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Supplementary Material

Photosynthesis of an Epiphytic Resurrection Fern (*Davallia angustata (*Wall, ex Hook. & Grev.).

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Theory of Measurement of Photosynthetic Parameters using PAM technology

The fluorescence yield is part of the WinControl software output as the effective quantum yield (Y or ΦPSII, Schreiber et al., 1995). Effective quantum yield have ranges from 0 to 1 (maximum usually no higher than ≈ 0.85). It is found experimentally that if Y is plotted against irradiance (E), it followsa simple exponential decay function of the form $y = e^{-kx}$,

$$Y = Y_{\max} \times e^{-k_y E} \qquad \text{Equation 1}$$

where, Y is the effective quantum yield,

$Y_{\max}$ is the effective quantum yield at theoretical zero irradiance,

$k_y$is a scaling constant, and E is the irradiance (µmol photons m^{-2} s^{-1}, PPFD).

The ETR is an estimate of the light reactions of photosynthesis and is defined as:

$$\mathrm{ETR} = \mathrm{Y} \times \mathrm{E} \times (\text{PSI/PSII allocation factor}) \times (\text{leaf absorptance factor}) \qquad \text{Equation 2}$$

where, Y is the effective quantum yield,

Eis the irradiance (mol quanta m^{-2} s^{-1} PPFD).

The PSI/PSII allocation factor is about 0.5 (Melis, 1989; Ritchie, 2008) and allows for absorption of ≈50% of quanta by PSII, and the leaf absorptance factor ($Abt_F = 0.84$) is the default absorptance factor for a variety of plants used by the Walz software.To properly estimate ETR it is necessary to allow for the Absorptance (Abt_λ) of the leaves and it is better to measure this experimentally (Ritchie and Runcie, 2014) rather than rely on a default value ($Abt_F = 0.84$). Absorptance of leaves is now known to bemore variable than previously thought and actual measurements of Abt at the blue wavelength used by a blue diode PAM (465 nm) are in general considerably higher than the default value (Ritchie and Runcie, 2014). Relative ETR (rETR) is calculated using the standard default absorptance factor (Abt_F). rETR as calculated by the software is converted into the actual ETR by multiplying rETR by the ratio of the experimentally measured Abt_{465nm} and the default value ($Abt_{465\,nm}/0.84$). POER can then be estimated as mol O_2 per meter squared per second (Ritchie, 2014; Ritchie and Runcie, 2014) by dividing ETR by 4 based on the basic relationship of the light reactions of photosynthesis, $2H_2O \rightarrow O_2 + 4e + 4H^+$. POER is a high estimate of gross photosynthesis because it does not take into account oxygen consumption by photorespiration and possible Mehler reactions (Atwell et al., 1999)

Since Yield is of the form $y = e^{-x}$, then since photosynthesis is proportional to the product of the yield and irradiance then an appropriate model for photosynthesis is of the form $y = x.e^{-x}$ (Ritchie, 2008). The equation $y = x.e^{-x}$ has a maximum at $x = 1$ and the slope of the line at $x = 0$ is 1 and there is a point of inflection ($d^2y/d^2x = 0$) at $x = 2$. A form suitable for modeling photosynthesis with experimentally determinable constants that are easily recognizable on a graphical representation of the data (Ritchie and Bunthawin, 2010a, b; Ritchie, 2012, 2014; Ritchie, 2015b) is,

$$\mathrm{ETR} = \frac{\mathrm{ETR}_{\max} \times \mathrm{E}}{\mathrm{E}_{\mathrm{opt}}} \times e^{1-\mathrm{E}/\mathrm{E}_{\mathrm{opt}}} \qquad \text{Equation 3}$$

where, ETR is electron transport rate as a measure of gross photosynthesis,

E is the Irradiance (µmol m^{-2} s^{-1} 400 – 700 nm PPFD),

E_{opt} is the optimum irradiance,

ETR_{max} is the maximum gross photosynthesis.

The maximum photosynthetic efficiency (α_0) is the initial slope of the curve at E = 0 ($\alpha = ETR_{max} \times e/E_{opt}$). It can be shown by analysis of Equation 3 that the half-maximum photosynthesis ($ETR_{half\text{-}max}$) occurs at $0.231961 \times E_{opt}$ and that photosynthesis is also inhibitedby 50% at $2.67341 \times E_{opt}$. Hence good rates of photosynthesis (>50% of maximum) are found under irradiances ranging from ¼ to 2.5 times the optimum irradiance. The asymptotic photosynthetic efficiency at zero irradiance (α_0) is theoretically useful but perhaps a more informative expression for productivity studies is the photosynthetic efficiency at optimum irradiance (α_{Eopt}). It can be shown that α × $E_{opt}$is equivalent to $\alpha_{Eopt} = \alpha_0/e$. For example, if $\alpha_0 = 0.2$ then the photosynthetic efficiency at Optimum Irradiance (α_{Eopt}) is 7.4%.

The equations used to estimate the two non-photochemical quenching parameters qN (known as Variable Fluorescence NPQ) and NPQ are calculated by the Walz software and are discussed by Ritchie and Bunthawin (2010a, b), Beckett et al. (2012) and Brestic and Zivcak (2013) and are based on Genty et al. (1989). qN and NPQ *vs.* irradiance best fit a simple exponential saturation curve of the form $y = y_{max}\,(1\text{-}e^{-kE})$ where y is qN or NPQ and E is the irradiance.

$$qN = qN_{max}\left(1 - e^{-k_{qN}E}\right) \quad \text{Equation 4}$$

$$NPQ = NPQ_{max}\left(1 - e^{-k_{NPQ}E}\right) \quad \text{Equation 5}$$

Equations 1 and 3, 4 and 5 curves are best fitted using non-linear least squares methods and the asymptotic estimates of the errors of the fitted parameters calculated by matrix inversion. EXCEL routines are available on the Internet (Ritchie 2015b).

Supplementary Material References

Atwell BT, Kreidermann, PE, Turnbull CGN (Eds.) 1999. ‘Plants in Action: adaptation in nature, performance in cultivation’. Chapter 1 p 23 – 44, Chapter 2 p 45 – 82, Chapter 12, p 380 – 415, Chapter 15, p 497-498. (MacMillan Education Publ., South Yarra, Victoria, Australia)

Beckett M, Loreto F, Velikova V, Brunetti C, Di Ferdinando M, Tattini M, Calfapietra C, Farrant JM (2012) Photosynthetic limitations and volatile and non-volatile isoprenoids in the poikilochlorophyllous resurrection plant *Xerophyta humilis* during dehydration and rehydration. Plant, Cell and Environment **35**, 2061–2074.

Brestic M, Zivcak M (2013) PSII Fluorescence techniques for measurement of drought and high temperature stress signal in plants: protocols and applications. In ‘Chapter 4 Molecular stress physiology in plants’. (Eds GR Rout, AB Das) pp. 87–131. (Springer Publ., Dordrecht), doi: 10.1007/978-81-322-0807-5

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta **990**, 87–92.

Melis A (1989) Spectroscopic methods in photosynthesis: photosystem stoichiometry and chlorophyll antenna size. Philosophical Transactions of the Royal Society B **323,** 397–409.

Ritchie RJ (2008) Fitting light saturation curves measured using PAM fluorometry. Photosynthesis Research **96**, 201–215.

Ritchie RJ (2012) Photosynthesis in the blue water lily (*Nymphaea caerulea* Saligny) using PAM fluorometry. International Journal of Plant Sciences **173**, 124–136.

Ritchie RJ (2014) Photosynthesis in an encrusting lichen (*Dirinaria picta* (Sw. Schaer.ex Clem., Physiaceae) and its symbiont, *Trebouxia sp*, using PAM fluorometry. International Journal of Plant Science **175**, 450–466.

Ritchie RJ (2015b) Photosynthetic Light Curve Fitting Models Excel File: October 2015 DOI: 10.13140/RG.2.1.2301.7680. Deposited - 2015-10-21 T 14:48:47 UTC on Research Gate [Accessed 26 May 2016]

Ritchie RJ, Bunthawin S (2010a) The use of PAM (pulse amplitude modulation) fluorometry to measure photosynthesis in a CAM orchid, *Dendrobium spp*. (D. cv. Viravuth Pink). International Journal of Plant Sciences **171,** 575–585.

Ritchie RJ, Bunthawin S (2010b) The use of PAM (pulse amplitude modulation) fluorometry to measure photosynthesis in pineapple (*Ananas comosus* [L.] Merr). Tropical Plant Biology **3:**193–203.

Ritchie RJ, Runcie JRW (2014) A portable reflectance absorptance transmittance (RAT) meter for vascular plant leaves. Photosynthetica **52**, 614–626.

Schreiber U, Bilger W, Neubauer C (1995) Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of *in vivo* photosynthesis. In ‘Ecophysiology of Photosynthesis Ecological Studies, Vol 100’. (Eds E-D Schulze, MM Caldwell) pp 49–70. (Springer Publ., Berlin)