Chlorophyll Fluorescence Monitoring at Selected LTER Network Sites

Richard L. Boyce[1,2,3,4]
Herman Sievering[1,3,5]

[1]Niwot Ridge LTER ;[2]Hubbard Brook LTER; [3]Institute of Arctic and Alpine Research; [4]Northern Kentucky University; [5]University of Colorado at Denver

Quick & Dirty Intro to Chl F

- When a leaf is exposed to PAR, emits light ~680-760 nm
  - Fluorescence
  - Mainly from PSII
- This light can be used for probing state photosynthetic machinery *in vivo*
- For ecophysiologists, useful for quantifying effect of plant stress on photosynthesis in the field

Fate of Light absorbed by Chlorophyll

- Energy channeled to reaction center of PSII, used for photosynthesis
  - Φ_P
- Thermal dissipation
  - Φ_D
- Fluorescent emission
  - Φ_F
- All quantum yields that sum to 1

$$\Phi_P + \Phi_D + \Phi_F = 1$$

Fate of Light absorbed by Chlorophyll

$$\Phi_P + \Phi_D + \Phi_F = 1$$

- Because 1st two processes are regulated by plant, and all 3 are competitive, fluorescence depends on photosynthesis & dissipation

Chlorophyll Fluorescence Terminology

Fluorescence Parameters

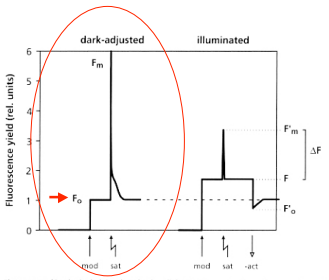


FIGURE 1. Fluorescence kinetics in dark-incubated (adjusted) and illuminated leaves in response to a saturating pulse of white light. mod = modulated measuring light on; sat = saturating pulse on; -act = actinic light off. For explanation of fluorescence symbols see text (after Schreiber et al. 1995).

Lambers et al. 1998

In Dark

- Dark-adapted leaf
  - Weak intermittent (modulated) light gives rise to F_o
  - Indicates open reaction centers, as the primary electron acceptor Q_A in PS II is fully oxidized

Fluorescence Parameters

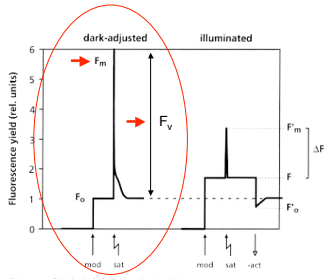


FIGURE 1. Fluorescence kinetics in dark-incubated (adjusted) and illuminated leaves in response to a saturating pulse of white light. mod = modulated measuring light on; sat = saturating pulse on; -act = actinic light off. For explanation of fluorescence symbols see text (after Schreiber et al. 1995).

Lambers et al. 1998

In Dark

- Dark-adapted leaf
  - Pulse of strong (saturating) light gives rise to F_m
  - Q_A is fully reduced, all reaction centers are closed
  - So no energy can go to photosynthesis
    - Thermal dissipation processes are usually minimal in the dark
    - So most energy goes to fluorescence

In Dark

- Dark-adapted leaf
  - Since no light goes to photosynthesis, $\Phi_{Dm} + \Phi_{Fm} = 1$
    - Φ_{Dm} = quantum yield of thermal dissipation at saturating light pulse
    - Φ_{Fm} = quantum yield of fluorescence at saturating light pulse

In Dark

- Dark-adapted leaf
  - Also assume that there's no change in relative quantum yields of dissipation & fluorescence during saturating pulse

$$\frac{\Phi_{Dm}}{\Phi_{Fm}} = \frac{\Phi_D}{\Phi_F}$$

  - Combining this with 3-process equation:

$$\Phi_P + \Phi_D + \Phi_F = 1$$

In Dark

- Dark-adapted leaf
  - Gives the following:

$$\Phi_{PSII} = \frac{\Phi_{Fm} - \Phi_F}{\Phi_{Fm}}$$

  - (where Φ_{PSII} is substituted for Φ_P, since originates mainly from PS II)
  - In dark, $\Phi_{Fm} = F_m$ and $\Phi_F = F_o$, **maximum quantum yield (Φ_{PSIIm} or F_v/F_m)** is

$$\Phi_{PSIIm} = \frac{F_m - F_o}{F_m} = \frac{F_v}{F_m}$$

Fluorescence Parameters

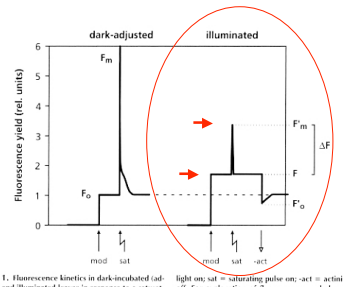


FIGURE 1. Fluorescence kinetics in dark-incubated (adjusted) and illuminated leaves in response to a saturating pulse of white light. mod = modulated measuring light on; sat = saturating pulse on; -act = actinic light off. For explanation of fluorescence symbols see text (after Schreiber et al. 1995).

Lambers et al. 1998

In Light

- Light-adapted leaf
  - Sub-saturating light level gives rise to constant fluorescent level F (also called F_s or F_t)
  - A pulse of saturating light gives rise to F'_m
    - Lower than F_m
  - Why?
    - Reaction centers are closed, but…
    - Thermal dissipation now competes with fluorescence

In Light

- Light-adapted leaf
  - Gives the following:

$$\Phi_{PSII} = \frac{\Phi_{Fm} - \Phi_F}{\Phi_{Fm}}$$

  - In light, $\Phi_{Fm} = F'_m$ and $\Phi_F = F$, **actual quantum yield (Φ_{PSII})** is

$$\Phi_{PSII} = \frac{F'_m - F}{F'_m} = \frac{\Delta F}{F'_m}$$

In Light

- Light-adapted leaf
  - Not all reactions centers in PS II are “open”
  - Some are reduced with excited electrons
  - The fraction that are open is given as:

$$qP = \frac{F'_m - F}{F'_m - F'_o} = \Delta F/F'_v$$

  - F'_o measured by exposing leaf to far-red light for a few seconds, then covering with cloth

Fluorescence Parameters

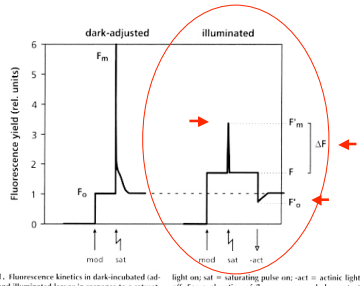


FIGURE 1. Fluorescence kinetics in dark-incubated (adjusted) and illuminated leaves in response to a saturating pulse of white light. mod = modulated measuring light on; sat = saturating pulse on; -act = actinic light off. For explanation of fluorescence symbols see text (after Schreiber et al. 1995).

Lambers et al. 1998

In Light

- Light-adapted leaf
  - You may also calculate a ratio similar to that seen in dark-adapted foliage, the **intrinsic quantum yield**:

$$F'_v/F'_m = \frac{F'_m - F'_o}{F'_m}$$

  - This is the quantum yield you would have gotten were all the reaction centers open (they aren’t)

In Light

- Light-adapted leaf
  - Actual quantum yield, qP and intrinsic quantum yield are all related:

$$F_v'/F_m' = \frac{\Phi_{PSII}}{qP}$$

Behavior of Fluorescence Parameters

- F_v/F_m (maximum quantum yield)
  - Typically ~ 0.8 in healthy, unstressed leaves
  - Decreases caused by **photoinhibition**
    - Shunting of light energy away from photochemistry caused by protective mechanisms and/or damage
  - Various stresses can cause photoinhibition
  - Usually changes slowly

Behavior of Fluorescence Parameters

- Φ_{PSII} (actual quantum yield)
  - $< F_v/F_m$
  - Decreases caused by
    - Decreases in F'_v/F'_m
    - Closure of reaction centers
  - Shunting of excess light energy away from photochemistry during the day
    - Generally decreases with increasing light
  - Changes quickly
  - Can be used to derive electron transport rate
    - Roughly proportional to photosynthetic rate

Behavior of Fluorescence Parameters

- F'_v/F'_m (intrinsic quantum yield)
  - $\geq \Phi_{PSII}$
  - Decreases caused by photoprotective xanthophyll cycle
    - Shunting of excess light energy away from photochemistry during the day
    - Generally decreases with increasing light
    - Usually correlated with de-epoxidation of xanthophylls
  - Papers by Demmig-Adams & Adams claim thermal dissipation of captured light is proportional to $1 - F'_v/F'_m$

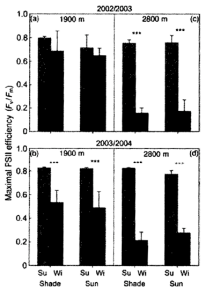
Behavior of Fluorescence Parameters

- qP (proportion of open reaction centers)
  - Decreases from 1 (in dark) to about 0.5-0.95 in light

Uses of Fluorescence Parameters

- F_v/F_m (maximum quantum yield)
  - Cold temperatures/freezing stress
  - Excess light (with cold stress)
  - UV light
  - Nutrient stress
    - N, P, Ca
  - Pollutants
  - Drought (extreme)
- Most responsive to stress?

Arctostaphylos in Summer & Winter

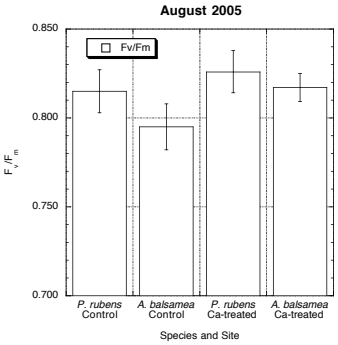


Zarter et al. 2006

Red Spruce & Balsam Fir at HBEF
Ca Fertilization Watershed Experiment

Ca-treated > Control
P = 0.002

P. rubens > *A. balsamea*
P = 0.004



Uses of Fluorescence Parameters

- Φ_{PSII} (actual quantum yield)
  - Nitrogen deficiencies
  - Temperature
  - Drought

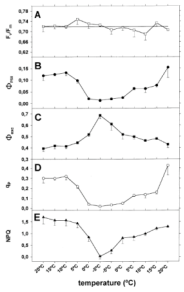
Uses of Fluorescence Parameters

- F'_v / F'_m (intrinsic quantum yield)
  - Nitrogen deficiencies
    - Foliar Uptake
  - Temperature
  - Drought

Uses of Fluorescence Parameters

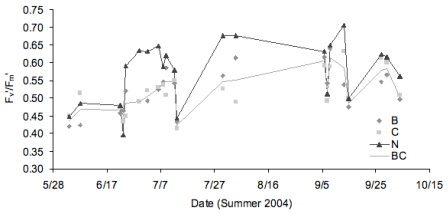
- qP (proportion of open reaction centers)
  - Nitrogen deficiencies
  - Temperature
  - Drought

Response of Chl F parameters to temperature



- *Quercus ilex*
- Corcuera et al. 2005

Effect of Canopy N Uptake



Tomaszewski and Sievering 2006

Proposed Work

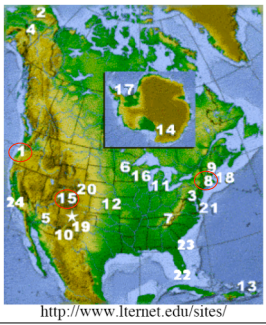
- Pre-dawn, mid-day (high light) measurements
  - Because we don't know which parameter will respond best to stress
- Do by ecosystem type
  - Coordinate with monitoring
  - Statistical modeling of potential stressors

Proposed Work

- Where should this be done?
  - Niwot
  - Andrews, Harvard Forest: interested
  - Elsewhere?

Proposed Work

- A good start for a longitudinal study
- But sparse!

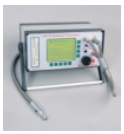


http://www.lternet.edu/sites/

Equipment Options

- Two instruments
  - Both use pulse amplitude modulation system
  - Both are portable and versatile enough for our uses
  - Both are relatively inexpensive

OS5-FL



- Manufactured by Opti-Sciences, Inc.
  - http://www.optisci.com/5fl.htm
- ~ $12.5 k
- Currently used by Boyce

PAM-2100

- Manufactured by Walz GmbH
  - http://www.walz.com/products/fluorescence/pam2100/index_pam2100.html
- Successor to popular PAM-2000
- ~ $14k
- Currently used by Sievering

Protocols

- Types of measurements to make
  - Predawn
    - Fv/Fm
  - Mid-day
    - Φ_{PSII}, F'_v/F'_m, qP
- When to make them
  - Once (twice?)/week during growing season
  - Once/month during dormant season
- What to make it on?
  - Dominant vegetation of ecosystem at each LTER