

Enhanced Employment of the Xanthophyll Cycle and Thermal Energy Dissipation in Spinach Exposed to High Light and N Stress¹

Amy S. Verhoeven, Barbara Demmig-Adams*, and William W. Adams III

Department of Environmental, Population, and Organismic Biology, University of Colorado, Boulder, Colorado 80309-0334

The involvement of the xanthophyll cycle in photoprotection of N-deficient spinach (*Spinacia oleracea* L. cv Nobel) was investigated. Spinach plants were fertilized with 14 mM nitrate (control, high N) versus 0.5 mM (low N) fertilizer, and grown under both high- and low-light conditions. Plants were characterized from measurements of photosynthetic oxygen exchange and chlorophyll fluorescence, as well as carotenoid and chlorophyll analysis. Compared with the high-N plants, the low-N plants showed a lower capacity for photosynthesis and a lower chlorophyll content, as well as a lower rate of photosystem II photosynthetic electron transport and a corresponding increase in thermal energy dissipation activity measured as nonphotochemical fluorescence quenching. The low-N plants displayed a greater fraction of the total xanthophyll cycle pool as zeaxanthin and antheraxanthin at midday, and an increase in the ratio of xanthophyll cycle pigments to total chlorophyll. These results indicate that under N limitation both the light-collecting system and the photosynthetic rate decrease. However, the increased dissipation of excess energy shows that there is excess light absorbed at midday. We conclude that spinach responds to N limitation by a combination of decreased light collection and increased thermal dissipation involving the xanthophyll cycle.

Plants growing in sunny environments experience excess light (light that is absorbed by the plant in excess of what can be utilized through the photosynthetic electron transport pathway) on a daily basis (Björkman and Demmig-Adams, 1994; Demmig-Adams and Adams, 1996a; Demmig-Adams et al., 1996a). Under conditions of environmental stress (e.g. N deficiency) the amount of excess light that a plant absorbs can increase due to stress-induced decreases in the capacity for photosynthesis.

In the case of limiting N there are numerous studies demonstrating that N-deficient plants exhibit decreases in photosynthetic capacity and the quantum yield of photosynthesis relative to N-replete controls (Sage and Percy, 1987; Terashima and Evans, 1988; Khamis et al., 1990; Sugiharto et al., 1990). Evans and Terashima found that N

deficiency does not alter the properties of spinach thylakoid membranes, but the amount of thylakoid proteins per chloroplast is decreased (Evans and Terashima, 1987; Terashima and Evans, 1988). These and other studies have shown that under N-deficient conditions there are decreases in both chlorophyll content and Rubisco content per unit leaf area, but that the ratio of Rubisco to chlorophyll also decreases with N deficiency (Wong, 1979; Ferrar and Osmond, 1986; Evans and Terashima, 1987; Seemann et al., 1987). This effect is more pronounced at higher light intensities (Seemann et al., 1987; Terashima and Evans, 1988). A key effect of N limitation, therefore, is a lowering in the capacity for carbon assimilation due, in part, to the limitations of synthesis of the Calvin cycle enzymes (Terashima and Evans, 1988; Sugiharto et al., 1990). This lowered capacity for carbon assimilation means that a given light intensity is potentially in greater excess under conditions of N deficiency.

Excess light can be harmlessly dissipated in the antennae complexes of PSII as heat in a process that involves the xanthophyll cycle as well as a low thylakoid pH. The xanthophyll cycle pigments Z and A are formed from V during conditions of excess light and are both thought to be involved in the photoprotective dissipation process (for recent reviews, see Björkman and Demmig-Adams, 1994; Demmig-Adams and Adams, 1996b; Demmig-Adams et al., 1996b; Horton et al., 1996; Yamamoto and Bassi, 1996). It is of interest to determine the role of the xanthophyll cycle in conditions of environmental stress when the amount of excess light increases.

The role of the xanthophyll cycle under conditions of cold-temperature stress has been extensively studied (Adams and Demmig-Adams, 1994, 1995; Gilmore and Björkman, 1994a, 1994b; Adams et al., 1995a, 1995b; Verhoeven

¹ The work was supported in part by the U.S. Department of Agriculture, Competitive Research Grants office, award no. 90-37130-5422, and by fellowships to B.D.-A. (from the David and Lucile Packard Foundation) and to A.S.V. (National Science Foundation predoctoral fellowship).

* Corresponding author; e-mail barbara.demmig-adams@colorado.edu; fax 1-303-492-8699.

Abbreviations: A, antheraxanthin; D, fraction of light absorbed by PSII that is dissipated in the antennae ($[F_v/F_m] - [F_v'/F_m']$); F , actual chlorophyll fluorescence emission during illumination; F_m , F_m' , maximal chlorophyll fluorescence yield in the dark or during energization, respectively; F_o , F_o' , minimal chlorophyll fluorescence yield in the dark or during energization, respectively; F_v , $F_m - F_o$; F_v/F_m , efficiency of PSII in the dark ($[F_m - F_o]/F_m$); NPQ, nonphotochemical fluorescence quenching ($F_m/F_m' - 1$); P, fraction of light absorbed by PSII that is utilized in PSII photochemistry ($[F_m' - F]/F_m'$); PFD, photon flux density; q_p , coefficient for photochemical quenching; V, violaxanthin; Z, zeaxanthin.

et al., 1996). The xanthophyll cycle has also been characterized under conditions of iron deficiency (Morales et al., 1990, 1994) and water stress (Demmig et al., 1988). In contrast, the characterization of the xanthophyll cycle under conditions of N deficiency has received little attention. A study by Khamis et al. (1990) that investigated N-deficient maize suggested an enhanced employment of xanthophyll cycle-dependent energy dissipation under these conditions. The goal of the present study was to perform a more detailed characterization of the xanthophyll cycle under N-deficient conditions using spinach (*Spinacia oleracea* L. cv Nobel). To do so we assessed the pool size and conversion state of the xanthophyll cycle under N-deficient conditions, relative to controls, at various growth and treatment PFDs, in addition to the corresponding fluorescence parameters and oxygen exchange.

MATERIALS AND METHODS

Spinach (*Spinacia oleracea* L. cv Nobel) plants were grown in growth chambers at 900, 500, and 190 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and in a naturally lit greenhouse in full sunlight (PFD at noon = 1400–1550 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), as well as under a shade cloth with a maximal PFD of 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Growth-chamber conditions consisted of a 10-h photoperiod and a temperature regime of 25/18°C day/night. Plants were fertilized with 14 mM nitrate (control, high N) versus 0.5 mM nitrate (low N) fertilizer in ample applications of about 0.5 L per pot each. The high-N fertilizer contained the following: 14 mM NO_3^- , 8 mM K^+ , 4 mM Ca^{2+} , 2 mM H_2PO_4^- , 2 mM Mg^{2+} , 2 mM $(\text{SO}_4)^{2-}$, and 0.042 mM Fe^{2+} . The low-N fertilizer contained only 0.5 mM NO_3^- , with the anionic balance being provided by 5 mM $(\text{SO}_4)^{2-}$ and 7.5 mM Cl^- . Low-N plants were watered with fertilizer twice each week, whereas high-N plants were fertilized four to five times each week.

Photosynthesis and Chlorophyll Fluorescence

Simultaneous measurements of photosynthetic oxygen exchange in a leaf-disc oxygen electrode chamber and of chlorophyll fluorescence (PAM-101, Walz, Effeltrich, Germany) (Schreiber et al., 1986) were performed in the presence of saturating CO_2 (5%) as described by Demmig-Adams et al. (1990). Leaves were collected from dark-adapted plants and kept in wet paper towels before insertion into the leaf-disc oxygen electrode (Hansatech, King's Lynn, UK). Fluorescence measurements were also performed on intact leaves under the appropriate growth PFD conditions with a portable fluorometer (PAM-2000, Walz). These measurements were performed in the actual growth environments, either both predawn (dark-adapted F_v/F_m) and at midday on the greenhouse plants, or at the end of the 14-h dark period (dark-adapted F_v/F_m) and during the light period (after no less than 3 h at growth PFD) on the growth-chamber plants. Leaves were given a 5-min treatment with far-red illumination (achieved in the oxygen electrode by a neutral density filter decreasing the actinic light to 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ [measured over

400–700 nm], in combination with a 710-nm cutoff filter) before the F_v/F_m measurements.

Pigment Analyses

Leaf discs (0.25 cm^2) were collected from intact leaves and stored in aluminum envelopes in liquid N until processing. The removal process of the disc from the leaves and submersion in liquid N took a maximum of 3 s. Extraction of the pigments and analysis of the extracts by HPLC was as described previously (Adams and Demmig-Adams, 1992; see also Thayer and Björkman, 1990), using a column described by Gilmore and Yamamoto (1991).

RESULTS AND DISCUSSION

PFD Response of Photosynthesis Rate and Chlorophyll Fluorescence Characteristics of Spinach Grown under Ample or Limiting N Supply

Figure 1 shows PFD response curves of photosynthetic oxygen evolution and various chlorophyll fluorescence parameters in spinach grown with an ample or a limiting N supply. The data demonstrate that the low-N plants had a considerably lower maximal capacity for photosynthesis on a leaf area basis than the plants grown at high-N levels (Fig. 1A), as has been observed previously (Sage and Pearcy, 1987; Terashima and Evans, 1988; Khamis et al., 1990; Sugiharto et al., 1990). However, the low-N plants also had much lower levels of chlorophyll on a leaf area basis relative to the high-N plants, and when oxygen evolution is calculated on a chlorophyll basis the capacities for photosynthesis were more similar (53.6 versus 40.1 $\text{mmol O}_2 \text{mol}^{-1} \text{Chl [a + b] s}^{-1}$ in high- and low-N leaves, respectively).

Figure 1B shows the actual PSII efficiency, $(F_m' - F)/F_m'$. This parameter is equivalent to the product of F_v'/F_m' (the efficiency of open PSII or "excitation capture efficiency") and of q_P (the coefficient for photochemical quenching $[F_m' - F]/[F_m' - F_o']$, or the fraction of PSII centers that are actually open) (Genty et al., 1989). $(F_m' - F)/F_m'$ reflects the fraction of light absorbed by PSII antennae that is utilized in PSII photochemistry at a given PFD. Figure 1B shows that this fraction utilized through PSII photochemistry was also lower in the low-N versus the high-N leaves. One should therefore expect a greater requirement for photoprotective dissipation of this excess energy in the low-N versus the high-N leaves. Our fluorescence analysis does indicate a greater level of energy dissipation at a given PFD in the low-N versus high-N leaves (Fig. 1C).

Figure 1C shows the NPQ of chlorophyll expressed as Stern-Volmer quenching ($F_m/F_m' - 1$; see Bilger and Björkman, 1990). For the low-N leaves we expressed NPQ in two ways, one using the actual measured levels of "control" F_m in darkened leaves, which correspond to a slightly depressed F_v/F_m value of 0.738 ± 0.034 . The "adjusted" NPQ values were calculated with a higher control F_m that corresponded to a higher control value of F_v/F_m of 0.799 ± 0.011 (i.e. the average F_v/F_m of the high-N control plants). The adjusted values were calculated based on the model of Kitajima and Butler (1975), assuming that a small increase

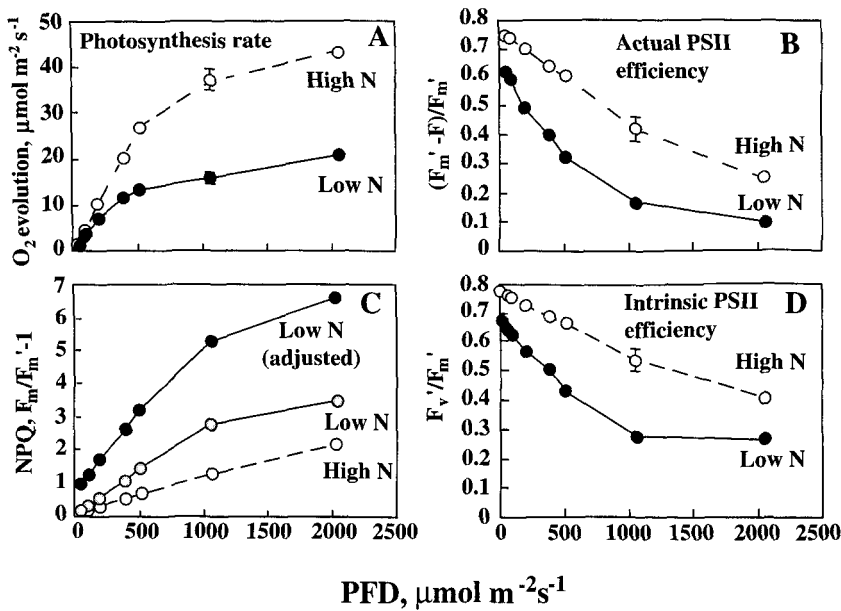


Figure 1. PFD response of photosynthesis rate and PSII characteristics for spinach leaves grown with ample (high-N) or limiting (low-N) N supply. Plants were grown in a growth chamber at $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Responses were generated by exposure of individual leaf discs to increasing PFDs, with at least 15 min of equilibration at each PFD. Data at $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ are means \pm SD from three leaf discs taken from three different leaves. Dark-adapted F_v/F_m ratios for the high-N versus low-N plants were 0.799 ± 0.011 and 0.738 ± 0.034 , respectively. Values are means \pm SD, $n = 3$. NPQ values for low-N leaves were calculated with either measured control F_m values, corresponding to a slightly depressed F_v/F_m of 0.738, or with higher assumed control F_m values, corresponding to an F_v/F_m of 0.799 (adjusted).

in the rate constant for thermal energy dissipation was responsible for the depressed F_v/F_m values, as described previously (Björkman, 1987; Demmig-Adams, 1990). Using the adjusted value for F_m resulted in greater levels of NPQ for the low-N leaves at each PFD. We feel that the slightly depressed levels of predawn F_v/F_m in the low-N leaves are most likely the result of a small extent of sustained NPQ that is associated with the engagement of A and Z that is nocturnally retained. We have reported evidence for such a sustained maintenance of high levels of Z-dependent energy dissipation leading to a sustained low PSII efficiency for overwintering plants in the field (Adams and Demmig-Adams, 1994, 1995; Adams et al., 1995a, 1995b; Verhoeven et al., 1996). Figure 2 shows that the slightly lower levels of

predawn F_v/F_m in the low-N leaves were mirrored by slightly higher levels of A and Z retained overnight in the low-N leaves.

In Figure 1 it is also apparent that F_v'/F_m' was lower at each given PFD in the low-N than in the high-N leaves (Fig. 1D). As indicated above, F_v'/F_m' reflects the efficiency of open PSII units, but can perhaps be better described as the "excitation capture efficiency" of PSII units (Schreiber et al., 1994). The latter term emphasizes that under many physiological conditions, changes in F_v'/F_m' have been shown to reflect changes in the efficiency with which excitation energy is made available to PSII centers; decreases in this excitation capture efficiency are apparently caused predominantly by increases in energy dissipation in the antennae (Adams et al., 1995a; Demmig-Adams et al., 1995; Demmig-Adams and Adams, 1996a). As has been found in these latter studies, the NPQ of F_m was associated with a proportional quenching of F_o in spinach leaves under N stress (Fig. 3). This same feature has been reported previously for xanthophyll cycle-dependent energy dissipation (e.g. Gilmore and Yamamoto, 1993) and is indicative of energy dissipation in the PSII antennae.

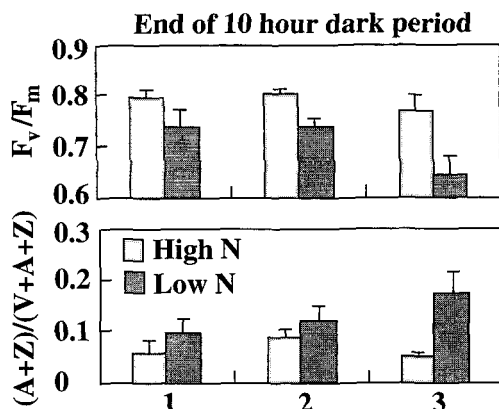


Figure 2. Pre-light F_v/F_m and the conversion state of the xanthophyll cycle expressed as $(A + Z)/(V + A + Z)$ at the end of the dark period in spinach plants grown with a high-N or low-N supply at three different growth PFDs. Measurements were performed in situ under the actual growth conditions in the growth chambers or the greenhouse. 1, Ten hours per day at $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; 2, natural sunlight in a greenhouse; and 3, 10 h per day at $900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Steady-State Response of PSII Characteristics and Composition of the Xanthophyll Cycle Pool under Ample or Limiting N Supply

It has also been suggested that decreases in F_v'/F_m' might be used to quantify the level of energy dissipation (Cornic, 1994; Demmig-Adams and Adams, 1996a; Demmig-Adams et al., 1996a). Such an analysis (Fig. 4) provides data on the fractions of light absorbed by PSII that are dissipated in the antennae (D, from $1 - F_v'/F_m'$) and utilized in PSII photochemistry (P, from $[F_m' - F]/F_m' = F_v'/F_m' \times q_p$; Genty et al., 1989). The small third portion of the absorbed light (white portion, represented by $F_v'/F_m' \times [1 - q_p]$) that remained at 11 to 13% independent of the

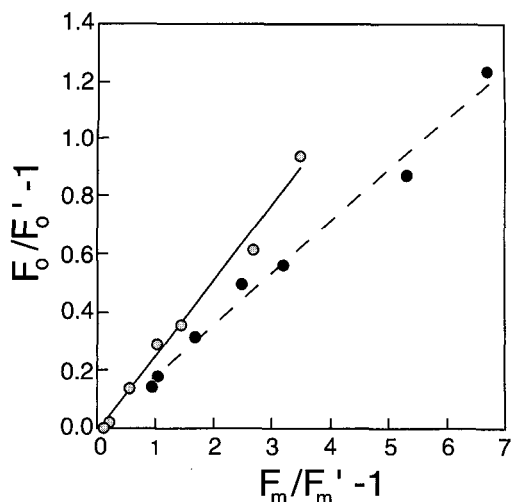


Figure 3. Relationship between Stern-Volmer quenching of F_m' ($F_m/F_m' - 1$) and F_o' ($F_o/F_o' - 1$) at different PFDs in low-N plants. The two lines reflect values from two sets of calculations. The gray circles represent values where the F_m and F_o used in the calculation were the actual values measured from dark-adapted spinach leaves. The black circles represent adjusted values of F_m and F_o that account for quenching that may be present even in the dark-adapted state as described in the text for Figure 1C. The linear regression equations are $[F_o/F_o' - 1] = -0.024 + 0.263 \times [F_m/F_m' - 1]$, and $[F_o/F_o' - 1] = -0.004 + 0.178 \times [F_m/F_m' - 1]$ for the unadjusted and adjusted lines, respectively.

N regime may reflect deexcitation of singlet excited chlorophyll via the triplet pathway (Demmig-Adams et al., 1996b). However, since it has been cautioned that $1 - q_p$ may overestimate the degree of the closed reaction centers (Schreiber et al., 1994), it is quite possible that much of this portion is actually part of P. Therefore, the line between P and the white portion (Fig. 4) is shown as a dashed line.

Figure 4 shows an analysis of the fractions of light absorbed in PSII going into different processes in a steady-state situation in the middle of the light period under the actual growing conditions in a growth chamber where low-N and high-N leaves were receiving a PFD of $900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Under these growth conditions, high-N leaves utilized a considerably greater portion of the light absorbed in PSII antennae for photochemistry (P) than the low-N leaves. In contrast, low-N leaves dissipated a much greater fraction of the light absorbed in PSII antennae (D), an estimated 64%, compared with only 36% in the more highly photosynthetically active high-N leaves (Fig. 4). This difference was associated with a pronounced difference in the levels of A + Z in these leaves as well (Fig. 4). A full 65% of the total V + A + Z pool was present as Z and A in the low-N leaves compared with 18% A + Z in the high-N leaves (Fig. 4). In addition, the total size of the V + A + Z pool on a Chl *a* + *b* basis was also greater in the low-N versus high-N leaves (Fig. 4), as represented by the relative areas of the pie graphs. These findings further support the conclusion that the low-N leaves dissipated a greater fraction of excitation energy in the antenna complexes via A + Z-dependent dissipation.

Relationship between the Xanthophyll Cycle Conversion State and Various Chlorophyll Fluorescence Characteristics from Spinach Grown with Ample or Limiting N Supply under Various PFD Regimes

Figure 5 shows the relationship between the ratio of A + Z to V + A + Z for all low-N and high-N leaves versus the percentage of light absorbed in PSII antennae that is utilized in PSII photochemistry ($[F_m' - F]/F_m' \times 100$) at various growth PFDs. Low percentages of light absorbed in the PSII antennae that are utilized in PSII photochemistry were associated with a higher degree of conversion of the xanthophyll cycle pool to A + Z (Fig. 5). Figure 6 shows NPQ (Fig. 6A) and F_v'/F_m' (Fig. 6B) as a function of the (A + Z)/(V + A + Z) ratio. A rather close relationship was

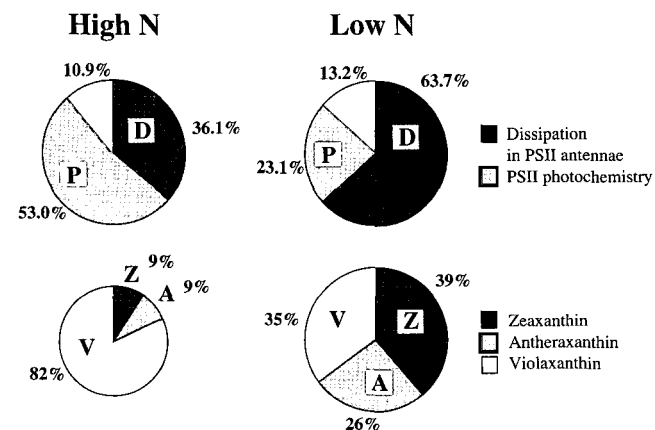


Figure 4. Comparison of the fluorescence characteristics and the conversion state of the xanthophyll cycle pigments in high-N and low-N plants grown at $900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. The pie graphs in the top half depict the fractions of light absorbed in PSII antennae that are dissipated via PSII electron transport, as well as the fractions dissipated thermally. The fraction of light absorbed in PSII antennae that is going into thermal energy dissipation (D) was calculated as $1 - F_v'/F_m'$ (for F_v'/F_m' , see Fig. 1D). This approach to estimate the fraction of thermally dissipated energy is analogous to the estimation of the fraction of light absorbed in PSII antennae that is utilized in photosynthetic electron transport (P) from $F_v'/F_m' \times q_p = F_m' - F/F_m'$ (Fig. 1C; see Genty et al., 1989) and is explained in detail by Demmig-Adams et al. (1996b). The parameter $1 - F_v'/F_m'$ includes any constitutive radiationless dissipation ($1 - F_v/F_m$) present at all PFDs (and presumably occurring by deexcitation of singlet chlorophyll via the triplet pathway) as well as the regulated, xanthophyll cycle-dependent dissipation ($F_v/F_m - F_v'/F_m'$). The white section represents the remaining fraction ($F_v'/F_m' \times [1 - q_p]$). If $1 - q_p$ was a quantitative indicator of the reduction state of the primary electron acceptor of PSII, this remaining portion would reflect an increased deexcitation of singlet excited chlorophyll via the triplet pathway at high PFD (and could therefore be combined with the other forms of dissipation in the PSII antennae). However, since $1 - q_p$ is likely to be overestimated at high PFDs at the expense of q_p (and thus of P), much of the small white section may actually be part of the fraction of absorbed light utilized in photochemistry. Values are means from three leaves. The pie graphs in the lower portion depict the composition of the xanthophyll cycle at the growth irradiance. The pie areas represent the relative pool size of the xanthophyll cycle pigments per chlorophyll. Actual values of the pool size were 131 ± 10 and $228 \pm 50 \text{ mmol V + A + Z (mol chlorophyll a + b)}^{-1}$ for high N and low N, respectively. Values are means \pm SD, $n = 3$.

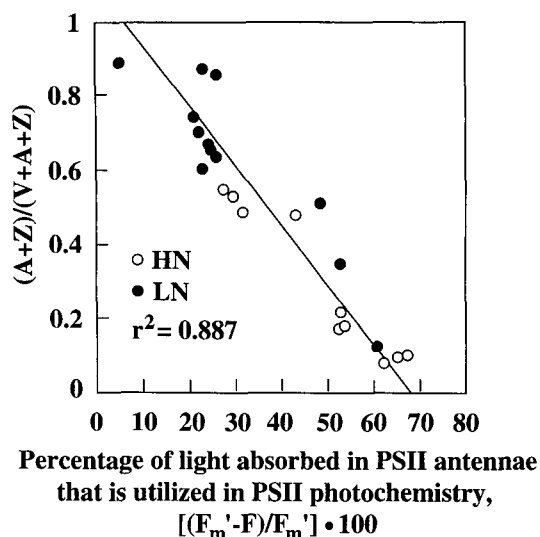


Figure 5. Relationship between the conversion state of the xanthophyll cycle $(A + Z)/(V + A + Z)$ and the percentage of light absorbed in PSII antennae that is utilized in PSII photochemistry $([(F_m' - F)/F_m'] \times 100)$. \circ , High-N plants (HN); \bullet , low-N plants (LN). The equation for the regression line (fitted using Kaleidograph [Abelbeck Software, Synergy Software, Reading, PA]) is $(A + Z)/(V + A + Z) = 1.024 - 1.433 \times [(F_m' - F)/F_m']/100$.

obtained for the combined data for low-N and high-N leaves.

Whereas NPQ increased in a curvilinear fashion as the percentage of $A + Z$ approached 100% of $V + A + Z$ (Fig. 6A), as had been reported previously for a number of other species (Demmig-Adams and Adams, 1996a), F_v'/F_m' could be fitted rather well to a linear relationship (Fig. 6B). High degrees of conversion of the xanthophyll cycle to $A + Z$ were thus strongly correlated with high levels of energy dissipation (from NPQ) and strong decreases in the excitation capture efficiency of PSII (F_v'/F_m'), which is again consistent with the assumption that the NPQ observed here reflects energy dissipation in the PSII antennae.

Chlorophyll and Carotenoid Content and Composition in Spinach Grown with Ample or Limiting N Levels under Various PFD Regimes

Figures 7 and 8 further characterize differences in pigment content and composition between low-N and high-N leaves. Figure 7 shows a compilation of the data from all of the various treatments, and Figure 8 shows a comparison of low-N versus high-N plants grown at two different light intensities (500 versus 900 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). In general, the low-N plants were depleted in all pigments, with the effect being more pronounced at higher light intensities (Figs. 7A and 8, A and B). Figure 7A demonstrates that the chlorophyll and carotenoid contents decreased largely proportionally to each other. However, when the chlorophyll content approached zero, there was still a small level of carotenoid present (Fig. 7A). This feature resulted in an increase in the molar ratio of total carotenoid to chlorophyll below about 150 $\mu\text{mol chloro-}$

phyll $(a + b) \text{ m}^{-2}$ (Fig. 7B). In spite of this increase of the ratio of carotenoid to chlorophyll, the composition of the carotenoid fraction did not differ much when the fractions of $V + A + Z$, neoxanthin, lutein, and β -carotene of the total carotenoid pool were considered (Fig. 7B).

A direct comparison of low-N and high-N leaves grown at PFDs of 500 versus 900 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in growth chambers reveals some interesting features. At the higher PFDs, the leaves of the low-N plants, but not of the high-N plants, were increasingly yellowed, i.e. showed a reduced chlorophyll content (Fig. 8A) as well as a decreased xanthophyll cycle pool per leaf area (Fig. 8B). However, the ratio of xanthophyll cycle components to chlorophyll was greater in the most strongly yellowed low-N leaves grown at the higher (900 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) PFD (Fig. 8C). In addition, a more pronounced conversion of the xanthophyll cycle pool to its photoprotectively active components ($A + Z$) was observed in the low-N versus high-N leaves at each growth PFD and with increasing growth PFD for each treatment (Fig. 8D). This greatly increased conversion state

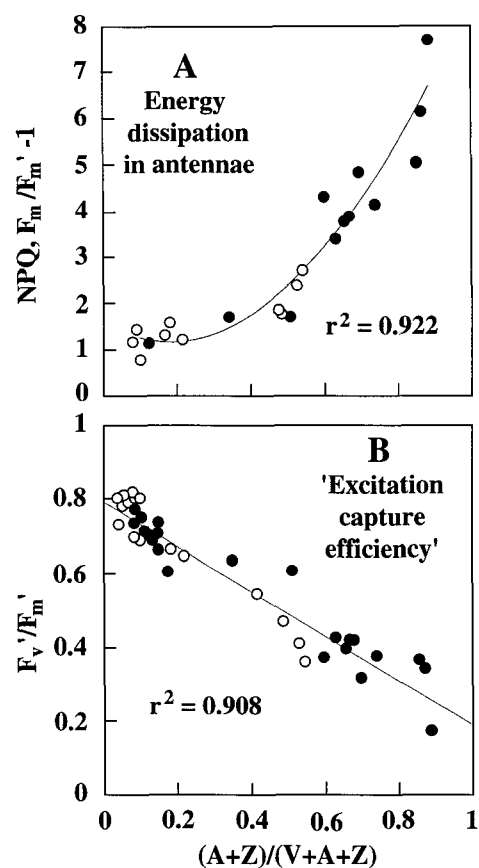


Figure 6. Relationship between the conversion state of the xanthophyll cycle $(A + Z)/(V + A + Z)$ versus Stern-Volmer quenching of F_m ($\text{NPQ} = F_m/F_m' - 1$) (A) and the excitation capture efficiency of PSII (F_v'/F_m') (B). The equation for the regression line shown in Figure 7A is $\text{NPQ} = 1.509 - 3.8008 \times [(A + Z)/(V + A + Z)] + 10.796 \times [(A + Z)/(V + A + Z)]^2$. The equation for the regression line in Figure 7B is $F_v'/F_m' = 0.795 - 0.629 \times [(A + Z)/(V + A + Z)]$. Both equations were fitted using Kaleidograph (Abelbeck Software, Synergy Software).

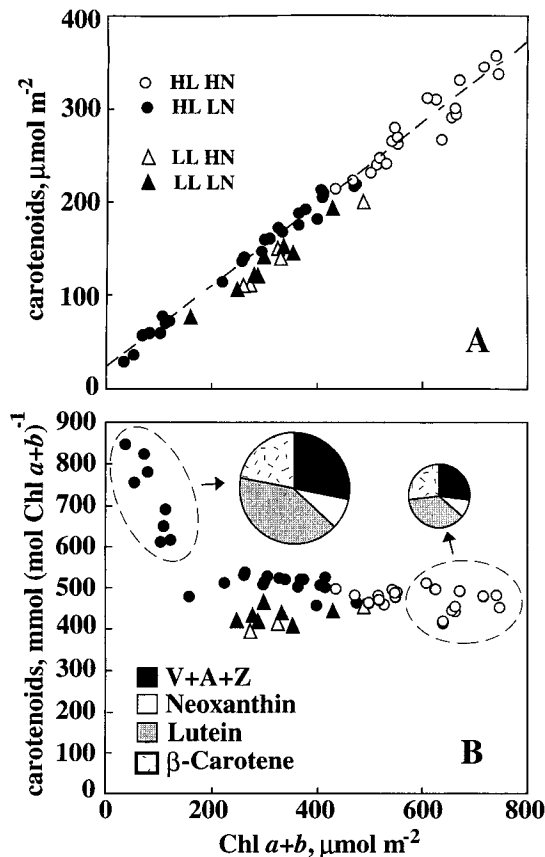


Figure 7. Relationship between carotenoid contents on a leaf area (A) or chlorophyll (B) basis and leaf chlorophyll content in spinach leaves grown at high-N or low-N levels under various PFD regimes. HL and LL stand for plants grown at a high or a low PFD, and HN and LN stand for high-N and low-N plants. LL plants were those grown at a PFD of $190 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 10 h per day in a growth chamber or with peak (maximal) PFDs of $75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ under a shade cloth in a greenhouse. HL plants were those grown under natural (full) sunlight in the greenhouse, or in growth chambers at 900 or $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 10 h per day. Pie graph insets in B show the fractions of the various carotenoids calculated as percentages of the total carotenoid pool per chlorophyll. The relative pie diameters represent the mean of the relative carotenoid pool size per chlorophyll at the extremes of the chlorophyll content for high-N ($n = 12$) and low-N ($n = 8$) plants. Pool sizes of total carotenoid in units of $\text{mmol carotenoid per mol chlorophyll}$ were 480 ± 26 and 710 ± 93 for high-N and low-N plants. Actual percentages for high N and low N, respectively, were: neoxanthin, $9.0 \pm 0.4\%$ and $8.9 \pm 1.5\%$; lutein, $37.8 \pm 2.1\%$ and $41.3 \pm 5.3\%$; β -carotene, $26.5 \pm 1.3\%$ and $22.2 \pm 7.2\%$; V + A + Z, $26.7 \pm 1.2\%$ and $27.6 \pm 3.6\%$. The dashed line in A is the best correlation calculated for the high-light samples for low-N and high-N plants combined.

of the xanthophyll cycle under combined N and high-light stress is consistent with a greater demand for photoprotective energy dissipation in these leaves, where photosynthetic electron transport consumes only a small fraction of the light absorbed in PSII antennae (compare Fig. 4).

Concerning the underlying reason for the pronounced chlorophyll bleaching observed in the low-N plants, this is

not predicted from the N limitation to carbon fixation capacity per se. However, there have been recent reports (Paul and Stitt, 1993) linking the decrease in chlorophyll content under N limitation to the known effects of limited sink strength on photosynthesis. When growth rates are limited by a variety of factors, a down-regulation of photosynthesis (e.g. Rubisco and chlorophyll binding protein levels) is observed, due to a decreased transcription of mRNAs coding for those proteins (Krapp et al., 1993; Krapp and Stitt, 1995; Stitt et al., 1995). It is quite possible that the low growth rates imposed by N limitation exert an effect on leaf chlorophyll content via the same mechanism (Paul and Stitt, 1993). However, it is clear from the results presented here that light absorption decreases less than photosynthetic utilization, resulting in a considerable level of excess absorbed light. This excess light is dissipated by an enhanced employment of xanthophyll cycle energy dissipation in PSII antennae under conditions of limiting N at high PFDs. We conclude that the acclimation of the light-processing reactions of spinach plants to N limitation involves a combination of a decreased absorption of light (due to a decreased chlorophyll content) and an increased thermal dissipation of absorbed light in the PSII antennae.

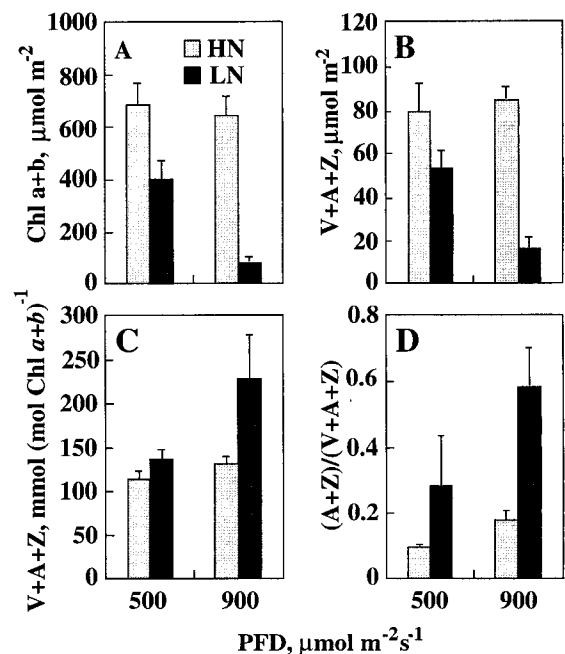


Figure 8. Effect of growth PFD (500 versus $900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and N supply on chlorophyll (A) and xanthophyll (B) cycle pool on a leaf area basis, as well as the ratio of xanthophyll cycle pool size per chlorophyll ($(V + A + Z)/\text{chlorophyll}$) (C) and the conversion state of the xanthophyll cycle at peak PFD ($(A + Z)/(V + A + Z)$) (D) in spinach leaves. All of these measurements were made on samples taken directly under the actual growth conditions in growth chambers. HN, High N; LN, low N. Values are means \pm SD, $n = 3$. Chl, Chlorophyll.

Received August 15, 1996; accepted November 26, 1996.
Copyright Clearance Center: 0032-0889/97/113/0817/08.

LITERATURE CITED

- Adams WW III, Demmig-Adams B** (1992) Operation of the xanthophyll cycle in higher plants in response to diurnal changes in incident sunlight. *Planta* **186**: 390–398
- Adams WW III, Demmig-Adams B** (1994) Carotenoid composition and down regulation of photosystem II in three conifer species during the winter. *Physiol Plant* **92**: 451–458
- Adams WW III, Demmig-Adams B** (1995) The xanthophyll cycle and sustained energy dissipation activity in *Vinca minor* and *Euonymus kiautschovicus* in winter. *Plant Cell Environ* **18**: 117–127
- Adams WW III, Demmig-Adams B, Verhoeven AS, Barker DH** (1995a) 'Photoinhibition' during winter stress: involvement of sustained xanthophyll cycle-dependent energy dissipation. *Aust J Plant Physiol* **22**: 261–276
- Adams WW III, Hoehn A, Demmig-Adams B** (1995b) Chilling temperatures and the xanthophyll cycle: a comparison of warm-grown and overwintering spinach. *Aust J Plant Physiol* **22**: 75–85
- Bilger W, Björkman O** (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in *Hedera canariensis*. *Photosynth Res* **25**: 173–185
- Björkman O** (1987) Low-temperature chlorophyll fluorescence in leaves and its relationship to photon yield of photosynthesis in photoinhibition. In DJ Kyle, CB Osmond, CJ Arntzen, eds, *Topics in Photosynthesis*, Vol 9. Elsevier Science Publishers, Amsterdam, pp 123–144
- Björkman O, Demmig-Adams B** (1994) Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. In E-D Schulze, MM Caldwell, eds, *Ecophysiology of Photosynthesis*. Springer-Verlag, Berlin, pp 17–47
- Cornic G** (1994) Drought stress and high light effects on leaf photosynthesis. In NR Baker, JR Bowyer, eds, *Photoinhibition of Photosynthesis from Molecular Mechanisms to the Field*. Bios Scientific Publishers, Oxford, UK, pp 297–313
- Demmig B, Winter K, Krüger A, Czygan F-C** (1988) Zeaxanthin and the heat dissipation of excess light energy in *Nerium oleander* exposed to a combination of high light and water stress. *Plant Physiol* **87**: 17–24
- Demmig-Adams B** (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. *Biochim Biophys Acta* **1020**: 1–24
- Demmig-Adams B, Adams WW III** (1996a) Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. *Planta* **198**: 460–470
- Demmig-Adams B, Adams WW III** (1996b) The role of the xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci* **1**: 21–26
- Demmig-Adams B, Adams WW III, Barker DH, Logan BA, Bowling DR, Verhoeven AS** (1996a) Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiol Plant* **98**: 253–264
- Demmig-Adams B, Adams WW III, Heber U, Neimanis S, Winter K, Krüger A, Czygan F-C, Bilger W, Björkman O** (1990) Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. *Plant Physiol* **92**: 293–301
- Demmig-Adams B, Adams WW III, Logan BA, Verhoeven AS** (1995) Xanthophyll cycle-dependent energy dissipation and flexible PSII efficiency in plants acclimated to light stress. *Aust J Plant Physiol* **22**: 249–260
- Demmig-Adams B, Gilmore AM, Adams WW III** (1996b) In vivo functions of carotenoids in higher plants. *FASEB J* **10**: 403–412
- Evans JR, Terashima I** (1987) Effects of nitrogen nutrition on electron transport components and photosynthesis in spinach. *Aust J Plant Physiol* **14**: 59–68
- Ferrar PJ, Osmond CB** (1986) Nitrogen supply as a factor influencing photoinhibition and photosynthetic acclimation after transfer of shade-grown *Solanum dulcamara* to bright light. *Planta* **168**: 563–570
- Genty B, Briantais J-M, Baker NR** (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* **990**: 87–92
- Gilmore AM, Björkman O** (1994a) Adenine nucleotides and the xanthophyll cycle in leaves. I. Effects of CO₂ and temperature-limited photosynthesis on adenylate energy charge and violaxanthin de-epoxidation. *Planta* **192**: 526–536
- Gilmore AM, Björkman O** (1994b) Adenine nucleotides and the xanthophyll cycle in leaves. II. Comparison of the effects of CO₂ and temperature-limited photosynthesis on photosystem II fluorescence quenching, the adenylate energy charge and violaxanthin de-epoxidation in cotton. *Planta* **192**: 537–544
- Gilmore AM, Yamamoto HY** (1991) Resolution of lutein and zeaxanthin using a nonendcapped, lightly carbon-loaded C-18 high-performance liquid chromatographic column. *J Chromatogr* **543**: 137–145
- Gilmore AM, Yamamoto HY** (1993) Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching: evidence that antheraxanthin explains zeaxanthin-independent quenching. *Photosynth Res* **35**: 67–78
- Horton P, Ruban AV, Walters RG** (1996) Regulation of light harvesting in green plants. *Annu Rev Plant Physiol Plant Mol Biol* **47**: 655–684
- Khamis S, Lamaze T, Lemoine Y, Foyer C** (1990) Adaptation of the photosynthetic apparatus in maize leaves as a result of nitrogen limitation. Relationships between electron transport and carbon assimilation. *Plant Physiol* **94**: 1436–1443
- Kitajima M, Butler WL** (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochim Biophys Acta* **376**: 105–115
- Krapp A, Hofmann B, Schäfer C, Stitt M** (1993) Regulation of the expression of *rbcs* and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis? *Plant J* **3**: 817–828
- Krapp A, Stitt M** (1995) An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. *Planta* **195**: 313–323
- Morales F, Abadía A, Abadía J** (1990) Characterization of the xanthophyll cycle and other photosynthetic pigment changes induced by iron deficiency in sugar beet (*Beta vulgaris* L.). *Plant Physiol* **94**: 607–613
- Morales F, Abadía A, Belkhdja R, Abadía J** (1994) Iron deficiency-induced changes in photosynthetic pigment composition of field-grown pear (*Pyrus communis* L.) leaves. *Plant Cell Environ* **17**: 1153–1160
- Paul MJ, Stitt M** (1993) Effects of nitrogen and phosphorus deficiencies on levels of carbohydrates, respiratory enzymes and metabolites in seedlings of tobacco and their response to exogenous sucrose. *Plant Cell Environ* **16**: 1047–1057
- Sage RE, Percy RW** (1987) The nitrogen use efficiency of C₃ and C₄ plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiol* **84**: 959–963
- Schreiber U, Bilger W, Neubauer C** (1994) Chlorophyll fluorescence as a noninvasive indicator for rapid assessment of in vivo photosynthesis. In E-D Schulze, MM Caldwell, eds, *Ecophysiology of Photosynthesis*. Springer-Verlag, Berlin, pp 49–70
- Schreiber U, Schliwa U, Bilger W** (1986) Continuous recording of photochemical and non-photochemical fluorescence quenching with a new type of modulation fluorometer. *Photosynth Res* **10**: 51–62
- Seemann JR, Sharkey TO, Wang JL, Osmond CB** (1987) Environmental effects on photosynthesis, nitrogen-use efficiency, and metabolite pools in leaves of sun and shade plants. *Plant Physiol* **84**: 796–802

- Stitt M, Krapp A, Klein D, Röper-Schwarz U, Paul M** (1995) Do carbohydrates regulate photosynthesis and allocation by altering gene expression? *In* MA Madore, WJ Lucas, eds, Carbon Partitioning and Source-Sink Interactions in Plants. American Society of Plant Physiologists, Rockville, MD, pp 68–77
- Sugiharto B, Miyata K, Nakamoto H, Sasakawa H, Sugiyama T** (1990) Regulation of expression of carbon-assimilating enzymes by nitrogen in maize leaf. *Plant Physiol* **92**: 963–969
- Terashima I, Evans JR** (1988) Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. *Plant Cell Physiol* **29**: 143–155
- Thayer SS, Björkman O** (1990) Leaf xanthophyll content andcom-
position in sun and shade determined by HPLC. *Photosynth Res* **23**: 331–343
- Verhoeven AS, Adams WW III, Demmig-Adams B** (1996) Close relationship between the state of the xanthophyll cycle pigments and photosystem II efficiency during recovery from winter stress. *Physiol Plant* **96**: 567–576
- Wong SC** (1979) Elevated atmospheric partial pressure of CO₂ and plant growth. I. Interaction of nitrogen nutrition and photosynthetic capacity in C₃ and C₄ plants. *Oecologia* **44**: 68–74
- Yamamoto HY, Bassi R** (1996) Carotenoids: localization and function. *In* DR Ort, CF Yocum, eds, Oxygenic Photosynthesis: The Light Reactions. Advances in Photosynthesis, Vol 4. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 539–563