing experiments for reliable results.

18. **Actinic light spectrum**. It was recently found that under natural field conditions, chloroplast migrations occured at higher actinic light levels in C_3 plants (Cazzaniga 2013) and C_4 plants (Maai 2011). *Furthermore, it was found that this light avoidance mechanism significantly affected chlorophyll fluorescence measurements.* Using wild and mutant Arabidopsis plants, the chloroplast migration, was responsible for up to ***30% of total NPQ*** (non-photochemical quenching) at high actinic light levels. This mechanism was found to be *regulated by intense white and intense blue actinic light. Chloroplast migration did not respond significantly to intense red actinic light.* As a result, **using a white actinic light source or a combination of red LEDs and blue LEDs that provide a *intense blue actinic light,* will prevent measuring errors due to chloroplast migration.** Sunlight provides an intense blue spectrum. (Cazzaniga 2013) (Dall'Osta 2014) The OS1p, the OS5p, the OS5p+ and the iFL have white actinic light sources with intense blue spectrums.

Y(II) vs. F_V/F_M -Y(II) is a more versatile measuring parameter than F_V/F_M, that is proven to measure more types of plant stress at more sensitive levels. It does require comparison to samples used as a standard, at the same PAR light level, and temperature. F_V/F_M offers the advantage that samples can be compared after being dark-adapted to the same known state. However, F_V/F_M, (the dark adapted test) is not sensitive to drought stress until about 7 days have passed without water (Bukhov & Carpentier 2004), heat stress

(Haldiman P, & Feller U. 2004), nitrogen stress (Baker 2004), sulfur stress (Baker 2004), nickel stress below 45°C (Joshi & Mohanty2004), zinc stress (Joshi & Mohanty2004), some types of chemical stress, and some types of herbicide stress. For more information about specific types of plant stress, *go to the table of contents.* Both are fast tests. A PAR Clip is a highly recommended accessory for the measurement of Y(II) as Y(II) varies with PAR light level and temperature.

Cookbook checklist before making NPQ and other quenching measurements.

Quenching measurement parameters, such as NPQ, are the least understood, and most often misused parameters that are available with advanced chlorophyll fluorometers. This Check list is designed to improve the understanding of proper quenching protocol usage.

There are a few quenching protocols to choose from: The Kramer lake model, Hendrickson lake model, puddle model, and quenching relaxation protocols. For an in depth discussion of the differences, and advantages of each, please request the *Opti-Sciences Quenching application note* at www.optisci.com.

To get reliable measurements, one should follow tested guidelines.

1. **Dark-adapt properly knowing the plant's light history.** It takes only a few minutes for the xanthophyll cycle and the Δph of the thylakoid lumen to return to a dark-adapted state. State transitions, however, take between fifteen to twenty minutes. These times can vary somewhat in field plants and can take slightly longer (Baker 2004). Under high actinic light conditions, it takes chloroplast migration from 20 to 35 minutes to relax to a dark adapted state. Chloroplast migrations will significantly affect fluorescence measurements if measurements are made before they are fully relaxed. (Cazzaniga S. 2013) (Dall'Osta). In addition, field plants and other plants that have been exposed to photoinhibition conditions for a number of hours, will retain a certain amount of NPQ for up to 30 to 60 hours (Lichtenthaler 2004). This means that even if dark adaptation is overnight, there will almost always be some residual NPQ built into summer field measurements of F_V/F_M, and other displayed quenching parameters. For this reason, it is important to only compare samples with a similar light history. ***When doing quenching measurements on field plants, it is common for researchers to use pre-dawn or overnight dark adaptation times*** (Maxwell & Johnson 2000). *(For more information, see dark adaptation application note.) If photoinhibition is your focus, Then you may want to partially shade samples from photoinhibitory light conditions for at least 60 hours to get a more reliable F_V/F_M and a more reliable q_I measurement. Before choosing a shorter dark adaptation time for lab work or growth chamber work, check with a reviewer from a target publication. They have strong feelings on the subject. However, overnight or pre-dawn values are generally accepted .*
2. **For quenching measurements, samples that are compared, must have the same F_V/F_M values.** Quenching measurements of different samples with different F_V/F_M values should not be compared (Baker 2008). F_V/F_M is used as the measuring standard for non-photochemical quenching measurements, and if the measuring standard is different, the quenching values are meaningless. Comparing values from samples with different F_V/F_M values is like measuring items with a ruler that has dimensions that change.
3. **Modulation light intensity setting** F_V/F_M is $(F_M-F_O)/F_M$. F_O, or minimum fluorescence is a dark-adapted value made by exposing the leaf antennae to a very low intensity modulated measuring light, that is not set high enough to drive photosynthesis or chemically reduce Q_A (Zhu 2005), but set high enough to make a measurement. The modulation light intensity must be set correctly for best accuracy and repeatability. If it is set too high, it will drive photosynthesis and provide an F_O value that is too high. The modulated light allows the measurement of pre-photosynthetic antennae fluorescence.

Maximum fluorescence is measured when exposing a leaf to a saturation flash with light intense enough to close all PSII reaction centers. The OS1p, the OS5p+, the iFL, and the OS30p+ all have automatic modulated light set up routines for ease of use. One can still use the manual method as well. This is done by placing the leaf in the leaf clip, PAR clip or leaf chamber and exposing the leaf to the modulated light. If the Ft value on the screen rises over a 10 to 20 second period it is set too high. If it is set too low, a too low message will appear on the screen.

4. **Leaves must be at steady state photosynthesis for most quenching measurement parameters.** Until recently it was thought that this process took between fifteen and twenty minutes at a lower and medium light levels (Maxwell and Johnson 2000) to reach steady state. However, recently it was discovered that chloroplast migration was responsible for the intermediate chlorophyll fluorescence change up to twenty to thirty five minutes, replacing state transitions and acute photoinhibition as the two sources of chlorophyll fluorescence change during these time scales. As a result actinic light levels should be on for at least 35 minutes to reach steady state photosynthesis. Chloroplast migrations will significantly affect fluorescence measurements at high actinic light levels if measurements are made before they are fully adjusted. (Cazziniga 2013) (Dall'Osta 2014). For example, if there are 18 saturation pulses spaced 2 minutes apart, the leaf will be exposed to the actinic light for 36 minutes after dark adaptation. Since an internal fluorometer artificial light source is normally used, the test allows one to compare Below canopy leaves as long as the F_V/F_M values are the same. According to Klughammer (2008), the only non-photochemical parameter that does not have to be taken at steady state photosynthesis is Y(NO) from Hendrickson or Kramer.
5. **Use a fluorometer with a stable actinic light output.** Depending on the brand and type of fluorometer, the intensity output of the actinic light can change over time. When an actinic light is on, it can heat the fluorometer and cause a lowering of the light output. The intensity of the actinic LED light source output changes as the heat from the LED changes the LED temperature. More advanced systems have ways to ensure a steady actinic light level, either by using a stable light source or monitoring light output with a PAR clip to maintain a constant light level. If light intensity changes significantly over a 20 -35 minute actinic illumination period, *the sample is no longer at steady state photosynthesis. The OS1p, the OS5p+ and the iFL use a PAR sensor to measure light irradiated onto the leaf surface and correct any changes in intensity. Corrections are made at least every 0.1 seconds or faster. All units have a stable actinic light output for the most reliable measurements.*
6. **Y(II) values and quenching values vary with light level, leaf angle to the light source and with temperature.** The higher the light level, the higher the NPQ value. When measuring NPQ in the field or the lab, it is extremely important to measure PAR leaf irradiation at the leaf, and leaf temperature. Light varies inversely with the square of the distance from the light source, and varies significantly with leaf angle to the source. Comparing Y(II) and quenching values taken at different light levels, different angles to the light source and different temperature levels, introduces a significant error, unless it is the change that is of interest. This is commonly done with a PAR Clip, a tripod, and a shroud over the sample for quenching measurements.
7. **Shade leaves vs. Sun leaves**. – The Y(II) ratio will be higher on Sun leaves than on shade leaves (Lichtenthaler 2004) for the same light intensity.
8. **Field plants should only be compared to field plants,** and green houseplants should be compared to green houseplants due to light history. (Lichtenthaler 2004)
9. **Leaf orientation is not important because an artificial actinic light source is used.**
10. **It is common to use the youngest fully mature leaf blade for diagnosis of deficiencies in plants (Reuter and Robinson 1997).**
11. **The duration of the saturation pulse** should be between 0.5 seconds and 1.5 seconds for higher plants, and 25 to 50 milliseconds for algae and cyanobacteria (Schreiber 1995). Times outside these ranges increase the error in Y(II) measurements. Shorter durations prevent complete saturation of PSII regardless of the light intensity (Roseqvist & van Kooten 2006). Longer durations create a form of saturation pulse NPQ that rounds the top trailing edge of the pulse maximum value, and reduces the average maximum saturation pulse value (Roseqvist & van Kooten 2006). Some fluorometer allow adjustment of this parameter, and others are preset at the factory at either. 0.8 seconds, or 1.0 seconds

for higher plants.. *This is not as important in the latest chlorophyll fluorometers from Opti-Sciences. They now use an eight point rolling average to determine F_M' at its highest point, regardless of saturation pulse duration, as long as the saturation pulse is wide enough to saturate the sample. The eight point rolling average prevents saturation pulse NPQ from being a problem. This feature exists on the OS5p, the OS5P+, the OS1p, the iFL the OS30p+, the Y(II) meter & the F_V/F_M meter*

12. **Saturation pulse intensity**. Saturation pulse intensity is more of an issue with Y(II) than with F_V/F_M. When dark adapting, shade leaves will saturate at a few hundred µmols, and sun leaves will usually saturate below 1,500µmols (Ralph 2004). However, a problem has been found when measuring Y(II) at high light levels. It has been discovered that at high actinic, or sun light levels, leaves resist the complete closure of all PSII reaction centers that is expected when using the most intense saturation pulse. Even with a 20,000 µmol saturation pulse, some reaction centers remain open. As a result up to a 41% error was found in Y(II) measurements using standard square saturation flash techniques at high actinic light levels (Loriaux 2006) (Loriaux 2013) when compared to gas exchange measurements. To correct for this issue, a method was developed using a multiple phased single saturation flash was used. The fluorescence intensity output was measured for each phase. The initial maximum saturation flash of 7,000 µmols for 0.3 seconds was made and then, a 20% down ramp in light intensity was created at a rate of 0.01 mol photons $m^{-2}s^{-2}$. Finally, a second 0.3 second flash at 7000 µmols was used to detect any saturation pulse NPQ. The measured fluorescence results were then subjected to east squares linear regression using PAR values of PAR/10,000. The Y axis intercept represented a fluorescence value with an infinitely intense saturation flash. The Loriaux 2013 paper was co-Authored by Bernard Genty, the creator of Y(II). The Loriaux 2013 method is an included option on the OS5p+, the iFL, the OS1p and the Y(II) meter chlorophyll fluorometers. One can still use the standard square topped flash if desired.
13. **The time between saturation pulses is important.** Rosenqvist and van Kooten (2006) state that between one to two minutes is required for complete relaxation of saturation pulse NPQ. If saturation pulses are not separated by this distance range, then an error caused by saturation pulse NPQ will result. Furthermore, It will accumulate with each saturation pulse. When in doubt, space saturation pulses two minutes apart or more. *We have found that when the actinic light is off it can take longer that two minutes* for saturation pulse NPQ to fully dissipate as seen during quenching relaxation measurements. If one sees the bottom of the fluorescence graph start to rise, it is either due to the modulated light intensity or a build up of saturation pulse NPQ after longer relaxation tests. In this case, we find that spacing the saturation flashes 3 to 4 minutes apart during the relaxation phase of the test works very well.
14. **Overlap of PSI fluorescence** -Part of the minimum fluorescence, the F_O parameter, in F_V/F_M $((F_M - F_O)/F_M)$, contains PSI fluorescence as well as PSII fluorescence. With F_V/F_M, one is trying to measure the maximum variable fluorescence of PSII in a dark-adapted state. PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap with PSII. This produces an error. In C_3 plants, about 30% of FO fluorescence is due to PSI, and in C_4 plants about 50% of Fo fluorescence is due to PSI fluorescence. PSI produces about 6% of the fluorescence found in Fm in C_3 plants, and about 12% in C_4 plants. (Pfundle 1998). This not a problem when comparing quenching measurements for plant stress because, PSI fluorescence does not change with light level or plant stress.
15. **PAR** is photosynthetically active radiation. Radiation on the leaf is measured Between the wavelengths of 400nm to 700 nm. PAR sensors and thermisters for measuring temperature are calibrated to other instruments that are traceable to the NIST. Since Y(II) and quenching parameters change with light and temperature, as well as plant stress levels, there are advantages to using a shrouded leaf and PAR Clip when making quenching measurements. In addition, it is important that the actinic light level does not change over the length of the measurement, because it will cause an error in quenching measurement results. This can be done with a stable internal light source, or a system that allows a PAR Clip that provides measurement feedback to maintain a constant light level required for steady state photosynthesis during the quenching measurement.
16. **Far-red pre-illumination**. Some fluorometers have the ability to pre-illuminate dark-adapted leaves

with far-red light. When this feature is used for 5 to 10 seconds before an F_V/F_M measurement takes place. It activates PSI, and ensures that all electrons have been drained from PSII before the measurement of F_O. While this feature ensures that PSII is completely re-oxidized, it does not relax the xanthophyll cycle, state transitions, chloroplast migration or photoinhibition. Time is still required in a darkened environment to relax all forms of NPQ and to obtain reliable quenching values.

17. **Far-red illumination.** This is usually used in the post actinic light mode to allow measurement of F_O' a parameter that reflects quenched F_O. This value is used in Kramer lake model parameters, and puddle model q_N and q_P. It is not used in Hendrickson simplified lake model parameters, or in NPQ.
19. **Light history** - Since chronic photoinhibition starts to relax at forty minutes and takes from thirty to sixty hours to relax (Lichtenthaler 2004), there can be un-relaxed photoinhibition built into all light adapted and dark-adapted measurements made on samples that have a "high" light history from the previous day or two. It is also likely that a few overcast days will allow complete relaxation of photoinhibition. For this reason, it is important to take this variable into account when comparing samples measured on different days and under different conditions. Light history should be considered when designing experiments for reliable results.

The best experiments are ones that take these issues into account. PSI fluorescence is involved in all measurements. It does not vary with light level or plant stress (Schreiber 2004). With this in mind, comparing samples with similar light histories allows comparison of many types of plant stress.
The Plant Stress Guide provides referenced papers that deal with specific types of plant stress and limitations of different chlorophyll fluorescence parameters for measuring plant stress.

Drought Stress:

Important Notes: Yield of PSII or Y(II), and ETR are effective tests for water stress in C_4 plants. In C_3 plants, the only method that reports good results for early water stress is the special Burke assay (2007, 2010) listed below. Fs/Fo only works for moderate water stress found in plants like grapes. It is not adequate for most other plants according to Flexas. In C_3 plants, photorespiration is thought to be the reason for less than adequate results in regard to early water stress testing. (Flexas 2000). Flexas 1999 and Flexas 2000 provide a good review of the limitations of standard chlorophyll fluorescence techniques for water stress measurement. The standard tests: F_V/F_M, and Y(II), will only work for *severe drought* stress measurement in C_3 plants due to photorespiration (Flexas 1999, 2000). F_V/F_M can be used for severe water stress after about 7 days without water. It should not be used for crops. (da Silva J. A. & Arrabaca M.C. 2008).

In addition, samples that are subject to drought stress display heterogeneous fluorescence from one place on a leaf to another. For more information on this topic see Baker (2008). *The Burke assays do not seem to be affected by this fact.* If the Burke assay is not being used for C_4 plants, then it is important to deal with this issue. To overcome this issue, it is recommended that measurements be made at multiple locations on the same leaf, and results may be averaged. Integrated fluorometer – gas exchange systems overcome this issue by averaging the fluorescence reading over the same area as gas exchange measurements. Imaging fluorescence displays the heterogeneity. Using several measurements on the same leaf, at different locations on the same leaf, with a non-imaging fluorometer provides a higher measurement resolution of the heterogeneity. See the Opti-Sciences application note on fluorescence heterogeneity for more information on the subject.

Best Tests C_3 plants

Y(II) in the Burke Special assay- (Designed for large and small plant populations). This is a light adapted test that can be used for ***very early water stress***. This test was designed for small or large populations of plants. At dawn, leaf disc samples are collected with a leaf punch, and kept moist in a tissue culture tray. They are transferred to a larger tray and covered with glad wrap. The samples are measured using Y(II) and then placed in an oven heated to 40°C for one half hour. The tray is removed from the oven, allowed to cool for thirty minutes, and reach steady state photosynthesis under room level lighting. Y(II) measurements are then made of each sample. It has been found that the non-water stressed leaves will measure lower than water stressed leaves even when irrigation had ended 24 hours earlier. This assay works because leaves that are under water stress retain sugars manufactured at night and so they are less susceptible to heat stress. It works for C_3 plants and C_4 plants. (Burke 2010), (Burke 2007) *For this test to work properly, either samples must be taken at or before sunrise, or whole plant dark adaptation by artificial means is required, before sample collection. When artificially dark adaptation is used, night length dark adaptation times should be used. Samples must always be kept wet. Momentary dark adaption is used before measurement. The newer 2010 paper procedure is the one to follow for best results regardless of whether the plants are C_3 or C_4 plants.*

F_S/F_O & F_S - F_O is from the dark-adapted F_V/F_M test, and FS is from the steady state Y(II) fluorescence test. This test is sensitive to moderate water stress and it is adequate for work with grapes but it is not sensitive enough for most other C_3 plant crops. Fs, a component of (YII) is not a normalize ratio but is has been found to be more sensitive to C_3 plant water stress than Y(II). Tested in C_3, C_4, and CAM plants. F_S/F_O is normalized ratio using F_S that allows comparison between samples. F_O is a predawn value of F_O.

Actinic light is used at saturating levels between 800 to 1250 µmls. (Flexas 2002), (Flexas 2000), (Flexas 1999).

Best Tests C_4 Plants

Yield or Y(II) - Fast light adapted test can also be used for water stress in **C_4 plants.** Good correlation between Y(II) and gas exchange measurements (da Silva J. A. & Arrabaca M.C. 2004).

ETR/A or *J*/A - Fast light adapted steady state fluorescence test. In C_4 Plants. The ratio of ETR to carbon assimilation, ETR/A, is known to be consistent in C_4 plants. **This is not true in C_3 plants**. *ETR requires a PAR Clip.* (J Cavender-Bares & Fakhri A. Bazzaz 2004) (Cerovic 1996). ETR/A requires a combined integrated fluorometer - gas exchange system. *When comparing ETR or J values on different leaves, leaf absorption should be measured and entered into the formula for ETR.*

Y(II) in the Burke Special assay- This is a light adapted test that can be used for ***very early water stress***. This test was designed for small or large populations of plants. At dawn, leaf disc samples are collected with a leaf punch and kept moist in a tissue culture tray. They are transferred to a larger tray and covered with glad wrap. The samples are measured using Y(II) and then placed in an oven heated to 40°C for one half hour. The tray is removed from the oven and allowed to cool and reach steady state photosynthesis under room level lighting. Y(II) measurements are then made of each sample. It has been found that the non-water stressed leaves will measure lower than water stressed leaves even when irrigation had ended 24 hours earlier. This assay works because leaves that are under water stress retain sugars manufactured at night and so they are less susceptible to heat stress. It works for C_3 plants and C_4 plants. (Burke 2010), (Burke 2007)

Other Tests

Combined Y(II) and Carbon Assimilation (A) –The combination of gas exchange and fluorescence is a powerful tool to use for water stress, as it shows how water stress affects different parts of light and dark reaction. The combined use of the two types of instruments has been found to be very useful for specific types of plant stress measurements, such as water stress, heat stress and cold stress. In these types of plant stress, the results of electron transport, as measured with a fluorometer, can show significant differences from carbon assimilation measurements, from gas exchange measurements.

F_S/F_O Light adapted test can also be used for water stress in steady state, Samples must be dark adapted to obtain F_O in F_V/F_M, and then samples must be brought to steady state photosynthesis to measure F_S. It is not as sensitive to water stress as the Burke assay but it may be used for plants like grapes (in C_3 plants). (Flexas 1999)

Light curve– Slow test that helps identify water as the cause of stress.
This is a longer light adapted test. F_S has been found to decrease as light intensity increases. (Flexas 2000)

NPQ – Slow test, increases with moderate to late water stress. This is a dark adapter test. (Cavender-Bares J. & Fakhri A. Bazzaz 2004)

-Continued -What tests are not sensitive to drought stress.

Non-Sensitive to early or moderate Drought Stress:

F_V/F_M - Fast dark-adapted test is <u>not</u> sensitive to early or moderate water stress, only severe stress

(Bukhov & Carpentier 2004) (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008) In some species F_V/F_M is more sensitive to water stress than in other species. (Deng X. Hu Z., Wang H., Wen X., Kuang T. 2003) It can be used for severe plant stress where drought lasts about 7 days. This may be adequate for long-term drought in forestry applications, however, is not adequate for crops.

F_V/F_M - Fast dark-adapted test is **not** sensitive to early or moderate water stress in **C_4 plants,** only severe stress. (da Silva J. A. & Arrabaca M.C. 2008). It can be used for severe water stress after about 7 days. It is not adequate for crops.

PI_{ABS} - Fast dark-adapted test for *detecting* water *stress after seven days* after cessation of irrigation on wheat using OKJIP protocol. It is not as sensitive as Y(II), ETR, *J*/A or the Burke assay in C_4 plants. This is a normalized OJIP parameter for comparing data between samples. The test correlates well with CO_2 gas exchange data during water stress measurements. (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008) (Thach 2007) , but it only works for severe drought stress after about 7 days (Thack 2007). It is not adequate for crops.

K Step - Fast dark-adapted test for water stress using OJIP protocol **(See PI_{ABS})** (Strasser 2004). Works only for severe drought stress.

F_V/F_M - Leaf treated with high light irradiation and polyethylene glycol to induce water stress.
20 mm leaf plugs are collected and treated with polyethylene glycol PEG at 6000 mol weight at various concentrations to induce water stress and exposed to 1500 to 1800 µmols for two hours before dark adaption . (Nair D. B., Alam B., Jacob J. 2005).

Light Stress:

While light stress can be measured effectively by most fluorescence protocols, it is common to study light stress using more elaborate chlorophyll fluorometers that allow longer quenching and quenching relaxation protocols.

To understand the effects of light stress on plants, the following papers provide a good start: (Lichtenthaler 1999, 2004), (Muller, Niyogi 2001), (Kramer 2004),(Cazzaniga S.2013) & (Dall'Osta 2014).

Best Tests

Quenching and Quenching Relaxation Test – Best test to study photo-protection mechanisms Including the ∆ph of the thylakoid lumen, and the xanthophyll cycle, as well as state transitions (where they exist), chloroplast migration, and photo-inhibition are quenching relaxation tests. Measuring parameters have been developed for each mechanism. q_E represents the fast acting photoprotective mechanisms that involve ∆ph of the thylakoid lumen, and the xanthophyll cycle (Muller, Niyogi 2001), q_T is a parameter for measuring state transitions where they exist, q_M is parameter for measuring chloroplast migration, a mechanism that affects chlorophyll fluorescence more at high actinic light levels (Cazzaniga S.2013) (Dall'Osta 2014). q_Z is a measuring parameter for an unknown mechanism thought to be related to the xanthophyll cycle. In many cases, q_Z is probably q_M (Cazzaniga S.2013). Chloroplast migration also exists in monocots (Maai 2011). Finally q_I is a measure of photoinhibition. Other quenching parameters have been developed as well to allow measurement of the effects of light stress They include: the Kramer lake model quenching protocol, the Hendrickson lake model quenching protocol that allows resurrection of NPQ from the puddle model of antennae –reaction center interaction to the newer lake model.. Kramer parameters include: Y(II), q_L, Y(NPQ), Y(NO). (Kramer 2004), Hendrickson parameters include: Y(II), Y(NPQ), Y(NO) and NPQ (Hendrickson 2004), (& Kluhammer and Schreiber 2008). Lake model parameters that include q_E, q_T, and q_I see (Ahn, Avenson 2008) For standardized definitions see (van Kooten O., & Snel J.F. 1990). For lake model parameters see Kramer (2004), Hendrickson (2004) and Ahn, Avenson (2008) NPQ , q_E, q_T, q_I (Muller, Niyogi 2001) for q_Z see (Nilkens 2010) and for q_M see (Cazzaniga S.2013). ***This is a longer dark-adapted test.***

***For definitions* of quenching parameters** q_E, q_T, q_I, with NPQ see (Muller P., Xiao-Ping L, Niyogi K. 2001). For q_E, q_T, q_I with q_N see (Lichtenthaler 1999) For lake model definitions Y(II), q_L, Y(NPQ), Y(NO) see Kramer D. M., Johnson G., Kiirats O., Edwards G. (2004). For simplified lake model parameters that include NPQ, see Hendrickson (2004), and Klughammer, Schreiber (2008). For division of lake model parameters into q_E, q_T, and q_I see Ahn, Avenson (2008). For standardized puddle model quenching definitions see (van Kooten O., & Snel J.F. 1990). For q_Z see (Nilkens 2010), and for q_M see (Cazzaniga S.2013).

For Quantum yield of PSII Y(II) or (∆F/Fm') correction in high light conditions see Earl (2004) and (Loriaux, 2006 & 2013) It has been found that under high actinic light conditions, a correction of quantum yield of PSII value is necessary to restore the correlation of ETR with Carbon assimilation measurements. Without this correction, it is not possible to close or completely chemically reduce all PSII reaction centers, a requirement for reliable Y(II) and ETR measurement. The methods are discussed in the papers and poster listed here.

Light Response Curves This is a longer test (usually dark-adapted and then a light adapted test) where actinic light levels are increased or decreased after steady state photosynthesis has been reached and measured. These are curves that show the results of light level on Y(II) and Electron

Transport Rate. The effects of light level increases and decreases can be studied easily. (Muller, Niyogi 2001), (Kramer 2004), (Hendrickson 2004).Automated fluorometer routines are programmed for desired light intensities, step time duration, the number of saturation pulses per step and the number of steps. With the addition of the Cazzaniga 2013 paper on chloroplast migration, the length of time to reach steady state chlorophyll fluorescence is 20 to 35 minutes for each step.

Yield of PSII or Y(II) - Fast light adapted test can also be used for light stress in steady state sensitive to light stress. (Cavender-Bares & Bazzaz 2004)

F_V/F_M - Fast dark-adapted test can be used to detect light stress. (Adams & Demming-Adams 2004) F_V/F_M correlates to carbon assimilation.

Other Tests –light stress

For under canopy work and aquatic plants

Rapid light Curves (RLC)– A longer dark-adapted, or momentary dark adapter test, that usual take takes less than five minutes, but may take longer. *Steady state photosynthesis is not reached.* Data from several measurements at different times of day are recommended by some, for reliable results (Rasher 2000). An internal fluorometer actinic illuminator is used to step light up, or down to determine ETR response at different times of day. This provides a diurnal light history of the sample, it also allows investigation of the saturation characteristics of plants and correlates well to Rubisco activity under variable light conditions *(Macintyre 1997), (Macintyre 1996)*. Rapid light curves are used for aquatic plants, and under canopy plants, where light is constantly variable, and other methods of testing can be difficult. (Ralph 2005)

The parameters ETR_{MAX}, or optimal ETR, the intensity where ETR_{MAX} occurs I_m, minimum saturation intensity I_k, and initial slope of the RLC curve α are made available in the OS5p+. *Light saturation rate as measured by rapid light curves highly correlates with the concentration and maximum activity of Rubico (Macintyre 1997), (Macintyre 1996). Measured steady state photosynthetic rates overestimate actual photosynthetic rates in a variable light environment (Macintyre 1997).*

Different researchers use different dark adaptation times, different step durations, different numbers of steps, and they step in different directions, up and down. They are light history dependant, and results change depending on the time of day that they are taken (Rasher 2000). Rapid Light Curves that uses 10 second steps have been found to have an unacceptably high level of error in benthic diatoms and longer steps are recommended (Perkins R.G, Mouget J-L, Lefebvre S., Lavaud J. 2006) The ability to saturate all reaction centers can be dependent on light history and method (Perkins R.G, Mouget J-L, Lefebvre S., Lavaud J. 2006). Rapid light curves are believed to provide relevant information on the saturation characteristics of electron transport (Schreiber 2004). Momentary dark adaptation for 5 to 10 seconds is covered by Ralph (2005). RLC as a way to measure full activation of Rubisco in a variable light environment see (MacIntyre 1997) contact Opti-Sciences for the RLC application note.

PI_{ABS} - Fast dark-adapted test sensitive to light stress using OKJIP protocol (Thach 2007). This parameter is a light stress detector but values do not correlate to gas exchange well.

Heat Stress:

The traditional method for measuring heat stress involves quenching measurements and non-photochemical quenching parameters such as NPQ. Chlorophyll fluorometers that perform this test are more expensive than basic systems. According to Haldiman (2004), NPQ will detect heat stress in Oak leaves at 35°C and higher. He also found that Y(II) will also detect heat stress at 35°C and higher. F_V/F_M will only detect heat stress at 45°C and higher. Gas Exchange has been shown to detect heat stress at 30°C or higher (Haldiman 2004).

Best Tests

Y(II) is a light adapted fast test that takes about two seconds. NPQ is a test that takes about twenty to thirty five minutes and overnight dark adaptation. F_V/F_O increase in the dark is a long test.

Yield of PSII or (YII) - Fast light adapted sensitive test for Moderate heat stress above **35°C** in Oak – *Q. pubescens* (Haldiman P, & Feller U. 2004), (Dascaliuc A., Ralea t., Cuza P., (2007) This is a two second test that can be used for small or large populations of plants.

Quenching Tests – Moderate heat stress above **35°C**, **NPQ** and **q_P** in Oak - *Q. pubescens* (Haldiman P, & Feller U. 2004) These are long, time-consuming tests suited to a small numbers of plants. More expensive fluorometers are required.

NPQ is sensitive to study moderate heat stress in Spinach plants. (Tang Y., Wen X., Lu Q., Yang Z., Cheng Z., & Lu C. 2007). This is a long, time consuming test suited to a small number of plants. More expensive fluorometers are required.

Combined Y(II) or ETR and Carbon Assimilation (A) –The combination CO_2 of gas exchange and chlorophyll fluorescence instrumentation is a powerful tool to use for heat stress, as it shows how heat stress affects different parts of the light and dark reactions. The combined use of the two types of instruments has been found to be very useful for specific types of plant stress measurements, such as water stress, heat stress and cold stress. In these types of plant stress, the results of electron transport, as measured with a fluorometer, can show significant differences from carbon assimilation measurements, in gas exchange measurements. Gas exchange detects heat stress at 30°C while Y(II) and NPQ detect heat stress at 35°C. (Haldiman P, & Feller U. 2004) These are longer, time consuming tests suited to a smaller number of plants. This is the most expensive type of instrumentation for measuring plant stress.

Other quenching parameters include **q_N, q_P**, (Schreiber U. 2004) *For definitions of quenching parameters* q_E, q_T, q_I, with NPQ see (Muller P., Xiao-Ping L, Niyogi K. 2001). For q_E, q_T, q_I with qN see (Lichtenthaler 1999) For lake model definitions q_L, Y(NPQ), Y(NO) see (Kramer D. M., Johnson G., Kiirats O., Edwards G. 2004). For simplified lake model parameters that include NPQ, see Hendrickson (2004), and Klughammer, Shreiber (2008). For division of lake model parameters into q_E, q_T, and q_I see Ahn, Avenson (2008). For standardized quenching definitions see (van Kooten O., & Snel J.F. 1990). For standardized puddle model quenching definitions see (van Kooten O., & Snel J.F. 1990) These are longer, time consuming tests suited to a small number of plants. More expensive fluorometers are required.

Other Tests – Heat stress

F_V/F_M – *Is not sensitive to moderate heat stress below* ***45^o C.***
(Haldiman P, & Feller U. 2004) (Schreiber U. 2004), (Baker and Rosenqvist 2004) (Crafts-Brander and Law 2000).

PI_{ABS} - Fast dark-adapted test sensitive to heat stress using OJIP protocol. This is a normalized parameter for comparing different samples.(Strasser 2004) results reported at **44^o C** and above. The test is not sensitive to heat stress below 44^{o}C

K Step - Fast dark adapted test sensitive to heat stress using OJIP protocol sensitive (Strasser 2004) **(see PI_{ABS} above)**

Nutrient Stress:

Using standard types chlorophyll fluorescence measurement for some types of nutrient stress works well, *however, non-standard methods are required for other types of nutient stress measurement including nitrogen and sulfur stress.* There is a special assay available by Cheng (2001) that incorporates high light levels to measure nitrogen stress listed below. However, the most cost effective tools for nitrogen and sulfer stress, and the most highly used methods involve chlorophyll content meters. They are available using two different methods. One type uses a light absorption techniques at two different light wavelengths. The second uses ratio fluorescence detection at two different wavelengths. Ratio fluorescence has added the advantages that it works well not only with larger leaves but also with very small samples, conifers, grasses, Arabidopsis, stems, or even cactus, because the measuring aperture does not need to be filled to get a reliable measurement. Ratio fluorescence also provides a larger reliable measuring range, especially at higher chlorophyll content levels, and direct read out in chlorophyll content level in mg^{-2} m^{-2} (Gitelson 1999). Gas exchange provides excellent results at a much higher price. For references and details, see the papers sited below.

Best Tests

Nitrogen

CCI or SPAD *These are absorbance – transmittance indices, not fluorescent parameters.* These instruments transmit light at two different wavelengths through leaves. One is in the red range that is very sensitive to chlorophyll content, and the other is in the far-red range. The far-red wavelength is not sensitive to chlorophyll content, but it is affected by leaf thickness and refractive index. The ratio of the two numbers provides CCI and SPAD. This type of instrument has been heavily used for nitrogen stress measurement, and nitrogen management protocols. Maize (Wang 2008), Maize under dry conditions (Mashego 2012), Maize (Bukan 2011), Maize and Wheat (Francis 1999), Maize (Shapiro C., Schepers J., Francis D., Shanahan J., 2006), Rice (Koontz 2011), Maple trees (van den Berg 2004), Asian Pear (GHASEMI 2011), Artichoke (Rodrigo 2011), Compares CCI and SPAD (Knighton 2005).

This is the most cost effective way to measure nitrogen stress at usable levels. Nitrogen stress and sulfur stress can not be distinguished. For this reason, it is common to add sulfur before the study of the effects of nitrogen stress. This is the most used, and most cost effective way to measure nitrogen stress.

Ratio Fluorescence - F_{735}/F_{700}. Various fluorescence ratios have been tried, but the F_{735}/F_{700} fluorescence ratio provides the best correlation to chlorophyll content results and the largest measuring range. CCI or SPAD work well for standard samples but they have problems with small leaves like immature crop plants, conifers, turf grasses, Arabidopsis, CAM plants such as cactus, or moss on rocks. The ratio fluorescence test offers an affordable solution for difficult samples. It has the advantage that the measuring aperture does not need to be covered for reliable measurement This ratio also has the advantage that it offers more than twice the chlorophyll content measuring range of absorption style chlorophyll content meters, 41 mg m^2 to 675 mg m^2 (Gitelson 1999) (Buschman 2007). Gitelson provides a formula for **direct readout in chlorophyll content** in mg m^2.

Continued on next page -

Other Tests – Nitrogen stress

Yield of PSII or Y(II) ***at high light levels*** - Fast light adapted test that can also be used for nitrogen stress at steady state for C_3 plants. Various nitrogen levels can be distinguished better using high light levels (Cheng 2001).This is a special assay that requires measuring Y(II) at high light levels to make nitrogen stress measurements at usable levels. It improves the resolution for nitrogen stress measurements to usable levels**.**

K Step – Fast dark adapted test that is sensitive at severe levels to nitrogen deficiency in soybean & maize (Strasser 2004), (Baker 2008) An OJIP fluorometer is required and the actinic light intensity should be at 3,000 µmols or 3,500 µmols because the K step changes with light level (Vredenburg 2011)

q_P - Slow modulated test that shows some nitrogen deficiency at severe levels, but not sulfur deficiency. (Baker and Rosenqvist 2004) A more expensive fluorometer is required.
Yield Y(II) - Fast light adapted test that can also be used for nitrogen stress at steady state. Nitrogen stress must be severe to detect nitrogen stress without high actinic light. (Cavender-Bares and Bazzaz 2004) (Baker and Rosenqvist 2004) High light levels are needed in combination with yield to measure nitrogen stress at usable levels. An intermediate priced fluorometer is required (Cheng 2001).

Boron

Yield of PSII or Y(II) and ETR – Fast Light adapted test sensitive to Boron deficiency in sunflowers (Kastori R., Plesnicar M., Pankovic D., Sakac Z., 1995) An intermediate priced fluorometer is required.

Calcium

F_V/F_M – Was found to detect C_a stress in tomato plants (Shmidts-Eiberger, Haefs, Noga) and apple trees (Shmidts-Eiberger, Haefs, Noga 2002). An inexpensive fluorometer is required

Chlorine

Yield of PSII or Y(II) & ETR , F_V/F_M are all sensitive test for Chlorine stress in watermelon (Zhang, Wang, Huang, Xing, Lin Wang 2010) An intermediate priced fluorometer is required.

CCI – Chlorophyll content meter (Cayanan 2008) (Cayanan 2009).

Cobalt

Yield of PSII or Y(II) - Cobalt. (Joshi & Mohanty2004)(Tripathy 1983) An intermediate priced fluorometer is required.

Copper

Yield of PSII or Y(II) - Copper. Sensitive test (Joshi & Mohanty2004) (Lanaras 1993) An intermediate priced fluorometer is required.

F_O/F_{5min} – A slow dark adapted test that is sensitive to copper deficit.
(Adams, Norvell, Philpot & Peverly 2000), (Kriedemann 1985) A more expensive fluorometer is required.

Iron

CCI – Chlorophyll content meter used to detect chlorosis due to sulfur and iron deficiency. (Christianson 2012)

K Step – Fast dark adapted test that is sensitive iron deficiency in soybean & maize (Jiang, Gao, & Zou 2006) An inexpensive priced fluorometer is required.

Yield of PSII or Y(II) – variation of 6% with a loss of up to 70% of chlorophyll. When chlorophyll loss exceeds 70%, changes in F_V/F_M are dramatic. Sugar beets (Beta vulgaris L.) (Morales F., Abadia A., Abadia J. 1991) An intermediate priced fluorometer is required.

Magnesium

PI_{ABS} – PI_{ABS} has been shown to be sensitive to Mg deficiency (Hermans C, Johnson GN, Strasser RJ, Verbruggen N, 2004) An intermediate priced fluorometer is required.

Manganese

F_V/F_O - A fast dark adapted test very sensitive to Manganese deficiency. (Adams, Norvell, Philpot & Peverly 2000), (Kriedemann 1985) (Hannam 1985) an inexpensive fluorometer is required

Molybdenum

CCI – Chlorophyll content meter (Biscaro 2009) Measures the effects of adding molybdenum and nitrogen uptake.

Nickel

ETR - Nickel. This also means that Y(II) is sensitive to Nickel stress. F_V/F_M is not a good indicator of Nickel stress. (Joshi & Mohanty2004), (Tripathy 1981) An intermediate priced fluorometer is required.

Phosphorus

F_V/F_M – Has been shown to be sensitive to phosphorus stress (Stark, Niemyska, Bogdan & Tawlbeh 2000)

PI_{ABS} - PI_{ABS} is sensitive to phosphorus stress in Sorghum (Ripley, Redfernand, Dames 2004)

Potassium

Yield of PSII or Y(II), NPO, and q_P - were effective in detecting K deficiency in rice plants. Experiments with K deficiency in reference to photoprotection mechanisms. (Weng, Zhen, Xu, Sun 2008)

Sulfur

CCI or SPAD in leaf absorption chlorophyll content meters. *These are not fluorescent parameters* that measure greenness of a leaf and leaf optical density. They are used in chlorophyll content meters for fertilizer and nitrogen management programs. Readings for sulfur stress and nitrogen stress are indistinguishable. (Yara fertilizer management guide on line). Fluorescence is not a good indicator of sulfur stress. (Baker and Rosenqvist 2004) (Christensen 2012) *This is a cost effective way to measure sulfur stress.*

F_V/F_M - was found to detect only starvation levels of sulfur stress in Chlamydomonas (Antal T. , Volgusheva A., Kukarskikh G., Krendelva T., Tusov V., Rubin A. 2005) (Baker 2008)

Zinc

F_S in Yield of PSII or Y(II) - Zinc - F_V/F_M is not a good indicator of zinc stress. (Joshi & Mohanty2004) (Tripathy & Mohanty 1980) (Krupa 1993)

Important Nutrient tests limitations:

F_V/F_M - Fast dark adapted test that is only sensitive to nitrogen content only *at very low levels, and Sulfur at starvation levels.* (Baker and Rosenqvist 2004). It is also not a good test for Zinc (Joshi & Mohanty2004). It is also not a good test for nickel. (Joshi & Mohanty2004)

Yield of PSII or Y(II)- Fast light adapted test is sensitive to Sulfur deficiency only *at starvation levels* (Baker and Rosenqvist 2004). It can be used for nitrogen stress at high light levels (Cheng 2006). However, absorption chlorophyll content meters work well for both Nitrogen and Sulfur stress. (Yara fertilizer management guide on line)

q_P - Slow modulated test is sensitive to Sulfur deficiency at starvation levels . (Baker and Rosenqvist 2004)

Gas exchange will work well for all types of nutrient plant stress, but tests are slow, making them suitable only for small populations. They are also the most expensive instruments.

Cold Stress: All tests below are important in Cold stress studies.

Important Notes: Cold stress provides unexpected results when using chlorophyll fluorescence. ETR measurements are three times higher than expected under cold stress (see the ETR/ CO_2 Assimilation test for more details).

In addition, samples that are subject to cold stress display *heterogeneous fluorescence* from one place on a leaf to another. For more information on this topic, see Baker (2008). To overcome this issue, it is recommended that measurements be made at multiple locations on the same leaf, and results may be averaged. Integrated fluorometer –gas exchange systems overcome this issue by averaging the fluorescence reading over the same large area as gas exchange measurements. Imaging fluorescence displays the heterogeneity. However, using a few measurements, at different locations on the same leaf, with a non-imaging fluorometer provides a higher measurement resolution of the heterogeneity. The results can be averaged for a reliable result (Bushmann 2008). See the Opti-Sciences application note on fluorescence heterogeneity for more information on the subject.

Recommended Tests

ETR/ CO_2 Assimilation or *J* /A - The ratio of ETR in PSII to CO_2 assimilation changes in cold stress indicating other electron sinks in cold stress. Under cold stress conditions, ETR is about three times higher than predicted by carbon assimilation measurements.(Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. 1998) *This test uses a combination fluorometer and CO_2 - H_2O gas exchange system. It has the added advantage that it is measuring fluorescence over the entire leaf chamber area, eliminating the heterogeneous fluorescence issue.*

Y(II) or $\Delta F'/F_M'$ - Yield of PSII - Fast light adapted sensitive test can also be used for moderate cold stress in steady state. (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams1994), (Adams1995), (Ball 1995).

F_V/F_M - Fast dark-adapted test can be used for moderate cold stress. (Oquit and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994, 1995), (Ball 1995).

Light Curves /Stepped Actinic Test – Light response curves and the effects of light level increases and decreases with cold stress can be studied easily. This is a longer light adapted test. (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams1994, 1995), (Ball 1995).

ETR - This is a short or long light adapted test related to yield and PAR or light level. A PAR clip is required. (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994, 1995), (Ball 1995).

Quenching and Quenching Relaxation Test – Test to study relaxation kinetics after exposure to light and chilling temperatures. Studies of the Δph of the thylakoid lumen, xanthophyll cycle, and photo-inhibition with NPQ, q_N, q_P, q_L, q_E, q_T, q_I, Y(NPQ), Y(NO). This is a longer dark adapted test. (Cavender-Bares J., Bazzaz F., 2004)

Over-Wintering Stress

Recommended Tests

Y(II) or ΔF'/F_M' - Yield of PSII - Fast light adapted sensitive test can also be used for moderate cold stress in steady state. (Adams & Demming- Adams 2004) (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994,1995), (Ball 1995).

F_V/F_M - Fast dark-adapted test can be used for moderate cold stress. (Adams & Demming- Adams 2004), (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994,1995), (Ball 1995).

Quenching and Quenching Relaxation Test – Test to study relaxation kinetics after exposure to light and over-wintering plants. Studies of qI mechanisms become possible as well as the ∆ph of the thylakoid lumen, xanthophyll cycle, and photo-inhibition with NPQ, q_N, q_P, q_L, q_E, q_T, q_I, Y(NPQ), Y(NO). This is a longer dark adapted test. (Adams & Demming- Adams 2004) (Cavender-Bares J., Bazzaz F.,2004)

Light Curves /Stepped Actinic Test – The effects of light level increases and decreases with cold stress can be studied easily. This is a longer light adapted test. (Adams & Demming- Adams 2004), (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994, 1995), (Ball 1995).

CO_2 Stress:

Best Tests

CO_2 stress causes *heterogeneous fluorescence* across the leaf, for this reason, a larger part of the leaf should be characterized with multiple measurements at multiple locations on the same leaf and averaged (Baker 2008), (Buschmann – email correspondence). Integrated chlorophyll fluorescence measurement and gas exchange measurement offer the best way to measure CO_2 stress. Chlorophyll fluorescence is averaged over the same large area as gas exchange measurements, eliminating the issue of patchy fluorescence response. Early on, Y(II) values actually increase while carbon assimilation decreases(Siffel & Braunova 1999)**. Furthermore, A/C_i curves or A/C_C curves can be used to characterize leaves at different CO_2 levels. A/C_C curves require an integrated system.**

A/C_i curves using a gas-exchange instruments with micro-environmental control are a good way to measure CO_2 stress (Sellin 2013).

F_V/F_M - Fast dark-adapted test is sensitive to early CO_2 stress. (Siffel & Braunova 1999)

PI_{ABS} - Fast dark adapted test sensitive to CO_2 stress using OJIP protocol. (Strasser 2004)

q_P - A longer slow light or dark adapted test that has been used in compound stress situations related to water and light stress with CO_2 stress (Bukov & Carpentier 2004), (Cornic 1989), (Brestic 1995)

Non-sensitive CO_2 Stress tests

Yield of PSII or Y(II) - Fast light adapted test that is not sensitive to CO_2 stress initially and has been show to actually increase early on. It will decline after a period of time. While it is not valuable to detect CO_2 stress, it may be valuable to identify it in conjunction with F_V/F_M, and NPQ. (Siffel & Braunova 1999)

NPQ - This is a longer dark adapted measurement. It has been shown there is no quenching in the total absence of CO_2. (Siffel & Braunova 1999).

Air Pollution Stress

F_V/F_M - Fast dark-adapted test is sensitive to ozone stress. (Mikkelsen 1994) (Calatayud, Pomares, and Barreno 2006)

Yield or Y(II) - Fast light adapted test can also be used for ozone stress in steady state. (Calatayud, Pomares, and Barreno 2006) (Carrasco-Rodriguez J. and del Valle-Tascon S., 2001)

q_P - Slow test. Ozone stress showed a lower q_P (Calatayud, Pomares, and Barreno 2006) (Carrasco-Rodriguez J. and del Valle-Tascon S., 2001)

NPQ – Slow test, ozone stress showed an increase in NPQ stress. This is a dark adapter test. (Calatayud, Pomares, and Barreno 2006) (Carrasco-Rodriguez J. and del Valle-Tascon S., 2001)

Herbicide Stress:

Different herbicides work in various ways. Some parameters are successful with certain types of herbicide stress and not for others.

For example: F_V/F_M is not sensitive to DCMU stress but VJ is sensitive to DCMU stress.

Herbicides are listed in alphabetical order and the test used to identify stress is listed on the left.

F_V/F_M , & NPQ - *Atrazine* , a PSII inhibitor. Both tests were sensitive to atrazine use in some different genotypes of sweet corn. (Kopsell 2010)

V_J –OJIP – *Atrazine* , a PSII inhibitor, by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise in J provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003) (Percival 2005)

Yield of PSII & NPQ -*Basta* (AgrEbo) is composed of 18.5 % *Glufosinate-ammonium* <Ammonium -DL-homoalanine-4-YL-(methyl)phosphinate> Yield and NPQ are sensitive tests for Basta herbicide stress. (Takayama K. , Konishi A., and Omasa K.. 2003)

V_J – *Bentazone*, a PSII inhibitor, V_J (or F_{VJ}) is the fluorescence rise from O to J in the OJIP

test, provides a sensitive test for stress. (Christiansen, Teicher and Streibig 2003)

V_J – OJIP - *DCMU* has little effect on Fv/Fm (Nedbal & Whitmarsh 2004). However by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise in J provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003), (Percival 2005)

NPQ - *DCMU*. A longer dark adapted test will provide stress information on DCMU. (Nedbal & Whitmarsh 2004)

NPQ – *DDT*. A sensitive test for DDT that is also dependent on zeaxanthin quantity in leaves. If there is little or no zeaxanthin production, NPQ can detect DDT stress. If zeaxanthin has been produced, NPQ is not affected by DDT. (Bilger & Bjorkman 1994)

V_J –OJIP – *Diuron* by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise In J provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003) (Percival 2005)

V_J – *Fluorochloridone* a PDS inhibitor, V_J (or F_{VJ}) is the fluorescence rise from O to J in the OJIP Test, provides a sensitive test for stress. (Christiansen, Teicher and Streibig 2003)

V_J – *Glycosate* an EPSPs inhibitor, V_J (or F_{VJ}) is the fluorescence rise from O to J in the OJIP test, provides a sensitive test for stress. (Christiansen, Teicher and Streibig 2003)

V_J –OJIP – *TU-1178* by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise in I provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003)

V_J –OJIP – *TU-1282* by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise in I provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003)

Herbicide effects on Arabidopsis at standard dose:

In F_V/F_M & F_V/F_O, F_O is minimum fluorescence measured with very low intensity modulated light of dark adapted sample before any Q_A is reduced by a saturation flash.

In other parameters listed below F_O is fluorescence at 40µs , F_P = P, F_I = J at 2ms, in the OJIP protocol

F_V/F_M, 1-(F_O/F_P), 1-(F_I/F_P) – *2,4D* in the phenoxy group, is a synthetic auxin herbicide. These parameters were sensitive to 2,4D use after 48 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)

F_V/F_M, 1-(F_O/F_P), 1-(F_I/F_P) – *Asulam.* These parameters were sensitive to Asulam use after 6 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)

F_V/F_M, 1-(F_O/F_P), 1-(F_I/F_P) – *Bifenox*. These parameters were sensitive to Bifenox use after 48 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)

F_V/F_M, 1-(F_O/F_P), 1-(F_I/F_P) – *Diclofop-methyl* . These parameters were sensitive to Diclofop-methyl use after 6 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)

F_V/F_M, 1-(F_O/F_P), 1-(F_I/F_P) – *Glycosate*. These parameters were sensitive to Glycosate use after 6 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)

F_V/F_M, 1-(F_O/F_P), 1-(F_I/F_P) – *Imazapyr* . These parameters were sensitive to Imazapyr use after 6 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)

F_O is minimum fluorescence, F_O is fluorescence at 40µs , F_P = P, F_I = J at 2ms, in the OJIP protocol

Pesticide Stress:

Different pesticides work in various ways. Some parameters are successful with certain types of pesticide stress and not for others.

Copper based Algicides and Fungicides – are main sources of Cu stress in plants, see Copper stress under Chemical Stress.

Mercury based Organo-mercury fungicides – A main source of Mg stress in plants, see Mercury stress under Chemical Stress.

PI_{ABS} , F_VF_M – Lindane. Sensitive test on cyanobacteria Anabaena (Bueno, Fillat, Strasser, Rodriguez, Marina, Smienk. Moreno, Barja 2004)

Yield of PSII or Y(II) – Trimax stress on Cotton Germ M.,
(Gonias E. D. Oosterhuis D.M., Bibi A.C. & Brown R.S. 2003)

Chemical Stress:

While some types of chemical stress can be measured by various parameters including F_V/F_M, some require specific parameters for measurement.

In F_V/F_M & F_V/F_O, F_O is minimum fluorescence measured with very low intensity modulated light of dark adapted sample before any Q_A is reduced by a saturation flash.

In other parameters listed below F_O is fluorescence at 40µs , F_P = P, F_I = J at 2ms, in the OJIP protocol

Listed by chemical. *Nitrogen, boron, calcium, chlorine, cobalt, copper, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, sulfur, and zinc <u>are listed under nutrient stress</u>.*

F_V/F_O - *Aluminum* (Joshi & Mohanty2004), (Pereira 2000)(Baker and Rosenqvist 2004)

$(F_P – F_I)/F_I$ - *Aluminum* (Baker and Rosenqvist 2004)

V_J - *Aluminum* Fi is = J in OJIP V_J = Fi-Fo/ Fm-Fo (Joshi & Mohanty2004),
(Moustakas 1993, 1995, 1997)

F_V/F_M - *Aluminum* (Joshi & Mohanty2004), (Moustakas 1996)
Not as sensitive as Fv/Fo (Baker and Rosenqvist 2004).

q_P , & q_N - *Aluminum* (Joshi & Mohanty2004), (Moustakas 1996)

q_N - *Cadmium*. qN is more sensitive to Cadmium concentration than Fv/Fm. (Joshi & Mohanty 2004) (Krupa 1993) Skorzynska and Baszynski 1997)

F_V/F_M - *Cadmium*. (Baker and Rosenqvist 2004), (Popovic et al., 2003).

Yield of PSII or Y(II) - *Cobalt*. (Joshi & Mohanty2004)(Tripathy 1983)

Yield of PSII or Y(II) - *Copper*. Sensitive test (Joshi & Mohanty2004) (Lanaras 1993)

F_V/F_M - *Copper* (Baker and Rosenquist 2004), (Popovic et al., 2003)

Rfd - *Copper*. Sensitive test (Joshi & Mohanty2004))

F_V/F_M - *Lead* (Joshi & Mohanty2004), (Parys 1998) (Romanowska 1998)

F_V/F_M - *Mercury* (Baker and Rosenqvist 2004), (Joshi & Mohanty2004), (Popovic et al., 2003)

q_N - *Mercury* (Joshi & Mohanty2004), (Lee 1995), (Xylander 1998)

J & I in OJIP -*Mercury* (Joshi & Mohanty2004), (Haldimann P., and Tsimilli-Michael M.2002)

ETR - *Nickel*. F_V/F_M is not a good indicator of Nickel stress. (Joshi & Mohanty2004), (Tripathy 1981)

NaCl (Salt) – NaCl measurement success appears to show variable results by plant type, C_3 or C_4, and in some cases, by species.

q_N – *NaCl (Salt)*. q_N is a very sensitive indicator of salt stress in *Rice*. F_V/F_M and yield were not sensitive to salt stress in Rice (Moradi & Ismail 2007)

q_N, q_P, F_V/F_M, Y(II), & ETR - *NaCl (Salt)* All parameters were sensitive to salt stress in Cereal Sorghum a C_4 plants (Moradi & Ismail 2007) (Netondo 2004)

F_V/F_M - *NaCl (Salt)* F_V/F_M was sensitive to salt stress in the red mangrove, *Rhizophora mangle* L. (Biber 2006)

F_V/F_M – *NaCl (Salt)* F_V/F_M was sensitive to salt stress in chickpea seedlings (Eyidogan 2007)

Fv/Fm - *NaCl (Salt)* not sensitive to salt stress in Rice (Moradi & Ismail 2007)

Yield or Y(II) – *NaCl (Salt)* Y(II) was sensitive to salt stress in chickpea seedlings (Eyidogan 2007)

Yield or Y(II)– *NaCl (Salt)* not sensitive to salt stress in Rice (Moradi & Ismail 2007)

CCI or chlorophyll content index with a chlorophyll meter - *NaCl (Salt)* was sensitive to salt stress in cotton a C_3 plant (Higbie 2010)

Yield or Y(II)– *Perchlorate* Y(II) is a very sensitive test for perchlorate stress in the aquatic plant, Alternanthera philoxeroides (Xie YF, Cai XL, Liu WL, Deng W 2009)

F_V/F_M, NPQ, ETR - *Perchlorate* These parameters will also detect perchlorate stress at different levels in the aquatic plant, Alternanthera philoxeroides (Xie YF, Cai XL, Liu WL, Deng W 2009)

Spad /CCI – *Perchlorate* is a sensitive test for perchlorate stress in the aquatic plant, Alternanthera philoxeroides (Xie YF, Cai XL, Liu WL, Deng W 2009)

F_S in Y(II) - *Zinc* - Fv/Fm is not a good indicator of zinc stress(Joshi & Mohanty2004) (Tripathy & Mohanty 1980). F_S is the steady state fluorescence level at a specific light intensity without the saturation flash information F_M. It takes between 20 minutes and 35 minutes to reach steady state photosynthesis at a specific light level (Cazzaniga 2013).

Ph Stress

F_V/F_M – Fv/Fm was found to detect severe acid rain stress at a ph of 1.8 or below. (Velikova, Yordanov 1996)

Biotic Stress: The fluorescence parameter best suited to the type of infection is dependent on the type of Infection (Nedbal & Whitmarsh 2004) Therefore it is important to have versatile capability

The tests listed in this category are not listed in order of sensitivity or effectiveness. While many references below involve fluorescence imaging, spot measurement can also be used for study.

Due to early site-specific infections, multiple point measurements on the same leaf in different areas are recommended. (Claus Buschmann 2008), or imaging fluorescence is recommended.

NPQ - This is a longer dark adapted measurement for crown rust on oat leaves (Sholes & Rolfe 1996)

NPQ - This is a longer dark adapted measurement for tobacco mosaic virus on tobacco (Osmond 1998), (Lohaus 2000)

F_V/F_M - Fast dark-adapted test can be used for Bean rust (Peterson & Aylor 1995)

Yield or Y(II)- Fast light adapted test used for cedar fungus (Ning 1995)

F_M -F_S /F_M - This is a longer dark-adapted test that requires several minutes to reach steady state photosynthesis. tobacco mosaic virus on tobacco (Osmond 1990). F_M is dark adapted and F_S is the light adapted value at steady state photosynthesis.

F_V/F_M - Fast dark-adapted test can be used for biotic stress chickpea leaves fungus (Esfield 1995) (Weiss 1998)

F_V/F_M - Fast dark-adapted test can be used for biotic stress lemons infected by Penicllium digitatum (Nebal 2000)

F_O/F_V - Fast dark-adapted test can be used for biotic stress Brassica Blackspot by destruxins (Buchwaldt & Green 1992)

NPQ - This is a longer dark adapted measurement recommended for virus infection in higher plants and algae. (Balachadran & Hurry 1997)

F_V/F_M - Fast dark-adapted test can be used for biotic stress recommended for virus infection in higher plants and algae. (Balachadran & Hurry 1997)

F_V / F_O - Fast dark-adapted test can be used for biotic stress Maize rust resistance. (Duraes 2001)

F_V/F_M - Fast dark-adapted test can be used for biotic stress. Maize rust resistance. (Duraes 2001)

Herbivory – (Animal Stress):

Yield of PSII or Y(II) – Fast light adapted sensitive test for Arthropod damage showing greater damage than the size of the hole indicates stress. (Aldea, Hamilton, Resti, Zangerl, Berenbaum, Frank and Deluca 2006), (Zangerl 2002)

F_V/F_M - Fast dark adapted test can be used to test for damage caused by insect larval foot hooks. (Hall, MacGregor, Nijsse, and Bown 2004)

Weed Stress

Maize – chlorophyll content measurement (Tollenaar M., Dibo A.A 1994), (Tollenaar M. 1994) (Tollenaar M. 1997)

Maize – using transcriptome analysis for weed stress. (Moriles J. 2012)

Rice - weed stress measured by high performance liquid chromatography (HPLC) for phenolic compounds (Hea 2012)

Radiation Stress

γ (gamma radiation stress) – on buckwheat - F_V/F_M, F_V/F_O,Y(II), ETR, photochemical quenching, and non-photochemical quenching are all sensitive to gamma radiation detection due to an increase in q_I or photoinhibition. (JIA C.F 2008)

Cosmic radiation during space flight – on Chlamdomonas reinhardtii. F_V/F_M and OJIP V_t showed that some mutants health after space flight performed better than others. (Masci S. 2011)

UVA and UVB sensitivity – on red algae – F_V/F_M was a sensitive test for measuring exposure of red algae to UBA and UVB radiation (DRING M.J. 1996)

X-ray exposure – Both F_V/F_M and Y(II) are sensitive measurements for measuring X-ray exposure in plants. Kurimoto (2010)

Neutron radiation – Exposure of plants to neutron radiation can be measured with F_V/F_M & Y(II) (Rea1 G 2008)

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