PHOTOSYNTHETICA 37 (2): 217-224, 1999

Photochemical and non-photochemical quenching coefficients of the chlorophyll fluorescence: comparison of variation and limits

C. BUSCHMANN

Botanical Institute, University of Karlsruhe, D-76128 Karlsruhe, Germany

Abstract

There are several types of quenching coefficients currently in use which describe the decrease of the chlorophyll fluorescence: the photochemical quenching coefficients q_P and $q_{(P)rel}$ and the non-photochemical quenching coefficients q_N, $q_{(N)rel}$, and NPQ. These five coefficients were calculated for a broad variety of cases of the fluorescence signals in a normal, realistic range and for determining the limits in a range with extremely low and high fluorescence values. The calculations showed that the quenching coefficients currently in use are not only numbers between 0 and 1 as one would expect when taking them as a relative measure of the quenching process. Most quenching coefficients must be regarded and interpreted carefully separated from each other. Each photochemical quenching coefficient and each non-photochemical quenching coefficient describe the same fluorescence signal in a different way. Only the relative quenching coefficients $q_{(P)rel}$ and $q_{(N)rel}$ match together and can be used to demonstrate a shift of the energy de-excitation from the photochemical to the non-photochemical route.

Additional key words: pulse-amplitude-modulation.

Introduction

When irradiating a leaf kept before in the dark, the chlorophyll (Chl) fluorescence varies with the changes in its photosynthetic activity (induction kinetics or Kautsky-effect: Kautsky and Hirsch 1931). In photosynthesis research the decrease of the yield of Chl fluorescence from its maximum is commonly termed "quenching". The same is done by physical chemists when describing the decrease of luminescence of solutions containing an organic or inorganic substance ("quencher") which reduces the luminescence by taking over energy from the fluorescing molecule in a bimolecular process. The thus excited quencher is de-excited *via* a radiationless, thermal transition into its ground state. In photosynthesis research, Duysens and

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Received 19 April 1999, *accepted* 16 August 1999.

Fax: (+49) 721 608 4874, e-mail: Claus.Buschmann@bio-geo.uni-karlsruhe.de

Sweers (1963) introduced the word "quencher" as a redox substance (nowadays abbreviated Q_A) which leads to the decrease of the Chl fluorescence in its oxidised state.

The Chl fluorescence which undergoes variation depending on photosynthetic activity is called "variable" fluorescence F_v, in contrast to the constant or "dead" fluorescence F_0 which is constant independent of the photosynthetic activity. The quenching of fluorescence from its maximum is commonly expressed by a quenching coefficient q which should give the percentage of quenched fluorescence as a value between 0 and 1. For the quenching of the maximum fluorescence F_M to the present measured fluorescence F the quenching coefficient q gives the proportion of the maximum variable fluorescence F_v (= F_M - F_0):

$$F_M - F = q \times F_v \quad \text{or} \quad q = (F_M - F)/F_v$$

When measuring the induction kinetics of a leaf by means of fluorescence excitation with a continuous radiation source one can describe the changes of the Chl fluorescence as inverse changes of the fluorescence quenching. The quenching of Chl fluorescence is high at the onset of irradiation, decreases rapidly (within less than a second) and increases again within several minutes. The interpretation of these induction kinetics has mainly been based on the redox state of Q_A (*e.g.*, Papageorgiou 1975). With the use of the "light-doubling method" (Bradbury and Baker 1981) fluorescence excitation with pulsed and continuous irradiation (Schreiber 1983) enables to separate the quenching into a photochemical part and a non-photochemical part. The resolution into different types of quenching allows to gain information about the function of the photosynthetic processes around photosystem 2 (PS2; for reviews see, *e.g.*, Karukstis 1991, Krause and Weis 1991). The photochemical quenching is defined as the decrease of fluorescence due to the photochemical charge separation in the reaction centre of PS2 (Schreiber *et al.* 1986). It is measured by comparing the fluorescence without and with a strong radiation pulse that saturates photosynthesis and closes the reaction centres. The non-photochemical quenching is more generally defined as the decrease of fluorescence not due to the photochemical process. It is induced by the pH-gradient at the thylakoid membrane, by state transitions, and by photoinhibition (Karukstis 1991, Krause and Weis 1991). The exact mechanism of the photochemical quench is still not fully understood (see, *e.g.*, Pospíšil 1997). It is usually assumed that the decrease of fluorescence due to non-photochemical quenching is caused by an increased loss of the energy taken up by absorption of photons *via* thermal dissipation. In case of photoinhibition an increased heat production could be directly measured (by means of the photoacoustic technique - Buschmann 1987, Havaux 1989) as earlier predicted from fluorescence studies, but it could also be shown that the kinetics of the non-photochemical quenching does not parallel the kinetics of heat production (Buschmann and Kocsányi 1989).

Several photochemical and non-photochemical quenching coefficients have been defined in the past (Table 1). In this study these coefficients are compared for a variety of cases of the induction kinetics leading to the detection of variation and limits of each coefficient. The description of different behaviour of these coefficients

Table 1. Quenching coefficients of the chlorophyll fluorescence. The definitions are given using the symbols of Fig. 1 with the generally agreed nomenclature (van Kooten and Snel 1990).
Types of radiation exciting chlorophyll fluorescence: AL = continuous medium-intensity actinic radiation, ML = pulsed low-intensity measuring radiation, SL = radiation pulse of high intensity saturating photosynthesis.
Types of chlorophyll fluorescence signals: F = chlorophyll fluorescence during actinic irradiation (ML and AL); F_0 = ground fluorescence before the induction of photosynthesis (ML); F_0' = ground fluorescence directly after turning off the actinic radiation used for the induction of photosynthesis (ML); F_M = maximum fluorescence upon saturating irradiation before the induction of photosynthesis (ML and SL); F_M' = signal upon saturating pulse irradiation during actinic irradiation (ML, AL, and SL).

Abbreviation	Definition	Reference
Photochemical quenching		
q_P	$(F_M' - F)/(F_M' - F_0') = (F_M' - F)/F_v'$	Bilger and Schreiber 1986
$q_{(P)rel}$	$(F_M' - F)/(F_M - F_0')$	Buschmann 1995
Non-photochemical quenching		
q_N	$1 - (F_M' - F_0')/(F_M - F_0)$ $= [(F_M - F_0) - (F_M' - F_0')]/(F_M - F_0)$ $= (F_v - F_v')/F_v$	Bilger and Schreiber 1986
$q_{(N)rel}$	$(F_M - F_M')/(F_M - F_0')$	Buschmann 1995
NPQ	$(F_M/F_M') - 1 = (F_M - F_M')/F_M'$	Bilger and Björkman 1990

will contribute to a better understanding of the interpretation by means of these parameters.

Materials and methods

The photochemical and non-photochemical quenching coefficients established in the literature for fluorescence measurements by means of pulse-amplitude modulation (Table 1) were calculated for fluorescence signals in a normal, realistic range and in

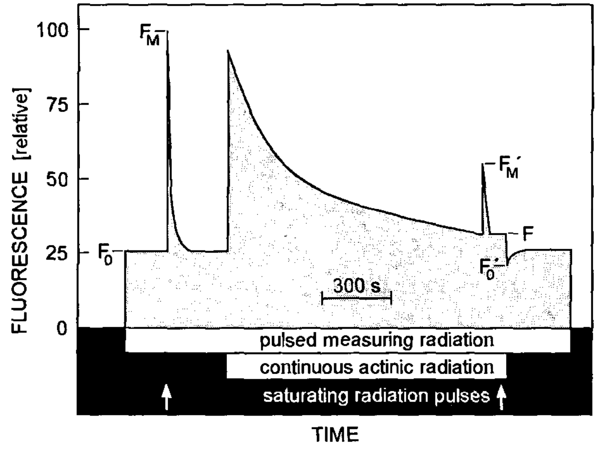

Fig. 1. Example of a recording of chlorophyll fluorescence measurement with the definition of fluorescence signals according to the generally agreed nomenclature (van Kooten and Snel 1990). The abbreviations for different points of the measurement and the parameters derived from the signals are given in Table 1.

a range with extreme low and high values. The abbreviation for the fluorescence signals was used as generally agreed (van Kooten and Snel 1990, Fig. 1). The fluorescence values were normalised to a constant maximum of 100 (= F_M). The fluorescence values, except for F_M, were varied from 1 to 100 with the realistic restriction that $F_M' = >F$, $F_0 = <F$, and $F_0' = <F_0$. No further experimental details can be given, since the calculation should be applicable for all plants under all experimental conditions (irradiances for the measuring radiation, actinic radiation, and saturating radiation, measuring climate, photosynthetic activities of the plants, *etc.*).

Results

q_P and q_N: The photochemical quenching coefficient q_P increases and the non-photochemical quenching coefficient q_N decreases with increasing difference between F_M' and F, *i.e.*, increasing effect of a saturating radiation pulse given during actinic irradiation (Fig. 2). The increase of q_P is a curve whereas the decrease of q_N is linear. When F_M' equals F (= minimum of the x-axis), q_P takes the value 0 and q_N a value somewhat below 1. When F_M' equals F_M (= maximum of the x-axis), q_P is 0.875 and q_N is somewhat below 0. When F_M' is close to F_M (*e.g.*, under low actinic irradiance), usually F_0' is close to F_0 in contrast to the assumption made for the calculation (F_0' being always 0.8 F_0). When taking this into account and calculating with equal values for F_0' and F_0, then q_P and q_N at the maximum of the x-axis increase somewhat to values of 0.933 and 0, respectively. The sum of q_P and q_N varies and, except for the lowest and highest values of the abscissa, becomes greater than 1.

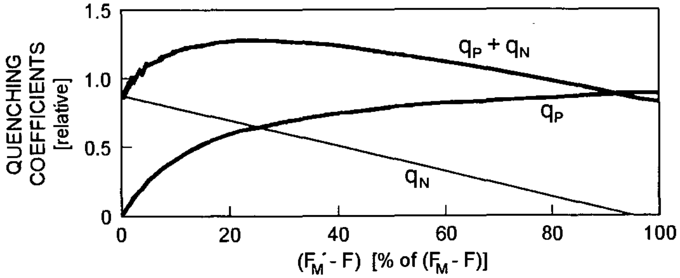

Fig. 2. Photochemical quenching coefficient q_P, non-photochemical quenching coefficient q_N, and the sum of both ($q_P + q_N$) in dependence of the relative difference between F_M' and F in percent of the constant value for F_M - F (*i.e.*, the effect of a saturating radiation pulse given during actinic irradiation). The calculation is based on the constant values: $F_M = 100$, $F = 30$, $F_0 = 25$, $F_0' = 20$. F_M' varied between 30 and 100.

$q_{(P)rel}$ and $q_{(N)rel}$: With increase of the difference between F_M' and F, the relative photochemical quenching coefficient $q_{(P)rel}$ rises whereas the relative non-photochemical quenching coefficient $q_{(N)rel}$ decreases (Fig. 3). Both coefficients show a maximum of 0.875. The changes with increasing difference between F_M' and

F are linear and the sum of these relative quenching coefficients is constant (= 0.875). When taking equal values for F_0' and F_0 (assuming that a small difference between F_M' and F_M goes along with a small difference between F_0' and F_0, see above for q_P and q_N), then $q_{(N)rel}$ increases slightly from 0.875 to 0.933 at the end of the x-axis.

NPQ: The non-photochemical quenching coefficient NPQ shows a declining curve with increasing effect of a saturating radiation pulse given during actinic irradiation, *i.e.*, increasing difference between F_M' and F. Under the conditions given for Fig. 3, NPQ declines from a value of 2.33 at 0 % to a value of 0 at 100 %.

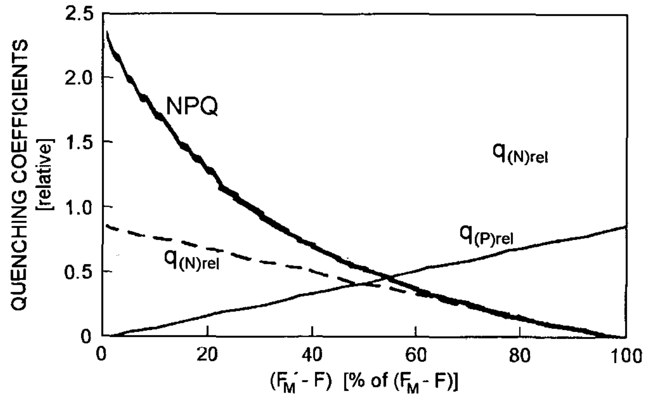

Fig. 3. Quenching coefficients defined in Table 1 in dependence of the relative difference between F_M' and F in percent of the constant value for F_M - F (*i.e.*, the effect of a saturating radiation pulse given during actinic irradiation). The calculation is based on the constant values: $F_M = 100$, $F = 30$, $F_0 = 25$, $F_0' = 20$. F_M' varied between 30 and 100.

Limits: Most quenching coefficients have a minimum of 0 and a maximum of 1 (Table 2). For the photochemical quenching coefficients the minimum is reached when F_M' equals F, a maximum is reached when F_M' equals F_M. The opposite is true for the non-photochemical quenching coefficients, they reach a maximum when the photochemical quenching coefficients are the lowest. The q_N and $q_{(N)rel}$ have a maximum value of 1, whereas the maximum for NPQ may lie between 0 and 100 depending on the values for F and F_0'. The minima for NPQ and $q_{(N)rel}$ are zero, but q_N may range between a slightly negative value and 1.

Discussion

The decrease of Chl fluorescence of leaves is generally termed quenching irrespective of the mechanism. This is in contrast to the original meaning of quenching still in use by physical chemists. Quenching in this sense is always the result of an energy transfer between two molecules to a (quenching) substance which later returns to its ground state by a loss of energy *via* thermal dissipation.

The separation of quenching into a photochemical and a non-photochemical part is based on the observation of changes of fluorescence due to closure of reaction centres induced by irradiation saturating the electron transport around PS2 (Schreiber *et al.* 1986). One would assume that the sum of the two quenching parameters would

Table 2. Minima and maxima of quenching coefficients of the chlorophyll fluorescence defined in Table 1. The values are reached under the extreme conditions given as head of each column: (*A*): minimum (F_M' - F) and (*B*): minimum (F_M - F_M'). *actually division by 0 (but otherwise close to 0).

		(*A*)	(*B*)
Variable (1 to 100):		$F_M' = F = F_0 = F_0'$	$F = F_0 = F_0'$
Constant:		$F_M = 100$	$F_M = F_M' = 100$
Photochemical quenching	q_P	0*	1.0
	$q_{(P)rel}$	0	1.0
Non-photochemical quenching	q_N	1.0	0
	$q_{(N)rel}$	1.0	0
	NPQ	100 to 0**	0

lead to 1, *i.e.*, that the photochemical quenching and the non-photochemical quenching would stand for the full quenching. This is not the case for the most frequently used quenching coefficients q_P and q_N (Fig. 2). The reason for the fact that q_P and q_N do not fit together is that q_P relates to the difference between F_M' and F_0' (= F_v') whereas q_N relates to the difference between F_M and F_0 (= F_v). Originally the photochemical quenching coefficient q_P was deduced describing the proportion of quenching the variable fluorescence F_v':

$F_M' - F = q_P \times F_v'$ this leads to $q_P = (F_M' - F)/(F_M' - F_0')$,

whereas the non-photochemical quenching coefficient q_N was deduced from the proportion of quenching the variable fluorescence F_v:

$F_v - F_v' = q_N \times F_v$ this leads to $q_N = (F_M - F_0) - (F_M' - F_0')/(F_M - F_0)$

Since F_0 and F_0' in most cases are close together, it is the difference between F_M and F_M' which is the main reason for this mismatch of q_P and q_N. In this way one can understand the unexpected result that upon high temperature or low irradiance stress the increase of q_N is not always complementary to the decrease of q_P (Laisk *et al.* 1997). Actually the mismatch between q_P and q_N was the reason for creating the relative quenching coefficients $q_{(P)rel}$ and $q_{(N)rel}$ (Buschmann 1995). These two quenching coefficients refer to the same fact (namely the full span of the variable fluorescence: F_M - F_0') and lead to a constant sum. Thus they can be taken as an indicator for the photochemical and non-photochemical parts of quenching and the shifts from the de-excitation of energy from the photochemical to the non-photochemical route. These relative quenching coefficients are linearly related to the difference between F_M' and F (Fig. 3) and thus describe directly the quenching of the Chl fluorescence.

The NPQ was established (Bilger and Björkman 1990) since it provides the advantage of being an indicator of the non-photochemical quench without a measurement of F_0 or F_0'. The NPQ is based on the Stern-Volmer equation and therefore some authors use the abbreviation SVN (Stern-Volmer non-photochemical quenching, Gilmore and Björkman 1994). The Stern-Volmer equation is used by photochemists for characterising the accessibility of quenching substances to fluorescing molecule and thus for their quenching efficiency:

$$\Phi_F/\Phi_Q = 1 + K \times [Q] \quad \text{or} \quad \Phi_F/\Phi_Q - 1 = K \times [Q]$$

where Φ_F = fluorescence without quencher, Φ_Q = fluorescence with quencher, K = Stern-Volmer quenching constant, [Q] = concentration of the quenching substance.

From the research on PS2 it is, however, obvious that the non-photochemical quench is not caused by one quenching substance but by a number of energy-consuming processes not directly involved in the charge separation at PS2 (Pospíšil 1997). The NPQ is totally different from the other non-photochemical quenching coefficients and thus does not match with any photochemical quenching coefficient. NPQ starts with a high value which may be up to 100 (Table 2) and declines in a curve down to 0 with increasing difference between F_M' and F (Fig. 3).

Conclusion: The calculations show that the quenching coefficients currently in use are not only numbers between 0 and 1 as one would expect when taking them as a relative measure of the quenching process. Most quenching coefficients must be regarded and interpreted carefully separated from each other. Each photochemical quenching coefficient and each non-photochemical quenching coefficient describe the same fluorescence signal in a different way. Only the relative quenching coefficients $q_{(P)rel}$ and $q_{(N)rel}$ match together and can be used to demonstrate a shift of the energy de-excitation from the photochemical to the non-photochemical route.

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