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REVIEW

Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships

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Abstract

Chlorophyll fluorescence parameters (Chl FPs) derived from the slow (long-term) induction kinetics of modulated Chl *a* fluorescence are reviewed and analysed with respect to their application in photosynthesis research. Only four mutually independent Chl FPs, calculated from values of five essential Chl fluorescence (ChlF) yields, are distinguished as the basic ones. These are: the maximum quantum yield of PS2 photochemistry (Φ_{P_0}), the photochemical quenching of variable ChlF (q_P), the non-photochemical quenching of variable ChlF (q_N), and the relative change of minimum ChlF (q_0). Φ_{P_0} refers to the dark-adapted state of a thylakoid membrane, q_P, q_N, and q_0 characterise the light-adapted state. It is demonstrated that all other Chl FPs can be determined using this quartet of parameters. Moreover, three FPs related to the non-radiative energy dissipation within thylakoid membranes are evaluated, namely: the non-photochemical ChlF quenching (NPQ), the complete non-photochemical quenching of ChlF (q_{CN}), and the effective quantum yield of non-photochemical processes in PS2 (Φ_N). New FPs, the total quenching of variable ChlF (q_{TV}) and the absolute quenching of ChlF (q_A) which allow to quantify co-action of the photochemical and non-photochemical processes during a light period are defined and analysed. The interpretation of Chl FPs and recommendations for their application in the photosynthesis research are also given. Some alternative FPs used in the laboratory practice have only an approximate character and can lead to incorrect conclusions if applied to stressed plants. They are reviewed and compared with the standard ones. All formulae and conclusions discussed herein are verified using experimental values obtained on young seedlings of the Norway spruce (*Picea abies* [L.] Karst.).

Additional key words: energy dissipation; fluorescence quenching; heat dissipation; photosystem 2; *Picea abies*; quantum yield.

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'*Abbreviations* (see also Tables 1 and 2): *A* – fraction of primary acceptor molecules in the oxidised state; AR – actinic radiation; *C* – multiplication constant; Chl – chlorophyll; ChlF – chlorophyll fluorescence; δ – relative deviation; *d* – dissipation factor; DAS – dark-adapted state; *f* – function; F_0 – minimum Chl fluorescence yield in the dark-adapted state; F_0' – minimum Chl fluorescence yield in the light-adapted state; F_M – maximum Chl fluorescence yield in the dark-adapted state; F_M' – maximum Chl fluorescence yield in the light-adapted state; F_P – maximum Chl fluorescence yield measured when the actinic radiation is switched on; – steady-state Chl fluorescence yield in the light-adapted state; F_V – maximum variable Chl fluorescence yield in the dark-adapted state; F_V' – maximum variable Chl fluorescence yield in the light-adapted state; F(t) – immediate Chl fluorescence yield; FIK – fluorescence induction kinetics; FP – fluorescence parameter; FR – far-red radiation; FY – fluorescence yield; k_D – rate constant for the thermal dissipation within the light-harvesting system associated with PS2; k_F – rate constant for the Chl *a* fluorescence; k_P – rate constant for the photochemistry at open PS2 reaction centres; λ – wavelength at the maximum of a radiation distribution curve; LAS – light-adapted state; LHC – light-harvesting chlorophyll-protein complex; MR – weak modulated measuring radiation; NRD – non-radiative energy dissipation; PAM – pulse amplitude modulation; PFD – photon flux density; PS – photosystem; Q_A – primary quinone-type electron acceptor of PS2; RC – reaction centre; SP – saturation pulse; t – time.

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Introduction

Recording of the fluorescence emitted from chlorophyll (Chl) molecules located in chloroplasts of photosynthesising organisms is a widely used non-destructive tool in the photosynthesis research. This technique has allowed an increased understanding of photochemical and non-photochemical processes occurring in thylakoid membranes of chloroplasts (for reviews, see Butler 1978, Walker 1987, Krause and Weis 1991, Govindjee 1995, Kramer and Crofts 1996, Lazár 1999, Maxwell and Johnson 2000). Availability of commercial devices enabled accurate measurements of the Chl fluorescence (ChlF), even under field conditions. Such fluorimeters, working on the principle of pulse amplitude modulation (PAM) of ChlF (Schreiber 1986, Schreiber *et al.* 1986) helped significantly to a worldwide expansion of the fluorescence methods. They allowed obtain an information on function of the photosynthetic apparatus by analysis of the so-called ChlF quenching which in a complex manner reflects photosynthetic activities of plants.

Although ChlF represents a minor deactivation process for excited Chl molecules, its time-course termed the Chl fluorescence induction kinetics (FIK) provides an excelent insight into utilisation of the excitation energy by photosystem 2 (PS2) and indirectly also by other complexes within the thylakoid membrane (Walker 1987). Increased flow of the excitation energy into a photochemical pathway leads to a decrease (quenching) of the Chl fluorescence yield (FY). In this way, ChlF reflects changes in the efficiency of photosynthetic processes (Kautsky and Hirsch 1934). A proper evaluation of this quenching depends on our ability to quantify contributions of the PS2 photochemistry (related to the photochemical quenching) and several distinct non-photochemical processes (related to the non-photochemical one) to the total ChlF quenching (Walters and Horton 1991). Generally, the photochemical quenching refers to the energy consumption by a charge separation in reaction centres (RCs) of PS2. The non-photochemical quenching is induced by a pH-gradient build-up as well as activation of numerous regulatory mechanisms which provide for an effective utilisation of the excitation energy and cope with over-excitation (photoinhibition) or other kinds of injury caused to the thylakoid membrane (see Krause and Weis 1991, Horton 1996). The resolution of both principal types of the ChlF quenching was allowed by a saturation pulse method (Schreiber 1986, Schreiber *et al.* 1986), originally introduced as a "light-doubling" technique (Bradbury and Baker 1981, 1984).

Useful qualitative and quantitative information on photosynthetic processes can be decoded from Chl FIK using a set of fluorescence parameters (FPs). These parameters were intensively sought within the last three decades and were consecutively introduced into the photosynthesis research, *e.g.*: the ratio of ChlF decrease (Rfd, Brown 1967, Lichtenthaler *et al.* 1984), F_V/F_M ratio (Kitajima and Butler 1975), photochemical (q_P) and non-photochemical (q_N) quenching of variable Chl FY (Bilger and Schreiber 1986, Schreiber *et al.* 1986), F_0-quenching coefficient (q_0, Bilger and Schreiber 1986), relative electron transport rate (ETR, Weis and Berry 1987), $\Delta\Phi_F/\Phi_{Fm}$ ratio (Genty *et al.* 1989), and non-photochemical quenching of ChlF (NPQ, Bilger and Björkman 1990). The necessary unification in a terminology was also given (van Kooten and Snel 1990). This article reviews and discusses the majority of Chl FPs currently used in the photosynthesis research. It is shown that only four parameters are mutually independent and the other can be expressed using this principal quartet.

Basic concepts

Photosynthetic activities of plants are ordinarily deduced from time changes of ChlF assuming an anti-parallel behaviour between photochemical processes (the charge separation in reaction centres of PS2 followed by an electron transport *via* a set of carriers) and non-photochemical ones (the thermal dissipation and Chl fluorescence). Both pathways participate in utilisation of the excitation energy by PS2 and PS1. At room temperature, ChlF originates almost exclusively from depopulation of the first excited singlet-state of Chl *a* molecules in the PS2 complexes (Govindjee 1995) and represents only about 3–5 % of the overall absorbed energy (Walker 1987). This radiative de-excitation process is characterised by the quantum yield of ChlF (Φ_F) and described by the following general equation (*e.g.* Krause and Weis 1991):

$$\Phi_F = \frac{I_F}{I_A} = \frac{k_F}{\Sigma k_i} \qquad (1)$$

Here I_F means the rate (photon flux) of ChlF emission, I_A the absorbed flux of radiation, k_F the rate constant for ChlF, and Σk_i represents the sum of rate constants for all competing processes that result in a return of the excited Chl molecule to its ground state. Only the relative quantum yield of ChlF can be reported in view of the homogenous ChlF emission (Govindjee 1995).

In the dark-adapted state (DAS), which is in view of electron transport processes the photochemically inactive state of a thylakoid membrane, generally all PS2 RCs and electron carriers of the PS2 acceptor side are re-oxidised. Also components of the non-photochemical quenching are relaxed to their minimum values (*cf.* Horton and Hague 1988, Hodges *et al.* 1989). Then minimum Chl FY in DAS (F_0) is recorded when a week modulated measuring radiation (MR) with an overall photon flux density (PFD) lower then 0.1 μmol m^{-2} s^{-1} is applied to a pre-darkened sample. The following equation describes the F_0 level (see Fig. 1) in terms of the rate constants of three main energy consuming processes (Oxborough and Baker 1997):

$$F_0 = C\frac{k_F}{k_F + k_D + k_P}. \tag{2}$$

In addition to k_F defined above, k_D represents the rate constant for thermal dissipation within the light-harvesting system associated with PS2, k_P the rate constant for photochemistry at open PS2 reaction centres, and the multiplication constant C accounts for a difference between proper Φ_F and the actually measured ChlF yield. In this state, when the maximum number of PS2 RCs is active (open), the efficiency of photochemical processes is dominant whereas the non-photochemical quenching processes are minimised. It means that $k_P \gg k_F + k_D$.

The approximation presented by Oxborough and Baker (1997) presupposes that the PS2 photochemistry is actually trap-limited (*cf.* Krause and Weis 1991, Lavergne and Trissl 1995) and not limited by the rate of exciton transfer from a light-harvesting complex to PS2 RC (the diffusion limitation), as proposed in the model by Kitajima and Butler (1975). In accordance with this model, it is postulated that the rate constant k_P at closed PS2 RCs is small enough to be ignored. It comes specifically when the sample is irradiated by a short pulse of a saturating polychromatic radiation (PFD up to 10 mmol m^{-2} s^{-1}). This saturation pulse (SP) causes a fast closure of all active PS2 RCs due to a full reduction of the primary quinone-type electron acceptor of PS2 (Q_A, Duysens and Sweers 1963) accompanied by a complete saturation of the photochemical processes in PS2. Then k_P converges to zero and maximum Chl FY in DAS (F_M) is recorded before the non-photochemical processes related to a linear electron transport can promote. The F_M level is characterised as follows (Oxborough and Baker 1997):

$$F_M = C\frac{k_F}{k_F + k_D}. \tag{3}$$

Correct determination of both F_M and F_0 is essential for the subsequent quantification of processes occurring during light period. All time changes of Chl FY during the light period are connected with fluorescence quenching mechanisms and are compared to those basic reference FY levels (see Fig. 1). A difference between F_M and F_0 is denominated maximum variable Chl FY in DAS (F_V) and it is (*e.g.* Kitajima and Butler 1975, van Kooten and Snel 1990):

$$F_V = F_M - F_0. \tag{4}$$

If the actinic radiation (AR) is switched on, the observed distinct changes in immediate Chl FY, F(t), are attributable to a simultaneous co-action of photochemical and non-photochemical processes. The former processes refer to a charge separation in PS2 RCs which is linked with variations in efficiency of the electron transport chain. The latter processes involve the pH-gradient build up, non-radiative energy dissipation (NRD) to heat within thylakoid membranes, LHC2 phosphorylation, photoinhibition of PS2 RCs, *etc.* (for reviews, see Krause and Weis 1991, Govindjee 1995, Maxwell and Johnson 2000). During the light period, the photosynthetic apparatus passes gradually from DAS to the light-adapted state (LAS). The LAS is characterised by a continuous synthesis of ATP, NADPH, and concurrent fixation of CO_2 (Walker 1987). When electron transport processes and coupled biochemical reactions in the carbon reduction cycle are equilibrated, the steady-state Chl FY level (F_S) is reached. Application of a pulse of the saturating radiation at this state enables to determine maximum Chl FY in LAS (F_M'); see Fig. 1. Once AR is switched off, minimum Chl FY in LAS (F_0') is recorded after a short

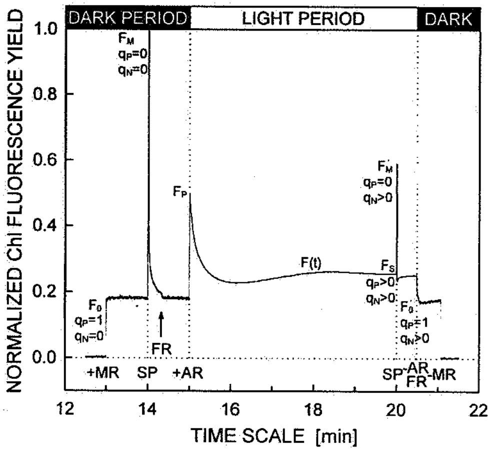


Fig. 1. Slow Chl *a* fluorescence induction kinetics recorded *in vivo* using a portable *PAM-2000* fluorometer in the saturation pulse regime on a dark-adapted (for 15 min) current-year branch of 4-year-old seedling of Norway spruce (*Picea abies* [L.] Karst.). The instrumental settings were as follows: measuring radiation (MR) PFD < 0.1 μmol m^{-2} s^{-1} (λ 650 nm), PAM-frequency of 600 Hz, switched on/off (+MR/-MR); actinic radiation (AR) PFD of 170 μmol m^{-2} s^{-1} (λ 655 nm), PAM-frequency of 20 kHz, switched on/off (+AR/-AR); saturation pulse (SP) PFD *ca.* 3 000 μmol m^{-2} s^{-1} (halogen lamp), pulse duration of 0.8 s, PAM-frequency of 20 kHz; far-red radiation (FR) PFD *ca.* 10 μmol m^{-2} s^{-1} (λ 735 nm), pulse duration of 3 s; the F_M level determined after 14 min of the dark adaptation; mean surface temperature of spruce needles during a record 22.9±0.1 °C. Dark/light adaptation periods were realised in a small ventilated chamber. Normalisation to F_M was used. *Additional abbreviations*: q_P, photochemical quenching of F_V ($0 \le q_P \le 1$); q_N, non-photochemical quenching of F_V ($0 \le q_N \le 1$).

pulse of the weak far-red radiation (FR) which accelerates a re-oxidation of the PS2 acceptor side. Then, analogously to Eq. 4, maximum variable Chl FY in LAS (F_V') is determined as:

$$F_V' = F_M' - F_0' . \tag{5}$$

Assuming that only the constant k_D varies during transition of the thylakoid membrane from DAS to LAS (Oxborough and Baker 1997) and the increase in a thermal decay in LAS is represented by the "dissipation factor" d ($d \geq 1$), three following equations describe F_0', F_M', and F_S in a representation of the rate constants (*cf.* a diverse access used by Laisk *et al.* 1997):

$$F_0' = C\frac{k_F}{k_F + d\,k_D + k_P}, \tag{6}$$

$$F_M' = C\frac{k_F}{k_F + d\,k_D}, \tag{7}$$

$$F_S = C\left[\frac{A\,k_F}{k_F + d\,k_D + k_P} + \frac{(1-A)k_F}{k_F + d\,k_D}\right]. \tag{8}$$

Parameter A ($0 \leq A \leq 1$) represents the fraction of primary acceptor molecules being oxidised in LAS (Kitajima and Butler 1975). As seen, Eq. 8 leads to Eq. 6 for $A = 1$ (fully re-oxidised Q_A, open PS2 RCs), and to Eq. 7 for $A = 0$ (fully reduced Q_A, closed PS2 RCs).

In general, five mutually independent Chl FY levels, F_M, F_0, $F_M'(t)$, $F(t)$, and $F_0'(t)$, suffice for definitions of most ChlF parameters at any time t of the light induction. In LAS, the time-dependent changes of $F_M'(t)$, $F(t)$, and $F_0'(t)$ are very small (< 1 % of F_M *per* 1 min) and can be neglected. At this condition, it is defined: $F_M'(t) = F_M'$, $F(t) = F_S$, and $F_0'(t) = F_0'$ (see Fig. 1). F_M and F_0 characterise DAS, F_M', F_S, and F_0' determine LAS. For a better understanding of the following text, all derivations presented in this paper are made under the assumption that LAS was reached. Therefore, the time-dependent formulae are not applied. As common now in fluorescence literature, the term 'coefficient' is omitted in names of fluorescence parameters given below, in spite of the fact that it would correspond better to a mathematical formalism applied herein (*cf.* van Kooten and Snel 1990, Krause and Weis 1991, Buschmann 1995).

Fluorescence parameters related to the dark-adapted state

Maximum quantum yield of PS2 photochemistry (Φ_{P_0}) is the most frequently used parameter. It is calculated using maximum (F_M) and minimum (F_0) Chl FYs measured in DAS (Fig. 1) according to the definition (Kitajima and Butler 1975):

$$\Phi_{P_0} = \frac{F_V}{F_M} = \frac{F_M - F_0}{F_M} = 1 - \frac{F_0}{F_M}. \tag{9}$$

Φ_{P_0} is often called the F_V/F_M ratio and/or maximum yield of primary photochemistry (Kitajima and Butler 1975), potential yield of PS2 photochemical reactions (Krause and Weis 1991), potential maximum PS2 quantum yield (Schreiber *et al.* 1995), and quantum efficiency of open PS2 centres (Maxwell and Johnson 2000). Its values are always less than 1 because $F_0 > 0$ and thus F_V is in all cases less than F_M. If Eqs. 2 and 3 are applied to Eq. 9 Φ_{P_0} can be expressed in the form (Kitajima and Butler 1975):

$$\Phi_{P_0} = \frac{F_M - F_0}{F_M} = \frac{k_P}{k_F + k_D + k_P} \tag{10}$$

which confirms the term used for this parameter.

Φ_{P_0} is almost constant for many different plant species measured under the non-stressed conditions and equals to 0.832±0.004 (Björkman and Demmig 1987). But for stressed plants Φ_{P_0} is markedly reduced. Therefore, the F_V/F_M ratio is frequently used as an indicator of the photoinhibition or other kind of injury caused to the PS2 complexes (*e.g.* Rees *et al.* 1990, Krause and Weis 1991, Buffoni *et al.* 1998, Lazár and Nauš 1998). Φ_{P_0} values can be lowered due to contribution of the ChlF emission from PS1 to the F_0 level (Lavergne and Trissl 1995). This effect might be significant, especially in the case of stressed plants, and has to be taken into account when analysing ChlF data (Pfündel 1998).

It is possible to define other FPs based on the F_M and F_0 levels: the ratios F_0/F_M and F_V/F_0. Of course, both parameters have a direct relation to Φ_{P_0}. It implies from Eq. 9 that:

$$\frac{F_0}{F_M} = 1 - \Phi_{P_0} = \Phi_{N_0}. \tag{11}$$

Applying Eq. 10 to Eq. 11, the meaning of the parameter Φ_{N_0} emerges from:

$$\frac{F_0}{F_M} = \frac{k_F + k_D}{k_F + k_D + k_P} = \Phi_{F_0} + \Phi_{D_0} = \Phi_{N_0}. \tag{12}$$

Here, the minimum quantum yield of ChlF in DAS (Φ_{F_0}) and the basal quantum yield of thermal dissipation within PS2 in DAS (Φ_{D_0}) were introduced, namely:

$$\Phi_{F_0} = \frac{k_F}{k_F + k_D + k_P}, \quad \Phi_{D_0} = \frac{k_D}{k_F + k_D + k_P}. \tag{13}$$

Φ_{F_0} is identical with F_0 (Eq. 2) if $C = 1$. As seen from Eq. 12, the F_0/F_M ratio might be termed the basal quantum yield of non-photochemical processes in PS2 (Φ_{N_0}). It covers influences of both ChlF and NRD in DAS when all PS2 RCs are open. For healthy, non-stressed plants, it is mostly $0.8 \leq \Phi_{P_0} \leq$

0.86 (Björkman and Demmig 1987) and therefore $0.14 \leq F_0/F_M \leq 0.2$. For stressed or damaged plants, the F_0/F_M ratio markedly increases (Bilger *et al.* 1987, Horton and Ruban 1992). Thus, Φ_{N_0} can also be used for indication of the plant stress.

The F_V/F_0 ratio means if adapted using expansion by F_M and substitution by Eqs. 9 – 11:

$$\frac{F_V}{F_0}=\frac{F_V}{F_M}\frac{F_M}{F_0}=\frac{\Phi_{P_0}}{1-\Phi_{P_0}}=\frac{\Phi_{P_0}}{\Phi_{N_0}}=\frac{k_P}{k_F+k_D}. \tag{14}$$

For the above Φ_{P_0} limits (0.8; 0.86), Eq. 14 implies that $4 \leq F_V/F_0 \leq 6$. If the referred value $\Phi_{P_0} = 0.832$ (Björkman and Demmig 1987) is used, this ratio equals to 4.95. It demonstrates the well known experimental finding that F_V exceeds five times F_0 in DAS (*e.g.* Krause and Weis 1984, Walker 1987). As shown in Fig. 2*A*, this Chl FP is very sensitive to actual Φ_{P_0} in a vicinity of the "optimum" value (*dotted*). Eq. 14 also indicates that this parameter reflects changes in the maximum ratio of quantum yields of photochemical and concurrent non-photochemical processes in PS2 related to DAS. Thus, the F_V/F_0 ratio might be used as a very sensitive indicator of the maximum efficiency of photochemical processes in PS2 and/or the potential photosynthetic activity of healthy as well as stressed plants (*cf.* Krause *et al.* 1982, Lichtenthaler *et al.* 1984, Babani and Lichtenthaler 1996).

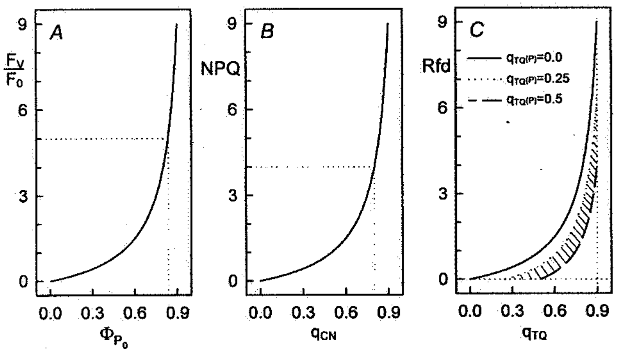


Fig. 2. Graphical relationships between some Chl fluorescence parameters: (*A*) F_V/F_0 *vs.* Φ_{P_0}, (*B*) NPQ *vs.* q_{CN}, and (*C*) Rfd *vs.* q_{TQ} (area of the most probable Rfd values is shaded). For the explanation, see text.

Basic fluorescence parameters related to the light-adapted state

The sufficiently long irradiation of a dark-adapted sample by continuous AR results in the change of initial Chl FYs F_M, F_0, and F_P (the maximum Chl FY recorded when AR is switched on) to the steady-state levels F_M', F_0', and F_S, respectively (Fig. 1). Transition of the sample from DAS to LAS can be described supposing a concurrent influence of the following three processes: the non-photochemical quenching of F_V to F_V', photochemical quenching of F_M' to F_S, and change of F_0 to F_0'. Let us denominate coefficients of proportionality of the individual processes q_N, q_P, and q_0, respectively. Employing the principle of superposition, the following equation is valid for F_M' (*cf.* Fig. 1):

$$F_M' = F_M - F_V q_N - F_0 q_0 . \tag{15}$$

If the formulae 4, 5, and the definition for q_0 (Bilger and Schreiber 1986):

$$q_0 = \frac{F_0 - F_0'}{F_0} = 1 - \frac{F_0'}{F_0} \tag{16}$$

are introduced into Eq. 15, then (*cf.* Schreiber *et al.* 1986, Buschmann 1999):

$$F_V' + F_0' = F_V + F_0 - F_V q_N - F_0 + F_0'$$
$$F_V' = (1 - q_N) F_V . \tag{17}$$

The definition for the coefficient of proportionality q_N called the non-photochemical quenching of variable ChlF (Schreiber *et al.* 1986, van Kooten and Snel 1990) follows from Eq. 17:

$$q_N = \frac{F_V - F_V'}{F_V} = 1 - \frac{F_V'}{F_V}. \tag{18}$$

Since generally $0 \leq F_V' \leq F_V$, it means that $0 \leq q_N \leq 1$. As seen, q_N is explicitly not dependent on q_0, in contrast with the definition published by Bilger and Schreiber (1986). This Chl FP is related to LAS and its values are found within the interval (0, 1).

The parameter q_N reflects activation of the non-photochemical processes during the light period, mostly leading to the non-radiative energy dissipation (NRD) to heat (Björkman and Demmig-Adams 1995). q_N is induced by changes in the trans-thylakoid pH-gradient, state transitions, photoinhibitory processes (inactivation of PS2 RCs, zeaxanthin formation), *etc.* (Krause *et al.* 1982, Horton and Hague 1988, Bilger and Björkman 1990, Krause and Weis 1991, Pospíšil 1997). q_N is defined as the relative change of F_V and has not to be replaced by other FPs which consider changes of F_M (see below).

Variable Chl FY in LAS ($F_S - F_0'$) is described by the equation (*cf.* Fig. 1):

$$F_S - F_0' = F_V' - F_V' q_P = (1 - q_P) F_V' , \tag{19}$$

from which the definition of the coefficient q_P called the photochemical quenching of variable ChlF (Schreiber *et al.* 1986, Genty *et al.* 1989) follows:

$$q_P = 1 - \frac{F_S - F_0'}{F_V'} = 1 - \frac{F_S - F_0'}{F_M' - F_0'} = \frac{F_M' - F_S}{F_M' - F_0'} = \frac{\Delta F}{F_V'} . \tag{20}$$

All expressions in Eq. 20 are equivalent. They only differ by introduction of the substitution $\Delta F = F_M' - F_S$ (Genty *et al.* 1989) and Eq. 5. Generally, $F_0' \leq F_S \leq F_M'$ and thus

$0 \le q_P \le 1$.

In case of q_P, the decrease (quenching) of ChlF is connected with the photochemical energy conversion by the charge separation in RCs of PS2. This Chl FP indicates the photochemical capacity of PS2 in LAS and quantifies the actual fraction of PS2 RCs that are in the open state, *i.e.* with re-oxidised Q_A (Duysens and Sweers 1963, Krause *et al.* 1982). Logically, the complement of q_P to 1:

$$1 - q_P = \frac{F_S - F_0'}{F_M' - F_0'} \tag{21}$$

approximates the reduction state of Q_A (Bilger and Björkman 1990). This Chl FP is called the degree of PS2 reaction centre closure (Björkman and Demmig-Adams 1995). By analogy with q_P, the parameter $1 - q_P$ is proportional to the fraction of PS2 RCs being closed in LAS.

The last from three mentioned coefficients, the relative change of minimum ChlF (q_0), Eq. 16, describes changes in the F_0 level during the induction by AR. Commonly, it is $0 < F_0' \le F_0$ and thus $0 \le q_0 < 1$. But events when $F_0' > F_0$ can also be observed. It is probably due to not fully re-oxidised PS2 acceptor side that results in the negative q_0 values, not exceeding -0.2 (Roháček and Šiffel 1995). There are also indications that pronounced changes of F_0 resulting in the large negative q_0 values can be recorded in the first minute of the light period (Roháček and Barták 1999). Therefore, q_0 is not denominated the 'quenching' of F_0 in this paper (*cf.* Bilger and Schreiber 1986).

As assumed, q_0 is of the non-photochemical nature, directly attributable to the high-energy-state ChlF quenching and has characteristics of the antenna quenching (Genty *et al.* 1990, Rees *et al.* 1990). q_0 has been connected with the electron flow regulating mechanisms (triggered by the pH-gradient), inactivation of PS2 RCs (photoinhibition, photodestruction), violaxanthin de-epoxidation, and conformation changes within pigment-protein complexes (state 1−2 transitions, aggregation of LHC2); *e.g.* Bilger and Björkman (1990), Horton and Ruban (1992), Šiffel and Vácha (1998). It was reported that q_0 is emission wavelength dependent and large differences can be found in pigment matrices contributing to F_0 at 730 nm (associated with PS1), compared to that at 690 nm (Genty *et al.* 1990).

In the frame of approximation used by Oxborough and Baker (1997), the definitions for q_0 (Eq. 16), q_N (Eq. 18), and q_P (Eq. 20) can be rewritten by means of Eqs. 2−8 into the forms:

$$q_0 = \frac{F_0 - F_0'}{F_0} = \frac{d-1}{d}\frac{dk_D}{k_F + dk_D + k_P} = \frac{d-1}{d}\Phi_D, \tag{22}$$

$$q_N = 1 - \frac{F_M' - F_0'}{F_M - F_0} = \frac{d-1}{d}\frac{dk_D}{k_F + dk_D}\left[1 + \frac{k_F + k_D}{k_F + dk_D + k_P}\right],$$

$$q_N = \frac{d-1}{d}\Phi_D^M\left[1 + \Phi_{F_0'} + \frac{1}{d}\Phi_D\right], \tag{23}$$

$$q_P = \frac{F_M' - F_S}{F_M' - F_0'} = A, \tag{24}$$

respectively. Eq. 22 implies that q_0 is proportional to the basal quantum yield of thermal dissipation within PS2 in LAS (Φ_D) supporting the premise on the non-photochemical nature of q_0. Eq. 23 reflects the assumption that q_N covers many processes of the non-photochemical nature which include besides Φ_D the maximum quantum yield of thermal dissipation within PS2 in LAS (Φ_D^M) and the minimum quantum yield of ChlF in LAS ($\Phi_{F_0'}$). Eq. 24 confirms the fact that q_P quantifies the fraction of PS2 RCs actually open in LAS. Definitions of the newly introduced parameters are as follows (see also Table 2):

$$\Phi_D = \frac{dk_D}{k_F + dk_D + k_P}, \quad \Phi_D^M = \frac{dk_D}{k_F + dk_D},$$

$$\Phi_{F_0'} = \frac{k_F}{k_F + dk_D + k_P}. \tag{25}$$

Parameters referring to the non-photochemical quenching of ChlF

The non-photochemical chlorophyll fluorescence quenching (NPQ) is another frequently used parameter. It quantifies processes leading to the decrease (quenching) of F_M during adaptation of the sample to AR. NPQ is often used for estimation of NRD within thylakoid membranes (Bilger and Björkman 1990). It was derived from the Stern-Volmer equation, based on the matrix model of antenna organisation, assuming the existence of non-photochemical quenching traps (Schreiber *et al.* 1995, Buschmann 1999). In LAS, the NPQ parameter is defined by the formula (Bilger and Björkman 1990):

$$\mathrm{NPQ} = \frac{F_M - F_M'}{F_M'} = \frac{F_M}{F_M'} - 1. \tag{26}$$

NPQ does not require knowledge of the F_0' level and can vary theoretically between 0 and +∞. Ordinarily, NPQ is less than 4 for measurements on the light-adapted samples (Fig. 2*B*).

It was reported that NPQ is linearly related to the excess radiation over a wide range of incident PFDs and the extent of its development in leaves correlates to a zeaxanthin formation (Björkman and Demmig-Adams 1995). Hence, NPQ is often used as an indicator of the excessradiant energy dissipation to heat in the PS2 antenna complexes (Bilger and Björkman 1990, Demmig-Adams *et al.* 1996, Gilmore 1997). This is absolutely correct if the quenching process is of the Stern-Volmer type (Horton 1996). Relation of NPQ to the NRD processes follows clearly from Eq. 26 after its adaptation using Eqs. 3 and 7:

$$\mathrm{NPQ} = \frac{(d-1)\,k_\mathrm{D}}{k_\mathrm{F} + k_\mathrm{D}} = (d-1)\,\Phi_{\mathrm{D_0}}^{\mathrm{M}}\,. \tag{27}$$

Here, $\Phi_{\mathrm{D_0}}^{\mathrm{M}}$ means the maximum quantum yield of thermal dissipation within PS2 in DAS. This situation comes in DAS when the acceptor side of PS2 is over-reduced using short SP. Then k_P tends to 0 and the thermal dissipation predominates. As follows from interrelationships of Eqs. 13, 25, and 27, it is true that $\Phi_{\mathrm{D_0}} < \Phi_\mathrm{D} < \Phi_{\mathrm{D_0}}^{\mathrm{M}} < \Phi_\mathrm{D}^{\mathrm{M}}$ if $k_\mathrm{D} > 0$, $k_\mathrm{P} > 0$, $k_\mathrm{P} > k_\mathrm{F}$, $d > 1$ (generally fulfilled requirements).

By analogy with $\mathrm{q_N}$, alternative Chl FP can be introduced not requiring knowledge of the $\mathrm{F_0}$ and $\mathrm{F_0'}$ levels but calculating only with $\mathrm{F_M}$ and $\mathrm{F_M'}$. Its definition is:

$$\mathrm{q_{CN}} = \frac{\mathrm{F_M} - \mathrm{F_M'}}{\mathrm{F_M}} = 1 - \frac{\mathrm{F_M'}}{\mathrm{F_M}}\,. \tag{28}$$

Here, it is termed the complete non-photochemical quenching of ChlF ($\mathrm{q_{CN}}$), in contrast to the non-photochemical quenching quantum yield (Φ_N, Buffoni *et al.* 1998). It refers to the energy dissipation processes connected with both the non-photochemical quenching of $\mathrm{F_V}$ ($\mathrm{q_N}$) and the relative change of $\mathrm{F_0}$ ($\mathrm{q_0}$). If rewritten using Eqs. 3, 7, and 25, the formula 28 leads to:

$$\mathrm{q_{CN}} = \frac{(d-1)\,k_\mathrm{D}}{k_\mathrm{F} + d\,k_\mathrm{D}} = \frac{d-1}{d}\,\Phi_\mathrm{D}^{\mathrm{M}}\,. \tag{29}$$

Hence, $\mathrm{q_{CN}}$ might be also used as the indicator of actual NRD within thylakoid membranes due to its proportionality to $\Phi_\mathrm{D}^{\mathrm{M}}$ (Eq. 25). In other words, $\mathrm{q_{CN}}$ can quantify the thermal dissipation processes within PS2 complexes. Because $0 < \mathrm{F_M'} < \mathrm{F_M}$ in LAS then $0 < \mathrm{q_{CN}} < 1$.

Relation of $\mathrm{q_{CN}}$ to $\mathrm{q_N}$ and $\mathrm{q_0}$ can be demonstrated applying Eqs. 9, 11, 16, and 18 to the definition 28 in the following way:

$$\mathrm{q_{CN}} = \frac{\mathrm{F_V} + \mathrm{F_0} - \mathrm{F_V'} - \mathrm{F_0'}}{F_\mathrm{M}} = \frac{\mathrm{F_V} - \mathrm{F_V'}}{\mathrm{F_V}}\,\frac{\mathrm{F_V}}{\mathrm{F_M}} + \frac{\mathrm{F_0} - \mathrm{F_0'}}{\mathrm{F_0}}\,\frac{\mathrm{F_0}}{\mathrm{F_M}}$$

$$\mathrm{q_{CN}} = \mathrm{q_N}\Phi_{\mathrm{P_0}} + \mathrm{q_0}(1 - \Phi_{\mathrm{P_0}}) = \mathrm{q_N}\Phi_{\mathrm{P_0}} + \mathrm{q_0}\Phi_{\mathrm{N_0}}\,. \tag{30}$$

Thus $\mathrm{q_{CN}}$ is the function (f) of three basic FPs, written formally $\mathrm{q_{CN}} = f(\Phi_{\mathrm{P_0}}, \mathrm{q_N}, \mathrm{q_0})$; *cf.* the diverse access used by Havaux *et al.* (1991) and Buschmann (1995).

At this moment, it is possible to compare NPQ with $\mathrm{q_{CN}}$ on the basis of a mathematical formalism. Using Eq. 28, the expression 26 is modified to:

$$\mathrm{NPQ} = \frac{\mathrm{F_M} - \mathrm{F_M'}}{\mathrm{F_M'}} = \frac{1 - \mathrm{F_M'}/\mathrm{F_M}}{\mathrm{F_M'}/\mathrm{F_M}} = \frac{\mathrm{q_{CN}}}{1 - \mathrm{q_{CN}}}\,. \tag{31}$$

It is evident that NPQ and $\mathrm{q_{CN}}$ are closely related. NPQ depends on the same triad of Chl FPs, namely $\mathrm{NPQ} = f(\Phi_{\mathrm{P_0}}, \mathrm{q_N}, \mathrm{q_0})$. Both parameters can be mutually converted in accordance with:

$$\mathrm{NPQ} = \frac{\mathrm{q_{CN}}}{1 - \mathrm{q_{CN}}}\,,\quad \mathrm{q_{CN}} = \frac{\mathrm{NPQ}}{1 + \mathrm{NPQ}}\,. \tag{32}$$

Due to $\mathrm{F_M'} < \mathrm{F_M}$ in LAS, it always is $\mathrm{q_{CN}} < \mathrm{NPQ}$. As seen from the plot in Fig. 2*B*, $\mathrm{q_{CN}}$ is in comparison with NPQ less sensitive to the increase in NRD at higher values.

Efficiency of the photochemical processes in PS2

Two Chl FPs have a close relation to the efficiency of photochemical processes in the thylakoid membranes in LAS. The first parameter, the effective quantum yield of PS2 photochemistry (Φ_P), quantifies an extent to which the photochemistry at PS2 is limited by a competition with the thermal decay processes (Oxborough and Baker 1997). Originally, Φ_P was called the efficiency of excitation energy capture by open ($\mathrm{q_P} = 1$) RCs of PS2 (Genty *et al.* 1989). Its definition is (see Fig. 1):

$$\Phi_\mathrm{P} = \frac{\mathrm{F_V'}}{\mathrm{F_M'}} = \frac{\mathrm{F_M'} - \mathrm{F_0'}}{\mathrm{F_M'}} = 1 - \frac{\mathrm{F_0'}}{\mathrm{F_M'}}\,. \tag{33}$$

By means of the rate constants (Eqs. 6, 7) Eq. 33 acquires the form (*cf.* Eq. 10):

$$\Phi_\mathrm{P} = \frac{\mathrm{F_M'} - \mathrm{F_0'}}{\mathrm{F_M'}} = \frac{k_\mathrm{P}}{k_\mathrm{F} + d\,k_\mathrm{D} + k_\mathrm{P}}\,. \tag{34}$$

As seen, values of Φ_P are always less then $\Phi_{\mathrm{P_0}}$ due to activation of the NRD processes during the light period ($d > 1$). Modification of Eq. 33 by the formulae 9, 18, 28, and 30 leads to:

$$\Phi_\mathrm{P} = \frac{\mathrm{F_V'}}{\mathrm{F_M'}} = \frac{\mathrm{F_V'}}{\mathrm{F_V}}\,\frac{\mathrm{F_V}}{\mathrm{F_M}}\,\frac{\mathrm{F_M}}{\mathrm{F_M'}} = \frac{(1 - \mathrm{q_N})\,\Phi_{\mathrm{P_0}}}{1 - \mathrm{q_{CN}}}$$

$$\Phi_\mathrm{P} = \frac{(1 - \mathrm{q_N})\,\Phi_{\mathrm{P_0}}}{1 - \mathrm{q_N}\Phi_{\mathrm{P_0}} - \mathrm{q_0}\Phi_{\mathrm{N_0}}}\,. \tag{35}$$

It implies that three principal Chl FPs contribute to Φ_P, namely $\Phi_\mathrm{P} = f(\Phi_{\mathrm{P_0}}, \mathrm{q_N}, \mathrm{q_0})$.

The other parameter is the effective quantum yield of photochemical energy conversion in PS2 (Φ_2). Many alternative terms exist for Φ_2 in literature, *e.g.*: the actual quantum yield of PS2 (non-cyclic) electron transport (Genty *et al.* 1989), efficiency of PS2 electron transport per quantum absorbed by PS2 complexes (ibidem), overall quantum yield of photochemical energy conversion in PS2 (Anonymous 1993), actual efficiency of energy conversion in PS2 (Björkman and Demmig-Adams 1995), quantum efficiency of PS2 photochemistry (Genty and Harbinson 1996), fraction of photons absorbed in PS2 antennae utilised in PS2 photochemistry (Demmig-Adams *et al.* 1996), and effective photochemical quantum yield of PS2 (Buffoni *et al.* 1998). The following definition is widely used for Φ_2 in LAS (Genty *et al.* 1989):

$$\Phi_2 = \frac{\Delta F}{F_M'} = \frac{F_M' - F_S}{F_M'} = 1 - \frac{F_S}{F_M'} . \tag{36}$$

As seen, Φ_2 does not require the knowledge of F_0', neither does it need the previous dark adaptation of the sample. Therefore, Φ_2 is often used for field investigations.

If the expression 36 is adapted using Eqs. 20 and 33 then:

$$\Phi_2 = \frac{\Delta F}{F_V'} \frac{F_V'}{F_M'} = q_P \Phi_P . \tag{37}$$

This well-known relationship (Genty *et al.* 1989) clarifies the dependence of Φ_2 on four basic Chl FPs. It means that $\Phi_2 = f(\Phi_{P_0}, q_P, q_N, q_0)$; see Table 1. Eq. 37 also signifies that Φ_2 can be interpreted as the effective quantum yield of PS2 photochemistry (Φ_P) related to the actual fraction of photochemically active PS2 RCs (q_P) in LAS. For $q_P = 1$, the actual efficiency of photochemical energy conversion in PS2 reaches its maximum and Φ_2 equals to Φ_P. It is valid universally that $0 < \Phi_2 < \Phi_P < \Phi_{P_0} < 1$, as follows from Eqs. 9, 33, and 36 (*cf.* Fig. 5*C*).

For completeness, the ratios F_0'/F_M' and F_V'/F_0' (both related to LAS) can be analysed by analogy with DAS. The modification using Eqs. 5, 6, 7, 25, and 33 results in:

$$\frac{F_0'}{F_M'} = 1 - \frac{F_V'}{F_M'} = 1 - \Phi_P = \Phi_N$$

$$\Phi_N = \frac{k_F + d\,k_D}{k_F + d\,k_D + k_P} = \Phi_{F_0'} + \Phi_D . \tag{38}$$

The F_0'/F_M' ratio is sometimes utilised in the ecophysiological studies and named the degree of thermal energy dissipation within PS2 complexes ($1 - F_V'/F_M'$; Demmig-Adams *et al.* 1996, Barker *et al.* 1998). But from Eq. 38 follows that this parameter contains besides Φ_D also $\Phi_{F_0'}$ (Eq. 25). Therefore, this parameter might be better termed the effective quantum yield of non-photochemical processes in PS2 (Φ_N); *cf.* Laisk *et al.* (1997) and Buffoni *et al.* (1998). Φ_N quantifies processes in LAS after that the PS2 acceptor side was quickly re-oxidised.

Because $\Phi_P < \Phi_{P_0}$, then $\Phi_N > \Phi_{N_0}$ according to Eqs. 11 and 38. It is due to integration of the non-photochemical processes in the overall excitation energy consumption during the light induction period, as demonstrated if the F_0'/F_M' ratio is expressed using Eqs. 11, 16, and 28:

$$\Phi_N = \frac{F_0'}{F_M'} = \frac{F_0'}{F_0} \frac{F_0}{F_M} \frac{F_M}{F_M'} = \frac{(1 - q_0)(1 - \Phi_{P_0})}{1 - q_{CN}} . \tag{39}$$

Numerical values calculated using Eqs. 18, 26, 28, and 38 are mutually comparable (see Fig. 5*B*). However, to obtain the complete information on the NRD processes within thylakoids, simultaneous measurements of ChlF and a thermal photoacoustic signal are necessary (see, *e.g.*, Buschmann and Kocsányi 1989).

The meaning of the F_V'/F_0' ratio can be illustrated using Eqs. 6, 7, 33, and 38 as follows:

$$\frac{F_V'}{F_0'} = \frac{F_V'}{F_M'} \frac{F_M'}{F_0'} = \frac{\Phi_P}{1 - \Phi_P} = \frac{\Phi_P}{\Phi_N} = \frac{k_P}{k_F + d\,k_D} . \tag{40}$$

Similarly to F_V/F_0 (Eq. 14), the F_V'/F_0' ratio might be termed the effective ratio of quantum yields of photochemical and concurrent non-photochemical processes in PS2 related to LAS. It reflects changes in the redistribution of the excitation energy between the photochemical and non-photochemical pathways during the light induction phase. The comparison of Eq. 14 with Eq. 40 shows that $F_V'/F_0' < F_V/F_0$ in all cases due to activation of many non-photochemical processes under the actinic irradiation (*e.g.* Krause and Weis 1991, Laisk *et al.* 1997).

Overall ChlF quenching in LAS

Variable Chl FY in LAS ($F_S - F_0'$) was described by the expression 19. Application of Eq. 17 to Eq. 19 results in another important relationship (*cf.* Schreiber *et al.* 1986):

$$F_S - F_0' = (1 - q_P)(1 - q_N) F_V . \tag{41}$$

It says that the quenching of F_V during the light induction period is caused both by the photochemical and non-photochemical processes. Now, it is possible to define by analogy with q_N new Chl FP, the total quenching of variable ChlF (q_{TV}):

$$q_{TV} = \frac{F_V - (F_S - F_0')}{F_V} = 1 - \frac{F_S - F_0'}{F_V} = 1 - \frac{F_S - F_0'}{F_M - F_0} . \tag{42}$$

Comparison of Eq. 41 with Eq. 42 leads to:

$$1 - q_{TV} = (1 - q_P)(1 - q_N) , \tag{43}$$

$$q_{TV} = q_P + q_N - q_P q_N . \tag{44}$$

Thus, q_{TV} reflects the simultaneous effects of both quenching mechanisms, $q_{TV} = f(q_P, q_N)$, which are activated during a photosynthetic utilisation of the excitation energy. This Chl FP can be interpreted as a measure of the maximum quenching of F_V by the photochemical and non-photochemical processes active in LAS. It ranges from 0 to 1. Although q_P and q_N are defined to the different reference levels (F_V' and F_V, respectively), it follows from Eq. 44 that the values of both FPs can be summed in principle; *cf.* the opposite assertion published by Buschmann (1995). Their negative product ensures that the sum does never exceed 1.

Similarly as q_{TV}, two other FPs result from a simultaneous co-action of the photochemical and non-photochemical processes during the light induction period. The first parameter is named here the total quenching of ChlF (q_{TQ}) and calculates only with the F_M and F_S levels; *cf.* the total quenching yield (Φ_{TOT}, Buffoni *et al.* 1998). Its definition is (see Fig. 1):

$$q_{TQ} = \frac{F_M - F_S}{F_M} = 1 - \frac{F_S}{F_M} \qquad (45)$$

This Chl FP integrates all quenching mechanisms (q_P, q_N, q_0) depressing F_M under the external irradiance. Hence, q_{TQ} serves also as a measure of the overall excitation energy consumption by the photochemical and non-photochemical processes in LAS. It ranges theoretically from 0 to 1. Relationship between q_{TQ} and principal FPs can be found using Eqs. 4, 9, 11, 16, and 42:

$$q_{TQ} = \frac{F_M - F_S}{F_M} = \frac{F_V + F_0 - F_S + F_0' - F_0'}{F_M}$$

$$q_{TQ} = \frac{F_V - (F_S - F_0')}{F_V}\frac{F_V}{F_M} + \frac{F_0 - F_0'}{F_0}\frac{F_0}{F_M}$$

$$q_{TQ} = q_{TV}\Phi_{P_0} + q_0(1 - \Phi_{P_0}) = q_{TV}\Phi_{P_0} + q_0\Phi_{N_0}\,. \qquad (46)$$

The substitution by Eq. 44 implies that $q_{TQ} = f(\Phi_{P_0}, q_P, q_N, q_0)$ because:

$$q_{TQ} = (q_P + q_N - q_P q_N)\Phi_{P_0} + q_0\Phi_{N_0}\,. \qquad (47)$$

The other parameter reflects effect of the maximum efficiency of photochemical as well as non-photochemical processes in LAS. This situation comes specifically if AR is switched off and the plastoquinone pool is quickly reoxidised, *i.e.* q_P converges to 1, q_N and q_0 are in their steady-state optimums. Then, the F_S level merges to F_0' and the absolute quenching of ChlF (q_A) is determined according to (see Fig. 1):

$$q_A = \frac{F_M - F_0'}{F_M} = 1 - \frac{F_0'}{F_M}\,. \qquad (48)$$

This new parameter, completing logically a set of "quenching" Chl FPs, quantifies the relative change between F_M ($q_P \cong 0$, $q_N \cong 0$, $q_0 \cong 0$) and F_0' ($q_P \cong 1$, q_N in optimum, q_0 in optimum) during the transition of a sample from DAS to LAS. q_A might be used as the measure of the maximum excitation energy consumption by the photochemical and NRD processes in LAS. This claim is supported by the adaptation of Eq. 48 using the formulae 3, 6, 25, and 34:

$$q_A = \frac{F_M - F_0'}{F_M} = \frac{(d-1)k_D + k_P}{k_F + d\,k_D + k_P} = \frac{d-1}{d}\Phi_D + \Phi_P\,. \qquad (49)$$

A simple modification of Eq. 48 using Eqs. 11 and 16 leads to:

$$q_A = 1 - \frac{F_0'}{F_0}\frac{F_0}{F_M} = 1 - (1 - q_0)(1 - \Phi_{P_0})$$

$$q_A = \Phi_{P_0} + q_0\Phi_{N_0}\,. \qquad (50)$$

q_A depends only on two basic FPs: $q_A = f(\Phi_{P_0}, q_0)$. Its values vary hypothetically from 0 to 1. Both q_{TQ} and q_A incorporate the total changes of F_M, as the reference level, and differ in this way significantly from the relative total quenching coefficient, $q_{(P+N)rel}$, introduced recently by Buschmann (1995); see the next section.

Last FP reflecting the overall changes of actual Chl FY in LAS is the ratio of ChlF decrease to steady-state ChlF (Rfd), also termed the "vitality index". Rfd was introduced to quantify the photosynthetic processes in plants (Brown 1967, Lichtenthaler *et al.* 1984, 1986). Its definition takes into account maximum Chl FY measured when AR is switched on in DAS (F_P) and steady-state Chl FY (F_S) reached in LAS (see Fig. 1):

$$\text{Rfd} = \frac{F_P - F_S}{F_S} = \frac{F_P}{F_S} - 1\,. \qquad (51)$$

The F_S level reflects not only changes of F_V during the induction period but also changes of F_0. Because there always is $F_S < F_P$, Rfd can range theoretically between 0 and $+\infty$. Typically, it is $1 < \text{Rfd} < 5$ when measured at the high irradiance (*e.g.* Haitz and Lichtenthaler 1988).

Rfd has been often used in the ecophysiological studies as a measure of the photosynthetic activity of higher plants, for estimation of the photosynthetic CO_2 assimilation rates and Chl content in leaves (*e.g.* Lichtenthaler *et al.* 1986, Lichtenthaler 1988, Babani and Lichtenthaler 1996). From its values, conclusions on the potential photosynthetic capacity, intactness, and functionality of leaves, as well as impact of different stress to the photosynthetic apparatus were made (Lichtenthaler *et al.* 1984, Lichtenthaler 1990, Lichtenthaler *et al.* 2000).

However, Rfd has not a simple physiological interpretation, irrespective of fact if measured under the low or high actinic irradiation. If the formula 51 is rewritten by means of Eq. 45 the result is:

$$\text{Rfd} = \frac{F_P - F_S}{F_S} = \frac{F_M - F_S - (F_M - F_P)}{F_M(1 - q_{TQ})}$$

$$\text{Rfd} = \frac{q_{TQ} - q_{TQ(P)}}{1 - q_{TQ}}\,, \quad q_{TQ(P)} = \frac{F_M - F_P}{F_M}\,. \qquad (52)$$

The parameter $q_{TQ}(P)$ was introduced into Eq. 52 as the application of Eq. 45 to the F_P peak (*cf.* Fig. 1). The incident PFD of AR is usually adjusted in such a way that the F_P level is located between the levels $^1/_2\,F_M$ and $^3/_4\,F_M$. Consequently, it is $0.25 \le q_{TQ}(P) \le 0.5$. Owing to $\text{Rfd} > 0$, there must be in all cases $q_{TQ} > q_{TQ}(P)$. Because $F_P > F_S$

and $q_{TQ} < 0.9$ at physiological conditions the Rfd values should be found predominantly between 0 and 6 (see Fig. 2*C*).

It is often emphasised that the F_P and F_S levels in Eq. 51 should be determined at the high irradiance (*e.g.* Haitz and Lichtenthaler 1988). But in this extreme case, when PFD of AR is close to its saturation value it is $F_P \cong F_M$, $F_S \cong F_M'$, $q_{TQ}(P) \cong 0$, and consequently Rfd is close to or identical with NPQ (*cf.* Eq. 26). NPQ is, however, linearly correlated with the rate of NRD to heat and the zeaxanthin formation (see the parameter NPQ). Disadvantage of this regime consists in the negative effect of the continuous excessive irradiation on the photosynthetic apparatus leading to a strong photoinhibition of photosynthesis. In spite of the fact that the F_S level measured after 5 min of that high irradiation cannot correspond to the steady-state ChlF level.

The relatively simple formula 52 becomes a very complex one after its adaptation by Eq. 47:

$$\mathrm{Rfd} = \frac{(q_P + q_N - q_P q_N)\Phi_{P_0} + q_0\Phi_{N_0} - q_{TQ(P)}}{1 - (q_P + q_N - q_P q_N)\Phi_{P_0} - q_0\Phi_{N_0}}\,. \tag{53}$$

As seen, $\mathrm{Rfd} = f(\Phi_{P_0}, q_P, q_N, q_0)$ and convolution of the photochemical and non-photochemical processes is evident. Therefore, the parameter Rfd can give any relevant information on the actual physiological state and photosynthetic activity of plants only in a context of the knowledge of other principal Chl FPs (*cf.* Lichtenthaler 1990). The short-term increase of Rfd observed often in stressed plants is connected most probably with a depression in the efficiency of PS2 photochemistry (lower Φ_{P_0}, q_P) and an amplification of NRD (higher q_N, q_0, NPQ). A direct connection of these effects with the overall photosynthetic processes, functional performance of the photosynthetic apparatus, and/or "vitality" of plants is controversial.

Alternative chlorophyll fluorescence parameters

Besides q_P, q_N, and q_0, similar Chl FPs are sometimes used in literature (*e.g.* Anonymous 1993, Buschmann 1995, Siebke and Weis 1995, Herrmann *et al.* 1997):

$$\mathrm{qP} = \frac{F_M' - F_S}{F_M' - F_0}\,,\ \mathrm{qN} = \frac{F_M - F_M'}{F_M - F_0}\,, \tag{54}$$

$$q_{(P)rel} = \frac{F_M' - F_S}{F_M - F_0'}\,,\ q_{(N)rel} = \frac{F_M - F_M'}{F_M - F_0'}\,,$$

$$q_{(F0)rel} = \frac{F_0 - F_0'}{F_M - F_0'}\,. \tag{55}$$

These formulae seem to respect the experimental finding that the increasing non-photochemical quenching of F_V is accompanied by a significant alteration (quenching) of F_0. It is a matter of opinion, how to describe this fact mathematically. The "hybrid approach" used in Eqs. 54 and 55 is rather ambiguous, especially in the case of $q_{(P)rel}$ and $q_{(F0)rel}$, because changes of F_M include the simultaneous changes of F_V and F_0, as well.

Comparison of the definitions 54 and 20 indicates that the parameter qP is systematically higher than q_P as long as $F_0 > F_0'$ which is the most probable case in measurements on higher plants. The numerical difference between both parameters will increase with the magnifying difference between the F_0 and F_0' levels. Similarly, it follows from definitions 4, 5, 18, and 54 that $q_N < \mathrm{qN}$ just if $F_0' < F_0$. Moreover, qN is always much higher than q_{CN} because the reference level $F_M - F_0$ (Eq. 54) is lower than the F_M one (Eq. 28); *cf.* Fig. 6*A,B*.

Buschmann's (1995) relative coefficient of photochemical quenching, $q_{(P)rel}$ in Eq. 55, combines both the photochemical and non-photochemical effects. The reason is that the reference level $F_M - F_0'$ includes changes of both F_V ($\sim q_P$, $\sim q_N$) and F_0 ($\sim q_0$). As well, $q_{(P)rel}$ is markedly reduced contrary to q_P because the reference level $F_M - F_0'$ is much higher than the $F_M' - F_0'$ one. Further, Buschmann's relative coefficient of non-photochemical quenching, $q_{(N)rel}$ (Buschmann 1995), is systematically higher than q_{CN} and q_N as follows from the comparison of Eq. 55 with Eq. 28 ($F_0' > 0$) and Eq. 18 ($F_0 > F_0'$, $F_M' > F_0'$), respectively. He had also introduced the relative coefficient of F_0-quenching, $q_{(F0)rel}$ in Eq. 55, referring to the same level $F_M - F_0'$. However, $q_{(F0)rel}$ is markedly reduced in comparison with q_0 because $F_M - F_0'$ is many times greater than F_0 (see Fig. 6).

As referred, the sum of all three "relative" quenching coefficients ranges between 0 and 1 (Buschmann 1995). It is not difficult to show from the formulae 55 that:

$$q_{(P)rel} + q_{(N)rel} + q_{(F0)rel} = 1 - \frac{F_S - F_0}{F_M - F_0'}\,. \tag{56}$$

The expression 56 is less than 1 only if $F_0 < F_S$. This premise is usually fulfilled. But under stress conditions, *e.g.* the CO_2-starvation (Vácha and Durchan 1995, Šiffel and Vácha 1998), the opposite case ($F_0 > F_S$) can occur and then the sum exceeds distinctly 1. Furthermore, the relative total quenching coefficient, $q_{(P+N)rel} = q_{(P)rel} + q_{(N)rel}$ (Buschmann 1995) if expressed using Eqs. 45, 46, 48, 50, and 55 means:

$$q_{(P+N)rel} = \frac{F_M - F_S}{F_M - F_0'} = \frac{F_M - F_S}{F_M}\frac{F_M}{F_M - F_0'}$$

$$q_{(P+N)rel} = \frac{q_{TQ}}{q_A} = \frac{q_{TV}\Phi_{P_0} + q_0\Phi_{N_0}}{\Phi_{P_0} + q_0\Phi_{N_0}} \tag{57}$$

As seen, $q_{(P+N)rel}$ differs significantly from previously defined FPs q_{TV}, q_{TQ}, and q_A. It is $q_{TQ} < q_A < q_{TV} < q_{(P+N)rel}$, as follows from Eqs. 9, 42, 45, 48, and 57 (see Fig. 6*D*).

Last "simplified" Chl FP discussed herein is the formula (*e.g.* Herrmann *et al.* 1997):

$$\Phi P = \frac{F_M' - F_0}{F_M'} = 1 - \frac{F_0}{F_M'}, \qquad (58)$$

sometimes used instead of Φ_P (Eq. 33). ΦP does not require knowledge of F_0' that seems to be advantageous. But only if $F_0' = F_0$ it is $\Phi P = \Phi_P$. In all other cases, the difference between ΦP and Φ_P increases. It follows from Eqs. 33 and 58 that $\Phi P < \Phi_P$ as long as $F_0' < F_0$.

As shown, none of the relationships 54, 55, and 58 is identical with the previously defined parameters q_P, q_N, q_0, and Φ_P which always require determination of the F_0' level even if it is sometimes problematic. The parameters qP, qN, and ΦP should not be used for measurements on the stressed plants because they might lead to incorrect results. In the case of $q_{(P)rel}$, contribution of the non-photochemical effects to its value should be taken into account as far as $q_{(P)rel}$ will be applied as the "measure of photochemistry" (*cf.* Buschmann 1995).

Application to experimental data

Herein, the derived relationships and theoretical claims are examined using experimental data. To this purpose, the Chl fluorescence induction kinetic curves recorded using a *PAM101-103* fluorometer (*H. Walz*, FRG) on spruce seedlings during their circadian cycle have been used (Roháček and Šiffel 1995). Six seedlings of the four-year-old Norway spruce [*Picea abies* (L.) Karst.] were grown and measured under controlled conditions in a climatic box *Sherer* (Kysor, USA). The day/night temperature was kept at +21/+11 °C, relative humidity ranged between 65–75 %, photoperiod with PFD of 470 μmol m^{-2} s^{-1} (08:00 – 19:00 h) was alternated periodically with darkness (21:00 – 06:00 h). The dawn and nightfall were simulated using sets of bulbs and fluorescent tubes switched on and off in three steps: 50 – 300 – 470 μmol m^{-2} s^{-1} (06:00 – 07:00 – 08:00 h) and 300 – 50 – 0 μmol m^{-2} s^{-1} (19:00 – 20:00 – 21:00 h), respectively. In these intervals, changeover between the day and night temperature regimes was realised. Seedlings were watered and dewed with distilled water during the nightfall and their positions were changed with the period of 5 – 7 d. Samples were measured *in vivo* by means of a small pre-darkening ventilated chamber. Experiments were performed on July 1994 (Roháček and Barták 1999).

All the parameters defined and discussed in the foregoing text are collected in two following tables. In Table 1, the principal ChlF parameters (Φ_{P_0}, q_P, q_N, q_0) and related ones (Φ_{N_0}, F_V/F_0, $1 - q_P$, q_{CN}, NPQ, q_{TV}, q_{TQ}, q_A, Rfd, Φ_P, Φ_2, Φ_N, F_V'/F_0'), their symbols, basic meaning, and definitions are listed. The relationship of individual parameters to DAS or LAS as well as their functional dependence on the principal foursome are indicated. Table 2 summarises the parameters introduced by a usage of the rate constants of three main processes participating in the utilisation of the excitation energy by PS2. They are valid just under the assumptions made by Oxborough and Baker (1997).

Table 1. Chlorophyll (Chl) fluorescence parameters related to the slow Chl *a* fluorescence induction kinetics. *Abbreviations*: DAS/LAS – dark-/light-adapted state; ChlF – chlorophyll fluorescence. ChlF levels are as defined in Fig. 1 and in the text.

Symbol State	*Meaning of the fluorescence parameter* Definition
Φ_{P_0} DAS	*Maximum quantum yield of PS2 photochemistry*; F_V/F_M ratio $\Phi_{P_0} = \frac{F_V}{F_M} = 1 - \frac{F_0}{F_M}$; $F_V = F_M - F_0$
q_N LAS	*Non-photochemical quenching of variable ChlF* $q_N = \frac{F_V - F_V'}{F_V} = 1 - \frac{F_V'}{F_V} = 1 - \frac{F_M' - F_0'}{F_M - F_0}$; $F_V' = F_M' - F_0'$
q_P LAS	*Photochemical quenching of variable ChlF* $q_P = \frac{\Delta F}{F_V'} = \frac{F_M' - F_S}{F_V'} = 1 - \frac{F_S - F_0'}{F_M' - F_0'}$; $\Delta F = F_M' - F_S$
q_0 LAS	*Relative change of minimum ChlF* $q_0 = \frac{F_0 - F_0'}{F_0} = 1 - \frac{F_0'}{F_0}$

Table 1 (continued).

Φ_{N_0} DAS	*Basal quantum yield of non-photochemical processes in PS2* $\Phi_{N_0} = \frac{F_0}{F_M} = 1 - \Phi_{P_0} \quad \Phi_{N_0}$
F_V/F_0 DAS	*Maximum ratio of quantum yields of photochemical and concurrent non-photochemical processes in PS2* $\frac{F_V}{F_0} = \frac{\Phi_{P_0}}{\Phi_{N_0}}$
$1 - q_P$ LAS	*Degree of PS2 reaction centre closure* $1 - q_P = \frac{F_S - F_0'}{F_M' - F_0'}$
q_{CN} LAS	*Complete non-photochemical quenching of ChlF* $q_{CN} = \frac{F_M - F_M'}{F_M} = 1 - \frac{F_M'}{F_M} = q_N \Phi_{P_0} + q_0 \Phi_{N_0}$
NPQ LAS	*Non-photochemical ChlF quenching* $NPQ = \frac{F_M - F_M'}{F_M'} = \frac{F_M}{F_M'} - 1 = \frac{q_{CN}}{1 - q_{CN}} = \frac{q_N \Phi_{P_0} + q_0 \Phi_{N_0}}{1 - q_N \Phi_{P_0} - q_0 \Phi_{N_0}}$
q_{TV} LAS	*Total quenching of variable ChlF* $q_{TV} = \frac{F_V - (F_S - F_0')}{F_V} = 1 - \frac{F_S - F_0'}{F_M - F_0} = q_P + q_N - q_P q_N$
q_{TQ} LAS	*Total quenching of ChlF* $q_{TQ} = \frac{F_M - F_S}{F_M} = 1 - \frac{F_S}{F_M} = (q_P + q_N - q_P q_N) \Phi_{P_0} + q_0 \Phi_{N_0}$
q_A LAS	*Absolute quenching of ChlF* $q_A = \frac{F_M - F_0'}{F_M} = 1 - \frac{F_0'}{F_M} = \Phi_{P_0} + q_0 \Phi_{N_0}$
Rfd LAS	*Ratio of ChlF decrease to steady-state ChlF*; "vitality index" $Rfd = \frac{F_P - F_S}{F_S} = \frac{F_P}{F_S} - 1 = \frac{q_{TQ} - q_{TQ(P)}}{1 - q_{TQ}} \; ; \; q_{TQ(P)} = 1 - \frac{F_P}{F_M}$
Φ_P LAS	*Effective quantum yield of PS2 photochemistry* $\Phi_P = \frac{F_V'}{F_M'} = 1 - \frac{F_0'}{F_M'} = \Phi_{P_0} \frac{1 - q_N}{1 - q_{CN}} = \frac{(1 - q_N) \Phi_{P_0}}{1 - q_N \Phi_{P_0} - q_0 \Phi_{N_0}}$
Φ_2 LAS	*Effective quantum yield of photochemical energy conversion in PS2* $\Phi_2 = \frac{\Delta F}{F_M'} = 1 - \frac{F_S}{F_M'} = q_P \Phi_P = \frac{q_P (1 - q_N) \Phi_{P_0}}{1 - q_N \Phi_{P_0} - q_0 \Phi_{N_0}}$
Φ_N LAS	*Effective quantum yield of non-photochemical processes in PS2* $\Phi_N = \frac{F_0'}{F_M'} = 1 - \Phi_P = \frac{1 - q_0}{1 - q_{CN}} \Phi_{N_0}$
F_V'/F_0' LAS	*Effective ratio of quantum yields of photochemical and concurrent non-photochemical processes in PS2* $\frac{F_V'}{F_0'} = \frac{\Phi_P}{\Phi_N}$

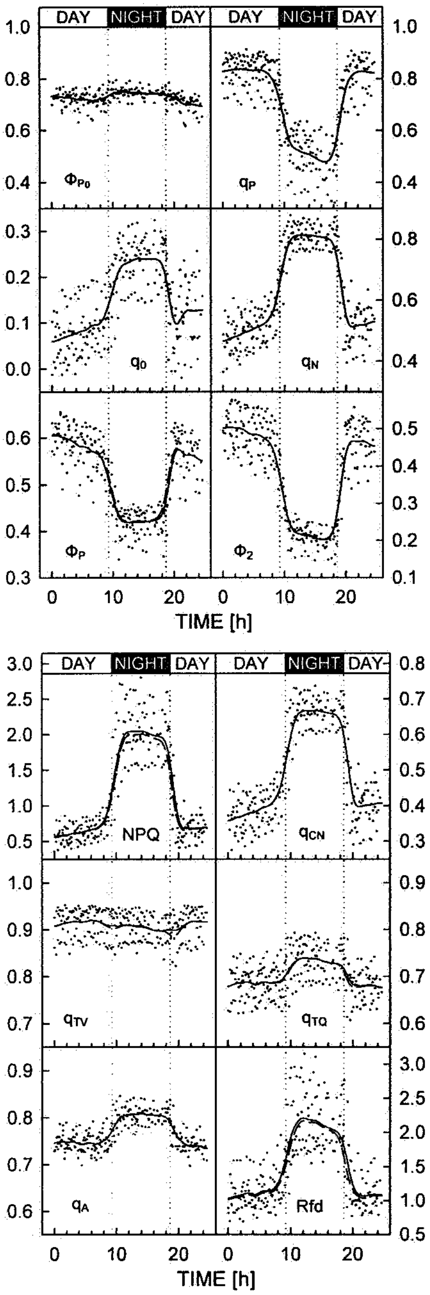
Table 2. List of non-photochemical parameters related to the approximation presented by Oxborough and Baker (1997). *Abbreviations*: d - dissipation factor; k_D – rate constant for the thermal dissipation within the light-harvesting system associated with PS2; k_F – rate constant for the Chl *a* fluorescence; k_P – rate constant for the photochemistry at open PS2 RCs.

Symbol State	*Meaning of the parameter* Definition
Φ_{D_0}	*Basal quantum yield of thermal dissipation within PS2 in DAS*
DAS	$\Phi_{D_0} = \dfrac{k_D}{k_F + k_D + k_P}$
Φ_D	*Basal quantum yield of thermal dissipation within PS2 in LAS*
LAS	$\Phi_D = \dfrac{d\,k_D}{k_F + d\,k_D + k_P}$
$\Phi_{D_0}^M$	*Maximum quantum yield of thermal dissipation within PS2 in DAS*
DAS	$\Phi_{D_0}^M = \dfrac{k_D}{k_F + k_D}$
Φ_D^M	*Maximum quantum yield of thermal dissipation within PS2 in LAS*
LAS	$\Phi_D^M = \dfrac{d\,k_D}{k_F + d\,k_D}$
Φ_{F_0}	*Minimum quantum yield of ChlF in DAS*
DAS	$\Phi_{F_0} = \dfrac{k_F}{k_F + k_D + k_P}$
$\Phi_{F_0'}$	*Minimum quantum yield of ChlF in LAS*
LAS	$\Phi_{F_0'} = \dfrac{k_F}{k_F + d\,k_D + k_P}$

Numerical values of 12 Chl FPs (for their definitions see Table 1) are presented in Figs. 3 and 4. The experimental points reflect variability of measured spruce seedlings in the course of their circadian cycle as well as a principal difference between the day and night physiological regimes of plants. It can be inferred from graphs that inactivation of the photosynthetic apparatus within the night period leads to the over-excitation of PS2 acceptor side during the short-term actinic irradiation. This situation manifests itself as a pronounced decrease in the efficiency of the photochemical processes (see q_P, Φ_P, Φ_2 in Fig. 3) and also a large activation of the non-photochemical ones (see q_0, q_N, NPQ, q_{CN}, Rfd in Figs. 3 and 4) which dissipate the excessive excitation energy mainly to heat (*cf.* Roháček and Barták 1999).

Large diurnal changes in values of most Chl FPs can serve very well for a verification of the all above discussed theoretical results. For that purpose, the values were processed in two ways: on the level of individual experimental points and on the level of smoothed curves. The individual values of related Chl FPs (Φ_P, Φ_2, NPQ, q_{CN}, q_{TV}, q_{TQ}, q_A, Rfd) were calculated on the basis of derived equations (Table 1) using experimental values found for the principal quartet of FPs (Φ_{P_0}, q_P, q_N, q_0). Resulted values were in an outright agreement with those obtained from the definitions based on the respective FY levels. It demonstrates unambiguously the validity of presented relationships on the level of individual points. If the same procedure was applied to averaged data the calculated and smoothed curves exhibited some deviations (see Figs. 3 and 4). Except of Φ_P, NPQ, and Rfd, the relative deviations (δ) between points of corresponding curves (solid *vs.* dashed) were lower than 2 % (values not shown). For Φ_P, NPQ, and Rfd, δ exceeded 4 % only in a vicinity of the dotted vertical lines plotted in Figs. 3 and 4. This effect was fully a product of the multiple smoothing and had not its origin in the derived formulae.

Graphs in Fig. 5 demonstrate the following theoretical conclusions: The finding that F_V'/F_0' is always less than F_V/F_0 is shown in Fig. 5*A*. Both ratios are more sensitive to changes in F_0' and F_0 than F_V'/F_M' and F_V/F_M can reflect. The capability of q_N, q_{CN}, and Φ_N (the F_0'/F_M' ratio) to estimate the efficiency of NRD processes in LAS is manifested in Fig. 5*B*. As seen, those curves are very similar to NPQ (Fig. 4) and q_0 (Fig. 3). The ratio F_0'/F_M' is greater than F_0/F_M ($\Phi_N > \Phi_{N_0}$) in all cases, as well as $F_0'/F_M' > 1 - q_P$, which is not evident from the definitions 38 and 21. The truth of inequalities $0 < \Phi_2 < \Phi_P < \Phi_{P_0} < 1$



Figs. 3–4. Circadian cycle of the Norway spruce (*Picea abies* [L.] Karst.) monitored as the diurnal courses of 12 Chl fluorescence parameters induced by simultaneous changes of the ambient temperature and irradiance. Each point in graphs (251 points *per* plot) corresponds to one fluorescence induction kinetics recorded *in vivo* using the *PAM101-103* fluorometer; for experimental settings see Roháček and Barták (1999). Experiments started at 11:30 (time zero) and were performed on the current-year spruce branches of 6 different 4-year-old seedlings. Solid curves in the graphs are results of 100-times repeated non-equidistant smoothing from 3 points. Dashed curves were calculated point-by-point according to derived formulae by means of the smoothed curves obtained for principal FPs Φ_{P_0}, q_P, q_N, and q_0.

and $\Phi_2 < q_P$ is shown in Fig. 5*C*. It is also easy to demonstrate, applying elementary algebra to Eqs. 16, 18, 28, 42, 45, that $q_0 < q_{CN} < q_{TQ}\,(q_N) < q_{TV} < 1$. These inequalities are validated graphically in Fig. 5*D*. Furthermore, the

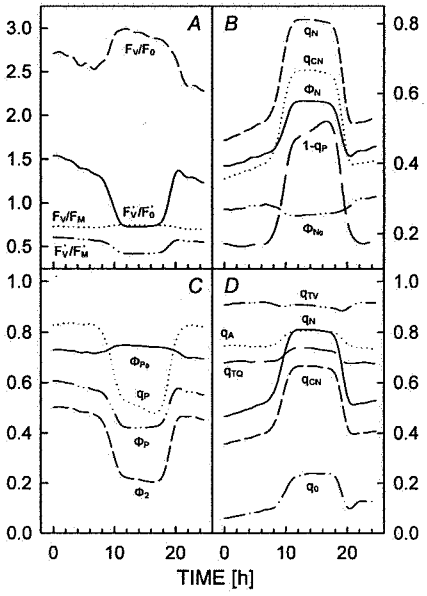


Fig. 5. Graphical verification of the inequalities and claims discussed theoretically in the text. Courses of all indicated Chl FPs (except Φ_{P_0}, q_P, q_N, and q_0) were calculated according to derived formulae (see Table 1) using smoothed values found for the principal parameters.

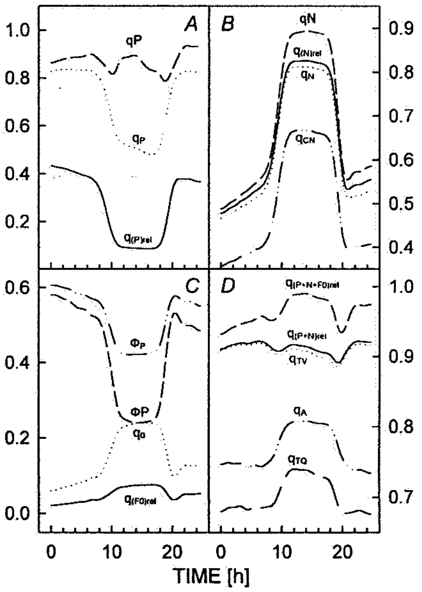


Fig. 6. Comparison of the alternative parameters qP, qN, $q_{(P)rel}$, $q_{(N)rel}$, $q_{(F0)rel}$, $q_{(P+N)rel}$, $q_{(P+N+F0)rel}$, and ΦP with principal (q_P, q_N, q_0) and some related (q_{CN}, q_{TV}, q_A, q_{TQ}, Φ_P) Chl FPs. The courses of alternative and related FPs were calculated according to the derived formulae (see Table 1 and the text).

course of q_{TV} (Fig. 4, Fig. 5*D*) indicates that the total quenching of F_V by photochemical and non-photochemical processes is almost constant both in the day and night physiological regimes of plants. Thus q_{TV} manifests clearly the fact that the excitation energy is redistributed steadily into the photochemical and non-photochemical pathways depending on the external conditions.

The conclusion that alternative Chl FPs qP and qN (Eqs. 54) are systematically higher than q_P and q_N, respectively, is demonstrated in Fig. 6*A,B*. As seen, the incorrect definition of qP results in a misleading distortion of the qP-course in the night period. From Fig. 6*B* follows that there is $q_{CN} < q_N <$ qN. The Buschmann's relative coefficient $q_{(P)rel}$ (in Eq. 55) is markedly reduced contraryto q_P (Fig. 6*A*) as well as $q_{(F0)rel}$ to q_0 (Fig. 6*C*). The coef-ficient $q_{(N)rel}$ (Eq. 55) is systematically higher but close to q_N (Fig. 6*B*). It follows from Fig. 6*D* that the Buschmann's parameter $q_{(P+N)rel}$ (Eq. 57) is higher than q_{TQ}, q_A, and q_{TV} but almost similar to q_{TV}. Further, the contribution of $q_{(F0)rel}$ to $q_{(P+N+F0)rel}$ (Eq. 56) is not negligible (*cf.* Buschmann 1995). At last, Fig. 6*C* indicates the dissimilarity of ΦP (Eq. 58) from Φ_P, mainly in the night period.

In summary, this article gives an overview of the Chl fluorescence parameters which are defined on the basis of six primary ChlF yields (F_M, F_0, F_P, F_M', F_0', F_S) determined from the slow ChlF induction kinetics recorded using the "modulated" fluorimeters. The possibility to express a majority of Chl FPs using the small set of parameters is clearly demonstrated. Herein, the quartet $\{\Phi_{P_0}, q_P, q_N, q_0\}$ is chosen as the principal set. From the view of a mathematical formalism, it is possible to construct other basic sets of Chl FPs which match up to that claim, *e.g.*: $\{\Phi_{P_0}, \Phi_2, q_{CN}, q_0\}$, $\{\Phi_{P_0}, q_{TQ}, q_{CN}, q_0\}$, *etc.* However, the determination of Φ_{P_0} and q_0 is essential in all cases, in spite of the fact that there are parameters not requiring knowledge of the F_0' level(NPQ, q_{CN}, q_{TQ}, Φ_2, Rfd). After the author's opinion, the most convenient FPs which are able to give a relevant insight into the photosynthetic processes in chloroplasts and can be used effectively in the photosynthesis research are: Φ_{P_0}, q_P, q_N, q_0, Φ_2, and NPQ. Finally, all Chl FPs based on F_0 and F_0' can be affected in their absolute values due to contribution of the ChlF emission from the PS1 pigment complexes to these levels. Although the ChlF emission from PS1 is insignificant at room temperature, this effect should be considered in the analysis of data obtained on samples measured under the strong-stress or non-physiological conditions (the very low/high temperatures, CO_2 starvation, excessive irradiance, *etc.*).

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