

Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method

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Abstract

Different fluorescence-based parameters are described in the literature for the quantum yields of non-photochemical energy conversion in PS II, ϕ_{NPQ} and ϕ_{NO} , which are complementary with the quantum yield of photochemical energy conversion, ϕ_{II} . With modern PAM fluorometers, like the Dual-PAM-100, these quantum yields are calculated online with every Saturation Pulse and displayed as the parameters Y(II), Y(NPQ) and Y(NO), the sum of which is always unity: $Y(II) + Y(NPQ) + Y(NO) = 1$. These quantum yields comprehensively describe the fate of excitation energy in PS II and allow deep insights into the plant's capacity to cope with excess excitation energy. While so far calculation of Y(NPQ) and Y(NO) by the PAM software has been based on rather complex expressions derived by Kramer *et al.* (2004) *Photosynth Res* 79: 209-218, more simple expressions derived by Genty *et al.* (1996) (abstract no P28 of Robert Hill Symposium, Sheffield, UK) were advocated by Hendrickson *et al.* (2004) *Photosynth Res* 82: 73-81, with Y(NO) and Y(NPQ) being expressed by the fluorescence yields F , F_m and F_m' , measured by PAM fluorometry and the Saturation Pulse method: $Y(NO) = F/F_m$ and $Y(NPQ) = F/F_m' - F/F_m$. Notably these expressions do not require knowledge of F_o' . Here we show that the expressions of Kramer *et al.* (2004) can be transformed into the simple expressions of Genty *et al.* (1996) and that these are not only valid for a lake model, but also for a puddle model, as already known for $Y(II) = (F_m' - F)/F_m'$. The meaning of Y(NO) is discussed and an example of characteristic changes of the complementary PS II quantum yields after heat-pretreatment is presented.

Abbreviations

ΔF	Increase of fluorescence yield, $F_m' - F$, induced by a Saturation Pulse
ϕ_{II}	Quantum yield of photochemical energy conversion in PS II, equivalent to Y(II)
ϕ_{NO}	Quantum yield of non-regulated non-photochemical energy loss in PS II, equivalent to Y(NO)
ϕ_{NPQ}	Quantum yield of regulated non-photochemical energy loss in PS II, equivalent to Y(NPQ)
F	Fluorescence yield measured briefly before application of a Saturation Pulse
F_m	Maximal fluorescence yield of dark-adapted sample with all PS II centers closed
F_m'	Maximal fluorescence yield of illuminated sample with all PS II centers closed
F_o	Minimal fluorescence yield of dark-adapted sample with all PS II centers open
F_o'	Minimal fluorescence yield of illuminated sample with all PS II centers open
F_v	Variable fluorescence of dark-adapted sample, $F_m - F_o$
k_D	Rate constant of radiationless decay to ground state not involving NPQ-mechanism
k_F	Rate constant of radiative deexcitation
k_i	Rate constants of competing first order decay reactions of excited singlet state in PS II
k_{NPQ}	Rate constant of deexcitation by non-photochemical trapping
k_P	Rate constant of deexcitation by photochemical trapping
NPQ	Non-photochemical quenching parameter describing regulated dissipation of excess energy
PAM	Pulse-Amplitude-Modulation
PAR	Photosynthetically active radiation measured in $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$
PS I	Photosystem I, which does not show variable fluorescence yield
PS II	Photosystem II, which shows variable fluorescence yield
q_L	Parameter estimating the fraction of open PS II centers based on a lake model
SP	Saturation Pulse serving for transient full closure of PS II centers
Y	Quantum yield calculated online with the help of PAM fluorometers

Introduction

PAM fluorometry and the Saturation Pulse (SP) method have been widely used to analyze the photosynthetic performance of green plants (for reviews, see Schreiber 2004; Baker 2008). In contrast to earlier approaches, based on dark-light induction kinetics (Kautsky effect), SP analysis can provide information during steady-state illumination, *i.e.* also under normal daylight conditions. The obtained information is based on two closely linked measurements of chlorophyll fluorescence yield, shortly before and during a pulse of saturating light. The SP induces maximal fluorescence yields, F_m or F_m' , respectively, depending on whether a dark-adapted or illuminated sample is used. Shortly before the SP, the fluorescence yields F_0 or F are measured, with dark-adapted or illuminated samples, respectively (for nomenclature see van Kooten and Snel 1990). Based on this basic information, various fluorescence parameters have been defined that have proven useful for characterization of photosynthetic performance. Particularly useful expressions were derived for the maximal Photosystem II (PS II) quantum yield of dark-adapted samples, $F_v/F_m = (F_m - F_0)/F_m$ (Kitajima and Butler 1975), the effective PS II quantum yield of illuminated samples, $\phi_{II} = (F_m' - F)/F_m' = \Delta F/F_m'$ (Genty *et al.* 1989), and the extent of "Stern-Volmer" non-photochemical fluorescence quenching $NPQ = (F_m - F_m')/F_m'$ (Bilger and Björkmann 1990).

Genty *et al.* (1996) first presented expressions based on basic fluorescence parameters that describe the partitioning of absorbed excitation energy in PS II between three fundamental pathways, expressed in terms of the complementary quantum yields of

- ϕ_{II} , photochemical conversion,
- ϕ_{NPQ} , regulated thermal energy dissipation related to NPQ,
- ϕ_{NO} , "primarily constitutive losses", corresponding to the sum of non-regulated heat dissipation and fluorescence emission:

$$\phi_{II} + \phi_{NPQ} + \phi_{NO} = 1 \quad (1)$$

$$\phi_{II} = (F_m' - F)/F_m' = \Delta F/F_m' \quad (2)$$

$$\phi_{NPQ} = F/F_m' - F/F_m \quad (3)$$

$$\phi_{NO} = F/F_m \quad (4)$$

As already pointed out by Genty *et al.* (1996), these three complementary quantum yields can be calculated without knowledge of F_0 and F_0' . Considering the fundamental information obtained by these expressions, it may be surprising that, except for the well-known "Genty-parameter" $\Delta F/F_m'$, their existence has not been well known even among experts. For example, they are not mentioned in a recent review article on chlorophyll fluorescence as a probe of photosynthesis *in vivo* (Baker 2008). The main reason for the unawareness of most users of PAM fluorometry is the fact that this information

was published in form of a one-page poster abstract, without any room for detailed derivations and background information on the underlying assumptions. Exactly the same abstract was published again half a year later by Cailly *et al.* (1996).

More recently, Kramer *et al.* (2004) derived expressions for ϕ_{NO} and ϕ_{NPQ} assuming a lake model (connected photosynthetic units, PS II reaction centers in a common pigment bed). Their derivation was based on a previous definition of the quenching coefficient q_L that describes the fraction of open PS II centers in a lake model:

$$q_L = \frac{(F_m' - F) \cdot F_0'}{(F_m' - F_0') \cdot F} \quad (5)$$

$$\phi_{NO} = \frac{1}{NPQ + 1 + q_L \cdot \left(\frac{F_m}{F_0} - 1\right)} \quad (6)$$

$$\phi_{NPQ} = 1 - Y(II) - \frac{1}{NPQ + 1 + q_L \cdot \left(\frac{F_m}{F_0} - 1\right)} \quad (7)$$

Since mid-2004 these expressions of complementary PS II quantum yields have been incorporated in the user software of several commercial PAM fluorimeters, like Imaging-PAM, PAM-2100 and Dual-PAM-100, as the developers of this software had not been aware of the more simple expressions of Genty *et al.* (1996). In any case, the concept of complementary PS II quantum yields proved quite useful for the analysis of the photosynthetic performance of plants based on the SP method. Furthermore, analogous expressions of complementary PS I quantum yields were developed, which can be determined by a similar SP method *via* P700 measurements (Klughammer and Schreiber 2008; Schreiber and Klughammer 2008). For the sake of presentation on the user surface of the PAM software, the complementary quantum yields were termed Y(I), Y(ND) and Y(NA) for PS I and Y(II), Y(NPQ) and Y(NO) for PS II. In the following we will use this terminology, with the Y standing for Yield.

A complicating aspect in the practical use of the complementary PS II quantum yields defined by Kramer *et al.* (2004) is the occurrence of q_L in the equations of Y(NO) and Y(NPQ), as the equation of q_L contains the parameter F_0' , reliable determination of which has been problematic. Hendrickson *et al.* (2004) re-discovered the simple fluorescence expressions of Genty *et al.* (1996), which do not contain F_0' , and presented detailed derivations based on a lake model. They empirically compared the complementary quantum yields obtained *via* the Kramer *et al.* and Genty *et al.* expressions. While there was close correlation between the Y(NPQ) values irrespective of temperature and light intensity, Y(NO) values tended to be lower when determined *via* the Kramer *et al.*

expressions. This was explained by Hendrickson *et al.* (2004) by possible errors in F_o' determination.

For the normal user of PAM fluorometry it is difficult to judge the practical relevance of the numerous papers published on competitive photochemical and non-photochemical energy conversion in PS II over the past 20 years (besides the already cited papers, see also Weis and Berry 1987, Schreiber and Bilger 1994, Demmig-Adams *et al.* 1996, Laisk *et al.* 1997). It is the purpose of the present communication to demonstrate that very fundamental information on the fate of excitation energy in PS II can be obtained with very simple means and that no detailed background knowledge on the complex dynamics of exciton equilibration in PS II is required in order to understand its significance. Without claiming any priority on new scientific insights, we want to present a consistent body of derivations of fluorescence expressions for the complementary quantum yields $Y(II)$, $Y(NPQ)$ and $Y(NO)$, which can be understood at undergraduate level. We present detailed step-by-step derivations and arithmetic transformation, which for experts may appear trivial, but can be a great help for non-specialists. We will show that the simple expressions of Genty *et al.* (1996) and Hendrickson *et al.* (2004) can be deduced from the complex expressions of Kramer *et al.* (2004) and are not only valid in the lake model, but in the puddle model as well.

Assumptions for derivations

Our derivations are based on a very general model of competitive first order deexcitation reactions in PS II, assuming a common pigment bed (Fig. 1), *i.e.* like Genty *et al.* (1996), Kramer *et al.* (2004) and Hendrickson *et al.* (2004), we first assume a lake model. Later we will show that the derived equations are valid in a puddle model as well.

Photochemical "trapping" of excitons in P680 *via* charge separation and stabilization formally is treated like non-photochemical "trapping" by zeaxanthin (Demmig-Adams 1990). Four different first order deexcitation pathways with the rate constants k_P (photochemical trapping), k_{NPQ} (regulated dissipation by non-photochemical trapping, NPQ-mechanism), k_F (radiative decay yielding fluorescence), and k_D (radiationless decay to ground state not involving NPQ-mechanism) are considered. The values of k_P and k_{NPQ} depend on the concentrations of photochemical and non-photochemical traps. It is assumed that $k_P = 0$ when all photochemical traps are closed by an SP and that $k_{NPQ} = 0$ when all non-photochemical traps have disappeared after dark-adaptation.

The rate constants k_D and k_F are considered to remain unchanged between measurements of F_o , F_m after dark-adaptation and measurements of F , F_m' , F_o' in the illuminated state. While k_{NPQ} , just like k_P , displays changes during continuous illumination, as the concentrations of photochemical and non-photochemical traps change, it is assumed that *during an SP* k_{NPQ} does not change.

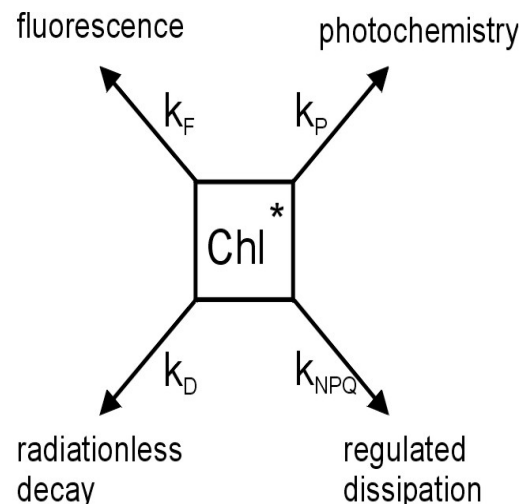


Fig. 1. Competitive first order deexcitation reactions in PS II.

The only change occurring during an SP is the suppression of k_P ($k_P = 0$). Photochemical trapping is considered an irreversible reaction. While it is known, that in reality the exciton dynamics are more accurately described by an exciton-radical-pair-equilibrium model for PS II (Schatz *et al.* 1988), Lavergne and Trissl (1995) have shown that a more sophisticated approach, in which reversibility is considered, essentially leads to the same expressions of fluorescence parameters as in the irreversible case. Furthermore, while we are aware of PS II heterogeneity (Lavergne and Briantais 1996) and contribution of PS I fluorescence to the measured overall fluorescence signals (Pfundel 1998, Gilmore *et al.* 2000), for the sake of clarity, these complicating aspects will not be considered in the presented definitions and derivations.

Definitions for derivations

First the quantum yield of fluorescence in general is defined, which reflects the probability of the radiative decay set in proportion to the probability of the total decay:

$$\phi_F = \frac{k_F}{k_F + k_D + k_{NPQ} + k_P} = \frac{k_F}{\sum k_i} \quad (8)$$

For the maximal fluorescence yields measured with the help of an SP in dark-adapted and illuminated samples it follows:

$$\phi_F(F_m) = \frac{k_F}{k_F + k_D}; \quad k_P = 0; \quad k_{NPQ} = 0 \quad (9)$$

$$\phi_F(F_m') = \frac{k_F}{k_F + k_D + k_{NPQ}}; \quad k_P = 0 \quad (10)$$

The complementary quantum yields $Y(II)$, $Y(NPQ)$ and $Y(NO)$ can be defined using the same rate constants:

$$Y(\text{II}) \equiv \phi_{\text{II}} = \frac{k_p}{\sum k_i} \quad (11)$$

$$Y(\text{NPQ}) \equiv \phi_{\text{NPQ}} = \frac{k_{\text{NPQ}}}{\sum k_i} \quad (12)$$

$$Y(\text{NO}) \equiv \phi_{\text{NO}} = \frac{k_F + k_D}{\sum k_i} \quad (13)$$

$$Y(\text{NO}) + Y(\text{NPQ}) + Y(\text{II}) = 1 \quad (14)$$

It should be noted that $Y(\text{NO})$ describes the combined pathways of radiative and non-radiative deexcitation reactions, which do not lead to photochemical energy conversion and are not involving the NPQ-mechanism.

Derivations

On the basis of these definitions, $Y(\text{NO})$, $Y(\text{NPQ})$ and $Y(\text{II})$ can be expressed in terms of fluorescence yield parameters:

$$\begin{aligned} Y(\text{NO}) &= \frac{k_F + k_D}{\sum k_i} = \frac{k_F \cdot (k_F + k_D)}{k_F \cdot \sum k_i} \\ &= \frac{k_F}{\sum k_i} = \frac{\phi_F}{\phi_F(F_m)} = \frac{F}{k_F + k_D} \end{aligned} \quad (15)$$

Hence, $Y(\text{NO})$ simply corresponds to the F_m -scaled fluorescence yield, as previously shown by Genty *et al.* (1996) and Hendrickson *et al.* (2004).

Analogously, the sum of $Y(\text{NO})$ and $Y(\text{NPQ})$ corresponds to F_m' scaled fluorescence yield:

$$\begin{aligned} Y(\text{NO}) + Y(\text{NPQ}) &= \frac{k_F + k_D + k_{\text{NPQ}}}{\sum k_i} \\ &= \frac{k_F \cdot (k_F + k_D + k_{\text{NPQ}})}{k_F \cdot \sum k_i} = \frac{\phi_F}{\phi_F(F_m')} = \frac{F}{F_m'} \end{aligned} \quad (16)$$

By combining equations (15) and (16), the following expressions for $Y(\text{NPQ})$ are obtained, as previously derived by Genty *et al.* (2004) and Hendrickson *et al.* (2004):

$$\begin{aligned} Y(\text{NPQ}) &= \frac{F}{F_m'} - Y(\text{NO}) = \frac{F}{F_m'} - \frac{F}{F_m} \\ &= Y(\text{NO}) \cdot \left(\frac{F_m'}{F_m} - 1 \right) = Y(\text{NO}) \cdot \text{NPQ} \end{aligned} \quad (17)$$

Finally, resolving equation (14) for $Y(\text{II})$ and inserting the fluorescence ratio expressions for $Y(\text{NO})$ and $Y(\text{NPQ})$ of equations (15) and (17), respectively, the well-known Genty equation (Genty *et al.* 1989) is obtained:

$$\begin{aligned} Y(\text{II}) &= 1 - (Y(\text{NO}) + Y(\text{NPQ})) \\ &= 1 - \frac{F}{F_m'} = \frac{F_m' - F}{F_m'} = \frac{\Delta F}{F_m'} \end{aligned} \quad (18)$$

As shown by Kramer *et al.* (2004), the validity of equation (18) is independent of the applied model. We previously showed that this equation can be derived without any assumptions on a particular model (Schreiber *et al.* 1995a). The only prerequisite was that during an SP the ratio between heat dissipation and fluorescence does not change. It can be shown that this is equivalent to the basic assumption of independent competitive first order deexcitation pathways, on which the present derivation is based.

Discussion

Comparison of the different expressions of $Y(\text{NO})$ and $Y(\text{NPQ})$

The question arises whether the largely different expressions for $Y(\text{NO})$ derived by Kramer *et al.* (2004) and Genty *et al.* (1996) are equivalent under all conditions. Hendrickson *et al.* (2004) just showed empirically that similar results are obtained. Here we will demonstrate that the complex expressions of Kramer *et al.* (2004) boil down to the simple expressions of Genty *et al.* (1996) when F_o' is expressed by the directly measurable fluorescence parameters F_o , F_m and F_m' .

First the quantum yields of F_o and F_o' have to be defined:

$$\phi_F(F_o) = \frac{k_F}{k_F + k_D + k_{po}}; \quad k_{\text{NPQ}} = 0 \quad (19)$$

$$\phi_F(F_o') = \frac{k_F}{k_F + k_D + k_{\text{NPQ}} + k_{po}} \quad (20)$$

k_{po} is the particular rate constant of photochemical energy conversion in PS II with all centers being open, which is assumed to be independent of the preillumination state:

$$k_{po} = k_p(F_o) = k_p(F_o') \quad (21)$$

When equations (15) and (16) are combined with equation (4), the following expression of $Y(\text{NPQ})$ is obtained:

$$\begin{aligned} \frac{F}{F_o'} - \frac{F}{F_o} &= \frac{\phi_F}{\phi_F(F_o')} - \frac{\phi_F}{\phi_F(F_o)} \\ &= \frac{k_{\text{NPQ}}}{k_F + k_D + k_{\text{NPQ}} + k_p} = \phi_{\text{NPQ}} \equiv Y(\text{NPQ}) \end{aligned} \quad (22)$$

We note that this constitutes a derivation of an expression of $Y(\text{NPQ})$ that is independent of that applied in the derivation of equation (17), so that by combining equations (17) and (22) the sought-after expression for F_o' can be obtained:

$$Y(\text{NPQ}) = \frac{F}{F'_m} - \frac{F}{F_m} = \frac{F}{F'_o} - \frac{F}{F_o}$$

$$\Rightarrow \frac{1}{F'_m} - \frac{1}{F_m} = \frac{1}{F'_o} - \frac{1}{F_o} \quad (23)$$

$$\frac{F_o}{F'_o} = \frac{F_o}{F'_m} + 1 - \frac{F_o}{F_m} = \frac{F_o}{F'_m} + \frac{F_m - F_o}{F_m} = \frac{F_o}{F'_m} + \frac{F_v}{F_m}$$

$$\Rightarrow F'_o = \frac{F_o}{\frac{F_v}{F_m} + \frac{F_o}{F'_m}} \quad (24)$$

The same expression for F'_o was previously derived in a slightly different way by Oxborough and Baker (1997).

Before this expression of F'_o can be entered into equation (6), the q_L term has to be expressed in terms of fluorescence yields:

$$q_L \cdot \left(\frac{F_m}{F_o} - 1 \right) = q_L \cdot \frac{F_m - F_o}{F_o} = q_L \cdot \frac{F_v}{F_o}$$

$$= \frac{(F'_m - F) \cdot F'_o}{(F'_m - F'_o) \cdot F} \cdot \frac{F_v}{F_o} = \frac{F'_m - 1}{\frac{F'_m}{F'_o} - 1} \cdot \frac{F_v}{F_o} \quad (25)$$

By replacing F'_o in (25) we obtain:

$$q_L \cdot \left(\frac{F_m}{F_o} - 1 \right) = \frac{\frac{F'_m - 1}{F} \cdot \frac{F_v}{F_o}}{\frac{F'_m}{F_o} \left(\frac{F_v}{F_m} + \frac{F_o}{F'_m} \right) - 1} \cdot \frac{F_v}{F_o}$$

$$= \frac{\frac{F'_m - 1}{F} \cdot \frac{F_v}{F_o}}{\frac{F'_m \cdot F_v}{F_o \cdot F_m} + 1 - 1} \cdot \frac{F_v}{F_o} = \frac{F'_m - 1}{\frac{F'_m}{F_m}} = \frac{F_m}{F} - \frac{F_m}{F'_m} \quad (26)$$

Using (26) and the definition of NPQ, we can transform the Kramer *et al.* (2004) equation for $Y(\text{NO})$:

$$Y(\text{NO}) = \frac{1}{\text{NPQ} + 1 + q_L \cdot \left(\frac{F_m}{F_o} - 1 \right)}$$

$$= \frac{1}{\frac{F_m}{F'_m} - 1 + 1 + \frac{F_m}{F} - \frac{F_m}{F'_m}} = \frac{1}{\frac{F_m}{F}} = \frac{F}{F_m} \quad (27)$$

As can be seen, the simple equation $Y(\text{NO}) = F/F_m$ (Genty *et al.* 2004) indeed can be obtained by transformation of the complex expression for $Y(\text{NO})$ derived by Kramer *et al.* (2004).

Validity in lake and puddle model

Kramer *et al.* (2004) showed that the expression for $Y(\text{II})$ (equation 18) is valid for the lake model as well as for the so-called puddle model. On the other hand, in view of equations (6) and (7) one may think that $Y(\text{NO})$ and $Y(\text{NPQ})$ are dependent on the chosen model because of their dependence on q_L . However, as we will show below, our expressions of $Y(\text{NO})$ and $Y(\text{NPQ})$ are valid not only for the lake model (as shown above) but for the puddle model as well.

In the case of the puddle model, photosynthetic units (PSU) with open and closed reaction centers have to be differentiated. If we call q the fraction of open centers, equation (8) must be replaced by equation (28), while equations (9) and (10) are still valid in the puddle model:

$$\phi_F = q \cdot \frac{k_F}{\sum k_i} + (1-q) \cdot \frac{k_F}{k_F + k_D + k_{\text{NPQ}}} \quad (28)$$

$$\phi_F(F_m) = q \cdot \frac{k_F}{k_F + k_D} + (1-q) \cdot \frac{k_F}{k_F + k_D}$$

$$= \frac{k_F}{k_F + k_D}; \quad k_P = 0; \quad k_{\text{NPQ}} = 0 \quad (29)$$

$$\phi_F(F'_m) = q \cdot \frac{k_F}{k_F + k_D + k_{\text{NPQ}}}$$

$$+ (1-q) \cdot \frac{k_F}{k_F + k_D + k_{\text{NPQ}}}$$

$$= \frac{k_F}{k_F + k_D + k_{\text{NPQ}}}; \quad k_P = 0 \quad (30)$$

Using equations (28) and (29), we find:

$$\frac{F}{F_m} = \frac{\phi_F}{\phi_F(F_m)}$$

$$= q \cdot \frac{k_F + k_D}{\sum k_i} + (1-q) \cdot \frac{k_F + k_D}{k_F + k_D + k_{\text{NPQ}}} = Y(\text{NO}) \quad (31)$$

Furthermore, by combining equations (28) and (30), we find:

$$\frac{F}{F'_m} = \frac{\phi_F}{\phi_F(F'_m)}$$

$$= q \cdot \frac{k_F + k_D + k_{\text{NPQ}}}{\sum k_i} + (1-q) \cdot \frac{k_F + k_D + k_{\text{NPQ}}}{k_F + k_D + k_{\text{NPQ}}}$$

$$= q \cdot \frac{k_F + k_D + k_{\text{NPQ}}}{\sum k_i} + (1-q) \quad (32)$$

The definition of $Y(\text{NPQ})$ in the puddle model is:

$$Y(\text{NPQ}) = q \cdot \frac{k_{\text{NPQ}}}{\sum k_i} + (1-q) \cdot \frac{k_{\text{NPQ}}}{k_F + k_D + k_{\text{NPQ}}} \quad (33)$$

Hence, for the sum of $Y(\text{NPQ})$ and $Y(\text{NO})$ we find:

$$\begin{aligned} Y(\text{NPQ}) + Y(\text{NO}) &= q \cdot \frac{k_{\text{NPQ}}}{\sum k_i} + (1-q) \cdot \frac{k_{\text{NPQ}}}{k_F + k_D + k_{\text{NPQ}}} \\ &+ q \cdot \frac{k_F + k_D}{\sum k_i} + (1-q) \cdot \frac{k_F + k_D}{k_F + k_D + k_{\text{NPQ}}} \\ &= q \cdot \frac{k_F + k_D + k_{\text{NPQ}}}{\sum k_i} + (1-q) \cdot \frac{k_F + k_D + k_{\text{NPQ}}}{k_F + k_D + k_{\text{NPQ}}} \\ &= q \cdot \frac{k_F + k_D + k_{\text{NPQ}}}{\sum k_i} + (1-q) = \frac{F}{F_m'} \end{aligned} \quad (34)$$

This is equivalent to equation (16) derived above on the basis of the lake model, so that the combination of equations (34) and (31) give the same expression for $Y(\text{NPQ})$. Hence, the simple expressions of $Y(\text{NO})$ and $Y(\text{NPQ})$ first derived by Genty *et al.* (1996) are more general than those derived by Kramer *et al.* (2004).

Finally it should be noted that equation (22) is not generally valid in the puddle model. We found an expression for $[(F/F_0') - (F/F_0)]$ which equals $Y(\text{NPQ})$ only when $q=1$ or $q=0$ (not shown here). Hence the expression for F_0' previously derived by Oxborough and Baker (1997) seems to be a consequence of the lake model and does not hold for the puddle model.

Meaning of $Y(\text{NO})$

While the quantum yields of photochemical and non-photochemical quenching, $Y(\text{II})$ and $Y(\text{NPQ})$, respectively, have been extensively discussed in the literature, not much information is available on the meaning of $Y(\text{NO})$. According to its original definition by Genty *et al.* (1996), $Y(\text{NO})$ corresponds to "the quantum efficiency of non-photochemical quenching of excitation via primarily constitutive losses (i.e. thermal energy dissipation that occurs even when potential photochemical yield is maximal and fluorescence emission)". In the approach of Kramer *et al.* (2004) the quantum yield $Y(\text{NO})$ "lumps together other light-induced processes that contribute to non-radiative decay including intrinsic (dark or basal) loss, and long-term quenching caused by photoinhibition or other processes including $^3\text{carotenoids}$, $^3\text{Chl}^*$, etc.". According to Hendrickson *et al.* (2004) $Y(\text{NO})$ corresponds to the sum of the quantum yields of fluorescence and "constitutive thermal dissipation", with the latter being "constitutive in the sense that short-term changes in light intensity do not alter its efficiency". These interpretations are somewhat misleading, as $Y(\text{NO})$ in principle can display large changes, complementary to simultaneous changes in $Y(\text{II})$ and $Y(\text{NPQ})$. While the rate con-

stants k_D and k_F are assumed to be invariable during illumination, the derived quantum yield $Y(\text{NO})$ obviously is not. When, for example, PS II is inhibited by a herbicide like Diuron (DCMU), the decrease in $Y(\text{II})$ is quantitatively compensated by a corresponding increase of $Y(\text{NO})$, which will approach unity. In the work of Genty *et al.* (1996), Kramer *et al.* (2004) and Hendrickson *et al.* (2004) only steady-state conditions are considered. In this case, $Y(\text{NO})$ indeed shows little variation only. Considering $Y(\text{NO}) = F/F_m$, this means that steady-state fluorescence yield, F , tends to be close to the dark-adapted yield, F_0 . As pointed out by Laisk *et al.* (1997), "this guarantees that the average lifetime of excitation stays constantly at a minimum, whether or not photochemistry is possible. Minimal lifetimes of excitation help to avoid the formation of triplet state chlorophylls, production of reactive oxygen species, and radicals." Hence, when considering steady-state conditions, the notion of $Y(\text{NO})$ characterizing "constitutive loss processes" appears justified. Strictly speaking, this applies to the dark-adapted state only, when $Y(\text{NO}) = F_0/F_m$, with healthy green leaves showing values of about 0.2. The quantum yield of the actual "constitutive loss" or "unavoidable loss" can be assumed to be lower than 0.2, as part of the measured fluorescence originates from PS I (Pfündel 1998, Gilmore *et al.* 2000). If the PS I contribution is known, the PAM user software allows to correct for this contribution.

We would like to note that there is no reason to restrict measurements of $Y(\text{NO}) = F/F_m$ to steady-state conditions. Actually, as this expression does neither contain F_0' nor F_m' , in contrast to the expression of Kramer *et al.* (2004), it can be quantified without SP measurements via the continuously measured fluorescence yield (parameter F_t in PAM user software), i.e. also during the course of a dark-light induction curve. This, however, is feasible only, if after the normalizing F_0 , F_m measurements, the rate constants k_F and k_D and, hence, also the ratio k_F/k_D do not change. Any increase of k_D , which e.g. occurs when the illumination is photoinhibitory, will be falsely interpreted as an increase of $Y(\text{NPQ})$. Such increase of k_D can be judged after dark relaxation of NPQ, as it will be reflected by a corresponding increase of $Y(\text{NO})$ under F_0 , F_m conditions, as compared to the value before illumination.

While stress induced damage of the photosynthetic apparatus often is reflected by an increase of $Y(\text{NO})$ measured under F_0, F_m conditions, this is not necessarily the case in the illuminated state. As long as the NPQ generating reactions are not affected, a high $Y(\text{NPQ})$ can compensate for a decrease of $Y(\text{II})$ and even cause a lowering of $Y(\text{NO})$. This is demonstrated in Fig. 2, which shows light response curves of $Y(\text{II})$, $Y(\text{NPQ})$ and $Y(\text{NO})$ of a rose leaf, different parts of which were heat-pretreated for 5 min at 46 and 50°C. The presented data originate from an experiment that was previously described by Schreiber and Klughammer (2008), where the corresponding P700 data were presented in terms of the complementary PS I quantum yields $Y(\text{I})$, $Y(\text{ND})$ and $Y(\text{NA})$.

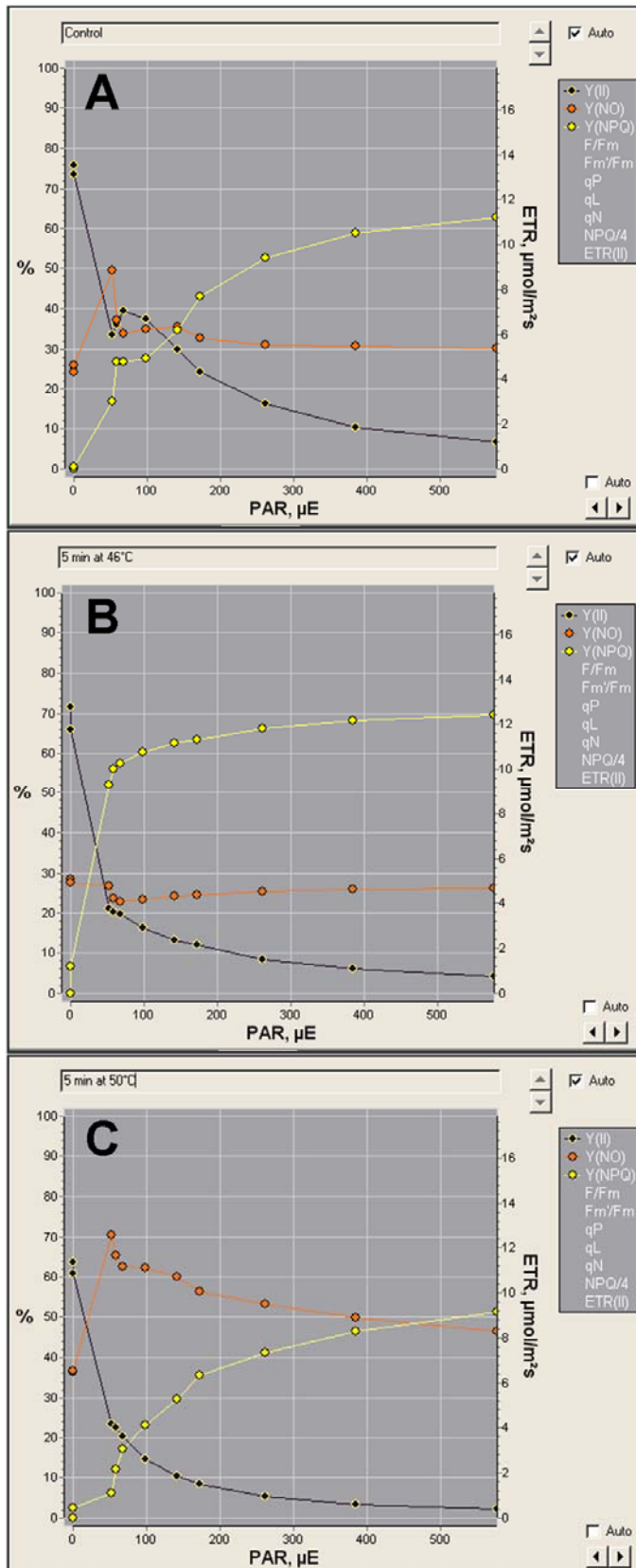


Fig. 2. Light intensity response curves of complementary quantum yields Y(II) (black), Y(NPQ) (yellow) and Y(NO) (orange) of rose leaf **A)** control, **B)** leaf part heat-pretreated for 5 min at 46°C, **C)** leaf part heat-pretreated for 5 min at 50°C. Illumination for 30 s at each intensity step. After mild heat-pretreatment (5 min at 46°C, Fig. 2B) an dominant increase of Y(NPQ) with respect to the control (Fig. 2A) is paralleled by decreases of Y(II) and Y(NO). Obviously, the NPQ-generating photoprotective reactions are not yet affected by the heating. More severe heat-pretreatment (5 min at 50°C, Fig. 2C) leads to a marked increase of Y(NO) mostly at the cost of Y(NPQ). This can be explained by heat damage of the NPQ-generating reactions, in particular of the Mehler-Ascorbate-Peroxidase cycle (Schreiber *et al.* 1995b; Asada 1999) which is mainly responsible for the generation of a transthylakoidal ΔpH after inactivation of CO_2 dependent electron flow. While the mild heat-pretreatment at maximal PAR results in an increase of Y(NPQ)/Y(NO) from 2.1 to 2.7, this ratio drops to 1.1 after 5 min at 50°C. Such drop in Y(NPQ)/Y(NO) is indicative of severe damage of the photoprotective reactions, which can be expected to lead to secondary damage by photoinhibition.

Conclusions

In conclusion, the partitioning of absorbed excitation energy in PS II can be comprehensively described by very simple and generally valid equations for the complementary quantum yields in terms of fluorescence yield parameters that can be readily determined by the SP method:

1.
$$Y(II) = \frac{F'_m - F}{F'_m} = \frac{\Delta F}{F'_m}$$
2.
$$Y(II) + Y(\text{loss}) = 1$$
3.
$$Y(\text{loss}) = 1 - Y(II) = Y(\text{NO}) + Y(\text{NPQ}) = \frac{F}{F'_m}$$
4.
$$Y(\text{NO}) = \frac{F}{F'_m}$$
5.
$$Y(\text{NPQ}) = \frac{F}{F'_m} - Y(\text{NO}) = \frac{F}{F'_m} - \frac{F}{F'_m}$$
6.
$$Y(\text{NO}) + Y(\text{NPQ}) + Y(II) = 1$$
7.
$$NPQ = \frac{Y(\text{NPQ})}{Y(\text{NO})} = \frac{F'_m - F}{F'_m}$$

Y(II) corresponds to the fraction of energy that is photochemically converted in PS II. The remaining fraction $1 - Y(II)$ constitutes the total quantum yield of all loss processes, Y(loss), which is split up into two distinct components, Y(NO) and Y(NPQ), the relative amounts of which provide valuable information on the photosynthetic performance of a plant. Y(NO) reflects the fraction of energy that is passively dissipated in form of heat and fluorescence, mainly due to closed PS II reaction centers. Y(NPQ) corresponds to the fraction of energy dissipated in form of heat *via* the regulated photoprotective NPQ-mechanism. When Y(II) values approach zero at high quantum flux densities, high values of Y(NPQ) are indicative of a high photoprotective capacity, whereas high values of Y(NO) reflect the inability of a plant to protect itself against damage by excess illumination. At a given set of environmental conditions, successful regulation generally is aimed at maximal values of Y(II), with the remaining Y(loss) aimed at a maximal ratio of Y(NPQ)/Y(NO). At saturating light intensity, high values

of Y(NO) and low values of Y(NPQ) or Y(NPQ)/Y(NO) reflect suboptimal capacity of photoprotective reactions, which eventually will lead to photodamage. High values of Y(NO) after dark-adaptation are indicative of such damage. Finally, it has to be emphasized that Fm determination after dark-adaptation is prerequisite for evaluation of Y(NO) and Y(NPQ). It always has to be kept in mind that the simple expressions of Y(NO) and Y(NPQ) are valid only, if between Fm measurement and the measurements of F and Fm' there are no changes in the rate constants k_D and k_F .

Acknowledgements

We thank Bernard Genty for drawing our attention to his 1996 abstract and other very relevant literature. Govindjee is thanked for helpful suggestions.

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