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A chlorophyll fluorescence analysis of the allocation of radiant energy absorbed in photosystem 2 antennae of cotton leaves during exposure to chilling

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Abstract

When dark-acclimated cotton (*Gossypium hirsutum* L. cv. Coker 312) leaves, pre-treated with lincomycin to inhibit chloroplast protein repair processes, were exposed to 10 °C and a PPFD of 500 μmol m^{-2} s^{-1}, the proportion of excitation energy entering photochemistry (P) increased, but only to 5 % of the total energy absorbed at steady state levels of P, which were reached at 40 min of irradiation. Thermal dissipation (D) of absorbed energy increased throughout the 360 min irradiation period and accounted for the greatest portion of absorbed energy at 10 °C. When D was partitioned into constitutive (D_{CON}), regulated (D_{REG}), and photoinhibitory (D_{PI}) components, it was primarily composed of D_{REG}, the readily reversible portion of D. However, the induction of D was slow at 10 °C. Sixty minutes were required for D to reach 70 % of the energy absorbed. Considerable absorption of energy in excess of that utilized in photochemistry or dissipated thermally (designated as E) occurred, especially during induction of P and D. Over the irradiation period, the time-dependent averaged E exhibited an inverse, linear relationship with the ratio of variable (F_v) to maximum (F_m) fluorescence (PS2 efficiency) and a linear relationship with D_{PI}. We propose that time-dependent averaged E may be useful for estimating the potential for damage to PS2 under stressful environmental conditions.

Additional key words: energy dissipation; *Gossypium hirsutum*; low temperature; photoinhibition.

Introduction

During exposure to low temperatures, even moderate PPFDs may lead to a slowly reversible reduction in the efficiency of energy utilization for photochemistry by PS2. This reduction is commonly referred to as photoinhibition. The extent of photoinhibition depends on the ability of the photosynthetic apparatus either to utilize absorbed radiant energy in photochemical reactions or to dissipate it safely in the light-harvesting complexes. These processes serve as sinks for excitation energy and reduce the vulnerability of PS2 to photoinhibition (Melis 1999, Allen and Ort 2001). Therefore, an assessment of the allocation of absorbed photon energy between different processes is of considerable importance to the investigation of the strategies employed by plants to protect PS2 from photoinhibition during exposure to environmental stress.

Analyses of chlorophyll (Chl) fluorescence emission can be used to gain information about the efficiency of photochemistry and thermal dissipation in PS2 complexes. Chl fluorescence, photochemistry, and thermal

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Abbreviations: Chl, chlorophyll; D, fraction of radiation absorbed in PS2 antennae that is dissipated *via* thermal dissipation; D_{CON}, D_{REG}, and D_{PI}, constitutive, regulated (reversible in the dark), and photoinhibitory (non-reversible or slowly reversible) thermal dissipation, respectively; E, fraction of radiation absorbed in PS2 antennae that is excessive (neither allocated to photochemistry nor to thermal dissipation); F, actual level of fluorescence; F_0, F_m, and F_v, initial, maximal, and variable chlorophyll fluorescence yield for dark-acclimated samples, respectively; F_0', F_m', and F_v', initial, maximal, and variable chlorophyll fluorescence yield for light-acclimated leaves, respectively; k_{PI}, rate constant of photoinhibition; P, fraction of radiation absorbed in PS2 antennae that is utilized in photosynthetic electron transport; PPFD, photosynthetic photon flux density; PS, photosystem; Q_A, primary quinone acceptor of PS2; q_E, high-energy-state quenching (ΔpH dependent); q_I, quenching associated with photoinhibition; q_P, coefficient of photochemical quenching; q_T, non-photochemical quenching associated with state transitions; S.D., standard deviation.
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dissipation serve as alternative routes for the consumption of excitation energy absorbed by PS2 antennae and can be viewed as in competition with each other. Thus, the intensity of Chl fluorescence depends on the efficiency of both photochemistry and thermal dissipation. For example, under conditions that decrease photon utilization by photochemistry or thermal dissipation, Chl fluorescence may increase, while an increase in photochemistry or thermal dissipation is said to quench Chl fluorescence. Maxwell and Johnson (2000) review the methodology used to study the functional characteristics of the photosynthetic apparatus by means of Chl fluorescence analysis.

The Chl fluorescence parameter, F_v'/F_m', can be used to estimate the intrinsic efficiency of PS2 photochemistry in irradiance-acclimated leaves. Demmig-Adams *et al.* (1996a) present a method to assess the fraction of absorbed photon energy allocated to thermal dissipation on the basis of changes in F_v'/F_m'. The novel parameter they derive, D, is calculated as $1 - F_v'/F_m'$, and is useful, primarily because it allows for the direct comparison of the allocation of photon energy to non-photochemical dissipation and to photochemistry, which is assessed *via* the parameter developed by Genty *et al.* (1989), $(F_m' - F)/F_m'$.

D was developed assuming that overall levels of energy dissipation could be attributed either to regulated xanthophyll cycle-dependent energy dissipation or to constitutive energy dissipation that lowers maximum photochemical efficiencies from unity to *ca.* 0.87 (Björkman and Demmig 1987, Demmig-Adams *et al.* 1996a). However, in addition to these two processes, photoinhibitory PS2 inactivation also leads to a decrease in F_v'/F_m' and acts as a third mechanism contributing to overall dissipation. Photoinhibitory dissipation complicates the interpretation of D, especially under conditions when high levels of photoinhibition may be sustained, such as after the imposition of an environmental stress to which the plant was not previously acclimated.

The aim of this study was to examine the changes in the allocation of excitation energy that occur during photosynthetic induction at moderate PPFD and low temperature for cotton, a chilling-sensitive species (Königer and Winter 1993, Payton *et al.* 1997, 2001). We extended the method of Demmig-Adams *et al.* (1996a) by using Chl fluorescence parameters to partition overall levels of energy dissipation into constitutive (D_{CON}), regulated (D_{REG}), and photoinhibitory (D_{PI}) components. Also, we derived a parameter that quantified the levels of absorbed photon energy neither allocated to dissipation nor to photochemistry. This parameter exhibited a strong, positive correlation with the extent of photoinhibition.

Materials and methods

Plants: Cotton plants, *Gossypium hirsutum* L. cv. Coker 312, were grown in 8 000 cm^3 pots in a greenhouse at ~30/26 °C (day/night) with a natural photoperiod. Plants were fertilized with Hoagland's solution twice a week. The upper, fully expanded leaf of 5 to 8 week-old plants was used for all analyses.

Experimental treatments: For most experiments, leaves were pre-treated with lincomycin to inhibit chloroplast protein synthesis (Klaff and Gruissem 1991), thus eliminating the influence of PS2 repair processes. For this treatment, the leaves were harvested before sunrise by cutting their petioles under water. They were immediately transferred to microcentrifuge tubes containing 1 kg m^{-3} lincomycin (863 units mg^{-1}) and kept in the dark for 3 h at room temperature. At the end of this dark incubation period, the concentration of lincomycin in the bulk leaf tissue (C_l) was 0.8 to 1.5 mM as estimated from the formula: $C_l = C_S(W_S/W_L)$, where C_S is the inhibitor concentration in the solution, W_S is the mass of the solution taken up by a leaf, and W_L is the fresh mass of the leaf (Bilger and Björkman 1994). Leaf discs that were removed from these leaves and subjected to photoinhibitory conditions exhibited no increase in F_v/F_m when subsequently exposed to conditions (3 h at a PPFD of 10 µmol m^{-2} s^{-1} and room temperature) that favored repair processes (values not shown).

Leaf discs (10 cm^2) were exposed to 10 °C for 10 min prior to irradiation at a PPFD of 500 µmol m^{-2} s^{-1} (at 10 °C) in the chamber of an oxygen electrode (*Hansatech*, King's Lynn, Norfolk, UK). For lincomycin-treated leaves, CO_2 was supplied by wetting a sponge in the electrode chamber with 1 M $NaHCO_3$. This solution was exchanged every 40 min during the first 2 h and every 30 min thereafter. For leaf discs that were not pre-treated with lincomycin (the analysis of temperature response), measurements were performed in humidified air containing 5.28 % CO_2.

Chl fluorescence emission from leaf discs was measured with a pulse amplitude-modulated fluorometer (*PAM 101/103, Heinz Walz*, Effeltrich, Germany) through a port in the leaf disc electrode chamber. The experimental protocol described by Schreiber *et al.* (1986) and nomenclature of van Kooten and Snel (1990) were used. Measurements of F_0 and F_0' were performed after application of low-intensity far-red radiation. The frequency of the modulated measuring radiation was 1.6 kHz when measurements were made in the absence of actinic radiation and 100 kHz in the presence of actinic radiation. Saturating pulses, 2 s in duration, were provided by a *KL 1500* source (*Schott*, Wiesbaden, Germany). Values of F_v/F_m for leaf discs prior to the chilling/moderate PPFD treatment averaged 0.78±0.02 (mean±S.D., n = 11). The ini-

tial measurements of F_v/F_m for non-stressed leaves were performed 5 min before beginning the continuous irradiation. As indicated above, these leaves were collected before sunrise and were maintained in the dark for the 3-h lincomycin treatment prior to F_v/F_m measurements and subsequent irradiation.

Following the approach of Demmig-Adams *et al.* (1996a), the portion of the absorbed photon energy that was thermally dissipated (D) was calculated as the difference between the maximal theoretical and actual levels of PS2 efficiency ($D = 1 - F_v'/F_m'$). The fraction of photon energy absorbed by PS2 antennae that was trapped by "open" PS2 reaction centers (centers with Q_A in the oxidized state) and utilized in PS2 photochemistry (P) was estimated as $P = F_v'/F_m' \times q_P = (F_m' - F)/F_m'$ (Genty *et al.* 1989), where q_P is the photochemical quenching coefficient calculated as $(F_m' - F)/F_v'$. The fraction of the absorbed radiation not attributed to P or D (denoted as "excess", E) and representing the photon energy absorbed by PS2 antennae and trapped by "closed" PS2 reaction centers (centers with Q_A in the reduced state) was estimated using $E = 1 - (D + P) = F_v'/F_m' \times (1 - q_P)$ (Demmig-Adams *et al.* 1996a).

The photon energy that is not used for electron transport ($1 - P = D + E$) can be considered to be excessive in relation to electron transport capacity. Of this additional energy absorption, D estimates the amount that is safely dissipated as heat, while E estimates absorbed energy that is in excess of that which can be thermally dissipated or used in photochemistry. E is thought to have the potential to damage PS2 complexes. Photon energy trapped by "closed" PS2 reaction centers can result in the formation of triplet P680, double reduction of Q_A, and the generation of singlet oxygen, thereby creating conditions that favor PS2 inactivation (Melis 1999, Oxborough and Baker 2000). Therefore, it is logical to suggest that the total amount of the photon energy trapped by "closed" PS2 reaction centers may be proportional to the extent of reduction in the activity of PS2 complexes during photoinhibition. To estimate the amount of the photon energy trapped by "closed" PS2 centers, we propose a new parameter, time-dependent averaged E:

Time-dependent averaged

$$E = \sum_{i=2}^{n}(E_i + E_{i-1})/2 \times t \times PPFD \times 0.75 \times 0.5) \qquad (1)$$

E_i and E_{i-1} are the levels of E measured at the current and previous time-points, respectively, t is the time between measurements, and 0.75 and 0.5 are the coefficients for cotton leaf absorbance (Björkman and Demmig 1987) and for the sharing of absorbed photons between PS1 and PS2, respectively. The value of the last coefficient (0.5) assumes an equal distribution of excitation between PS1 and PS2 (Krall and Edwards 1992). F_v/F_m is used for E_1 at $t = 0$, because at the beginning of a chilling treatment, q_P is approximately 0.

The time-dependent averaged E represents the extent of exposure to excessive energy absorption by PS2 antennae. In order to investigate the relationship between the time-dependent averaged E and the extent of PS2 inactivation measured as F_v/F_m, the values for time-dependent averaged E were calculated for different time points during the irradiation. At the same time points, 1 cm^2 leaf discs were removed from the 10 cm^2 disc to determine F_v/F_m for stressed samples [denoted as $(F_v/F_m)_{PI}$ in the text below] after 3 h of dark acclimation at room temperature. No significant differences were observed in the magnitude of $(F_v/F_m)_{PI}$ between 3 and 6.5 h of dark recovery (values not shown).

Results

The induction kinetics of P, D, and E from lincomycin-treated cotton leaf discs upon irradiation with 500 μmol(photon) m^{-2} s^{-1} at 10 °C are depicted in Fig. 1*A*. Whereas P increased only slightly, D increased rapidly during the first 60 min of irradiation and then gradually, thereafter, reaching 0.8 by 360 min. Thus, approximately 80 % of the photon energy absorbed by the PS2 antennae was dissipated as heat 360 min after the initiation of irradiation at 10 °C. Coincident with increases in P and D, a decline occurred in E, that portion of the absorbed photon energy not attributable to P or D.

Steady state values of P and D, which were reached after 1.5 h of irradiation at 15-30 °C and after 3 h at 5 and 10 °C, were strongly temperature dependent (Fig. 1*B*). As temperature increased, P increased while D declined, indicating that more absorbed energy was utilized in photochemistry, presumably because of an increase in rates of CO_2 fixation. A decline in E occurred with an increase in temperature, as well, but the change was small compared to that for P and D.

The parameter D is actually the sum of different processes that account for thermal energy dissipation. Calculating these components allows one to determine their behavior during exposure to environmental stress. One can separate D into constitutive (D_{CON}), regulated (D_{REG}), and photoinhibitory (D_{PI}) thermal energy dissipation:

$$D = D_{CON} + D_{REG} + D_{PI} \qquad (2)$$

Even in dark-acclimated, non-stressed leaves, thermal dissipation in the antennae occurs, leading to a decrease in the quantum yield of photochemistry from 1.0 (maximal theoretical value) to about 0.8. To account for this constitutive thermal dissipation, which is intrinsic to the structural characteristics of the PS2 light-harvesting

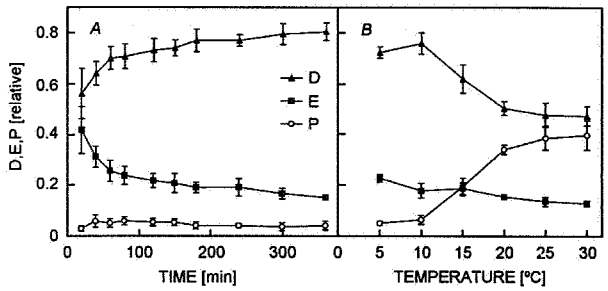

Fig. 1. Changes in the allocation of the photon energy absorbed by photosystem 2 antennae of cotton leaves during dark-to-light transitions at 500 μmol(photon) m^{-2} s^{-1} and 10 °C (*A*) and the temperature dependency of the allocation (*B*). P (proportion to photochemistry), D (proportion to thermal dissipation), and E [excess energy trapped by "closed" PS2 reaction centers (reaction centers with Q_A in the reduced state)] in *B* were determined after 90 min of irradiation at temperatures of 15-30 °C and after 180 min of irradiation at 5 and 10 °C. The leaves were harvested before sunrise and treated with lincomycin for 3 h in the dark prior to irradiation to inhibit chloroplast repair processes. Mean±S.D., n = 4-11 (*A*) or 3-5 (*B*).

system, one can use the following equation for dark-acclimated leaves:

$$D_{CON} = 1 - F_v/F_m \quad (3)$$

Applying a theoretical approach based on the analysis of energy fluxes within the photochemical apparatus of photosynthesis, Havaux *et al.* (1991) showed that the quantum yield of non-photochemical energy dissipation in PS2 is equal to F_0/F_m ($F_0/F_m = 1 - F_v/F_m$) when there is no energy exchange between PS2 units. Since D_{CON} is a proportion of the total photon energy absorbed, the magnitude of D_{CON} is affected by the development of other forms of thermal dissipation. For example, a sustained increase in thermal dissipation occurs after a photoinhibitory treatment, resulting in a decrease of the dark-acclimated F_v/F_m value. One can use the ratio $(F_v/F_m)_{PI}/(F_v/F_m)$ to account for the changes in the share of constitutive thermal dissipation:

$$D_{CON} = (1 - F_v/F_m) \times (F_v/F_m)_{PI}/(F_v/F_m) \quad (4)$$

$(F_v/F_m)_{PI}$ is the level of F_v/F_m for dark-acclimated leaves after a photoinhibitory treatment. In our experiments, $(F_v/F_m)_{PI}$ was determined after the chilling exposure and a 3-h incubation in darkness at room temperature.

In the calculation of D_{CON} for light-acclimated leaves, one must account for the level of regulated thermal dissipation, which plays a role in adjusting the efficiency of the energy capture by PS2 reaction centers. The development of the regulated component of thermal dissipation results in the reversible decrease in the quantum efficiency of the charge separation in PS2 reaction centers. Therefore, the ratio $(F_v'/F_m')/(F_v/F_m)_{PI}$ can be applied as a coefficient for the calculation of D_{CON} in the light-acclimated sample:

$$D_{CON} = (1 - F_v/F_m) \times (F_v/F_m)_{PI}/(F_v/F_m) \times (F_v'/F_m')/(F_v/F_m)_{PI} = (1-F_v/F_m) \times (F_v'/F_m')/(F_v/F_m) \quad (5)$$

To estimate the proportion of photoinhibitory thermal dissipation, one can use the following equations:

$$D_{PI} = 1 - (F_v/F_m)_{PI}/(F_v/F_m)$$
for dark-acclimated, stressed leaves (6)

$$D_{PI} = [1 - (F_v/F_m)_{PI}/(F_v/F_m)] \times (F_v'/F_m')/(F_v/F_m)_{PI}$$
for light-acclimated leaves (7)

The expression for D_{REG} can be obtained from Eqs. 2, 5, and 7:

$$D_{REG} = D - D_{CON} - D_{PI} = 1 - (F_v'/F_m')/(F_v/F_m)_{PI} \quad (8)$$

D_{REG} is the readily reversible component that can be attributed to ΔpH-dependent energy dissipation involving the xanthophyll cycle (for review see Demmig-Adams *et al.* 1996b).

Since the parameters D_{CON}, D_{REG}, and D_{PI} are the quantum yields of the processes resulting in different forms of thermal dissipation (*i.e.*, the probabilities that an individual exciton is utilized through the process controlled by a certain mechanism of thermal dissipation), each can be expressed as the ratio of the rate constant for an individual form of thermal dissipation to the sum of the rate constants for all processes taking part in the utilization of the photon energy absorbed by PS2 (Kornyeyev *et al.* 2001). The measured levels of Chl fluorescence are proportional to the quantum yields of Chl fluorescence of PS2 complexes at different stages and can also be expressed in terms of the rate constants. Therefore, we can verify the validity of the equations used for estimating the share of thermal dissipation for each component. Our calculations are based on the assumption that the pool of PS2 complexes and their antennae are an integrated system.

Photon energy absorbed by the system can be reemitted as Chl fluorescence, thermally dissipated, or used for photochemistry (rate constants k_f, k_d, and k_p, respectively). The three components of overall thermal dissipation (constitutive, regulated, and photoinhibitory) have the rate constants k_{con}, k_{reg}, and k_i, respectively ($k_d = k_{con} + k_{reg} + k_i$). All rate constants presented here are applied to the leaf pool of PS2 complexes contained in the sample, not to individual complexes. Combining the expressions for the intrinsic efficiency of the charge separation in PS2 in terms of the Chl fluorescence yields (Harbinson *et al.* 1989, Schreiber *et al.* 1998) and rate constants (Kitajima and Butler 1975) gives the following equations:

$$F_v/F_m = k_p/(k_{con} + k_f + k_p)$$
for a dark-acclimated non-stressed sample (9)

$$(F_v/F_m)_{PI} = k_p/(k_{con} + k_f + k_p + k_i)$$
for a dark-acclimated stressed sample (10)

$$F_v'/F_m' = k_p/(k_{con} + k_f + k_p + k_i + k_{reg})$$
for an irradiance-acclimated sample (11)

Replacing the Chl fluorescence parameters in equations 5-8 with the corresponding ratios of the rate constants (Eqs. 9-11) and assuming that k_f is relatively small, so that the approximation $k_{con} \approx k_{con} + k_f$ is possible, one can obtain the expressions for D_{CON}, D_{REG}, and D_{PI} in terms of these rate constants for leaves in the irradiance-acclimated state:

$$D_{CON} = (k_{con} + k_f)/(k_{con} + k_f + k_p + k_i + k_{reg}) \approx k_{con}/(k_{con} + k_f + k_p + k_i + k_{reg}) \quad (12)$$

$$D_{PI} = k_i/(k_{con} + k_f + k_p + k_i + k_{reg}) \quad (13)$$

$$D_{REG} = k_{reg}/(k_{con} + k_f + k_p + k_i + k_{reg}) \quad (14)$$

Therefore, we conclude that the proposed expressions for components of D in terms of the Chl fluorescence yields are valid.

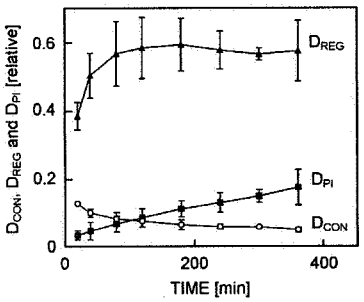

Fig. 2. The time course of constitutive (D_{CON}), regulated (D_{REG}), and photoinhibitory (D_{PI}) thermal dissipation of absorbed photon energy for cotton leaves at 500 μmol(photon) m^{-2} s^{-1} and 10 °C. The leaves were harvested before sunrise and pre-treated with lincomycin in the dark for 3 h prior to irradiation to inhibit chloroplast repair processes. At different times during irradiation, F, F_m', and F_0' values were determined, and then small leaf discs were immediately removed for determining $(F_v/F_m)_{PI}$ after a 3-h dark acclimation period at room temperature. Mean±S.D., n = 4-7.

When dark-acclimated cotton leaf discs treated with lincomycin were exposed to 10 °C and a PPFD of 500 μmol m^{-2} s^{-1}, D_{REG} increased rapidly, reaching 85 % of the maximum in 40 min and essentially reaching maximum in about 80 min (Fig. 2). In contrast, D_{PI} increased more gradually and continued to increase throughout the 360-min irradiation period. A 4.5-fold decline in D_{CON} was observed as a result of irradiation (D_{CON} = 0.22±0.02, n = 11, for dark-acclimated leaves before the treatment).

To explore the possibility that the extent of photoinhibition sustained by leaf discs was dependent on levels of E, we calculated a new parameter, time-dependent averaged E. A linear relationship was obtained between the time-dependent averaged E and a common measure of photoinhibition, the decrease in F_v/F_m (obtained after 3 h of dark acclimation) over the chilling period (Fig. 3*A*). In addition, the relationship between the time-dependent averaged E and D_{PI} was also linear (Fig. 3*B*).

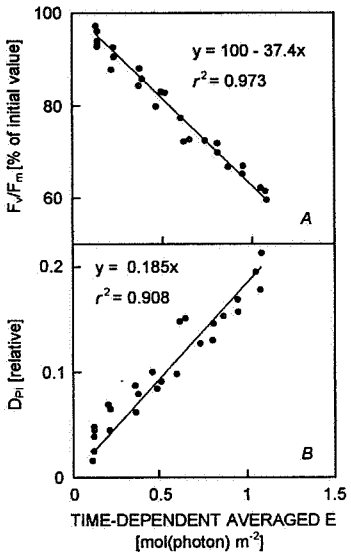

Fig. 3. The relationship between time-dependent averaged E [excess energy trapped by "closed" PS2 reaction centers (reaction centers with Q_A in the reduced state)] and the decline in photosystem 2 activity measured as F_v/F_m for cotton leaves (*A*) or the photoinhibitory component of thermal dissipation (D_{PI}) (*B*) at 500 μmol(photon) m^{-2} s^{-1} and 10 °C. The leaves were harvested before sunrise and pre-treated with lincomycin for 3 h in the dark prior to irradiation to inhibit chloroplast repair processes. (*A*) The initial values of F_v/F_m measured at the end of the dark period averaged 0.78±0.02 (mean±S.D., n = 11). (*B*) At different times during irradiation, F, F_m', and F_0' values were determined, and then small leaf discs were immediately removed for determining $(F_v/F_m)_{PI}$ after a 3-h dark acclimation period at room temperature.

The decrease in E during the chilling treatment (Fig. 1*A*) and the correlation between the time-dependent averaged E and the extent of PS2 inactivation (F_v/F_m) suggested that the rate constant of photoinhibition (k_{PI}), which reflects the probability for PS2 to be damaged, should decrease as a result of acclimation to irradiance, as well. Indeed, k_{PI} calculated for the period between 300 and 360 min was significantly lower (based on a Student's t-test, p<0.05) than that calculated for the first 40 min of the treatment (-0.098±0.059 $h^{-1}$and -0.190 ±0.041 h^{-1}, respectively; mean±S.D., n = 4-7).

Discussion

The analysis of Chl fluorescence emission is a non-invasive method of assessing the allocation of absorbed photons to assimilatory and dissipative processes. Demmig-Adams *et al.* (1996a) developed a parameter D that represents the thermally dissipated fraction of absorbed photons. In the present study, we have extended this method by developing a means of deconvoluting D into its principal components, constitutive, regulated, and photoinhibitory dissipation (D_{CON}, D_{REG}, and D_{PI}, respectively). This approach of separating D into its components is analogous to the approach applied to the analysis of Chl fluorescence quenching that is not associated with photochemistry. The following forms of non-photochemical fluorescence quenching have been identified: (*a*) a form associated with state transitions (q_T) that is due to the reduction in the absorption cross-section of PS2 after phosphorylation of its light-harvesting complex, (*b*) high-energy-state quenching (ΔpH-dependent) (q_E), and (*c*) quenching associated with photoinhibition (q_I) (Walters and Horton 1993, Maxwell and Johnson 2000, Müller *et al.* 2001). The processes that control q_T and q_E combine to influence D_{REG}. These processes reflect regulatory changes in the fate of excitation energy that can be reversed in the dark. In other words, D_{REG} may be used to assess the so-called "down-regulation" of PS2 activity. q_T contributes significantly to overall quenching only under low PPFDs (Walters and Horton 1993, Lichtenthaler and Burkart 1999, Müller *et al.* 2001). Consequently, we suggest that under the conditions of our experiments, D_{REG} is principally a measure of ΔpH-dependent q_E that is associated with zeaxanthin and antheraxanthin of the xanthophyll cycle (Demmig-Adams *et al.* 1996b).

D_{PI} is analogous to q_I. Both parameters quantify sustained thermal dissipation that often develops under environmental stress. At the same time, they are independently determined characteristics. It is not necessary to measure q_I in order to determine D_{PI}. The slowly relaxing component of non-photochemical quenching, q_I, is thought to represent the extent of PS2 photodamage (Lichtenthaler and Burkart 1999). However, it is possible that other slowly reversible changes in PS2 efficiency may also contribute to q_I. q_I is determined by Chl fluorescence relaxation kinetics. This process is time-consuming and difficult to perform in the field. However, D_{PI} reflects changes in thermal dissipation that are not readily reversible in the dark, and its determination requires only one measurement of $(F_v/F_m)_{PI}$ that can be easily obtained from dark-acclimated samples. It is important to insure that the period of dark acclimation prior to the measurements of $(F_v/F_m)_{PI}$ is sufficient for complete relaxation of the reversible components of thermal dissipation. We observed no change in this parameter between 3 and 6.5 h of dark acclimation at room temperature, indicating that the values determined after 3 h represent photoinhibition that is not readily reversible.

Quantifying the energy dissipation as non-photochemical quenching does not allow one to compare the flux of photon energy into thermal dissipation processes with the flux into photochemistry (P) (Demmig-Adams *et al.* 1996a). Moreover, as was pointed out by Maxwell and Johnson (2000), measurements of the forms of non-photochemical Chl fluorescence quenching (q_T, q_E, and q_I) yield thermal dissipation relative to the dark-acclimated state. Therefore, accurate interpretation of these measurements depends upon knowledge of the condition of the photosynthetic apparatus in the dark-acclimated state. The ability to directly compare D with P even without knowledge of the previous status of the leaf is among the principal advantages of the method utilized in our study.

Using this method of Chl fluorescence analysis, we have been able to quantitatively compare the different processes of photon energy utilization during exposures of cotton leaves to chilling temperatures at a moderate PPFD. Low temperature dramatically suppresses the portion of absorbed photon energy utilized in photochemistry (P) (Fig. 1). Presumably, the increase in P during the initial phase of irradiance acclimation (up to 80 min after the initiation of irradiation) at 10 °C reflects the activation of CO_2 fixation, while its slow decrease after 180 min may be the result of an inhibition of the activity of the photosynthetic apparatus (*i.e.*, photoinhibition). With so little absorbed energy utilized by photochemistry at 10 °C (about 4 % after 3 h of irradiation), thermal dissipation (D) is critical for the safe dissipation of the remaining absorbed photon energy.

The calculation of the components of D during irradiance acclimation reveals the changes in the contribution of the different mechanisms to thermal dissipation (Fig. 2). For cotton leaves at 10 °C, the majority of energy dissipation is accomplished by a readily reversible mechanism (D_{REG}). As stated above, D_{REG} is most likely a reflection of zeaxanthin-dependent thermal energy dissipation. Our results are consistent with the observation that cotton under salt stress and natural irradiance exhibits a large increase in non-photochemical quenching of Chl fluorescence concomitant with a large accumulation of de-epoxidized xanthophylls (Björkman 1994). The induction kinetics for D_{REG} and D_{PI} for cotton at chilling temperatures in this study are similar to those reported for the related components of non-photochemical fluorescence quenching in rice exposed to chilling (Xu *et al.* 2000). This observation strengthens the analogy between the deconvolution of non-photochemical quenching and the partitioning of D into its components.

The induction of thermal dissipation causes a profound decrease in E during the irradiation (Fig. 1*A*). E re-

presents the absorbed photon energy that is neither allocated to P nor to D. This portion of total absorbed photon energy has the greatest potential to cause PS2 photoinactivation, because it represents photon energy that is trapped by "closed" PS2 reaction centers (*i.e.*, those with Q_A in the reduced state). The calculation of E includes the reduction state of Q_A ($1 - q_P$) and the efficiency of the charge separation in PS2 complexes in the light-acclimated state (F_v'/F_m'), which depends on the level of thermal dissipation (Demmig-Adams *et al.* 1996a). The reduction state of Q_A and the extent of thermal dissipation are the factors affecting the probability of PS2 photoinactivation (Öquist *et al.* 1993, Maxwell *et al.* 1995, Huner *et al.* 1996, Demmig-Adams *et al.* 1996a). In our experiments, the relationship between accumulated excessive photons and the extent of PS2 inactivation is demonstrated by the observation that time-dependent averaged E, an integrated measure of overall E, is linearly correlated with a decrease in photochemical efficiency (F_v/F_m) (Fig. 3*A*) and an increase in D_{PI} (Fig. 3*B*), two parameters that estimate PS2 photoinactivation.

During photosynthetic induction at 10 °C, cotton leaves increase D rapidly with a concomitant decrease in E. Irradiance-acclimated cotton leaves maintain high D at a PPFD of 500 μmol m^{-2} s^{-1} and 10 °C. At higher temperatures, maximum values of D are much lower, while P is much higher. At 25 or 30 °C, the activity of metabolism dependent on photochemistry is high and there is less need for thermal dissipation at moderate PPFD. However, P and D do not account for all of the energy absorbed even at the optimum temperature for P. A small amount of excess energy absorption still occurs even at a moderate PPFD.

Low temperature retards the induction of the mechanisms that protect PS2 (Demmig-Adams *et al.* 1989, Bilger and Björkman 1994, Adams and Demmig-Adams 1995). For cotton leaves exposed to 10 °C, not only are 40 min required to reach maximum efficiency of photochemistry, 80 min are required to reach nearly maximum protective thermal dissipation of absorbed photon energy (Fig. 1*A*). Consequently, the rate constant of photoinactivation is higher during photosynthetic induction at 10 °C than during steady-state conditions. Therefore, at low temperature, cotton leaves are most vulnerable to photooxidation when they are suddenly exposed to a large increase in PPFD.

In conclusion, thermal dissipation (D) plays a major role in the acclimation of the photosynthetic apparatus of cotton leaves to the photoinhibitory conditions imposed by low temperatures that reduce Calvin-Benson cycle activity. This finding is consistent with the notion that the development of thermal energy dissipation is crucial for the protection of photosynthetic capacity at high PPFD and low temperature for chilling-sensitive plants (Xu *et al.* 2000). By partitioning D into its regulated and photoinhibitory components, we have determined that most of the thermal dissipation is controlled by the leaf and is readily reversible. We propose that the new parameter, time-dependent averaged E, is useful for estimating the potential for damage to PS2 given its linear relationship with the inactivation of PS2 during irradiation at low temperature. Time-dependent averaged E is advantageous, because it allows one to predict the level of PS2 photoinactivation on the basis of the Chl fluorescence parameters measured solely during the irradiation.

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