Light Absorption and Partitioning in Relation to Nitrogen Content in 'Fuji' Apple Leaves

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ABSTRACT. Bench-grafted 'Fuji' apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] trees on Malling 26 (M.26) rootstocks were fertigated for 6 weeks with N concentrations ranging from 0 to 20 mM. These treatments produced levels of leaf N ranging from 0.9 to 4.3 g·m⁻². Over this range, leaf absorptance increased curvilinearly from 74.8% to 92.5%. The light saturation point for CO₂ assimilation expressed on the basis of absorbed light increased linearly at first with increasing leaf N, then reached a plateau at a leaf N content of ≈3 g·m⁻². Under high light conditions (photosynthetic photon flux of 1500 μmol·m⁻²·s⁻¹), the amount of absorbed light in excess of that required to saturate CO₂ assimilation decreased with increasing leaf N. Chlorophyll fluorescence measurements revealed that the maximum photosystem II (PSII) efficiency of dark-adapted leaves was relatively constant over the leaf N range, except for a slight decrease at the lower end. As leaf N increased, nonphotochemical quenching declined under high light, and there was an increase in the efficiency with which the absorbed photons were delivered to open PSII centers. The photochemical quenching coefficient remained high except for a decrease at the lower end of the leaf N range. Actual PSII efficiency increased curvilinearly with increasing leaf N, and was highly correlated with light-saturated CO₂ assimilation. The fraction of absorbed light potentially going into singlet oxygen formation was estimated to be ≈10%, regardless of leaf N status. It was concluded that there was more excess absorbed light in low N leaves than in high N leaves under high light conditions. Nonphotochemical quenching was enhanced with decreasing leaf N to reduce both the PSII efficiency and the probability of damage from photooxidation by excess absorbed light.

Carbon dioxide assimilation is closely related to leaf nitrogen content because the large number of enzymes or proteins involved in the process of photosynthesis account for the majority of leaf N. A curvilinear relationship has been found between leaf N and light-saturated CO₂ assimilation in apple leaves (*Malus sylvestris* var. *domestica*) (Cheng and Fuchigami, 2000). The decreased activation state of ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) with increasing leaf N accounts for this curvilinearity (Cheng, 1999). Low total rubisco activity and high rubisco activation state in low N leaves indicate that light-saturated CO₂ assimilation is mainly limited by the amount of rubisco. However, low N leaves have low chlorophyll content. Thus, it is unclear whether decreased light absorption also limits CO₂ assimilation in low N leaves.

The input of light energy into the photosynthetic system involves processes whereby quantum energy is captured by antenna pigments and excess excitation energy is dissipated. Finally, a proportion of the absorbed quantum energy is used in photosynthetic electron transport (Horton et al., 1996). At steady state photosynthesis, light input flux is coordinated with CO₂ input in such a way that the capacity to make triose phosphate by photosynthetic carbon reduction balances the capacity to use triose phosphate in end-product synthesis (Sharkey, 1990; Woodrow and Berry, 1988). When photosynthetic photon flux (*PPF*) is in excess of that required for photosynthetic electron transport, photosystem II (PSII) must be down-regulated to match rubisco activity. Both electron transport and total rubisco activity are correlated linearly to leaf N, indicating that light and dark reactions are altered equally by leaf N (Evans, 1996). However, the input of *PPF* fluctuates dramatically during a

clear day, with maximum *PPF* occurring at midday under cloudless conditions.

Chlorophyll content is correlated linearly with N content in apple leaves (Cheng and Fuchigami, 2000). Decreased light absorption by antenna pigments in low N leaves, however, may not be sufficient to deal with the large fluctuation of *PPF* under natural conditions. Quantum efficiency must be maximized under low *PPF*, yet leaves must be protected from excessive light damage under high *PPF*. Because low N leaves have low rubisco activity, which requires low electron transport, there may be more excess excitation energy in low N leaves than high N leaves under high *PPF* conditions.

When plants are exposed to *PPFs* in excess of those that can be used in photosynthetic electron transport, excess excitation energy can be dissipated as heat in the antenna pigments of PSII (Demmig-Adams et al., 1997; Gilmore, 1997). Analyzing changes in chlorophyll fluorescence emission via a pulse-modulated fluorescence monitoring system (Schreiber et al., 1994) has been used increasingly to estimate the activity of thermal dissipation processes, as well as photosynthetic electron transport in leaves (Demmig-Adams et al., 1996; Genty et al., 1989). Thermal dissipation of excess excitation energy has been studied extensively under high light conditions (Adams and Demmig-Adams, 1992; Demmig-Adams and Adams, 1996; Demmig-Adams et al., 1996) and low temperature stress (Adams and Demmig-Adams, 1995; Adams et al., 1995a, 1995b; Gilmore and Björkman, 1994a, 1994b; Verhoeven et al., 1996). However, information is limited about thermal dissipation of excitation energy in response to leaf N status (Khamis et al., 1990; Verhoeven et al., 1997). Therefore, the objectives of this study were to determine, under high *PPF* conditions, if there is more excess absorbed *PPF* in low N leaves than in high N leaves and how absorbed *PPF* is partitioned into photochemical and nonphotochemical processes in response to N content in apple leaves.

Materials and Methods

PLANT MATERIAL. 'Fuji' apple trees on Malling 26 (M.26) rootstocks were used in this study. Bench-grafting was done in late

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March 1997 and each grafted tree was planted immediately into a 3.8-L pot containing a medium of 1 peat : 2 pumice : 1 sandy loam soil (by volume). The plants were grown in a lathhouse until early June. During this period, beginning from budbreak in early May, they were fertigated every 2 weeks with 150 mg·L⁻¹ N, using Plantex 20N–10P₂O₅–20K₂O water-soluble fertilizer with micronutrients (Plantex Corp., Ontario, Canada). When the scion shoots were ≈15 cm tall, plants were selected for uniformity, and moved to full sun. Thereafter, they were fertigated weekly with Plantex for 3 weeks. Beginning 30 June, plants were fertigated twice weekly with N at 0, 2.5, 5.0, 7.5, 10.0, 15.0 or 20.0 mM (from NH₄NO₃) by applying 300 mL of a modified Hoagland's solution to each pot (Cheng and Fuchigami, 2000). There were four replications for each N treatment in a completely randomized design. Plants were subirrigated with a saucer placed at the bottom of each pot. After 6 weeks, recent fully expanded leaves were selected for gas exchange and chlorophyll fluorescence measurements.

MEASUREMENTS OF LEAF ABSORPTANCE, GAS EXCHANGE, AND CHLOROPHYLL FLUORESCENCE. A spectroradiometer with the 1800-12S integrating sphere attachment (LI-1800; LI-COR Inc., Lincoln, Nebr.) was used to measure leaf reflectance and transmittance. For each leaf, both a reference scan and a sample scan of reflectance or transmittance were made from 400 to 700 nm, at 1-nm intervals. The sample scan was divided by its corresponding reference scan, and integrated over the wavelength range to obtain the average reflectance or transmittance. Leaf absorptance was calculated as 1 – reflectance – transmittance.

Carbon dioxide assimilation and PSII efficiency were monitored concurrently with a system that combined a CIRAS-1 gas exchange system (PP Systems, Hitchin, Herts., U.K.) and an FMS-1 pulse-modulated fluorometer (Hansatech Instruments Ltd., King's Lynn, Norfolk, U.K.). The light- and temperature-controlled cuvette of the CIRAS-1 system was modified so that the fiber optic of the FMS-1 was inserted into the cuvette at a 60° angle. This did not significantly interfere with *PPF* distribution at the leaf surface, yet it allowed delivery of a saturation pulse of actinic light and detection of fluorescence signals. Measurements of CO₂ assimilation and PSII efficiency in response to *PPF* were made in descending order, at incident *PPFs* of 1500, 1150, 850, 600, 400, 250, 150, 75, and 0 μmol·m⁻²·s⁻¹, at ambient CO₂ (350 mg·L⁻¹) and O₂ (21%). The two light sensors in the cuvette were not located exactly at the leaf surface, therefore, the actual corresponding incident *PPFs* at the leaf surface level were 1645, 1225, 907, 640, 425, 267, 160, 80, and 0 μmol·m⁻²·s⁻¹, as measured with a recently calibrated quantum sensor (LI-190SA; LI-COR). At each *PPF*, CO₂ assimilation and actual PSII efficiency were recorded after reaching steady state. Actual PSII efficiency was measured in essentially the same way as under natural *PPF* conditions described below.

Total electron transport was calculated as incident *PPF* × absorptance × 0.5 × PSII efficiency (Krall and Edwards, 1992). Responses of CO₂ assimilation and actual PSII efficiency to *PPF* were measured on 20 leaves with different N contents. Each photosynthetic light response curve was fitted to the following negative exponential model by nonlinear regression analysis, using the SAS procedure NLIN (SAS Institute Inc., Cary, N.C.) with the Marquardt option (Hampson et al., 1996): $A = R_d + S(1 - e^{-G \times PPF})$, where *A* is CO₂ assimilation, *R_d* is dark respiration, *S* is the asymptotic maximum, *e* is the base of natural log, *G* is the rate of approach to the maximum, and *PPF* is photosynthetic photon flux. The light saturation point for CO₂ assimilation was estimated as the *PPF* at which 95% of the light-saturated assimilation rate was attained.

The relationship between leaf N and chlorophyll fluorescence

parameters was studied on 28 leaves with a wide range of leaf N contents either predawn or at a *PPF* of 1500 ± 50 μmol·m⁻²·s⁻¹ at noon, under natural conditions, using the FMS-1 fluorometer. The fiber optic of the FMS-1 was positioned using the *PPF*/temperature leaf clip at a 60° angle from the upper surface of the leaf, and the distance between the fiber optic and the leaf surface was kept constant for both predawn and noon measurements. Maximum fluorescence (*F_m*) and minimum fluorescence (*F_o*) of dark-adapted leaves were measured predawn. For leaves exposed to natural sunlight, steady state fluorescence (*F_s*) was monitored to ensure it was stable before a reading was taken. Maximum fluorescence (*F_m'*) under natural light exposure was obtained by imposing a 1-s saturating flash of ≈18,000 μmol·m⁻²·s⁻¹ *PPF* at the end of the fiber optic to the leaf in order to reduce all the PSII centers. To determine the minimum fluorescence (*F_o'*) under natural light exposure, a black envelope was placed around the leaf while a far-red light was

Fig. 1. Leaf absorptance in relation to (A) chlorophyll content and (B) nitrogen content, and (C) the relationship between leaf N and chlorophyll content in apple leaves. Regression equations: (A) $Y = 27.94(1 - e^{-4.594X}) + 64.58$ ($R^2 = 0.99$, $P = 0.0001$), (B) $Y = 48.39(1 - e^{-1.153X}) + 44.18$ ($R^2 = 0.99$, $P = 0.0001$), and (C) $Y = -0.122 + 0.256X$ ($R^2 = 0.98$, $P = 0.0001$).

Fig. 2. (A) Carbon dioxide assimilation, (B) actual PSII efficiency, and (C) electron transport of apple leaves in response to incident *PPF*. Measurements were made at a CO_2 concentration of $350 \pm 2 \text{ mg} \cdot \text{L}^{-1}$, a leaf temperature of $25 \pm 0.2^\circ\text{C}$, and an ambient water vapor pressure of $1.55 \pm 0.2 \text{ kPa}$. Leaf N content ($\text{g} \cdot \text{m}^{-2}$) is 1.06 (●), 1.35 (○), 1.82 (▼), 2.77 (▽), and 4.02 (■).

switched on to rapidly oxidize PSII by drawing electrons from PSII to PSI. The maximum PSII efficiency of dark-adapted leaves was calculated as $F_v/F_m = (F_m - F_o)/F_m$ (For fluorescence nomenclature see van Kooten and Snel, 1990).

For leaves exposed to natural sunlight, thermal dissipation was estimated from nonphotochemical quenching as $F_m/F_m' - 1$ (Stern-Volmer quenching, or NPQ; see Bilger and Björkman, 1990) or nonphotochemical quenching coefficient: $q_N = 1 - (F_m' - F_o')/(F_m - F_o)$. The efficiency of open PSII centers under natural light exposure (F_v'/F_m') was calculated as $(F_m' - F_o')/F_m'$. This is also referred to as the efficiency with which excitation energy is delivered to open PSII centers, or the efficiency of excitation capture by PSII. The photochemical quenching coefficient q_P was $(F_m' - F_s)/(F_m' - F_o')$. The degree of closure of PSII reaction centers was estimated as $1 - q_P$. Actual PSII efficiency was $(F_v'/F_m') \times q_P = (F_m' - F_s)/F_m'$ (Genty et al., 1989). The percentage of absorbed light potentially going into singlet oxygen formation was estimated as $(F_v'/F_m') \times (1 - q_P)$ (Demmig-Adams et al., 1996).

LEAF N AND CHLOROPHYLL ANALYSIS. After all the above measurements, leaf area was determined with a leaf area meter (LI-3000, LI-COR). Leaves were frozen in liquid nitrogen and stored at -80°C until freeze-dried. Leaf N content was determined by the Kjeldahl

procedure (Schuman et al., 1973). Leaf chlorophyll content was measured according to Arnon (1949).

Results

LEAF ABSORPTANCE IN RESPONSE TO LEAF N. A curvilinear relationship was found between leaf chlorophyll content and leaf absorptance (Fig. 1A). Leaf absorptance increased almost linearly with increasing leaf chlorophyll content up to $\approx 0.3 \text{ g} \cdot \text{m}^{-2}$, then began to level off with a further rise in leaf chlorophyll content. Leaf absorptance also showed a curvilinear response to leaf N (Fig. 1B) because chlorophyll content was correlated linearly with leaf N (Fig. 1C). Although leaf N content varied from 0.9 to $4.3 \text{ g} \cdot \text{m}^{-2}$, leaf absorptance increased only from 74.8% to 92.5%.

RESPONSE OF CO_2 ASSIMILATION AND ELECTRON TRANSPORT TO *PPF* AND EXCESS ABSORBED *PPF* IN RELATION TO LEAF N. For all leaves, CO_2 assimilation increased with increasing *PPF* up to a saturation point, then showed little response to any further rise in *PPF* (Fig. 2A). Actual PSII efficiency decreased with increasing *PPF* (Fig. 2B). Calculated total electron transport showed a response to *PPF* similar to that of CO_2 assimilation (Fig. 2C). At each given incident *PPF*, low N leaves had lower CO_2 assimilation, actual PSII efficiency, and total electron transport than high N leaves. A positive correlation was found between actual PSII efficiency and CO_2 assimilation under light-saturated conditions (Fig. 3A), and also between total electron transport and CO_2 assimilation (Fig. 3B).

After accounting for leaf absorptance, CO_2 assimilation in response to absorbed *PPF* was still dependent on leaf N (Fig. 4).

Fig. 3. Carbon dioxide assimilation in relation to (A) actual PSII efficiency and (B) total electron transport in apple leaves at an incident *PPF* of $1645 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Regression equations: (A) $Y = 0.98 + 38.28X$ ($R^2 = 0.97$, $P = 0.0001$), and (B) $Y = 2.11 + 0.0476X$ ($R^2 = 0.97$, $P = 0.0001$). Measurement conditions were the same as in Fig. 2.

Fig. 4. Carbon dioxide assimilation of apple leaves in response to absorbed *PPF*. Leaf N content ($\text{g}\cdot\text{m}^{-2}$) is 1.06 (●), 1.35 (○), 1.82 (▼), 2.77 (▽), and 4.02 (■). Measurement conditions were the same as in Fig. 2.

Fig. 5. Relationship between leaf N content and (A) the light saturation point expressed as absorbed *PPF*, and (B) excess absorbed *PPF* at an incident *PPF* of $1500\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Excess absorbed *PPF* was calculated as the difference between the actual absorbed *PPF* and the light saturation point expressed as absorbed *PPF*. Measurement conditions were the same as in Fig. 2.

Quantum yield for CO_2 assimilation remained relatively unchanged, except for a slight decrease at the lower end of the leaf N range (data not presented). The light saturation point, expressed as absorbed *PPF*, increased at first with increasing leaf N, then leveled off at a leaf N content of $\approx 3\ \text{g}\cdot\text{m}^{-2}$ (Fig. 5A). At an incident *PPF* of $1500\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the amount of excess absorbed light (calculated as the difference between the actual absorbed *PPF* and the light saturation point expressed as absorbed *PPF*) decreased with increasing leaf N (Fig. 5B).

CHLOROPHYLL FLUORESCENCE PARAMETERS IN RELATION TO LEAF N. Maximum PSII efficiency (F_v/F_m) of dark-adapted leaves remained relatively unchanged, except for a slight decrease at the

lower end of the leaf N range (Fig. 6A). Thermal dissipation of excitation energy, indicated by both nonphotochemical quenching (NPQ) and the nonphotochemical quenching coefficient (q_N) decreased with increasing leaf N (Fig. 6B and C). Correspondingly, the efficiency of excitation capture (F_v'/F_m') by open PSII reaction centers increased with increasing leaf N up to nearly $3\ \text{g}\cdot\text{m}^{-2}$, then leveled off with a further rise in leaf N (Fig. 6D). Photochemical quenching (q_P) remained high except for a decrease at the lower end of the leaf N range (Fig. 6E). This corresponded with a low degree of closure of PSII ($1-q_P$) over the leaf N range, except in extremely low N leaves (Fig. 6G). Actual PSII efficiency increased with increasing leaf N at first, then leveled off with further increases in leaf N (Fig. 6F). The fraction of absorbed light potentially going into singlet oxygen formation remained at $\approx 10\%$ over the leaf N range examined (Fig. 6H).

Discussion

Leaf light absorption and partitioning into different pathways may be summarized as in Fig. 7. Part of the incident light is either reflected or transmitted; only a proportion is absorbed by light-harvesting pigments. Depending on the amount of absorbed *PPF*, either most or only a proportion of it is delivered to open PSII centers. At low *PPFs*, most of the absorbed light is used in photosynthetic electron transport; any dissipation of excitation energy via an alternative pathway would decrease the efficiency of PSII. At high *PPFs*, however, not all the absorbed light can be used in photosynthetic electron transport. The excess excitation energy must then be dissipated either through a harmless thermal process or by formation of toxic, activated-oxygen species (Demmig-Adams et al., 1996, 1997).

Apple trees do not have the rapid, active leaf movement to evade excessive light that plant species with pulvinar tissues have (e.g., Leguminosae and Oxalidaceae; reviewed in Koller, 1990). Decreasing the absorption of light by reducing chlorophyll content is the first line of defense for protecting leaves from photooxidation in response to low N availability. This was suggested in studies with maize (*Zea mays* L.) (Khamis et al., 1990) and spinach (*Spinacia oleracea* L.) (Verhoeven et al., 1997), although leaf absorbance was not measured in either experiment. A decrease in chlorophyll content provides a coarse regulatory mechanism for reducing light absorption at each given incident *PPF*. Decreased light absorption alone, however, is not enough for low N leaves to cope with high light because light absorption is not reduced proportionally when leaf N decreases (Fig. 1B). In our experiment, under high light conditions, low N leaves had more excess absorbed light than high N leaves (Fig. 5B) because photosynthetic electron transport used only a small percentage of the absorbed light.

Actual PSII efficiency is determined by both the photochemical quenching (q_P) and the efficiency of excitation capture (F_v'/F_m') by open PSII centers (Genty et al., 1989). When there is excess absorbed light, actual PSII efficiency can be reduced by two processes to match photosynthetic electron transport. One process is to decrease the efficiency with which excitation energy is delivered to open PSII centers via thermal dissipation of excitation energy in the antenna pigment complexes (Demmig-Adams et al., 1995, 1996; Genty et al., 1990). The alternative process is to close PSII reaction centers by overreducing the electron acceptors of PSII. In the latter case, a transient backlog of excitation energy occurs. This leads to increased formation of triplet excited chlorophyll, which has the potential to react with oxygen to form toxic singlet oxygen (Asada, 1996).

Fig. 6. Chlorophyll fluorescence parameters in relation to N content in apple leaves. (A) Maximum PSII efficiency (F_v/F_m) of dark-adapted leaves was measured predawn on 11 Aug. 1997. The remaining parameters were measured at a PPF of $1500 \pm 50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and air temperature of $29 \pm 1^\circ\text{C}$ at noon. (B) NPQ = nonphotochemical quenching ($(F_m/F_m' - 1)$), (C) qN = nonphotochemical quenching coefficient [$1 - (F_m' - F_o')/(F_m - F_o)$], (D) F_v'/F_m' = efficiency of excitation capture by open PSII centers [$(F_m' - F_o')/F_m'$], (E) qP = photochemical quenching coefficient [$(F_m' - F_s)/(F_m' - F_o')$], (F) $PSII$ = actual PSII efficiency [$(F_m' - F_s)/F_m'$], (G) $1 - qP$ = degree of closure of PSII centers, and (H) $(F_v'/F_m')(1 - qP)$ = the fraction of absorbed PPF potentially going into singlet oxygen formation.

Thermal dissipation can safely remove excess excitation energy before it reaches PSII reaction centers, thus protecting these centers by preventing an accumulation of reduced electron acceptors of PSII (Demmig-Adams et al., 1996, 1997). In our experiment, thermal dissipation of excitation energy, indicated by nonphotochemical quenching, was enhanced in low N leaves in response to more excess absorbed light, compared with high N leaves under high light (Figs. 6B and C). This increase in thermal

dissipation was so effective in decreasing the efficiency for delivering excitation energy to open PSII centers (Fig. 6D) that the fraction of absorbed light potentially going into singlet oxygen formation remained at $\approx 10\%$ across the leaf N range (Fig. 6H). In response to N deficiency, enhancement of thermal dissipation of excitation energy in maize leaves overcompensated for the decrease in photosynthetic electron transport, such that the primary electron acceptor of PSII remained more oxidized in N-

Fig. 7. Leaf light absorption and partitioning (modified from Demmig-Adams et al., 1996, 1997). Thermal dissipation of excitation energy is indicated by NPQ (nonphotochemical quenching of chlorophyll fluorescence). F_v'/F_m' is the fraction of absorbed PPF that is delivered to open PSII centers. Of the PPF delivered to PSII, the proportion used in photosynthetic electron transport is qP (photochemical quenching coefficient); the rest $(1 - qP)$ represents the fraction that potentially goes into singlet oxygen formation. The percentage of absorbed PPF used in electron transport is the actual PSII efficiency $(F_v'/F_m')qP$ (Genty et al., 1989). The percentage of absorbed light potentially going into singlet oxygen formation is estimated as $(F_v'/F_m')(1 - qP)$ (Demmig-Adams et al., 1996).

deficient plants than in N-replete controls (Khamis et al., 1990).

Under slowly developed water stress, nonphotochemical quenching increased in apple leaves (Massacci and Jones, 1990). Considerable evidence proves that increased thermal dissipation of excitation energy is an important photoprotective mechanism for plants responding to excess absorbed PPF, whether this results from increasing PPF or decreased electron transport caused by environmental stress. In a natural setting, the plant's thermal dissipation of excess excitation energy was sufficient to remove excess absorbed PPF and protect leaves from photooxidation (Demmig-Adams et al., 1995, 1996, 1997).

The photoprotective effect of thermal dissipation in response to N deficiency was also reflected in the maximum PSII efficiency of dark-adapted leaves. Except for a slight decrease at the lower end of the leaf N range, F_v/F_m of dark-adapted leaves remained relatively unchanged across the leaf N range (Fig. 6A). This decrease in F_v/F_m may have been caused by sustained xanthophyll cycle-dependent thermal dissipation during the night; PSII centers are not necessarily damaged by excess absorbed light (Adams and Demmig-Adams, 1994, 1995; Adams et al., 1995a, 1995b; Verhoeven et al., 1996, 1997). When plants growing in natural gaps of a tropical forest were exposed to direct sunlight for 1 to 2 h at midday, the decline in F_v/F_m of dark-adapted leaves and subsequent recovery in the dark were correlated closely with the amount of zeaxanthin in leaves. This indicates that the decrease in F_v/F_m of dark-adapted leaves after midday direct sun exposure may also result from sustained xanthophyll cycle activity (Thiele et al., 1998).

F_v/F_m of dark-adapted apple leaves was not affected by water stress, but CO_2 assimilation decreased (Massacci and Jones, 1990). Measuring F_v/F_m of dark-adapted leaves alone may not provide enough information on CO_2 assimilation of the photosynthesis process. The linear relationship between actual PSII efficiency $(F_m' - F_s)/F_m'$ and CO_2 assimilation suggests that actual PSII efficiency may better indicate the rate of CO_2 assimilation at a fixed PPF level if partitioning of total photosynthetic electron transport into CO_2 assimilation and photorespiration remains unchanged.

Thermal dissipation of excitation energy involves a xanthophyll cycle and a transthylakoid pH difference (Demmig-Adams et al., 1997; Gilmore, 1997). The xanthophyll cycle consists of light-dependent conversion between three xanthophylls in a cyclic reaction (Demmig-Adams et al., 1997). Under high PPF conditions, diopside violaxanthin (V) is deepoxidized via the monoepoxide

antheraxanthin (A) to the epoxide-free form zeaxanthin (Z). When PPF decreases, epoxidation of Z occurs in the reverse direction to form V via A. Nonphotochemical energy dissipation is correlated closely with the level of Z or A+Z; this relationship is species-independent (Demmig-Adams and Adams, 1996). A transthylakoid pH difference is required for activating the deepoxidase enzyme that converts V to A and Z, and for protonation of lumen-exposed carboxyl groups of minor chlorophyll binding proteins (CPs) of the PSII inner antenna. The interaction of Z+A and protonated CPs leads to an increased rate constant of heat dissipation for excitation energy in the PSII antenna (Gilmore, 1997; Gilmore et al., 1995).

In maize (Khamis et al., 1990) and spinach (Verhoeven et al., 1997), low N leaves had a greater proportion of the xanthophyll cycle as Z and A at midday and a higher ratio of xanthophyll cycle pigments to total chlorophyll. This supports the general conclusion found in other environmental stresses that thermal dissipation of excitation energy is xanthophyll cycle-dependent. Nevertheless, N supply did not affect F_v/F_m of dark-adapted leaves, the pool size and the deepoxidation state of the xanthophyll cycle at midday in 'Traveler's Joy' clematis (*Clematis vitalba* L.) grown at several PPFs (Bungard et al., 1997). Thermal dissipation, however, was not measured in that experiment. The involvement of the xanthophyll cycle in thermal dissipation of excitation energy in apple leaves in response to leaf N warrants further research.

In summary, light absorption is less in apple leaves with low N content, but those leaves still have more excess absorbed light than high N leaves under high light conditions. This is because low N leaves have low rates of electron transport. As leaf N content decreases, thermal dissipation of excitation energy is enhanced. This reduces the efficiency with which excitation energy is delivered to open PSII reaction centers, whereby protecting leaves from photooxidation by excess absorbed light.

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