

Short communication

New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes

David M. Kramer^{1,*}, Giles Johnson², Olavi Kiirats³ & Gerald E. Edwards³

¹*Institute of Biological Chemistry, Washington State University, 289 Clark Hall, Pullman, WA 99163-6340, USA;*

²*School of Biological Sciences, University of Manchester, Manchester, UK;* ³*School of Biological Sciences, Washington State University, 289 Clark Hall, Pullman, WA 99164-6340, USA; *Author for correspondence (e-mail: dkramer@wsu.edu; fax: +1-509-335-7643)*

Received 30 July 2003; accepted in revised form 3 November 2003

Key words: antenna, chlorophyll *a* fluorescence, electron transport, fluorimeter, photosynthetic unit, quantum yield

Abstract

A number of useful photosynthetic parameters are commonly derived from saturation pulse-induced fluorescence analysis. We show, that q_p , an estimate of the fraction of open centers, is based on a pure ‘puddle’ antenna model, where each Photosystem (PS) II center possesses its own independent antenna system. This parameter is incompatible with more realistic models of the photosynthetic unit, where reaction centers are connected by shared antenna, that is, the so-called ‘lake’ or ‘connected units’ models. We thus introduce a new parameter, q_L , based on a Stern–Volmer approach using a lake model, which estimates the fraction of open PS II centers. We suggest that q_L should be a useful parameter for terrestrial plants consistent with a high connectivity of PS II units, whereas some marine species with distinct antenna architecture, may require the use of more complex parameters based on intermediate models of the photosynthetic unit. Another useful parameter calculated from fluorescence analysis is ϕ_{II} , the yield of PS II. In contrast to q_L , we show that the ϕ_{II} parameter can be derived from either a pure ‘lake’ or pure ‘puddle’ model, and is thus likely to be a robust parameter. The energy absorbed by PS II is divided between the fraction used in photochemistry, ϕ_{II} , and that lost non-photochemically. We introduce two additional parameters that can be used to estimate the flux of excitation energy into competing non-photochemical pathways, the yield induced by downregulatory processes, ϕ_{NPQ} , and the yield for other energy losses, ϕ_{NO} .

Abbreviations: ϕ_{II} – the quantum efficiency of Photosystem II estimated by fluorescence yield measurements based on a lake model for the PSU; ϕ_{IIi} – the intrinsic yield of open PS II centers in a puddle model; fQ_{Aox} – fraction of Q_A in its oxidized state, also implying the fraction of PS II centers in open states; NPQ – light induced photoprotection through thermal dissipation of energy; PS – photosystem; PSU – photosynthetic unit; q – a generic term for the fraction of open PS II centers; q_{cu} – a parameter estimating the fraction of PS II centers in open states based on a connected units model for the PSU; q_L – a parameter estimating the fraction of PS II centers in open states based on a lake model for the PSU; q_p – a parameter estimating the fraction of PS II centers in open states based on a puddle model for the PSU

Introduction

Steady-state chlorophyll *a* fluorescence yield has been a convenient and powerful tool for the study of higher plant photosynthesis. The vast majority of chlorophyll fluorescence studies focus on PS II and its related

antenna. Events related to PS I photochemistry and its antenna are obscured by the fact that ‘closed’ PS I centers quench excitation energy as well as open centers, owing to the fact that P_{700}^+ is as good a photochemical trap as P_{700} . The steady-state chlorophyll fluorescence has been described as a ‘rich’ signal

because it responds to a large number of factors, and can potentially provide information about each of them (e.g., Bradbury and Baker 1983; Weis and Lechtenberg 1989; Govindjee 1995; Kramer and Crofts 1996; Adams and Demmig-Adams 2004). At the same time, the multitude of contributions can confound simple interpretation. Luckily, very elegant (i.e., straightforward, and for the large part effective) techniques have been introduced to distinguish among many of these contributions, yielding very useful parameters (Bradbury and Baker 1983; Schreiber et al. 1986; Genty et al. 1989; Krause and Weis 1991). The most commonly used of these parameters include the quantum yield of Photosystem (PS) II photochemistry, or ϕ_{II} , the degree photons absorbed by PS II associated antenna are utilized in photochemistry *versus* quenching by so-called non-photochemical processes, that is, the degree to which 'non-photochemical quenching' is engaged.

Many authors have also employed the so-called q_p parameter, photochemical quenching of chlorophyll fluorescence, the subject of this communication. A number of nomenclatures for fluorescence parameters were introduced in the 1980s and early 1990s (see van Kooten and Snel 1990), so it is important to point out that, in the context of this work, q_p refers to the parameter developed by Krause et al. (1982), Bradbury and Baker (1984), Quick and Horton (1984), Schreiber et al. (1986) and Genty et al. (1990) and recently reviewed in Maxwell and Johnson (2000). The q_p parameter was conceived as an 'indicator' of the degree of openness of the PS II reaction centers, or more specifically, the fraction of Q_A in its oxidized state (fQ_{Aox}). Butler described the efficiency of capture of excitation energy by open reaction centers by fluorescence analysis (Kitajima and Butler 1975). Genty et al. (1989) developed a useful simple procedure through fluorescence analysis to measure the yield of PS II (ϕ_{II}), which is defined as the product of the fraction of open PS II centers (estimated by q_p) times the quantum yield of photochemistry at open PS II centers (ϕ_{qp}).

In this paper, we reinforce earlier work (Schreiber et al. 1994; Baker et al. 2001) indicating not only that the q_p parameter, as currently determined from fluorescence analysis, is not a linear function of the fraction of open centers, but as we point out below, is mechanistically incompatible with other fluorescence measurements, and is influenced in a complex way by a number of factors. In the course of this discussion, we derive a new, easily-obtained parameter, q_L , which is based on the same model for the photosynthetic unit

as other commonly used fluorescence parameters, and thus is arguably a more appropriate indicator of Q_A redox state.

Derivations, results and analysis

The typical steady-state fluorescence yield experiments consist of measuring fluorescence excited by a weak (relatively non-actinic) modulated measuring beam, while superimposing either actinic (background) light and/or intense pulses (typically 3–10 times full sunlight) to saturate PS II photochemistry (e.g., Schreiber 1986). The measurement circuitry only amplifies the signal induced by the modulated probe beam, so the background light is not observed. Under ideal conditions, fluorescence measurements with different background illuminations can give estimates of the rate constants for the various processes affecting fluorescence yield. For example, the quantum yield of photochemistry or the relative rate constant for non-photochemical quenching can be estimated in this way (Genty et al. 1989, 1990).

We start with an analysis of the ϕ_{II} parameter – though this has been discussed extensively in earlier work (e.g., Genty et al. 1989) – because it illustrates a major difficulty in using the q_p parameter as an indicator of the redox state of Q_A . It was recognized early on that the effectors of chlorophyll fluorescence yield could be divided into two major categories, that related to productive photochemistry in the reaction centers, and those related to processes which dissipate excitation energy by non-productive processes, termed 'non-photochemical' quenching (Weis and Berry 1987). The fluorescence yield is the result of competition for excitation energy among several decay pathways, indicated by their respective rate constants, fluorescence (k_f), photochemistry (k_p), the basal rate of non-radiative decay (k_d), intersystem crossing to form triplets (k_{isc}), and induced or photoprotective non-photochemical quenching, which is termed NPQ (k_{NPQ}). The main component of NPQ is thought to be due to down regulation of PS II antenna efficiency mediated by high lumen $[H^+]$ and the xanthophyll cycle. An equation can be derived for the yield of any of these processes, provided that a suitable model for the photosynthetic unit (PSU) is chosen (for a nice review, see Blankenship 2002). On one extreme, the PSU may consist of a relatively large number of reaction centers embedded in a common 'lake' (or matrix) of antenna. In this case, termed the 'lake model,' all open reaction centers compete for excitons in the pigment bed, and

the photochemical efficiency of an open center will depend upon fQ_{Aox} . On the other extreme, one can imagine a system where each reaction center has its own independent antenna system. In other words, each PSU is composed of one reaction center and its associated antenna. This later case is termed the 'puddle' model. In the puddle model, there is no effect of fQ_{Aox} on the efficiency of a nearby PSU.

It is clear from extensive research (Butler 1978; Lavergne and Trissl 1995; Lazár 1999) that the PSU do not act as a puddle. In fact, it is now known that PS II reaction centers form functional dimers in the membrane (Barber 2003), so a formal puddle model is not really possible. A number of elegant measurements have shown that the PSU are fairly well approximated by the lake model, but are most accurately described by intermediate models, a number of which have been proposed (reviewed in Lavergne and Trissl 1995).

We now demonstrate (by re-deriving the equation) that the ϕ_{II} parameter can be derived on the basis of a pure lake model. In this type of model, since each dissipative process competes at the level of bulk antenna, we treat them simultaneously, with the following competitive yield equation:

$$\phi_f = \frac{k_f}{k_f + k_d + k_{isc} + k_{NPQ} + k_p}. \quad (1)$$

It is convenient to introduce a new term,

$$k_{NP} = k_f + k_d + k_{isc} + k_{NPQ}, \quad (2)$$

where k_{NP} is the sum of rate constants for all non-photochemical losses. So that in the steady-state

$$\frac{F_S}{S} = \phi_f(F_S) = \frac{k_f}{k_{NP} + k_p}, \quad (3)$$

where F_S is the relative steady-state fluorescence yield measured by the modulated fluorimeter, and S is a sensitivity factor, relating the instrument response and measuring beam intensity to yield.

The fluorescence level termed F'_M is obtained if, from the steady-state conditions, a short, but intense pulse of light is given, so that productive photochemistry is completely saturated, that is, $k_p \rightarrow 0$, but all other rate constants remain constant (see Genty et al., 1989, and Kramer and Crofts, 1996, for discussion). In this case,

$$\frac{F'_M}{S} = \phi_f(F'_M) = \frac{k_f}{k_{NP}}. \quad (4)$$

Because the sum of photochemical (ϕ_{II}) and non-photochemical yields (ϕ_n) must be unity,

$$\phi_{II} = 1 - \phi_n, \quad (5)$$

where ϕ_n is the total yield of non-photochemical processes. Considering Equations (1) and (2), the following is obtained

$$\phi_n = \frac{k_{NP}}{k_{NP} + k_p}, \quad (6)$$

then,

$$\phi_{II} = 1 - \frac{k_{NP}}{k_{NP} + k_p}. \quad (7)$$

From Equations (3) and (4),

$$k_{NP} + k_p = \frac{S \cdot k_f}{F_S}, \quad (8)$$

$$k_{NP} = \frac{S \cdot k_f}{F'_M}, \quad (9)$$

and

$$\phi_{II} = 1 - \frac{S \cdot k_f / F'_M}{S \cdot k_f / F_S} = 1 - \frac{F_S}{F'_M} = \frac{F'_M - F_S}{F'_M}, \quad (10)$$

which, indeed, is the published equation for estimating ϕ_{II} .

We next show that the same equation can also be derived from a pure puddle model. Because the reaction centers in the puddle model do not interact, the yield of photochemistry is simply the fraction of open centers, q , multiplied by ϕ_{III} , the intrinsic yield of open centers,

$$\phi_{II} = q \cdot \phi_{III}. \quad (11)$$

The overall yield of fluorescence in the puddle model would be the sum of that from open and closed PSUs, which because they are independent, they are treated separately, so that

$$\frac{F_S}{S} = \phi_f = q \cdot \frac{k_f}{k_{NP} + k_p} + (1 - q) \cdot \frac{k_f}{k_{NP}}. \quad (12)$$

At F'_M , $q \rightarrow 0$, so that

$$\frac{F'_M}{S} = \phi_f(F'_M) = \frac{k_f}{k_{NP}}. \quad (13)$$

We insert Equations (12) and (13) into Equation (10), defining the ϕ_{II} parameter, to get

$$\phi_{II} = \frac{\frac{k_f}{k_{NP}} - \left(q \cdot \frac{k_f}{k_{NP} + k_p} + (1 - q) \cdot \frac{k_f}{k_{NP}} \right)}{\frac{k_f}{k_{NP}}}, \quad (14)$$

$$\phi_{II} = q - \frac{q \cdot (k_f / (k_{NP} + k_p))}{k_f / k_{NP}}, \quad (15)$$

$$\phi_{II} = q \left(1 - \frac{k_{NP}}{k_{NP} + k_p} \right) = q(1 - \phi_n) = q \cdot \phi_{III}. \quad (16)$$

This simple derivation illustrates that the ϕ_{II} parameter is not dependent on whether we chose a lake or puddle model (but see Lavergne and Trissl 1995, for a discussion of intermediate models). This robustness is consistent with success of ϕ_{II} in predicting electron transfer rates at least under most conditions (e.g., Genty et al. 1989; Krall and Edwards 1990; Krall et al. 1991; Edwards and Baker 1993; Baker et al. 1995; Kramer and Crofts 1996; Ruuska et al. 2000).

We will next deal with the q_P parameter, and show that it is based on a pure puddle model. This parameter was described by Schreiber et al. (1986), essentially as it is now routinely measured, but it is clearly derivable from earlier work (e.g., Vredenberg and Duysens 1965; Kitajima and Butler 1975; Duysens 1986). The parameter requires three measurements, F_S , F'_M and F'_O , the latter of which is obtained when the actinic light is switched off from steady-state, and a weaker, PS I-specific (i.e., $\lambda > 720$ nm) light is switched on to oxidize Q_A , so that $fQ_{Aox} \rightarrow 1$. (Schreiber's parameter used the F_O taken in the dark, but without the far red light, rather than F'_O .) The currently commonly used q_P parameter is defined as

$$q_P = \frac{F'_M - F_S}{F'_M - F'_O}. \quad (17)$$

Schreiber et al. (1986) suggested this is an indicator of Q_A redox state, 'in a first approximation, assuming a linear relationship between fluorescence yield and the percentage of Q_A '. Indeed, the derivation below will show that the q_P parameter, used as an indicator of open PS II centers, can only be valid within the puddle model.

We start with a puddle model as above, and define q_P as being the fraction of centers in open states, that is, $q_P = q$. At F'_O , when $q_P \rightarrow 1$,

$$\frac{F'_O}{S} = \phi_f(F'_O) = \frac{k_f}{k_{NP} + k_p}. \quad (18)$$

By combining with Equations (12) and (13), it is straightforward to show that $q_P = (F'_M - F_S)/(F'_M - F'_O)$, Equation (17). By inserting terms for a lake model into the q_P equation, it can be shown that, within a lake model, q_P will not be a continuous function of fQ_{Aox} , but will also depend on other factors, most notably NPQ. This implies that q_P provides a valid estimate of the fraction of open centers *only* for the puddle PSU model. Hereafter, we will refer to q_P as photochemical quenching based on the puddle model.

In this section, we will derive a simple equation for estimating photochemical quenching and the fraction of open centers using a lake model for the PSU, employing the same fluorescence parameters used to estimate q_P . We will call this estimate q_L (to indicate that it is derived using a lake model). In this derivation, the magnitude of k_p is determined by two parameters, an 'intrinsic' rate constant (k_{pi}) for capture of excitation energy by the reaction centers, which is assumed to be constant, and the fraction of 'open' reaction centers (q_L), so that

$$k_p = k_{pi}q_L, \quad (19)$$

and using a Stern-Volmer type approach (see e.g., Section 5.1.7 of Hoppe et al. 1983),

$$\phi_f = \frac{k_f}{k_f + k_d + k_{isc} + k_{NPQ} + k_{pi} \cdot q_L}. \quad (20)$$

In the steady-state the fluorescence yield is affected by all of the rate constants discussed above, so that

$$\frac{F_S}{S} = \phi_{fs} = \frac{k_f}{k_f + k_d + k_{isc} + k_{NPQ} + k_{pi} \cdot q_L}, \quad (21)$$

which, referring to Equation (2),

$$= \frac{k_f}{k_{NP} + k_{pi} \cdot q_L}. \quad (22)$$

Again, at F'_M , that is, $q_L \rightarrow 0$, so

$$\frac{F'_M}{S} = \frac{k_f}{k_{NP}}, \quad (23)$$

and at F'_O , q_L is defined as unity, and

$$\frac{F'_O}{S} = \frac{k_f}{k_{NP} + k_{pi}}. \quad (24)$$

From these three equations, it is easy to show that

$$q_L = \frac{(1/F_S) - (1/F'_M)}{(1/F'_O) - (1/F'_M)}, \quad (25)$$

and further rearrangement shows

$$q_L = \frac{F'_M - F_S}{F'_M - F'_O} \cdot \frac{F'_O}{F_S}. \quad (26)$$

Compared to Equation (17) which fits the puddle model,

$$q_L = q_P \cdot \frac{F'_O}{F_S}. \quad (27)$$

Therefore, as illustrated later, the fraction of open centers will be lower when calculated by the lake model,

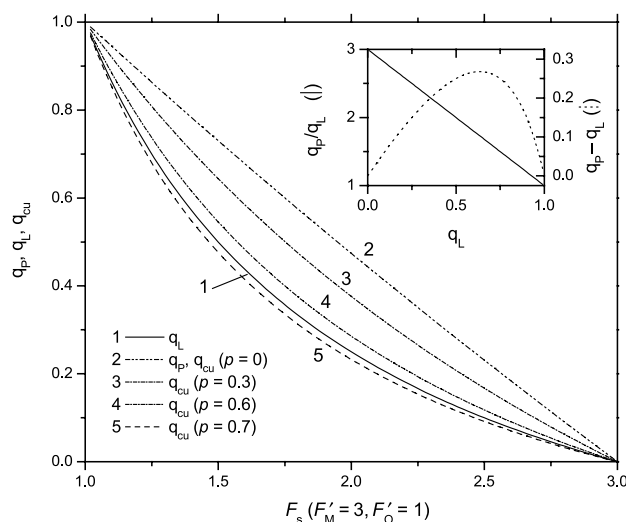


Figure 1. Comparison of estimators of the fraction of Q_A oxidized. An example is given, where F'_O was set at 1, F'_M was set at 3, and F_S was varied from 1 to 3. The fraction of open centers was estimated by three equations, yielding q_L (trace 1), q_p (trace 2) and q_{cu} (traces 3–5) as described in the text. Three values of p , the probability of exciton transfer (see text), were used for calculation of the q_{cu} parameter, 0.3 (trace 3), 0.6 (trace 4) and 0.7 (trace 5). Inset: comparison of q_p with q_L for the same calculations presented in the main figure. The solid line represents the ratio of q_p/q_L and the dotted line represents the difference between q_p and q_L .

except at the extremes with all centers open where $F'_O = F_S$, or closed where $F_S = F'_M$.

One can also derive similar equations based on PSU models intermediate between lakes and puddles. There are a large number of these, nicely reviewed and analyzed in Lazár (1999), partly because a complete description of the structure of the antenna systems is not available. For illustration, we chose the 'connected unit' model presented by Lavergne and Trissl (1995), see also Vavilin et al. (1998), in which each RC possesses its own antenna (like the puddle model), but with a defined probability for transfer of excitation energy from one antenna system to another (similar to the lake model). Rearranging Equation (31) from Lavergne and Trissl, assuming that it applies to all steady-state conditions, and using our nomenclature, an expression for the fraction of open centers, which we term q_{cu} (the 'cu' stands for connected units) is derived:

$$q_{cu} = \frac{F'_M - F_S}{J(F_S - F'_O) + F'_M - F'_O}, \quad (28)$$

where J is a connectivity parameter, related to the exciton transfer probability parameter, p , presented by Joliot and Joliot (1964), by:

$$J = \frac{p}{1 - p}. \quad (29)$$

When p is equal to zero, that is, for the puddle model, Equation (28) reduces to Equation (17). As

will be seen below, at appropriate values of p , $q_{cu} \approx q_L$.

Figure 1 compares the calculated fraction of Q_A in the oxidized state (i.e., fraction of open centers) as a function of F_S , for a hypothetical case where $F'_O = 1$ and $F'_M = 3$, based on the three different estimators (q_p , q_L and q_{cu}). In the case of q_{cu} , the relationship is shown at a range of values of p . As expected, both the q_p parameter and q_{cu} with $p = 0$, give identical, linear relationships between F_S and calculated fQ_{Aox} . Using either q_{cu} with $p > 0$ or q_L results in a hyperbolic relationship between F_S and fQ_{Aox} . Theoretically, Equation (29) allows for any value of p below 1 (J approaches infinity as p approaches unity), but in reality exciton transfer between connected units competes with deexcitation, and thus is restricted to values below about 0.7.

What parameter should be used to estimate Q_A redox state? It is clear, given the established non-puddle nature of the PSU in higher plants, that the q_p relationship will always overestimate the fraction of open centers except at the extreme boundary conditions, where $fQ_{Aox} = 0$ and $fQ_{Aox} = 1$. As illustrated in the inset to Figure 1, the differences in estimations can be quite large, especially at low fractions of open centers, conditions that are incurred under stress, where photoprotective mechanisms break down. There are many open questions about the regulation and limitations of photosynthesis under such extreme con-

ditions, where knowledge about the fraction of open centers would be vital to further understanding.

One could argue that the photosynthesis community should use parameters based on models such as the connected units model, which are better able to explain fluorescence induction experiments (Lazár 1999). (To remain consistent, this would technically require us to use a new estimator for ϕ_{II} , like the one described in Lavergne and Trissl (1995) but given that ϕ_{II} is insensitive to whether a puddle or lake model is chosen, it is unlikely to be much affected by choosing an intermediate model.) A value of p of about 0.6 has been suggested for higher plants, based on fluorescence experiments (reviewed in Lazár 1999), where for practical applications, the q_{cu} and q_L treatments should give quite similar relationships (see Figure 1). Thus, we suggest that the q_L be used for terrestrial higher plants. On the other hand, significantly smaller values for p (of about 0.3) have been suggested for some marine microalgae, especially under iron limitation (Vassiliev et al. 1995). In these cases, a parameter like q_{cu} as suggested in Lavergne and Trissl (1995) should be used.

We now extend our analysis to include estimates of photon fluxes down both photochemical and non-photochemical pathways. As noted earlier, the quantum yield of PS II photochemistry, ϕ_{II} , can be decomposed into two components, the fraction of open centers times the yield of open centers (see Genty et al. 1989). This has been used as a means of assessing whether overall yield decreases could be attributed to changes in antenna or reaction centers (Baker 1991; Andrews 1995; Oxborough and Baker 1997). Previously this was written as:

$$\phi_{II} = q_P \cdot \phi_{qp} \quad (30)$$

where ϕ_{qp} is the photochemical yield of open PS II centers. In fluorescence terms, Equation (30) is expressed as

$$\frac{F'_M - F_S}{F'_M} = \frac{F'_M - F_S}{F'_M - F'_O} \cdot \frac{F'_M - F'_O}{F'_M} \quad (31)$$

As shown above, this is in conflict mechanistically since q_P is only valid for a puddle model. Here, we suggest an alternate equation to estimate the fraction of open centers for terrestrial plants, and the quantum yield of open centers, using the lake model where

$$\phi_{II} = \frac{F'_M - F_S}{F'_M} = q_L \cdot \phi_{qL} \quad (32)$$

$$\phi_{qL} = \frac{F'_M - F_S}{F'_M - F'_O} \cdot \frac{F'_O}{F_S} \cdot \frac{F'_M - F'_O}{F'_M} \cdot \frac{F_S}{F'_O}$$

Also, the yield of open centers is considered dependent on thermal dissipation of energy by downregulation, and the fraction of absorbed light which is dissipated thermally (D) has been expressed as: 1 minus the yield of open centers (Demmig-Adams et al. 1996; Kato et al. 2003), where

$$D = 1 - \frac{F'_M - F_O}{F'_M} \quad (33)$$

but, this may overestimate thermal dissipation since according to the lake model the yield of open centers is defined in Equation (32), giving

$$D = 1 - \frac{F'_M - F_O}{F'_M} \cdot \frac{F_S}{F'_O} \quad (34)$$

However, this presents a problem because the ϕ_{qp} term is not independent from q_L , that is, ϕ_{qp} by itself does not tell us the fraction of excitons dissipated via the radiative pathway, and thus D is not useful in this context.

We start with the definition that the sum of all yields for dissipative processes for the energy absorbed by PS II is unity:

$$\phi_{II} + \phi_{NPQ} + \phi_{NO} \equiv 1, \quad (35)$$

where ϕ_{II} is the yield of photochemistry, ϕ_{NPQ} is the yield for dissipation by downregulation and ϕ_{NO} is the yield of other non-photochemical losses. In this approach, ϕ_{NO} reflects non-light induced (basal or dark) quenching processes. ϕ_{NPQ} can be calculated to reflect either all light-induced – q_E , photoinhibition (q_I), 3 carotenoid, 3 Chl, etc. – or only the rapidly reversible processes, considered to be regulatory (see below). Our approach also assumes that antenna associated with closed centers are identical to that associated with open centers (Lavergne and Trissl 1995). The approach is to solve for each yield so that the flux of photons down each pathway can be estimated. In other words, one might want to know what fraction of the excitons go via the NPQ pathway, whether a particular change in photochemical yield resulted primarily from changes in antenna downregulation or the fraction of open reaction centers.

Using the above Stern–Volmer approach, and now separating the rate constants for non-radiative decay by basal processes, k_d , and regulatory processes, k_{NPQ} ,

$$\phi_{II} = \frac{q_L k_{pi}}{k_f + k_d + k_{NPQ} + q_L k_{pi}}, \quad (36)$$

$$\phi_{NPQ} = \frac{k_{NPQ}}{k_f + k_d + k_{NPQ} + q_L k_{pi}}, \quad (37)$$

and

$$\phi_{NO} = \frac{k_f + k_d}{k_f + k_d + k_{NPQ} + q_L k_{pi}}. \quad (38)$$

Since ϕ_{II} is easily assessed using fluorescence, we separate that term:

$$1 - \phi_{II} = \phi_{NPQ} + \phi_{NO} = \frac{k_f + k_d + k_{NPQ}}{k_f + k_d + k_{NPQ} + q_L k_{pi}}, \quad (39)$$

$$1 - \phi_{II} = \phi_{NPQ} + \frac{k_f + k_d}{k_f + k_d + k_{NPQ} + q_L k_{pi}}, \quad (40)$$

and

$$\begin{aligned} \phi_{NPQ} &= 1 - \phi_{II} - \phi_{NO} \\ &= 1 - \phi_{II} - \frac{k_f + k_d}{k_f + k_d + k_{NPQ} + q_L k_{pi}}. \end{aligned} \quad (41)$$

Now we derive an equation for ϕ_{NO} . We take advantage of the fact that NPQ is also readily estimated using fluorescence. In fluorescence terms, NPQ is empirically defined as

$$NPQ = \frac{F_M - F'_M}{F'_M}. \quad (42)$$

Again using a Stern–Volmer approach, it can be shown that

$$NPQ = \frac{k_{NPQ}}{k_f + k_d + k_{isc}}. \quad (43)$$

By taking the reciprocal of ϕ_{NO} , we can easily eliminate the k_{NPQ} term:

$$\frac{1}{\phi_{NO}} = \frac{k_f + k_d + k_{isc} + k_{NPQ} + q_L k_{pi}}{k_f + k_d + k_{isc}}, \quad (44)$$

$$\frac{1}{\phi_{NO}} = NPQ + \frac{k_f + k_d + k_{isc} + q_L k_{pi}}{k_f + k_d + k_{isc}}, \quad (45)$$

$$\frac{1}{\phi_{NO}} = NPQ + 1 + q_L \frac{k_{pi}}{k_f + k_d + k_{isc}}. \quad (46)$$

At F_O , $q_L = 1$, and $NPQ = 0$, whereas at F_M , both $q_L = 0$ and $NPQ = 0$. From this, we can obtain the values of $(k_f + k_d + k_{isc})$ and k_{pi} .

$$\frac{F_M}{S} = \phi_f(F_M) = \frac{k_f}{k_f + k_d + k_{isc}}, \quad (47)$$

$$\frac{F_O}{S} = \phi_f(F_O) = \frac{k_f}{k_f + k_d + k_{isc} + 1 \cdot k_{pi}}, \quad (48)$$

$$\begin{aligned} \frac{F_M}{F_O} - 1 &= \left(\frac{1}{F_O} - \frac{1}{F_M} \right) F_M \\ &= \frac{k_{pi}}{k_f} \cdot \frac{k_f}{k_d + k_f + k_{isc}} = \frac{k_{pi}}{k_d + k_f + k_{isc}}, \end{aligned} \quad (49)$$

$$\frac{1}{\phi_{NO}} = NPQ + 1 + q_L \left(\frac{F_M}{F_O} - 1 \right), \quad (50)$$

$$\phi_{NO} = \frac{1}{NPQ + 1 + q_L (F_M/F_O - 1)}. \quad (51)$$

From this we can calculate ϕ_{NPQ} , as:

$$\phi_{NPQ} = 1 - \phi_{II} - \frac{1}{NPQ + 1 + q_L (F_M/F_O - 1)}. \quad (52)$$

Inputs for calculation of ϕ_{NPQ} include F_M and F'_M (Equations (42) and (52)). If F_M is taken from the completely dark adapted state, ϕ_{NPQ} will reflect all light-induced quenching mechanisms. Alternatively, to calculate that part of non-photochemical quenching due to down-regulation, F_M can be taken following illumination after a short period (e.g., 15 min in the dark, a measurement termed F''_M), which indicates the fast relaxing component of non-photochemical quenching (i.e., q_E). If this is the primary means of dissipating excess energy, then ϕ_{NPQ} will account for most non-photochemical losses and ϕ_{NQ} will be largely due to the basal intrinsic non-radiative decay. However, if energy dependent quenching is low, and other non-photochemical losses occur, then ϕ_{NPQ} will be lower and ϕ_{NQ} will increase. An excellent illustration of the latter situation is in the npq4 mutants of Arabidopsis which lack PsbS protein and energy dependent quenching (Li et al. 2002).

In Figure 2, we present results showing the fraction of photons which are absorbed by PS II and utilized by photochemical and non-photochemical means in a light response curve with tobacco calculated for ϕ_{II} , ϕ_{NPQ} , and ϕ_{NO} , the sum of which equals to 1, as well as q_L . Figure 2A is under sub-atmospheric levels of CO_2 (100 μ bar) and 2% O_2 , where photochemistry is more limited by the regeneration of ADP and NADP by the dark reactions, compared to Figure 2B under supra-atmospheric levels of CO_2 (460 μ bar) and 2% O_2 .

The results show that under lower CO_2 with increasing light there is a rapid drop in the yield of PS II and a corresponding rapid rise in the yield of NPQ, together with a decrease in q_L . Under high CO_2 the capacity for photochemistry is higher which is indicated by the slower drop in the yield of PS II and q_L with increasing light, and slower rise in the yield of NPQ with increasing light intensity. In both cases the yield of ϕ_{NO} was similar and changed little with varying light (consistent with a basal non-photochemical loss), indicating compensatory changes in ϕ_{II} and ϕ_{NPQ} . An

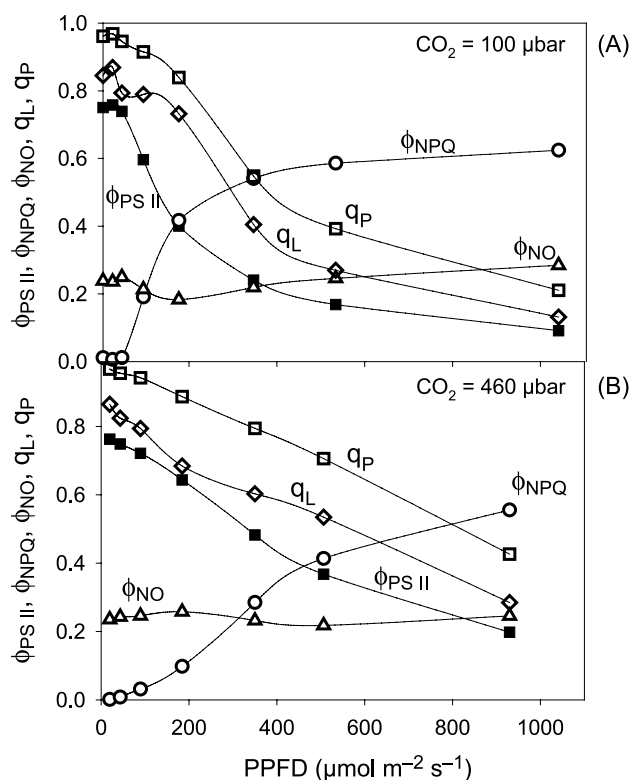


Figure 2. Effects of changing light intensities on the relationships among Φ_{II} , Φ_{NPQ} and Φ_{NO} . The results are with *Nicotiana tabacum* (tobacco). Leaf temperature was 25 °C and O_2 was 2%. PPFD = photosynthetic photon flux density incident on the leaf. Gas exchange measurements were with a two-channel fast-response system (Fast-Est, Tartu, Estonia) including fiber optics for chlorophyll fluorescence measurements and a PAM 101 chlorophyll fluorometer (Heinz Walz, Effeltrich, Germany) as described by Laisk and Edwards (1997). Φ_{II} , Φ_{NO} and Φ_{NPQ} were calculated by Equations (10), (51) and (52), respectively.

alternative method of calculating the yield of non-photochemical quenching, introduced by Genty and coworkers has been utilized by Laisk et al. (1997) where, $Y_N = F_S/F'_M - F_S/F_M$, or $Y_N = NPQ (F_S/F_M)$. Calculations of Y_N , compared to Φ_{NPQ} , gave similar yields of non-photochemical quenching (results not shown).

Particularly interesting features of the data in Figure 2 are deviations between Φ_{II} and q_L upon large changes in Φ_{NPQ} . This is especially noticeable in Figure 2A at PPFD between 0 and 200 PPFD where a large increase in Φ_{NPQ} induces a decrease in Φ_{II} with little change in q_L , that is, under these conditions, the PS II reaction centers stay essentially completely open and photosynthetic yield is determined largely by changes in NPQ. At higher light intensities, Φ_{II} and q_L approach each other, indicating that the capacity of NPQ to regulate light capture is saturated. Also interesting is the relative stability of Φ_{NO} , which results from compensatory changes in Φ_{II} and Φ_{NPQ} .

Figure 2 also provides a real data comparison of the q_P and q_L parameters. q_L is consistently lower than q_P over the entire range of light intensities, and at both CO_2 levels. The effect was substantial, particularly at high light, where q_P was about twice q_L . This analysis demonstrates that choosing the correct model will often have very significant effects on the interpretation of such fluorescence data.

Conclusions

In summary, we suggest that the simple q_L parameter is appropriate for estimation of the fraction of open centers, and q_{qP} for the yield of open centers, under a wide range of conditions, where the Φ_{II} parameter has been shown to give reasonable estimates of PS II quantum efficiency. Of course, more complexity may be merited under special conditions. We have also presented new equations for calculating Φ_{NPQ} and

ϕ_{NO} , the fraction of excitons dissipated via the two competing non-productive pathways, that induced by activation of downregulatory processes *versus* other non-photchemical losses.

As mentioned above, light harvesting by PSU may be better approximated by connected-units model. Also, it is assumed that k_{pi} is constant, but it may be variable or heterogeneous, depending upon conditions. On the other hand, adherence to Occam's Razor requires that we avoid whenever possible the multiplication of free-fitting parameters, especially without the means for independent assessment of their values, so we prefer the simpler models unless we have evidence that a more complex model is needed.

Acknowledgements

The authors would like to thank Mr Thomas Avenson, Dr J. Berry, Dr J. Cruz, Dr B. Demmig-Adams, Dr P. Falkowski, Dr K. Niyogi, Dr B. Green and Dr P. Juneau for stimulating discussions and critical readings of the manuscript. This work was supported by grants from the US Department of Energy (DE-FG03-98ER20299) and the NSF (IBN 0131098).

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