AN ABSTRACT OF THE DISSERTATION OF

Lailiang Cheng for the degree of Doctor of Philosophy in Horticulture presented on June 17, 1999. Title: Photosynthesis in Relation to Nitrogen in Apple (*Malus domestica* Borkh.) Leaves.

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Leslie H. Fuchigami

Four aspects of the relationship between N and photosynthesis in apple leaves were examined: 1) gas exchange; 2) rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) activities and activation state; 3) light absorption and partitioning; and 4) quantum yield for CO_2 assimilation (Φ_{CO2}) in relation to actual photosystem II efficiency (Φ_{PSII}). A wide range of leaf N content was achieved by fertigating bench-grafted 'Fuji/M_{26}' trees, grown in full sun, with different N concentrations using a modified Hoagland's solution.

Curvilinear relationships were found between leaf N content and 1) light-saturated CO_2 assimilation at ambient CO_2 (A_{350}); 2) the initial slope (IS) of CO_2 assimilation (A) in response to intercellular CO_2 concentration (Ci); and 3) CO_2-saturated photosynthesis. All three initially increased linearly with leaf N, then reached a plateau at a leaf N of approximately 3 g m^{-2}. Analysis of A/Ci curves indicated that A_{350} fell within the linear region of the A/Ci curve regardless of leaf N status.

Total rubisco activity increased linearly with leaf N, whereas initial activity showed a curvilinear response to leaf N. Rubisco activation state decreased with

increasing leaf N. Both A_{350} and IS were linearly related to initial rubisco activity, but curvilinearly related to total activity. The ratio of total rubisco activity to leaf N increased with leaf N. As leaf N increased, photosynthetic N use efficiency declined with decreasing rubisco activation state.

Leaf absorptance increased curvilinearly from 74.8 to 92.5% over a leaf N range of 0.9 ~ 4.3 g m^{-2}. The amount of absorbed light in excess of that required to saturate photosynthesis increased with decreasing leaf N under high light. Non-photochemical quenching was enhanced with decreasing leaf N to reduce Φ_{PSII}. The fraction of absorbed light potentially going into singlet oxygen formation remained at about 10% across the leaf N range.

A single curvilinear relationship was found between Φ_{CO2} and Φ_{PSII} for leaves with different N contents under non-photorespiratory conditions. Both the rate of linear electron flow and the rate to CO_2 or O_2 increased with increasing leaf N at any given Ci, but the partitioning of electron flow between CO_2 assimilation and photorespiration was not affected by leaf N.

PHOTOSYNTHESIS IN RELATION TO NITROGEN IN APPLE (*Malus domestica* Borkh.) LEAVES

by

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APPROVED:

Major Professor, representing Horticulture

Chair of Department of Horticulture

Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Lailiang Cheng, Author

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DEDICATION

This dissertation is dedicated to my mother,
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PHOTOSYNTHESIS IN RELATION TO NITROGEN IN APPLE (*Malus domestica* Borkh.) LEAVES

CHAPTER 1

INTRODUCTION

Photosynthesis provides the ultimate carbon supply for plant growth and development. Nitrogen is an essential macroelement, an important resource in the environment, and the most heavily used fertilizer for increasing plant production. Photosynthesis is closely related to leaf N because the photosynthetic process depends on the activity and coordination of many proteins, which account for the majority of the N in a leaf (Evans, 1989, 1996; Makino and Osmond, 1991). Therefore, understanding the relationship between leaf N and photosynthesis is critical not only for optimizing carbon production relative to N input, but also for elucidating the mechanisms involved in the regulation of photosynthesis.

The relationship between leaf N content and photosynthesis has been studied extensively over the last 20 years (Evans, 1989, 1996). Major advances have been made in understanding, 1) partitioning of leaf N between rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) and thylakoid proteins in response to leaf N; and 2) CO_2 assimilation in relation to total rubisco activity and CO_2 transfer conductance between intercellular air spaces to carboxylation sites in chloroplasts. Many aspects of the mechanisms for regulating photosynthesis in response to leaf N, however, remain to be elucidated. Three of them are of particular interest. First, the long-standing idea that rubisco is not fully active in leaves with a high N content, thus serving as a storage

protein (Huffaker and Peterson, 1974), has not been rigorously tested. If this hypothesis were true, it could explain the curvilinear relationship between leaf N and photosynthesis observed in many species (Evans, 1983, 1989; DeJong and Doyle, 1985; Sinclair and Horie, 1989). Second, electron transport and rubisco activity are coordinated to match triose phosphate utilization in end-product synthesis (Woodrow and Berry, 1988; Sharkey, 1990). It is unclear how this coordination is achieved in low N leaves under high light conditions. Low N leaves have low rubisco activity, which requires low electron transport. Although chlorophyll content decreases proportionally with decreasing leaf N content, the decreased light absorption alone may not be sufficient to coordinate electron transport and rubisco activity. Third, photosynthetic electron transport not only provides reducing power for photosynthetic carbon reduction and oxidation, but it also supplies electrons for other processes such as nitrate reduction and the Mehler reaction (Badger, 1985). It is not well understood how leaf N affects the partitioning of electron flow to rubisco-supported photosynthesis relative to alternative electron sinks. If this partitioning were altered by leaf N, the relationship between quantum yield for CO_2 assimilation and actual PSII efficiency would be changed.

Most of the research on the photosynthesis-N relationship has concentrated on model systems using annual herbaceous plants (Evans, 1989, 1996). Very little is known about this relationship in woody perennials. Woody perennials differ from herbaceous annuals in terms of photosynthetic capacity, N use efficiency, and N storage strategy. For example, herbaceous plants can accumulate substantial amounts of nitrate in leaf vacuoles under an excessive nitrate supply (Maynard et al., 1976; Millard, 1988). In contrast, nitrate-N accounts for only a very small proportion of total N in leaves of apple

(Lee and Titus, 1992) and poplar (Bañados, 1992) even under a very high nitrate supply (30 to 50 mM). This difference could be manifested in the relationship between leaf N content and photosynthetic activities.

In this research, we used apple, a woody perennial, to study the relationship between leaf N and photosynthesis. Apple is an important deciduous tree fruit crop in the Pacific Northwest and worldwide. Although considerable efforts have been made to understand the environmental regulation of photosynthesis in this species (Flore and Lakso, 1989; Lakso, 1994), much remains to be learned about photosynthetic capacity of apple leaves in relation to leaf N status.

The objectives of this research were to determine 1) the relationship between leaf N and CO_2 assimilation capacity; 2) rubisco activities and activation state in relation to leaf N; 3) light absorption, partitioning, and electron transport in relation to leaf N; and 4) the relationship between quantum yield for CO_2 assimilation and actual PSII efficiency.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of C_3 photosynthesis

2.1.1 Structure of the C_3 photosynthetic system

The C_3 photosynthetic system has a structure of both a convergent metabolic pathway in terms of its inputs and a divergent metabolic pathway in terms of its end-product synthesis (Woodrow and Berry, 1988; see Figure 2-1). The two inputs into the system are CO_2 from the atmosphere and the quantum energy for photosynthetic carbon reduction. The input of CO_2 is mediated by a series of diffusion processes (through boundary layer, stomata, intercellular air space, mesophyll cell wall, and liquid phase to the carboxylation site within chloroplasts) and the largely irreversible reaction catalyzed by ribulose-1, 5-bisphosphate carboxylase/oxygenase (rubisco; Sharkey, 1985). The input of light energy involves processes in which quantum energy is captured by antenna pigments. Excess excitation energy is then dissipated and, finally, a proportion of the absorbed quantum energy is utilized in photosynthetic electron transport to produce ATP and reducing equivalents (Horton et al., 1996). CO_2 and light inputs converge at the reduction phase of the Calvin cycle.

End-product synthesis of C_3 photosynthesis is characterized by a divergent metabolic pathway: triose phosphates formed in the photosynthetic carbon reduction either remain in the chloroplast for starch synthesis, or are transported across the chloroplast envelope to the cytosol for sucrose synthesis. In many species of Rosaceae,

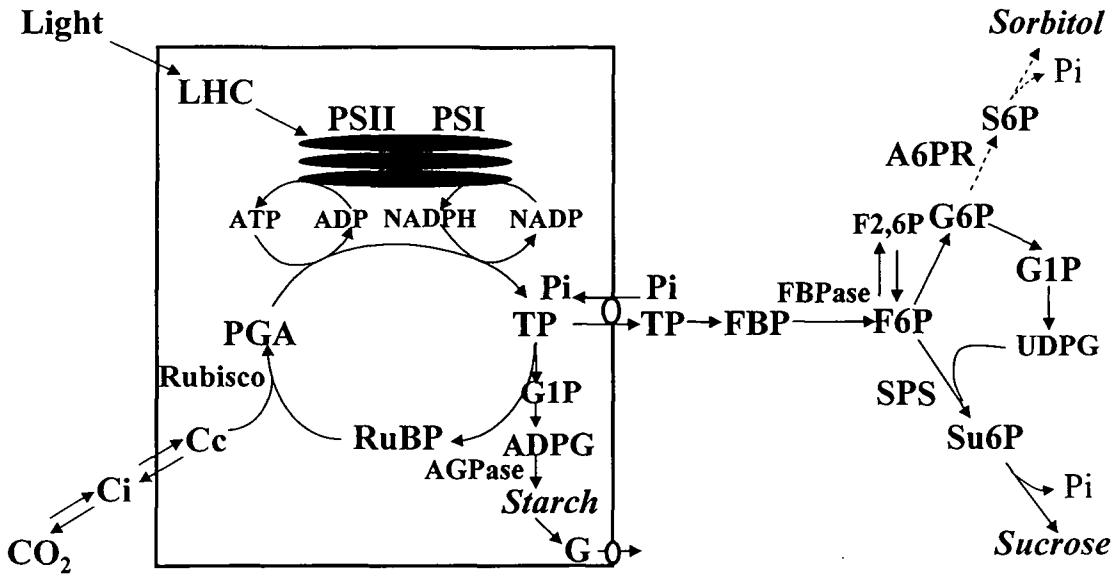

Figure 2-1. The structure of the C_3 photosynthetic system. The box represents chloroplasts. Inputs of light and CO_2 converge at the photosynthetic carbon reduction cycle. Triose phosphates (TP) either remain in the chloroplast for starch synthesis or are transported out via TP translocator to the cytosol for sucrose synthesis. Ca: CO_2 in the atmosphere; Ci: intercellular CO_2; Cc: CO_2 at the carboxylation site within chloroplasts; Rubisco: ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase; PGA: 3-phosphoglyceric acid; LHC: light harvesting complex; PSII and PSI: Photosystem II and I reaction centers; G1P: glucose-1-phosphate; ADPG: ADP-glucose; AGPase: ADP-glucose pyrophosphorylase; Pi: inorganic phosphate; FBP: fructose-1,6-bisphosphate; FBPase: fructose-1,6-bisphosphatase; F6P: fructose-6-phosphate; F2,6P: Fructose-2,6-bisphosphate; SPS: sucrose-6-phosphate (Su6P) synthase; G6P: glucose-6-phosphate; A6PR: aldose-6-phosphate reductase; S6P: sorbitol-6-phosphate. UDPG: UDP-glucose. (Modified from Woodrow and Berry, 1988)

sorbitol is also synthesized from triose phosphate in the cytosol in addition to sucrose (Loescher, 1987), which adds another branch to the end-product synthesis pathway (Figure 2-1).

2.1.2 Regulation of C_3 photosynthesis

The structure of the C_3 photosynthetic system requires that at steady state CO_2 and light energy inputs conform to a specific stoichiometry such that the input fluxes to make triose phosphate by photosynthetic carbon reduction match the capacity to use triose phosphate in end-product synthesis (Woodrow and Berry, 1988; Sharkey, 1990). Because of the divergent nature of the end-product synthesis pathway and the transitory storage property of starch, it is possible that carbon partitioning between end-products can be altered without affecting photosynthesis. Only under certain circumstances is the maximum capacity to use triose phosphate in end-product synthesis approached, a feedback mechanism is then triggered to reduce the rate of energy input and rubisco activity (Woodrow and Berry, 1988; Sharkey, 1990).

The regulation of photosynthesis has been studied extensively (Sharkey, 1985; Woodrow and Berry, 1988; Stitt and Schulze, 1994; Stitt, 1996). The following four mechanisms will be reviewed here. First, at low photon flux density (PFD), rubisco activity is down-regulated to balance the input of quantum energy. Second, at high PFD, photosystem II (PSII) efficiency is down-regulated to match rubisco activity. Third, starch synthesis is regulated by sucrose synthesis to sustain the overall utilization of triose phosphate without affecting photosynthesis. Fourth, feedback regulation of photosynthesis is provided by end-product synthesis.

2.1.2.1 Down-regulation of rubisco activity at low PFD

Rubisco catalyzes the initial reactions in photosynthetic CO_2 reduction and photorespiratory carbon oxidation. In order to be catalytically competent, rubisco must

be activated. Rubisco activation involves the reversible reaction of a CO_2 molecule with a lysine residue within the active site to form a carbamate. This is followed by a rapid binding of a magnesium ion to create an active ternary structure (Portis, 1992). A special protein, rubisco activase, catalyzes this activation process *in vivo* (Portis et al., 1986; Portis, 1990). Antisense suppression of rubisco activase expression in tobacco plants significantly decreases rubisco activation and CO_2 assimilation (Mate et al., 1993, 1996).

Rubisco activase counteracts with tightly binding inhibitors of rubisco, sugar phosphates, to regulate rubisco activity (Portis, 1990, 1992; Salvucci and Ogren 1996). These sugar phosphates include ribulose-1,5-bisphosphate (RuBP), caboxyarabinitol-1-phosphate (CA1P), xylulose-1,5-bisphosphate (XuBP) and 3-keto-arabinitol-1,5-bisphosphate (KABP). In addition to serving as a substrate for rubisco in the carboxylation and oxygenation reactions, RuBP binds to the inactive form of rubisco, restricting access of the activator CO_2 and Mg^{2+} to the site of carbamylation (Jordan and Chollet, 1983). CA1P is a nocturnal inhibitor of rubisco, which binds to the RuBP site on the activated rubisco to form a stable rubisco-CA1P complex (Berry et al., 1987; Seemann et al., 1990). CA1P concentration in dark-treated leaves is quite variable among species, ranging from less than 0.009 moles per mole of rubisco catalytic site in spinach, wheat and *Arabidopsis* to 1.5 ~1.8 moles per mole rubisco catalytic site in bean and petunia (Moore et al., 1991). In species that contain large amounts of CA1P, its concentration increases in the dark and decreases in the light. Both XuBP and KABP are formed during carboxylation by a stereochemically incorrect protonation of the 2,3-enediolate intermediate bound at the catalytic site. This causes slow inactivation of rubisco during catalysis (Edmondson et al., 1990). Rubisco activase promotes rubisco

activation by releasing RuBP from RuBP-bound rubisco (Wang and Portis 1992), and up-regulates rubisco activity by releasing CA1P from activated rubisco in the light (Mate et al., 1993; Hammond et al., 1998). It also maintains the catalytic activity of rubisco by preventing inactivation by XuBP and KABP during catalysis (Robinson and Portis 1990). Because ATP hydrolysis is the required energy source for rubisco activase, rubisco activase is modulated by light via the stromal ATP/ADP ratio (Portis, 1990; Salvucci and Ogren 1996).

Rubisco activity is down-regulated in response to a decrease in PFD below the saturation point (Kobza and Seemann, 1988; Salvucci, 1989; Sage et al., 1990; Seemann et al., 1990). The regulation of rubisco activity by PFD ranges from an almost complete regulation by CA1P concentration to regulation only by changes in activation (Kobza and Seemann, 1988). For species with large amounts of CA1P in the dark (e.g., bean), regulation of rubisco activity in response to changing PFD is accomplished by CA1P; activation may not be important for light regulation of the enzyme. For species with little CA1P in the dark (e.g., spinach), rubisco activity is modulated by activation in response to PFD. For species with CA1P levels between those extremes, rubisco activity is regulated in response to PFD by both activation and CA1P concentration. As a result, rubisco activity is adjusted to the prevailing PFD.

2.1.2.2 Down-regulation of PSII efficiency at high PFD

Light drives photosynthetic electron transport to provide reducing equivalents for photosynthesis. Partitioning of absorbed light into different pathways in leaves of C_3 plants is shown in Figure 2-2. Under low PFD conditions, most of the absorbed light is

delivered to open PSII centers and utilized in the photosynthetic electron transport. The actual PSII efficiency operates close to its maximum.

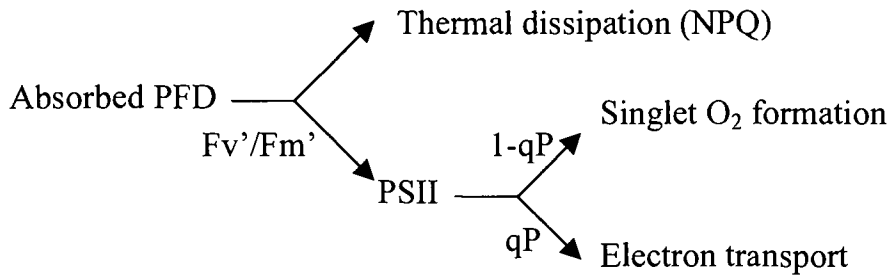

Figure 2-2. Light partitioning in leaves. Thermal dissipation of excitation energy is indicated by NPQ (non-photochemical quenching of chlorophyll fluorescence). Fv'/Fm' is the fraction of absorbed PFD that is delivered to open PSII centers. Of the PFD delivered to PSII, the proportion that is used in photosynthetic electron transport is qP (photochemical quenching coefficient); the remainder (1-qP) represents the fraction that potentially goes into singlet oxygen formation. The percentage of absorbed PFD used in electron transport is the actual PSII efficiency (Fv'/Fm')qP (Genty et al. 1989). The percentage of absorbed light potentially going into singlet oxygen formation is estimated as (Fv'/Fm')(1-qP) (Demmig-Adams et al. 1996).

Under high PFD conditions, rubisco operates at its full activity (von Caemmerer and Edmondson, 1986; Evans and Terashima, 1988; Kobza and Seemann, 1988; Sage et al., 1990, 1993). However, even for species with high photosynthetic capacity, such as cotton and sunflower, rubisco-supported CO_2 assimilation and photorespiration can use only 40-50% of the total absorbed PFD at midday on a clear day (Björkman and Schafer, 1989; Demmig-Adams et al., 1996). The actual PSII efficiency must be down-regulated under excess PFD to match photosynthetic electron transport set by rubisco activity.

There are two ways in which the actual PSII efficiency can be down-regulated (Figure 2-2). One is to decrease the efficiency with which excitation energy is delivered to open PSII reaction centers by thermal dissipation. The alternative is to close some of the PSII reaction centers. Closure of the PSII reaction centers results in formation of toxic activated oxygen species (Asada, 1996). In contrast, thermal dissipation can safely remove excess excitation energy before it reaches PSII reaction centers, thus protecting the photosynthetic apparatus from photo-oxidation (Demmig-Adams et al., 1996, 1997).

Thermal dissipation of excitation energy is activated in response to excessive absorbed PFD to down-regulate PSII efficiency and protect leaves from photo-oxidation (Demmig-Adams and Adams, 1992). This dissipation process has been measured by analyzing changes in chlorophyll fluorescence emission (Schreiber et al., 1994; Demmig-Adams et al., 1996). Activation of the thermal dissipation process leads to a decrease in fluorescence emission, known as non-photochemical quenching (NPQ). As excess PFD increases, thermal dissipation as indicated by NPQ increases, which reduces the efficiency with which excitation energy is delivered to PSII reaction centers (Adams and Demmig-Adams, 1992; Demmig-Adams and Adams, 1996; Demmig-Adams et al., 1996). For plants in natural habitats, thermal dissipation of excess excitation energy is sufficient to remove excess absorbed PFD (Demmig-Adams et al., 1995, 1996, 1997).

Thermal dissipation of excitation energy involves both a xanthophyll cycle and a transthylakoid pH difference (Demmig-Adams et al., 1995; Gilmore, 1997). The xanthophyll cycle consists of light-dependent conversion between three xanthophylls in a cyclic reaction (Demmig-Adams et al., 1997). Under high PFD conditions, diepoxide violaxanthin (V) is de-epoxidized via the monoepoxide antheraxanthin (A) to the

epoxide-free form zeaxanthin (Z). When PFD decreases, epoxidation of Z occurs in the reverse direction to form V via A. A close correlation has been found between non-photochemical energy dissipation and 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 de-epoxidase 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 et al., 1995; Gilmore 1997).

2.1.2.3 Regulation of starch synthesis by sucrose synthesis

Sucrose is synthesized from triose phosphates produced in the chloroplast. Triose phosphates are transported across the chloroplast envelope to the cytosol by a triose phosphate translocator (TPT) in counter exchange for inorganic phosphate (Pi) from the cytosol (Flügge and Heldt, 1991). The key regulatory steps in sucrose biosynthesis are the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate catalyzed by fructose 1,6-bisphosphatase (FBPase), and the formation of sucrose 6-phosphate from fructose 6-phosphate and UDP-glucose catalyzed by sucrose phosphate synthase (SPS; Daie, 1993; Huber et al., 1985). Both FBPase and SPS are allosteric enzymes. FBPase activity is inhibited by the signal metabolite fructose 2, 6-bisphosphate (F2,6P; Stitt, 1990). SPS activity is subject to allosteric regulation by metabolites and post-translational modification via reversible protein phosphorylation (Huber and Huber, 1992, 1996). SPS is activated by glucose 6-phosphate and is inhibited by Pi (Doehlert and Huber, 1983). Sucrose synthesis is coordinated with photosynthetic CO_2 fixation by a feedforward

mechanism. When the triose phosphate concentration rises above a threshold level (below which sucrose synthesis is inhibited to maintain a minimum triose phosphate concentration for the Calvin cycle turnover) as photosynthesis increases, cytosolic FBPase activity is stimulated by a decrease in the concentration of F2,6P (Stitt, 1990), and SPS is activated by dephosphorylation of the SPS protein (Huber and Huber, 1992), resulting in increased substrate affinity. Because both activated FBPase and SPS have sigmoidal substrate kinetics (Stitt and Heldt, 1985; Siegl and Stitt, 1990), a small increase in the triose phosphate concentration strongly activates sucrose synthesis without requiring large changes in the steady state metabolite concentration. This minimizes the risk that phosphorylated intermediates accumulate to the point where photosynthesis becomes Pi-limited (Woodrow and Berry, 1988; Stitt, 1996).

In leaves, starch is synthesized in the chloroplast during the day. The key enzyme in starch synthesis is ADP-glucose pyrophosphorylase (AGPase), whose activity is allosterically regulated by the PGA/Pi ratio (Stitt, 1991; Preiss and Sivak, 1996). When sucrose synthesis decreases, phosphorylated intermediates accumulate and the Pi concentration in the cytosol falls. This results in a decrease in the Pi concentration and an increase in the PGA concentration in the chloroplast. The activity of AGPase is up-regulated by decreased Pi and increased PGA concentrations (Stitt, 1991; Preiss and Sivak, 1996). Starch in source leaves plays a transitory storage role in supplying carbon for sinks at night. Starch can be degraded either hydrolytically to glucose and exported to the cytosol via a hexose tranporter, or phosphorylytically to glucose 1-phosphate, followed by glycolysis to triose phosphate and PGA, then transport out by TPT (Ziegler and Beck, 1989; Stitt, 1990; Schleucher et al., 1998).

The reciprocal regulation of starch synthesis by sucrose synthesis has been studied extensively by using transgenic plants with altered capacity for sucrose synthesis. Biochemical and physiological analyses of transgenic plants with decreased expression of TPT (Riesmeier et al., 1993; Heineke et al., 1994; Häusler et al., 1998), FBPase (Zrenner et al., 1996) and SPS (Krause, 1994) have revealed that, 1) sucrose synthesis is inhibited, resulting in elevated PGA/Pi ratio, and up-regulated starch synthesis; 2) amylolytic degradation of starch and subsequent export of glucose via the hexose transporter are accelerated to compensate for the decrease in TPT activity (Heineke et al., 1994; Häusler et al., 1998); and 3) the compensating change in starch synthesis is so effective that photosynthesis remains unchanged under normal conditions at ambient CO_2. Studies of transgenic plants with 3- to 6-fold increase in SPS activity in potato (Galtier et al., 1993, 1995) and *Arabidopsis* (Signora et al., 1998) show that sucrose synthesis is increased, and starch synthesis is down-regulated. A strong positive correlation exists between the sucrose to starch ratio and SPS activity in their leaves. SPS, therefore, plays a pivotal role in carbon partitioning between sucrose and starch. Photosynthesis at ambient CO_2 conditions is not significantly increased by elevated SPS activity.

2.1.2.4 Feedback inhibition by end-product synthesis

When triose phosphate utilization in end-product synthesis can not keep pace with triose phosphate production, such as in high CO_2, low O_2 conditions, or shortly after a transition to low temperatures, a feedback mechanism is triggered to down-regulate light energy input and rubisco activity (Sharkey, 1990). Inorganic phosphate (Pi) plays a central role in triggering this feedback sequence. When feedback-limited photosynthesis occurs, phosphate-containing molecules accumulate at the expense of Pi, resulting in low

Pi in the stroma (Sharkey and Vanderveer, 1989). The reduced concentration of Pi limits ATP formation, causing decarbamylation of rubisco through rubisco activase (Portis, 1990) and reduction in photosynthetic electron transport by increasing NPQ (Sharkey et al., 1988). Feedback limited photosynthesis shows loss of sensitivity to O_2 (Sharkey et al., 1986; Sharkey, 1990).

This biochemical mechanism has been confirmed by studying mutants of *Flaveria linearis* with reduced cytosolic FBPase activity (Sharkey et al., 1988b; Micallef and Sharkey, 1996) and transgenic plants with altered capacity for sucrose synthesis (Micallef et al., 1995). The *Flaveria linearis* mutant has similar photosynthesis as controls at ambient CO_2 and O_2, but carbon partitioning is altered in favor of starch synthesis at the expense of sucrose (Sharkey et al., 1992). Photosynthesis shows loss of O_2 sensitivity (Sharkey et al., 1988, 1992), which is diagnostic of feedback limitation (Sharkey, 1990). Transgenic plants with decreased capacity for sucrose synthesis generally have lower CO_2-saturated photosynthesis (Riesmeier et al., 1993; Häusler et al., 1998). Those with increased capacity for sucrose synthesis have higher CO_2-saturated photosynthesis (Galtier et al., 1993, 1995; Micaleff et al., 1995; Signora et al., 1998), which indicates that the overall utilization of triose phosphate in end-product synthesis approaches its maximum at high CO_2. When grown under elevated CO_2 conditions, transgenic plants with increased capacity for sucrose synthesis have less down-regulation of photosynthesis compared with control plants (Micallef et al., 1995; Signora et al., 1998).

2.1.3 Mechanistic models

Mechanistic models based on the biochemical regulation of photosynthesis have been developed to, 1) analyze and interpret rate limitations of gas exchange behavior of

intact leaves in biochemical terms, and 2) predict CO_2 assimilation in response to a changing environment.

The widely accepted model of C_3 photosynthesis was first proposed by Farquhar and his colleagues (Farquhar et al., 1980; Farquhar and von Caemmerer, 1982), and later modified by Sharkey (1985) and Sage (1990). In this model, CO_2 assimilation is limited by one of three general processes: 1) the enzymatic capacity of rubisco; 2) the capacity of electron transport to supply NADPH and ATP for RuBP regeneration; and 3) the capacity for triose phosphate utilization in starch and sucrose synthesis to regenerate Pi for photophosphorylation. When one process becomes limiting, the non-limiting processes are regulated to match the limiting process. When rubisco limits photosynthesis under saturating light and low CO_2 partial pressure, electron transport and triose phosphate use are down-regulated so that the rate of RuBP regeneration matches the rate of RuBP consumption by rubisco. Both CO_2 assimilation and electron transport have strong responses to changes in CO_2 under rubisco-limited photosynthesis. At subsaturating light, electron transport limits photosynthesis and both rubisco activity and triose phosphate use are adjusted downward. CO_2 assimilation responds strongly to light under electron transport-limited photosynthesis. At high light and elevated CO_2 partial pressure, when starch and sucrose syntheses limit photosynthesis, both rubisco activity and electron transport are down-regulated to balance the limitation of triose phosphate utilization. CO_2 assimilation responds strongly to temperature, as does sucrose synthesis.

This model assumes that rubisco is fully activated at ambient (350 ppm) to low CO_2 under saturating light conditions (Farquhar et al., 1980). The initial slope of the response of CO_2 assimilation (A) to intercellular CO_2 concentration (Ci) is predicted to

be linearly correlated with the total rubisco activity under light saturating conditions (Farquhar et al., 1980). Measurements of rubisco activity and gas exchange in response to light and CO_2 have generally confirmed the assumption and predictions of the model (von Caemmerer and Farquhar, 1981; von Caemmerer and Edmondson, 1986; Evans and Terashima, 1988; Sage et al., 1988, 1990, 1993; Hudson et al., 1992; von Caemmerer et al., 1994). Any curvilinear relationships found between total rubisco activity and the initial slope of the A/Ci curves have been attributed to the existence of a CO_2 transfer resistance between the intercellular air space to the carboxylation site within the chloroplast (Evans, 1983, 1989; Evans and Seemann, 1984; Evans and Terashima, 1988; von Caemmerer and Evans, 1991; Makino et al., 1994a). The only apparent deviation from the model is that when Ci drops below 100 ppm, rubisco deactivates instead of being fully activated as assumed in the model. This may be because carbamylation of rubisco by rubisco activase is limited by low availability of CO_2 (Portis, 1990; Sage et al., 1990).

Flux control analysis of photosynthesis using transgenic tobacco plants with decreased expression of rubisco also generally supports the model (Stitt, 1996). When transgenic tobacco plants are grown under high PFD and high temperature conditions, flux control coefficient of rubisco for photosynthesis is 0.8 to 0.9 (Krapp et al., 1994). This indicates that photosynthesis is predominantly limited by rubisco, although the flux control coefficient is still <1. When the plants are grown under a low PFD (350 μmol m^{-2} s^{-1}), photosynthesis does not respond to a decrease in rubisco until the rubisco content is reduced to 60% of the control (Quick et al., 1991), because electron transport is limiting under low PFD.

Because photosynthesis exhibits characteristic responses to CO_2 and O_2, gas exchange behavior can be analyzed in biochemical terms (von Caemmerer and Farquhar, 1981; Sharkey, 1985; Sage, 1994). Based on the C_3 photosynthesis model described above, an A/Ci curve constructed under saturating light can be divided into three regions: the linear region at low Ci, the curvilinear region at intermediate Ci, and the plateau at high Ci (Sharkey, 1985; Sage, 1990, 1994). In the linear region, CO_2 assimilation is mainly limited by rubisco activity. Both CO_2 assimilation and electron transport increase with increasing Ci. In the curvilinear region, photosynthesis is mainly limited by electron transport. CO_2 assimilation increases with increasing Ci because photorespiration is decreased, but electron transport remains constant with increasing Ci. In the plateau region, photosynthesis is mainly limited by triose phosphate utilization. Here, CO_2 assimilation remains the same with increasing Ci, but both electron transport and rubisco activation state decrease.

Analysis of A/Ci curves based on the C_3 photosynthesis model has been used extensively as a tool to analyze the limitations of photosynthesis in response to developmental constrains and environmental factors (see Sage, 1994, for an example). The model has also been used to simulate photosynthesis in response to CO_2, leaf nitrogen and light exposure within the canopy (Harley et al., 1986, 1992; Chen et al., 1993). Developing the model and testing its predictions of the model have greatly increased our understanding of C_3 photosynthesis.

2.2 Photosynthesis in relation to leaf nitrogen

2.2.1 Leaf N and proteins

2.2.1.1 Composition of leaf N

Nitrogen in leaves is either inorganic or organic. Nitrate is the main form of inorganic N in leaves. In most cases, nitrate comprises only a small proportion of total N. Under an excess nitrate supply, some herbaceous plants can accumulate up to 15-40% of the total leaf N as nitrate (Maynard et al., 1976; Millard, 1988). For woody plants, e.g. poplar and apple, nitrate-N accounts for only a very small proportion (<2-3%) of total N in leaves, even under an excess nitrate supply (Bañados, 1992; Lee and Titus, 1992).

Organic nitrogen accounts for the majority of total leaf N, and includes proteins, amino acids and amides, nucleic acids, and other nitrogenous compounds. Typically, 70-80% of the organic N in a leaf is in proteins, 10% in nucleic acids, 5-10% in chlorophyll and lipoproteins, and the remainder in free amino acids (Chapin and Kedrowski, 1983).

All of the photochemical and biochemical processes of photosynthesis require nitrogenous compounds, especially proteins. Approximately 75-80% of the leaf N is associated with chloroplasts in C_3 plants (Morita and Kono, 1975; Morita, 1980; Makino and Osmond, 1991). Photosynthesis depends on the activity of many different proteins. The amount, activities, and coordination of these proteins determine the photosynthetic capacity of a leaf.

Based on their relationship with photosynthesis, leaf proteins are first categorized into two classes: thylakoid proteins and soluble proteins, which correspond to the light and dark reactions of photosynthesis, respectively (Evans, 1989, 1996). Thylakoid proteins include pigment-protein complexes involved in light harvesting, and other proteins that participate in electron transport and photophosphorylation. Soluble proteins that function in photosynthesis include primarily rubisco and other enzymes in the photosynthetic carbon reduction and oxidation cycles as well as those involved in

processes such as membrane transport, carbohydrate metabolism, nitrate reduction, and protein and nucleic acid synthesis.

2.2.1.2 Experimental alteration of leaf N

A range of leaf N content is required for studying its relationship with photosynthesis. The following approaches have been used individually or in combination to alter leaf N content: 1) controlling N supply during leaf development; 2) using natural variation in the leaf senescence process; or 3) exposing leaves to different levels of PFD or using natural light exposure in tree canopies.

Manipulating the N supply during leaf development under well-exposed conditions is the most widely used experimental approach. Causal relationships between leaf N and photosynthesis can be inferred from this type of manipulation. However, when the N supply is reduced, the initial impact is on plant growth, which results in a marked reduction in total leaf area whereas the leaf N content of newly expanded leaves remains above a certain minimum. This is frequently observed in nutrient studies (Evans, 1996). Altering the N supply under well-exposed conditions does not change leaf anatomy (Longstreth and Nobel, 1980).

The natural variation in N content for leaves of different ages has been used to relate photosynthesis to leaf N content, especially for wild-grown plants in an ecological context (Field and Mooney, 1986). N mobilization and export occurs during the leaf senescence process, so that leaf N content decreases with increasing leaf age. This provides a natural source of variation in leaf N. However, leaf senescence is a complex process and other aspects related to photosynthesis (e.g., chloroplast integrity) may covary with leaf N (Thomas and Stoddart, 1980).

Leaf N content decreases with decreasing light exposure during leaf development (Evans, 1996). This is because light influences leaf anatomy. Leaves developed under shaded conditions have less dry mass per unit area and are thinner than leaves exposed to full sunlight. This is true both for leaves treated with different light exposures and for leaves within a tree canopy under natural light exposure. The variation caused by light exposure has been used to study the relationship between leaf N and photosynthesis for both herbaceous plants and woody perennials (DeJong and Doyle, 1985; DeJong et al., 1989; Evans, 1996).

The relationship between leaf N and photosynthesis obtained involving different light exposure is very useful for understanding photosynthetic acclimation to light via leaf N partitioning, and N distribution within the entire tree canopy with respect to light exposure (DeJong and Doyle, 1985; Evans, 1996). However, this relationship should be used cautiously for predicting the distribution of leaf photosynthetic capacity in the canopy. This is because there is a difference in partitioning of leaf N into different groups of leaf proteins caused by light exposure and N supply, and this difference influences the relationship between leaf N and photosynthesis (DeJong et al., 1989; Evans, 1989).

2.2.2 The relationship between leaf N and photosynthesis

Because the large number of proteins involved in photosynthesis account for the majority of leaf N, close relationships have been found between leaf N and photosynthesis (Field and Mooney, 1986; Evans, 1989; Sinclair and Horie, 1989). Field and Mooney (1986) found a single linear relationship between leaf N and light-saturated CO_2 assimilation expressed on a leaf dry weight basis for a wide range of C_3 species.

They argued that this represents a fundamental relationship independent of species and environment. However, closer examination of the relationship between leaf N and photosynthesis reveals that when there is a sufficiently wide range of leaf N, a curvilinear relationship is found. The linear response at low leaf N levels is followed by a curvilinear response at higher leaf N (Evans, 1983, 1989; DeJong and Doyle, 1985; Sinclair and Horie, 1989). In addition, the response functions are markedly different among species in terms of N use efficiency (Evans, 1989; Sinclair and Horie, 1989).

2.2.2.1 Photosynthetic N use efficiency

Photosynthetic N use efficiency (PNUE) has been measured as the rate of CO_2 assimilation expressed on a leaf N basis (A/N), or the slope of the response of CO_2 assimilation to leaf N (dA/dN) (Sage and Pearcy, 1987). All the factors that influence both CO_2 assimilation capacity and N content and its partitioning within the leaf will affect PNUE.

Species differ markedly in their PNUE. At the same leaf N content, C_4 plants have higher photosynthetic capacity and PNUE than do C_3 plants (Brown, 1978; Sage and Pearcy, 1987; Sinclair and Horie, 1989). This is because the C_4 concentrating mechanism leads to CO_2 saturation of rubisco. In addition, high CO_2 partial pressure in the bundle sheath cells nearly eliminates rubisco oxygenase activity in C_4 plants. In contrast, substantial rubisco oxygenase activity reduces the efficiency of rubisco for CO_2 assimilation in C_3 plants. Moreover, rubisco specific activities may be significantly higher for C_4 as compared with C_3 plants (Seemann et al., 1984).

Among the C_3 plants, rapidly growing annuals generally have the highest CO_2 assimilation rate and the greatest response of CO_2 assimilation to leaf N. Deciduous trees

and shrubs have an intermediate rate and response, while the evergreen trees have the lowest rate and response (Field and Mooney, 1986; Wullschleger, 1993). This difference may have several causes. First, the fraction of leaf N not involved in photosynthesis may vary between species. Although direct proof of this is very limited, the intercept in the relationship between leaf N and photosynthesis represents this pool (Field and Mooney, 1986), and varies among species (Field and Mooney, 1986; Evans, 1989; Sinclair and Horie, 1989). Second, the partitioning of leaf N between thylakoid and soluble proteins differs among species. This is indicated by the ratio of thylakoid to soluble proteins and the ratio of whole chain electron transport rate to leaf N at full sunlight (Evans, 1996). Shade plants typically have lower rates of CO_2 assimilation and N use efficiency (Seemann et al., 1987). Third, specific rubisco activity varies among species (Seemann and Berry, 1982; Evans and Seemann, 1984; Makino et al., 1988). The difference in specific rubisco activity in C_3 dicots has been correlated with PNUE (Seemann and Berry, 1982). Finally, CO_2 transfer resistance from the intercellular airspace to the carboxylation site in the chloroplast differs among species (von Caemmerer and Evans, 1991; Epron et al., 1995; Syvertsen et al., 1995). CO_2 transfer resistance generally increases in order from annual herbaceous plants to deciduous tree species to evergreen trees. Lower concentrations of CO_2 at the carboxylation site caused by larger CO_2 transfer resistance may limit CO_2 assimilation capacity and reduce the overall N use efficiency of the leaf (Evans, 1996).

Even for species with similar CO_2 assimilation capacity and PNUE, the partitioning of leaf N between rubisco and thylakoid proteins, and their specific rubisco activities may still be different. Specific rubisco activity in rice leaves is lower than in

wheat leaves (Makino et al., 1988). Interestingly, a larger proportion of the leaf N is partitioned to rubisco in rice than in wheat, and rice also has higher stomatal conductance (Makino et al., 1988, 1992). As a result, rice and wheat leaves have similar rates of photosynthesis at a given leaf N content (Makino et al., 1988).

For a given species, the way leaf N is manipulated affects both CO_2 assimilation capacity for a given leaf N and the slope of the response of CO_2 assimilation to leaf N. When light exposure is the source of variation for leaf N, more N is invested into pigment-protein complexes as light levels during growth decrease; less N is partitioned into the electron transport chain, coupling factors and rubisco (Evans, 1989, 1996). Thus, for a given leaf N, leaves acclimated to low light exposure have lower CO_2 assimilation capacity compared with leaves developed under full sunlight. This may explain why the relationship between leaf N and photosynthesis in peach tree canopies supplied with high N has a steeper slope than that obtained on those supplied with low N (DeJong et al., 1989).

2.2.2.2 The curvature in the relationship

The curvilinear response of CO_2 assimilation to leaf N indicates that CO_2 assimilation is less limited by N at high leaf N levels. The physiological mechanisms whereby this curvilinear response occurs, however, are not completely clear and may differ between species. Several possibilities exist:

(1) The partitioning of leaf N into the fraction that is not involved in photosynthesis may increase with increasing leaf N. Herbaceous plants are able to store N as nitrate in their vacuoles under an excess nitrate supply (Maynard et al., 1976; Millard, 1988). As the nitrate supply increases, a larger proportion of total N is

accumulated as nitrate in leaves (Zhen and Leigh, 1990). Failing to account for nitrate content may result in a curvilinear relationship between total leaf N and CO_2 assimilation (Sage and Pearcy, 1987).

(2) CO_2 transfer resistance between intercellular air spaces to carboxylation sites in chloroplasts may cause a curvilinear relationship between total rubisco activity and the initial slope of the A/Ci curve (Evans, 1983, 1989; Evans and Seemann, 1984; Evans and Terashima, 1988; Makino et al., 1994a). However, whether this curvilinear relationship translates into the curvature in the relationship between leaf N and the initial slope of the A/Ci curves depends on the partitioning of leaf N into rubisco in response to leaf N.

(3) Increased stomatal limitation with increasing leaf N may cause relatively less CO_2 to be available for carboxylation of rubisco in high N leaves. Stomatal conductance typically increases with increasing leaf N, and is well correlated with the rate of CO_2 assimilation. The intercellular CO_2 concentration, however, tends to decrease with increasing leaf N (DeJong, 1982; Makino et al., 1988). Lower Ci in combination with CO_2 transfer resistance may restrict CO_2 supply to the carboxylation sites of rubisco, reducing its efficiency. DeJong and Doyle (1985) observed a more pronounced curvature in the relationship between leaf N and CO_2 assimilation than that between leaf N and the initial slope of A/Ci curve in peach leaves. They suggest that stomatal limitation contributes to the curvilinear relationship between leaf N and CO_2 assimilation.

(4) Measurement under subsaturating light will cause CO_2 assimilation to be underestimated in high N leaves. Those leaves have more rubisco and higher electron transport capacity than low N leaves. To reach light saturation, a progressively higher PFD is required with increasing leaf N (Evans, 1989, 1996). If measurements are done at

a PFD that is above the saturation point for low N leaves, but below light saturation for high N leaves, photosynthetic capacity of high N leaves will be underestimated (Evans, 1989).

2.2.3 Rubisco

Rubisco catalyzes the first key reaction in CO_2 assimilation in C_3 plants, but is not very efficient. Rubisco has a low rate of catalysis, and the rate of carboxylation is further reduced by its poor affinity to CO_2 and the competing oxygenation reaction (Andrews and Lorimer, 1987). In C_3 leaves, rubisco operates at only about 25% of its maximum capacity (von Caemmerer and Farquhar, 1981). This is compensated for by the large amount of rubisco protein, typically about 20 to 30% of the total N in leaves of C_3 plants (Evans, 1989). Because the N cost for rubisco is very high, N availability affects its amount, activities, and its relationship with photosynthesis.

2.2.3.1 Rubisco content and total activity

Rubisco content and total activity increase linearly with increasing leaf N on all species that have been examined (Evans, 1983, 1986, 1989; Evans and Seemann, 1984; Sage et al., 1987; Makino, 1992, 1994a, b, 1997; Nakano et al., 1997). However, partitioning of leaf N into rubisco with increasing leaf N varies among species. The proportion of leaf N partitioned to rubisco is constant in wheat regardless of leaf N content (Evans, 1983; Makino et al., 1992). In contrast, the ratio of rubisco to total leaf N increases with increasing leaf N content in rice (Makino et al., 1992, 1994a, 1997), spinach (Makino et al., 1992), pea (Makino and Osmond, 1991; Makino et al., 1992) and other species (Evans, 1989; Makino et al., 1992). For example, the proportion of leaf N

partitioned to rubisco increased from 28% to 37% in rice as leaf N increased approximately from 50 to 125 mmol m^{-2} (Makino et al., 1992).

2.2.3.2 Total rubisco activity and CO_2 assimilation

Although other proteins involved in photosynthesis inevitably depend on leaf N, total rubisco activity (or content) is closely related to the initial slope of the A/Ci curve under saturating light conditions (von Caemmerer and Farquhar, 1981; Evans, 1983, 1986, 1989; Evans and Seemann, 1984; Sage et al., 1987; Makino et al., 1988, 1994a, b, 1997). The shape of this relationship depends on CO_2 transfer conductance from the intercellular air space to the carboxylation site in the chloroplasts (von Caemmerer and Evans, 1991). If CO_2 transfer conductance increases to the same degree as total rubisco activity with increasing leaf N, rubisco will operate at the same CO_2 partial pressure at the carboxylation site. This results in a linear relationship between rubisco and the initial slope of the A/Ci curve. If CO_2 transfer conductance increases to a lesser degree than total rubisco activity with increasing leaf N, rubisco will operate at a lower efficiency *in vivo* because of the lower CO_2 partial pressure at the carboxylation site. This will lead to a curvilinear relationship between total rubisco activity and the initial slope of the A/Ci curve (von Caemmerer and Evans, 1991).

Using concurrent gas exchange and carbon discrimination measurements, von Caemmerer and Evans (1991) found that CO_2 transfer conductance increased with increasing N content in wheat leaves. The species difference in CO_2 transfer resistance between *Triticum aestivum* and *T. monococcum* explains why the former has 30% greater specific rubisco activity than the latter *in vitro*, but the same initial slope of the A/Ci

curve for a given leaf N (Evans and Seemann, 1984). As leaf N increases, the ratio of CO_2 transfer conductance to rubisco activity may, or may not, remain constant.

For species such as wheat, rubisco accounts for a constant proportion of leaf N with increasing leaf N (Evans, 1983; Makino et al., 1992). The curvature in the relationship between total rubisco activity and the initial slope of the A/Ci curve caused by CO_2 transfer resistance leads to the curvature in the relationship between leaf N and the initial slope of the A/Ci curve (Evans, 1983; Evans and Seemann, 1984). Even in this case, however, the curvature in the latter relationship should be less pronounced than that between total rubisco activity and the initial slope of the A/Ci curve, if CO_2 transfer resistance is the only cause. This is because the linear relationship between leaf N and total rubisco activity has a slope >1 (μmol CO_2 $mmol^{-1}$ N s^{-1}) for wheat (Evans, 1983).

For species such as rice, partitioning of leaf N into rubisco increases with increasing leaf N (Makino et al., 1992, 1994a, 1997). The curvature in the relationship of the initial slope of the A/Ci curve with total rubisco activity caused by CO_2 transfer resistance does not necessarily translate into the curvature in its relationship with leaf N. CO_2-limited photosynthesis (at Ci=20 Pa) is curvilinearly related to total rubisco content, but linearly related to leaf N in rice leaves (Makino et al., 1994a). This is because the increased ratio of rubisco to leaf N compensates for the curvature in the relationship between rubisco activity and CO_2-limited photosynthesis.

2.2.3.3 Rubisco activation state

Surprisingly, there are few reports on the rubisco activation state in response to leaf N. Evans and Terashima (1988) showed that there was no apparent deactivation of rubisco in spinach leaves under a high N supply. In contrast, Mächler et al. (1988) found

that rubisco activation state in wheat leaves decreased as the N supply increased. These plants, however, were grown at a relatively low PFD level (540 μmol m^{-2} s^{-1}), and leaf N and CO_2 assimilation were not measured. Lawlor et al. (1987) showed that CO_2 assimilation of wheat leaves did not respond to any increase in rubisco in excess of 3.0 g m^{-2}. They calculated that about 50% of the rubisco was not activated in high N leaves but, again, plants were grown at a relatively low PFD (around 550 μmol m^{-2} s^{-1}). The relationship between leaf N and rubisco activation state under high PFD is unclear. In all other studies of the relationship between rubisco activity and CO_2 assimilation, only total rubisco activity was measured and rubisco was assumed to be fully active under high PFD conditions.

This assumption that rubisco is fully active under high light is generally supported by experimental data under normal conditions (medium N supply). Considering that rubisco is so expensive in terms of N investment, it would arguably represent a waste of resources if rubisco were present in great excess. Indeed, based on the rubisco kinetics, many experiments have shown that CO_2 assimilation can be explained only when it is assumed that rubisco is fully active (von Caemmerer and Farquhar, 1981; von Caemmerer and Edmondson, 1986; Makino et al., 1988). When curvilinear relationships are found between total rubisco activity and the initial slope of the A/Ci curve, CO_2 transfer resistance is factored in to explain the apparent deviation from rubisco's expected behavior (Evans, 1983; Evans and Seemann, 1984; Evans and Terashima, 1988; von Caemmerer and Evans, 1991, Makino et al., 1994a).

Under high N supply conditions, however, accumulating an excessive amount of rubisco may be beneficial for plant resource acquisition and reutilization. Nitrogen is a

very important resource for plant growth and its availability in the soil is often limited under natural conditions. Rubisco breaks down during leaf senescence, then N is mobilized from leaves to reproductive organs in herbaceous plants (Millard, 1988), or to bark and root tissues in woody perennials (Titus and Kang, 1982). Because the proportion of nitrate-N is minimal in leaves, especially in woody plants (Bañados, 1992; Lee and Titus, 1992), rubisco may serve as a storage protein in leaves under excessive N supply conditions. Compared with a strictly storage protein, an excessive amount of rubisco in leaves may also enable plants to best utilize high PFD, and marginally improve water use efficiency (Quick et al., 1991). If so, rubisco activation state should decrease at high leaf N levels. This could also explain the curvature of the relationship between leaf N and CO_2 assimilation.

2.2.4 Light absorption, partitioning and electron transport

2.2.4.1 Thylakoid proteins

When leaf N content of spinach (Evans and Terashima, 1987; Terashima and Evans, 1988) and pea (Makino and Osmond, 1991) is altered by N supply under high PFD conditions, the amount of thylakoid proteins increases with increasing leaf N. However, the proportion of leaf N partitioned into thylakoid proteins remains constant and the photosynthetic property of the thylakoid membranes is not dependent on leaf N content. As thylakoid N is proportional to chlorophyll content (50 mol thylakoid N mol^{-1} chl), comparison of published results on the ratio of chlorophyll to total N indicates that the proportion of leaf N associated with thylakoids remains constant with increasing leaf N (Terashima and Evans, 1988; Evans, 1989).

When light exposure is the source of variation in leaf N, the response of partitioning of leaf N into thylakoid proteins varies among species. For most species, the proportion of N partitioned into thylakoid proteins typically increases with a corresponding decrease in soluble proteins as growth PFD decreases (For a review see Evans, 1996). For pea (Evans, 1987b) and spinach (Terashima and Evans, 1988), with decreasing PFD during growth, however, partitioning of N between thylakoid proteins and soluble proteins remains constant. More N is invested in pigment-protein complexes at the expense of proteins in the electron transport chain and the coupling factor. The net result from either response is that more N is partitioned to pigment-protein complexes, and less N is partitioned to components of electron transport with decreasing growth PFD (Evans, 1989, 1996).

2.2.4.2 Light absorption and partitioning

Because chlorophyll content, electron transport components and rubisco activity are closely related to leaf N, light absorption and partitioning between thermal dissipation and electron transport are consequently altered by leaf N.

When leaf N is decreased by N supply, chlorophyll content decreases proportionally (Evans, 1989). Light absorption then decreases, but the relationship between leaf chlorophyll and leaf absorptance is not linear (Björkman and Demmig, 1987; Evans, 1996). When leaf chlorophyll content changed during the various developmental stages in *Hedera canariensis*, a 59% decrease in chlorophyll content resulted in only a 10 to 13% reduction in leaf absorptance (Björkman and Demmig, 1987). This is because decreasing chlorophyll content has a relatively small effect on light absorption at the absorption peaks of chlorophyll, but has a large effect in the green

and far-red region where the chlorophyll absorption coefficient is low. Chlorophyll content is always decreased by N deficiency, but the reduction in light absorption is not proportional to the decrease in chlorophyll content.

Electron transport capacity is closely related to leaf N content calculated from gas exchange measurements *in vivo* (Harley et al., 1992) or assayed using thylakoid preparations (Evans and Terashima, 1987). Low electron transport capacity of low N leaves may result in more excessive excitation energy under high PFD conditions if the decrease in antenna pigments is not enough to reduce light absorption. When maize (Khamis et al., 1990) and spinach (Verhoeven et al., 1997) plants were subjected to N deficiency, NPQ increased, with a corresponding increase in the proportion of zeaxanthin in the xanthophyll pool under high PFD. This indicates there is more excessive excitation energy in low N leaves than in high N leaves at high PFD, and xanthophyll cycle dependent thermal dissipation of excitation energy is up-regulated to remove more excessive absorbed light in low N leaves. In contrast, Bungard et al. (1997) did not observe any changes in xanthophyll cycle components in response to N deficiency in *Clematis vitalba*. They argued that reduced chlorophyll content and the concomitant reduction in light absorption were sufficient to prevent excessive excitation energy and protect low N leaves from light damage. Both decreased light absorption and increased thermal dissipation, therefore, play roles in coping with high light in response to N limitation, but their relative importance is still unclear.

2.2.4.3 Balance between thylakoid proteins and rubisco

For most species, when leaf N is increased either by N supply or light exposure, the ratio of rubisco to thylakoid N increases. When the N supply is the source of

variation in leaf N, the proportion of leaf N partitioned to rubisco increases with increasing leaf N (except in wheat); the proportion of thylakoid N remains unchanged (Section 2.2.3.1 and 2.2.4.1). When light exposure is the source of variation, the proportion of leaf N partitioned into thylakoid proteins (except in pea and spinach) increases with decreasing growth PFD while total leaf N decreases with decreasing growth PFD (Section 2.4.1). In other words, the ratio of soluble protein N to thylakoid N increases with increasing growth PFD. This increased partitioning of leaf N to rubisco relative to thylakoid proteins with increasing leaf N prompts questions as to the cause and effect of N partitioning changes on the *in vivo* balance between electron transport and rubisco activity.

The *in vivo* balance between rubisco activity and electron transport has been assessed by analyzing the A/Ci curves of leaves with different N contents. Electron transport determines the rate of CO_2 assimilation at the curvilinear region of the A/Ci curve, while rubisco activity determines the initial slope of the A/Ci curve. Therefore, the ratio of CO_2 assimilation at the curvilinear region (60 Pa) to that at the linear region (20 Pa) reflects the balance between electron transport and rubisco activity *in vivo*. This ratio, or the relationship between the initial slope of the A/Ci curve and the rate of whole chain electron transport, is constant when leaf N is altered by N supply (Evans, 1983; Evans and Terashima, 1987, 1988; Sage et al., 1990; Makino et al., 1992, 1994a). Similarly, the ratio is not altered by light exposure (Sims and Pearcy, 1989; Thompson et al., 1992). The *in vivo* balance between rubisco activity and electron transport, therefore, is maintained when leaf N is altered by N supply or light exposure.

One of the main reasons that the ratio of rubisco to thylakoid N increases is because high N leaves have relatively large CO_2 transfer resistance between the intercellular air space and the carboxylation site in the chloroplasts (Evans and Terashima, 1987, 1988). Chloroplasts in high N leaves are larger than in low N leaves (Terashima and Evans, 1988). A larger chloroplast area means a longer path for CO_2 transfer in the liquid phase, and potentially lower CO_2 partial pressure at the carboxylation site. Evans (1986) found that doubling rubisco content from 3 to 6 μmol m^{-2} in wheat increased *in vivo* activity by only 64% for a given Ci. Therefore, if the balance between electron transport and rubisco activity were to be maintained *in vivo*, rubisco would have to increase to a greater extent with increasing leaf N. In wheat, however, this explanation is hard to reconcile with the following facts. 1) The partitioning of leaf N between rubisco and thylakoid proteins remains constant, yet the *in vivo* balance between rubisco and electron transport activities is maintained with increasing leaf N (Makino et al., 1992). 2) CO_2 transfer resistance is relatively large enough at high N levels to cause the curvilinear relationship between total rubisco activity and the initial slope of the A/Ci curve (Evans, 1983, 1989; Evans and Seemann, 1984).

2.2.4.4 The relationship between quantum yield for CO_2 assimilation and actual PSII efficiency

Genty et al. (1989) found a linear relationship between quantum yield for CO_2 assimilation and the product of photochemical quenching (qP) and the efficiency of excitation capture by open PSII centers (Fv'/Fm') under non-photorespiratory conditions. As a result, qP×Fv'/Fm' (now referred to as actual PSII efficiency) is used to monitor

electron transport *in vivo*. This relationship has been studied in many species, and the quantitative relationship has been used as a calibration curve to estimate the rate of electron flow associated with rubisco and partitioning of electron flow between CO_2 assimilation and photorespiration under photorespiratory conditions (Cornic and Briantais, 1991; Cornic and Ghashghaie, 1991; Ghashghaie and Cornic, 1994; Habash et al., 1995).

The relationship between quantum yield for CO_2 assimilation and actual PSII efficiency depends on the partitioning of electron flow to rubisco-associated CO_2 assimilation relative to other processes, such as nitrate reduction and the Melher reaction. For any given absorbed PFD, if other electron sinks consume more reducing equivalents, the quantum yield for CO_2 assimilation would be reduced. Apparently, the relationship between quantum yield for CO_2 assimilation and actual PSII efficiency is very conserved. Seaton and Walker (1990) found a single curvilinear relationship between the apparent quantum yield for O_2 evolution and the actual PSII efficiency at saturated CO_2 for 16 species including C_3, C_4 and CAM plants. Water stress or elevated CO_2 treatment did not change the relationship between quantum yield for CO_2 assimilation and actual PSII efficiency (Cornic and Ghashghaie, 1991; Habash et al., 1995). The effect of leaf N on this relationship has not been closely examined. The only report is by Khamis et al. (1990) on maize. They found that N deficiency caused a small decrease in quantum yield for CO_2 assimilation at any given PSII efficiency. However, the decrease in light absorption caused by N limitation may not have been considered.

2.2.5 End-product synthesis

Because CO_2 assimilation increases with increasing leaf N, a corresponding increase is required in the capacity for end-product synthesis. The activities of cytosolic FBPase and SPS, the two key enzymes in sucrose synthesis, increased with increasing leaf N, and correlated with CO_2-saturated photosynthesis in rice (Makino et al.,1994a, b; Nakano et al., 1997). However, the activity of both FBPase and SPS expressed on a leaf N basis decreased with increasing leaf N, indicating that a lower proportion of leaf N was partitioned into FBPase and SPS with increasing leaf N (Makino et al., 1994a).

The relationship between leaf N and the capacity for end-product synthesis has been studied by analyzing the A/Ci curves of leaves with different N contents. The CO_2 saturation point for CO_2 assimilation decreased with increasing leaf N content in leaves of *Chenopodium album* (Sage et al. 1990). Makino et al. (1994a) reported that CO_2-limited photosynthesis (at Ci of 20 Pa) in rice leaves was linearly related to leaf N, but CO_2-saturated photosynthesis (at Ci>60 Pa) was curvilinearly dependent on leaf N. As leaf N increased, the capacity for sucrose and starch synthesis increases to a lesser extent than do rubisco and electron transport. As a result, photosynthesis in high N leaves may be limited by end-product synthesis at a lower CO_2 partial pressure than in low N leaves. However, this limitation by end-product synthesis with increasing leaf N did not operate at normal ambient air CO_2 unless leaf temperature decreased well below the growth temperature (Sage et al., 1990; Makino et al., 1994a).

CHAPTER 3

CO_2 ASSIMILATION IN RELATION TO NITROGEN IN APPLE LEAVES

3.1 Abstract

Bench-grafted Fuji/M_{26} apple (*Malus domestica* Borkh.) trees were fertigated with different nitrogen concentrations by using a modified Hoagland's solution for 45 days. A wide range of leaf N content was noted in recent fully expanded leaves (from 0.9 - 4.4 g m^{-2}). Apparent quantum efficiency for CO_2 assimilation was relatively constant except for a slight decrease at the lower end of the leaf N range. The light saturation point for CO_2 assimilation increased with increasing leaf N. Curvilinear relationships were found between leaf N and 1) light-saturated CO_2 assimilation at ambient CO_2, 2) the initial slope of the response of CO_2 assimilation to intercellular CO_2 concentration, and 3) CO_2-saturated photosynthesis. All three initially increased linearly with increasing leaf N, then reached a plateau at a leaf N content of approximately 3 g m^{-2}. The relationship between leaf N and stomatal conductance was similar to that of CO_2 assimilation with leaf N. Calculated intercellular CO_2 concentration, however, tended to decrease with increases in leaf N, indicating non-stomatal limitation of photosynthesis in leaves with low N content. Light-saturated CO_2 assimilation expressed on a leaf N basis decreased with increasing leaf N. In conclusion, there is a curvilinear relationship between leaf N content and photosynthetic capacity in apple leaves. Photosynthetic N use efficiency decreases with increasing leaf N content.

3.2 Introduction

Photosynthesis is one of the primary resource acquisition processes in plants, providing the ultimate carbon supply for plant growth and development. Nitrogen is an essential element for plant growth, an important resource in the environment and the most heavily used fertilizer for improving plant production. Because nitrogen is one of the main constituents in the photosynthetic apparatus, understanding the relationship between leaf N and photosynthesis is critical not only for optimizing carbon production relative to N input, but also for elucidating the mechanisms involved in the regulation of photosynthesis.

There is often a good correlation between leaf N content and photosynthetic capacity (Field and Mooney, 1986; Evans, 1989; Sinclair and Horie, 1989). Field and Mooney (1986) found a single linear correlation between leaf N and light-saturated CO_2 assimilation expressed on a leaf dry weight basis for a wide range of C_3 species. They argued that this represented a fundamental relationship independent of species and environment. However, Evans (1989) compiled data from photosynthesis-N relationship studies and showed that the relationship between leaf N and photosynthetic capacity varied among different types of plants. This variation reflected the differences in N partitioning between thylakoid and soluble proteins, electron transport capacity, and specific activities of rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase). In general, when a wide range of leaf N content has been examined, curvilinear relationships have been found between leaf N and photosynthetic capacity (Evans, 1983, 1989; DeJong and Doyle, 1985; Sinclair and Horie, 1989).

Most of the research on the photosynthesis-N relationship has been conducted on herbaceous plants; much less information is available for deciduous tree fruit crops. Of the few studies on fruit crops, DeJong (1982) found that both CO_2 assimilation and mesophyll conductance were highly correlated to N content in peach leaves under natural light exposure within the tree canopy. A similar relationship also was found between leaf N content and CO_2 assimilation among five *Prunus* tree fruit species (DeJong, 1983). Although considerable efforts have been made to understand the environmental regulation of photosynthesis in apple (Lakso, 1994), very little is known about its photosynthetic capacity in relation to leaf N. From a practical standpoint, optimum leaf N content for nursery apple trees has not been established, although standard leaf N contents are well-defined for bearing trees in the orchard. Because nursery apple trees have only vegetative growth, leaf CO_2 assimilation may be a good criterion for determining optimum leaf N. The objectives of this study were, therefore, to characterize the relationship between leaf N and photosynthetic capacity, and determine how photosynthetic N use efficiency responds to leaf N.

3.3 Materials and Methods

3.3.1 *Plant material*

'Fuji' apple (*Malus domestica* Borkh.) trees on M_{26} rootstocks were used. Bench-grafting was done in late March, and each grafted tree was immediately put into a 3.8-L pot containing a mixture of peat moss, pumice and sandy loam soil (1:2:1 by volume). The plants were grown in a lathhouse until early June. During this period, they were fertigated with 150 ppm N using Plantex® 20-10-20 with micronutrients (Plantex Corp.,

Ontario, Canada) every two weeks, beginning from budbreak in early May. When new shoots were approximately 15 cm tall, plants were selected for uniformity, and moved out to full sunlight. Thereafter, they were fertigated weekly with Plantex® for three weeks. Beginning on June 22, plants were fertigated twice weekly with one of six concentrations of nitrogen (0, 3.0, 6.0, 9.0, 15.0, or 21.0 mM) by applying 300 ml of a complete nutrient solution to each pot. The nutrient solution was modified from Hoagland's solution No. 2 (Hoagland and Arnon, 1950) to allow N supplied solely from NH_4NO_3. Irrigation was provided from a saucer placed at the bottom of each pot. After 45 days, recent fully expanded leaves were chosen for gas exchange measurements.

3.3.2 *Gas exchange measurements*

Measurements were made via a CIRAS-1 portable photosynthesis system (PP Systems, Herts., UK). For all measurements, photon flux density (PFD), leaf temperature, and ambient water vapor pressure were kept at 1650 ± 35 μmol m^{-2} s^{-1}, 26.0 ± 1.0 °C, and 1.35± 0.10 kPa, respectively. Light-saturated CO_2 assimilation was measured at an air CO_2 concentration of 350 ± 2 ppm. The initial slope of the response of CO_2 assimilation (A) to intercellular CO_2 concentration (Ci) was estimated as the slope of the linear regression between Ci and A, from at least three points measured at Ci below 200 ppm. A/Ci curves (at 21% O_2) were constructed at air CO_2 concentrations of 110, 190, 270, 350, 450, 600, 800, 1000,1200 or 1420 ppm, from low to high concentrations, until Ci reached approximately 1000 ppm. At incident PFD of 1800, 1400, 1000, 750, 500, 250, 150, 100 or 50 μmol m^{-2} s^{-1}, responses of CO_2 assimilation were measured in order from high to low densities. At each CO_2 concentration or PFD, CO_2 assimilation and stomatal conductance were recorded after allowing enough time for them to reach

steady state. CO_2-saturated photosynthesis was measure at a CO_2 concentration of 1300 ppm.

3.3.3 *Leaf N and chlorophyll analysis*

After measuring gas exchange, leaf area was determined with a LI-3000 Area Meter (Li-Cor Inc., Lincoln, NE, USA). Leaves were frozen at –80 °C, then freeze-dried. Nitrogen content was determined by the Kjeldahl method (Horneck et al., 1989). Leaf chlorophyll content was measured according to Arnon (1949).

3.4 Results

3.4.1 *CO_2 assimilation in response to light and intercellular CO_2 concentrations*

As PFD increased, CO_2 assimilation increased almost linearly first, then reached a saturation point (Figure 3-1). Both the light saturation point for CO_2 assimilation and light-saturated CO_2 assimilation increased with increasing leaf N. In contrast, the initial slope of the light response curve remained relatively unchanged except for a slight decrease in extremely low N leaves.

Figure 3-2 shows CO_2 assimilation in response to calculated intercellular CO_2 concentration in leaves with different N contents. The dependence of A/Ci curves on leaf N is reflected in the initial slope of the A/Ci curve, and the rate of CO_2 assimilation at both ambient CO_2 and saturated CO_2 concentrations. CO_2 assimilation at ambient CO_2 concentration fell into the linear region of the A/Ci curve regardless of the N status of the leaves, indicating that activity of rubisco limited CO_2 assimilation under ambient CO_2 and saturated PFD conditions.

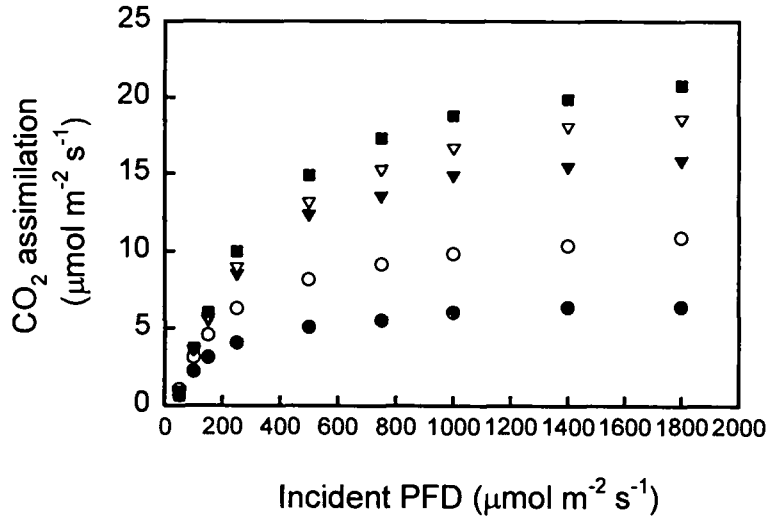

Figure 3-1. CO_2 assimilation in response to incident PFD in apple leaves. Leaf N content (g m^{-2}) is: 1.00 (●), 1.42 (○), 2.46 (▼), 3.12(▽), and 4.34 (■).

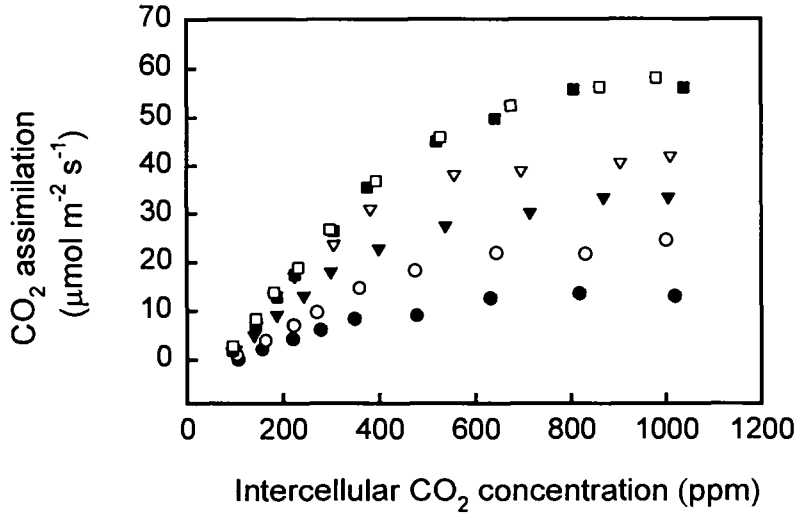

Figure 3-2. CO_2 assimilation in response to intercellular CO_2 concentration in apple leaves. Leaf N content (g m^{-2}) is: 1.02 (●), 1.42 (○), 1.82 (▼), 2.55 (▽), 3.27 (■), and 4.25 (□).

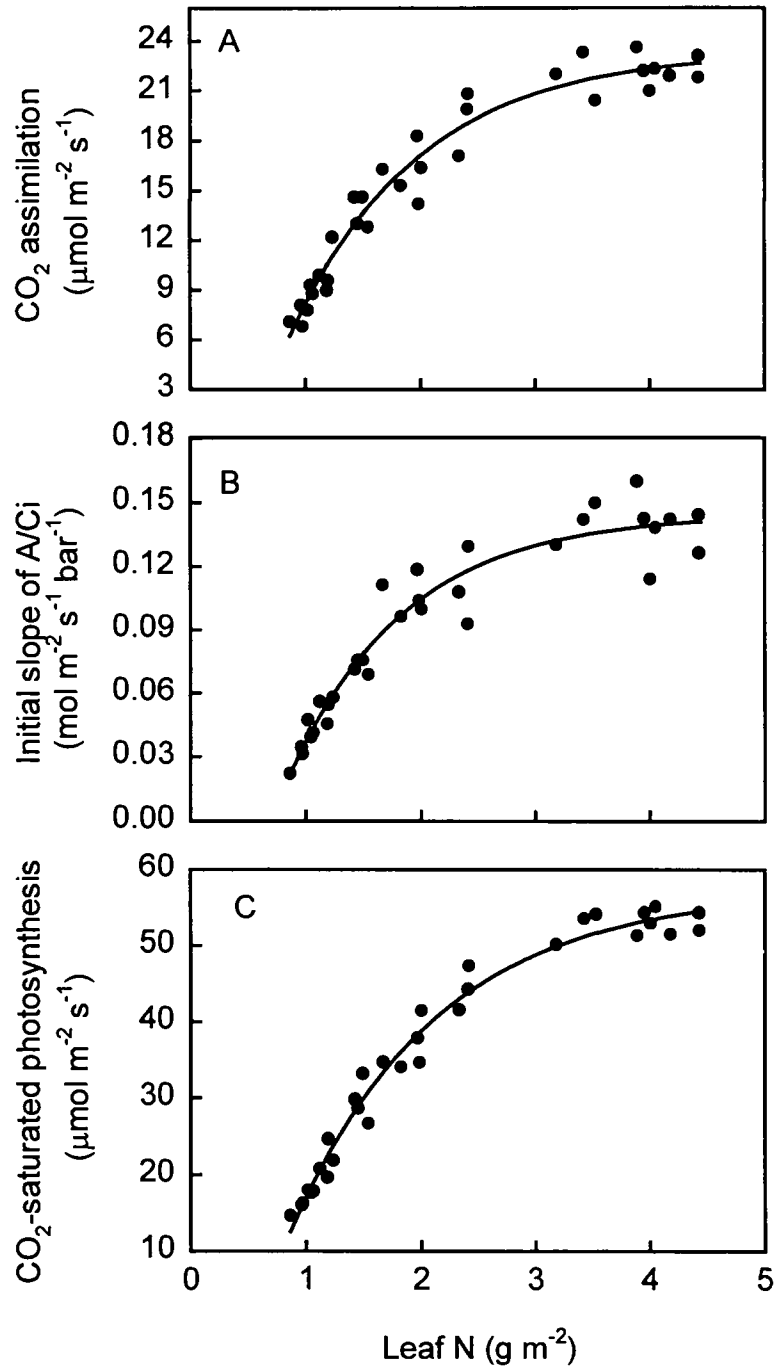

Figure 3-3. Light-saturated CO_2 assimilation at ambient CO_2 (A), the initial slope of the response of CO_2 assimilation to intercellular CO_2 concentration (B), and CO_2-saturated photosynthesis (C) in relation to N content in apple leaves. Regression equations: (A) $Y = 37(1 - e^{-0.8937X}) - 13.67$ ($R^2 = 0.958$, $p = 0.0001$); (B) $Y = 0.289(1 - e^{-0.9882X}) - 0.144$ ($R^2 = 0.933$, $p = 0.0001$); (C) $Y = 87.39(1 - e^{-0.7747X}) - 30.03$ ($R^2 = 0.980$, $p = 0.0001$).

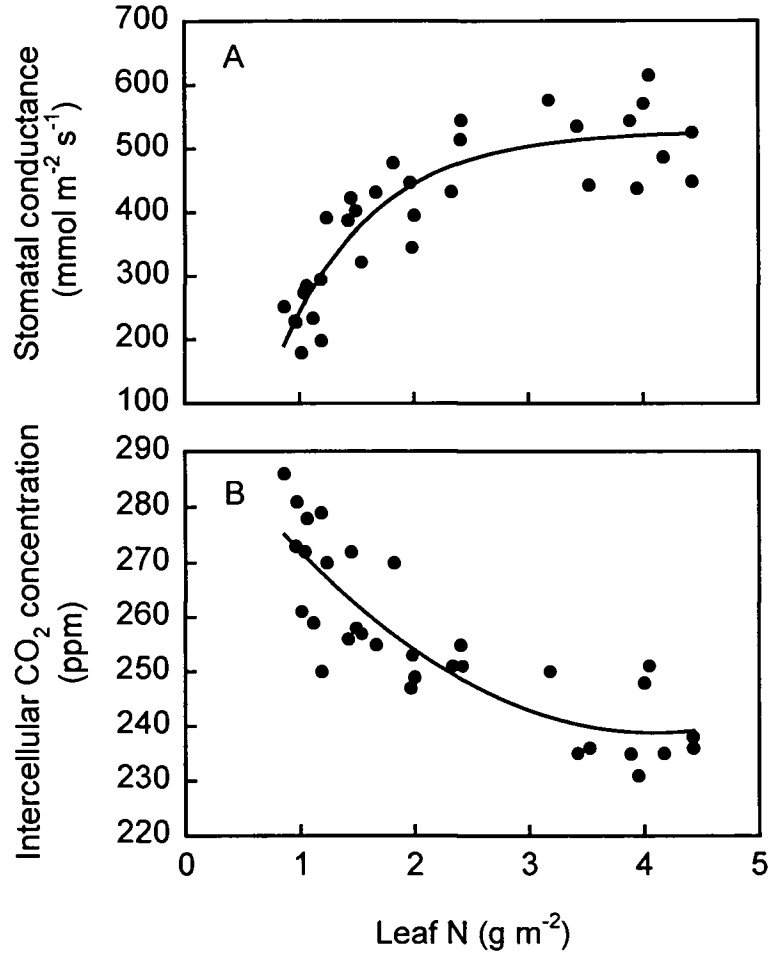

Figure 3-4. Stomatal conductance (A) and intercellular CO_2 concentration (B) in relation to N content in apple leaves. Regression equations: (A) $Y = 977.3(1 - e^{-1.237X}) - 449.9$ ($R^2 = 0.794$, $p = 0.0001$); (B) $Y = 297.11 - 28.52X + 3.49X^2$ ($R^2 = 0.740$, $p = 0.0001$).

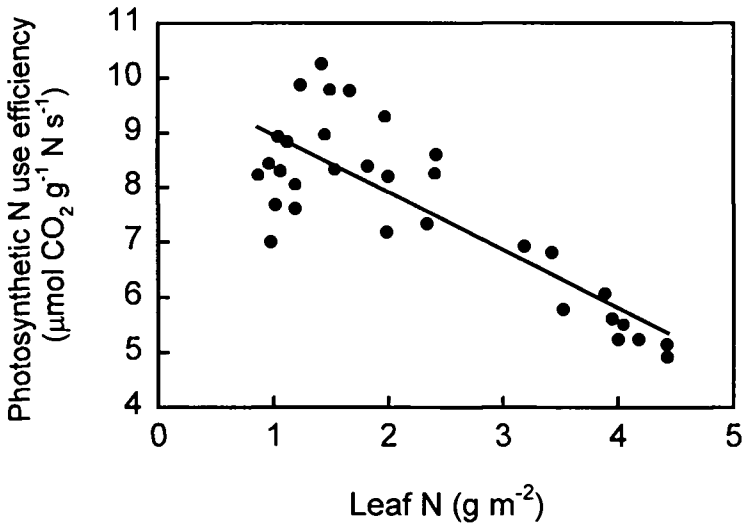

Figure 3-5. Photosynthetic N use efficiency in relation to N content in apple leaves. The regression equation: $Y = 10.01 - 1.05X$ ($R^2 = 0.684$, $p = 0.0001$).

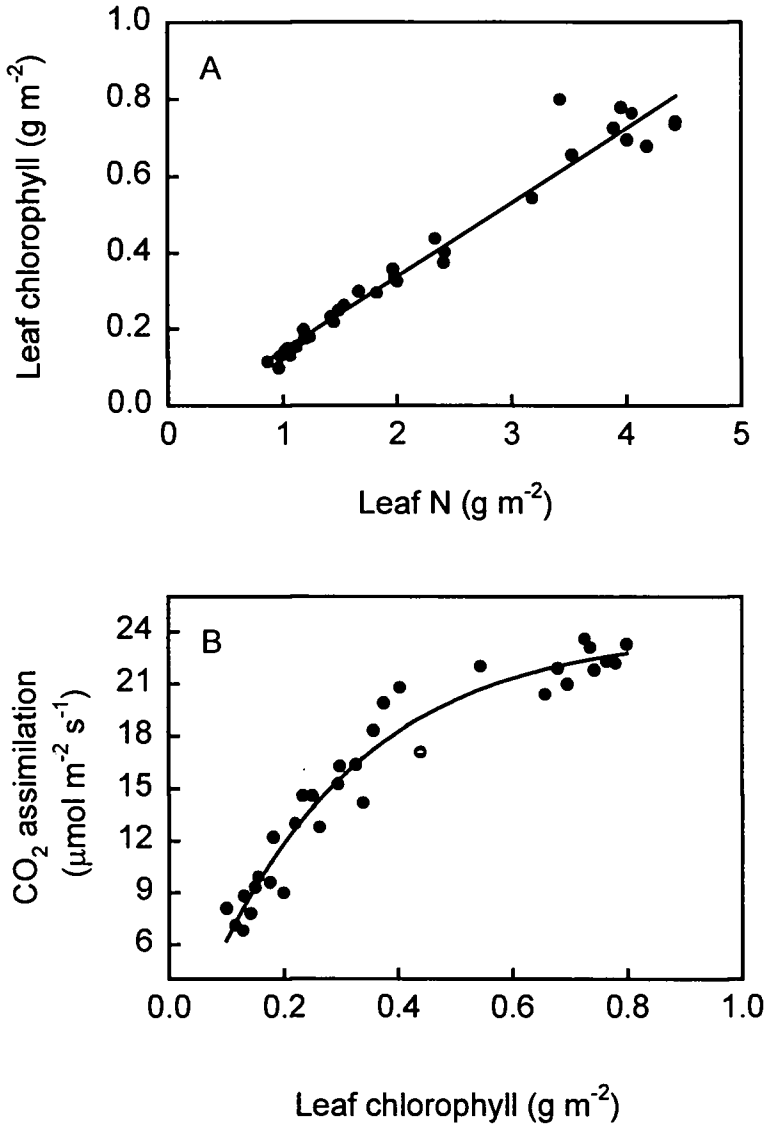

Figure 3-6. The correlation between leaf N and chlorophyll content (A) and the relationship of light-saturated CO_2 assimilation with leaf chlorophyll content (B) in apple leaves. Regression equations: Y = -0.05 + 0.194X (R^2 = 0.963, p = 0.0001); (B) Y = $25.77(1 - e^{-3.732X}) - 1.71$ (R^2 = 0.946, p = 0.0001).

3.4.2 *The relationship between leaf N and CO_2 assimilation*

Over the wide range of leaf N content that we examined (from 0.9-4.4 g m^{-2}), curvilinear relationships were found between leaf N and 1) light-saturated CO_2 assimilation at ambient CO_2, 2) the initial slope of the A/Ci curves, and 3) CO_2-saturated photosynthesis (Figure 3-3). All three initially increased linearly with increasing leaf N, then reached a plateau at a leaf N content of approximately 3 g m^{-2}. Stomatal conductance showed a response to leaf N similar to that of CO_2 assimilation (Figure 3-4A). Calculated intercellular CO_2 concentration, however, decreased with increasing leaf N (Figure 3-4B). Photosynthetic N use efficiency also decreased with increasing leaf N (Figure 3-5).

Leaf chlorophyll content increased linearly with increasing leaf N content (Figure 3-6A). Because of this, a curvilinear relationship was also found between leaf chlorophyll and light-saturated CO_2 assimilation (Figure 3-6B)

3.5 Discussion

We found a close relationship between N content and CO_2 assimilation in apple leaves in this study. This was expected, considering the large number of enzymes or proteins involved in the process of CO_2 assimilation. Kang and Titus (1980) reported that rubisco alone accounted for over 50% of all the soluble proteins in apple leaves. Although stomatal conductance showed a response to leaf N similar to that of CO_2 assimilation, decreased intercellular CO_2 concentrations with increasing leaf N clearly indicate that low stomatal conductance is not the cause of low photosynthetic capacity in low N leaves (Farquhar and Sharkey, 1982). Instead, the correlation between leaf N and

CO_2 assimilation results from the close relationship between leaf N and the initial slope of the A/Ci curves (Figure 3-3B). Because the initial slope reflects the increase in carboxylation per unit increase in intercellular CO_2 concentration (*i.e.*, carboxylation efficiency), it is a measure of CO_2 assimilation capacity after accounting for stomatal conductance.

The curvilinear nature of the relationship between leaf N and CO_2 assimilation appears to be similar to other species where a wide range of leaf N has been examined (Evans, 1983, 1989; DeJong and Doyle, 1985; Sinclair and Horie, 1989). The curvilinear relationship itself indicates that the limitation imposed by leaf N on CO_2 assimilation decreases with increasing leaf N, but the exact causes for this curvilinear relationship are not clear. There are several possible explanations. First, partitioning of leaf N into non-photosynthetic N storage may increase with increasing leaf N. Herbaceous plants can store N as nitrate in the vacuole of leaf cells when N supply is in excess (Millard, 1988). This would cause a curvilinear relationship between leaf N and CO_2 assimilation if nitrate-N were not taken into account. However, this is unlikely the case in apple leaves, because nitrate-N accounts for only a very small proportion of total N in apple leaves, and it is not affected by N supply (Lee and Titus, 1992).

Second, the total amount of rubisco, or the amount of rubisco involved in CO_2 assimilation, reaches a plateau at a certain leaf N level. Analysis of our A/Ci curves of apple leaves with different N contents indicated that light-saturated CO_2 assimilation at ambient CO_2 fell within the linear region of the A/Ci curve regardless of leaf N status (Figure 3-2), indicating that CO_2 assimilation is mainly limited by rubisco activity, according to the C_3 photosynthesis model of Farquhar et al. (1980). If the initial slope of

the A/Ci curve represents the total amount of rubisco in apple leaves, as has been demonstrated in herbaceous plants (von Caemmerer and Farquhar, 1981; Hudson et al., 1992; von Caemmerer et al., 1994), one would predict that the amount of N partitioned into rubisco reaches a plateau at leaf N of 3 g m^{-2} in apple leaves. Alternatively, it could be that the total amount of rubisco increases with increasing leaf N, as shown in other plants (Evans, 1983, 1989; Makino et al., 1994). The amount of rubisco that is involved in CO_2 assimilation, however, reaches a plateau at leaf N of 3 g m^{-2} because an activation process is required for rubisco to be engaged in CO_2 assimilation.

Third, a CO_2 transfer resistance exists between the intercellular air space and the carboxylation site within the chloroplast (Evans, 1983; von Caemmerer and Evans, 1991). This resistance may have caused the curvilinear relationship between leaf N and the initial slope of the A/Ci curve in wheat (*Triticum aestivum*) leaves, assuming all the rubisco was involved in photosynthesis (Evans, 1983). Obviously, more research is needed for identifying the main cause of the curvilinear response of CO_2 assimilation to N in apple leaves. From a practical standpoint, leaf N of approximately 3 g m^{-2} is the optimum for CO_2 assimilation, regardless of the exact mechanism by which the curvilinear relationship occurs.

Photosynthetic N use efficiency of apple leaves decreased with increasing leaf N in our study. This result differs from that of herbaceous plant studies, where photosynthetic N use efficiency increased initially, then began to level off as leaf N increased further (Sage and Pearcy, 1987). Apparently, this is caused by the difference in the response of CO_2 assimilation to leaf N between apple and herbaceous plants. The slope of CO_2 assimilation in response to leaf N in apple leaves is smaller than that of

most C_3 annuals. Typically, the slope of CO_2 assimilation in response to leaf N of C_3 annuals ranges from 0.2-0.3 μmol $mmol^{-1}$ N s^{-1} (Lugg and Sinclair, 1981; Evans, 1983; Sage and Pearcy, 1987). The value found for apple in this experiment was about 0.12 μmol $mmol^{-1}$ N s^{-1} (Figure 3-3A). This is consistent with the general trend that deciduous trees and shrubs have a lower response of CO_2 assimilation to leaf N compared with rapidly growing annuals (Field and Mooney, 1986).

The maximum rate of CO_2 assimilation that apple leaves could reach at the highest leaf N content under ambient CO_2 concentration in this experiment was about 22 -23 μmol m^{-2} s^{-1}. For rapidly growing annuals, this value can be as high as 35 - 40 μmol m^{-2} s^{-1} (Lugg and Sinclair, 1981; Sage and Pearcy, 1987; Reddy et al., 1996; Makino et al., 1997).

The smaller slope of CO_2 assimilation in response to leaf N in combination with a lower maximum CO_2 assimilation at ambient CO_2 concentration provides the immediate explanation for the decrease in photosynthetic N use efficiency with increasing leaf N. The ultimate cause for a decrease in photosynthetic N use efficiency, however, lies in the mechanism by which the curvilinear relationship between leaf N and CO_2 assimilation occurs.

The relationship between leaf N and CO_2 assimilation found in apple leaves is in general agreement with that of peach leaves (DeJong, 1982; DeJong and Doyle, 1985; DeJong et al., 1989). Minimum rates of CO_2 assimilation were achieved at about 0.9 -1.0 g m^{-2} leaf N and maximum rates were achieved at approximately 3 g m^{-2}. However, photosynthetic N use efficiency of peach leaves increased with increasing leaf N

(DeJong, 1982). The different responses of photosynthetic N use efficiency to leaf N between apple and peach leaves were likely caused by the way leaf N was manipulated.

In our experiment, the wide range of leaf N was achieved by altering N supply, with all the leaves exposed to full sunlight. In the peach experiment, leaf N was altered by natural light exposure within the tree canopy (DeJong, 1982). Peach leaf N content was linearly correlated with the amount of light exposure within the tree canopy (DeJong and Doyle, 1985). When the response of CO_2 assimilation to leaf N in peach leaves (under natural canopy light exposure) was compared under low and high N supply conditions, the slope of the relationship between leaf N and light-saturated CO_2 assimilation was greater for trees with high rather than low N supplies (DeJong et al., 1989). This was because trees with a high N supply had more shoot growth, resulting in denser canopies and more shaded leaves than trees with a low N supply (DeJong et al., 1989).

Under shaded conditions, leaves respond to reduced PFD by investing more N in pigment-protein complexes and less into soluble proteins, especially rubisco, to maximize light absorption and quantum efficiency (Evans, 1996). In contrast, under well-exposed conditions, when the N supply is the only limiting factor, both light and dark reactions of photosynthesis are equally affected (Evans, 1996). So, with the same leaf N content per unit leaf area, well-exposed leaves should have a higher CO_2 assimilation capacity than shaded leaves (Evans, 1989). This was confirmed in peach trees. At the same N content per leaf area basis, higher rates of leaf CO_2 assimilation were observed for trees with a low N supply but better light exposure than for trees with a high N supply but poor light exposure (DeJong et al., 1989). Testing this hypothesis in apple trees would require a

direct comparison of the response of CO_2 assimilation to leaf N, where leaf N would be altered only by light exposure or by N supply under well-exposed conditions.

Light response curves of CO_2 assimilation showed that the apparent quantum yield remained relatively unchanged except for a decrease at the lower end of the leaf N range in this experiment (Figure 3-1). Because no attempt has been made to measure leaf absorptance, it is unclear how leaf light absorption and partitioning between different pathways respond to leaf N, and whether extremely low N leaves have a lower quantum yield on the absorbed light basis. Leaf chlorophyll content, however, is linearly correlated with leaf N (Figure 3-6A).

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CHAPTER 4

RUBISCO ACTIVATION STATE DECREASES WITH INCREASING LEAF NITROGEN CONTENT IN APPLE LEAVES

4.1 Abstract

Based on the curvilinear relationship between leaf nitrogen content and the initial slope of the response of CO_2 assimilation (A) to intercellular CO_2 concentrations (Ci) in apple leaves, we hypothesized that rubisco activation state decreases with increasing leaf N content, and that this decreased activation state accounts for the curvilinear relationship between leaf N and CO_2 assimilation. A wide range of leaf N content (1.0 to 5.0 g m^{-2}) was achieved by fertigating bench-grafted Fuji/M_{26} apple (*Malus domestica* Borkh.) trees for 45 days with different N concentrations using a modified Hoagland's solution. Analysis of A/Ci curves under saturating light indicated that CO_2 assimilation at ambient CO_2 fell within the rubisco limitation region of the A/Ci curves regardless of leaf N status. Initial rubisco activity showed a curvilinear response to leaf N. In contrast, total rubisco activity increased linearly with increasing leaf N throughout the leaf N range. As a result, rubisco activation state decreased with increasing leaf N. Both light-saturated CO_2 assimilation at ambient CO_2 and the initial slope of the A/Ci curves were linearly related to initial rubisco activity, but curvilinearly related to total rubisco activity. The curvatures in the relationships of both light-saturated CO_2 assimilation at ambient CO_2 and the initial slope of the A/Ci curves with total rubisco activity were more pronounced than in their relationships with leaf N. This was because the ratio of total rubisco activity to leaf N increased with increasing leaf N. As leaf N increased, photosynthetic N use efficiency declined with decreasing rubisco activation state.

4.2 Introduction

Whenever a sufficiently wide range of leaf N content has been examined, the relationship between leaf N and light-saturated CO_2 assimilation has been curvilinear (Evans, 1983, 1989; DeJong and Doyle, 1985; Sinclair and Horie, 1989). This indicates that CO_2 assimilation is less limited by nitrogen as leaf N content increases. However, the mechanism that causes the curvature in the relationship between leaf N and CO_2 assimilation is not well understood.

Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) content and its total activity increase linearly with leaf N (Evans, 1983,1986; Sage et al., 1987; Makino et al., 1992, 1994a, b, 1997; Nakano et al., 1997). In wheat (*Triticum aestivum*; Evans, 1983) and spinach (*Spinacia oleracea*; Evans and Terashima, 1988), the initial slope of the response of CO_2 assimilation (A) to intercellular CO_2 concentrations (Ci) is curvilinearly related to total rubisco activity. This curvature was attributed to CO_2 transfer resistance from the intercellular air space to the carboxylation site in chloroplasts. Based on the C_3 photosynthesis model by Farquhar et al. (1980), it has been deduced that if the ratio of CO_2 transfer conductance to the maximum activity (V_{cmax}) of rubisco does not hold constant, a nonlinear relationship between V_{cmax} and the initial slope of the A/Ci curves is expected (von Caemmerer and Evans, 1991; also see the appendix).

von Caemmerer and Evans (1991) found that CO_2 transfer conductance increased with increasing N content in wheat leaves. As leaf N increases, the ratio of CO_2 transfer conductance to rubisco activity may, or may not, remain constant, depending on species. However, when rubisco is fully activated *in vivo*, the curvature in the relationship

between total rubisco activity and the initial slope of the A/Ci curves does not necessarily translate into the curvature in the relationship between leaf N and the initial slope of the A/Ci curves, if the ratio of rubisco to leaf N increases with increasing leaf N. In rice (*Oryza sativa*) leaves, CO_2-limited photosynthesis (at Ci=20 Pa) was curvilinearly related to total rubisco content, but linearly related to leaf N (Makino et al., 1994a). This was because the ratio of total rubisco to leaf N increased with increasing leaf N. The increased ratio of rubisco to leaf N compensated for the curvature in the relationship between rubisco activity and CO_2-limited photosynthesis (Makino et al., 1994a). As leaf N increases, the response of the ratio of rubisco to total leaf N varies among C_3 species. This ratio was independent of leaf N content in wheat leaves (Evans, 1983; Makino et al., 1992), but it increased with increasing leaf N content in rice (Makino et al., 1992; 1994a; 1997), spinach (Makino et al., 1992), pea (*Pisum sativum*; Makino and Osmond, 1991; Makino et al., 1992) and other species (Evans, 1989; Makino et al., 1992).

Rubisco must be activated to catalyze the carboxylation and oxygenation reactions (Portis, 1990, 1992). If only a proportion of the total rubisco is activated *in vivo* in high N leaves, the relationship between leaf N and photosynthesis will be curvilinear. Evans and Terashima (1988) showed that there was no apparent deactivation of rubisco in spinach leaves under a high N supply. In contrast, Mächler et al. (1988) found that rubisco activation state in wheat leaves decreased as the N supply increased. However, plants were grown at a relatively low photon flux density (PFD of 540 $\mu mol\ m^{-2}\ s^{-1}$), and photosynthesis and leaf N were not measured in that experiment. In addition, Lawlor et al. (1987) showed that rubisco accounted for almost a constant proportion of the soluble proteins in wheat leaves, but CO_2 assimilation remained almost constant with increasing

amounts of rubisco in excess of 3 g m^{-2}. Their calculations suggested that approximately 50% of the rubisco protein was not activated in high N leaves under low PFD conditions.

Both light-saturated CO_2 assimilation at ambient CO_2 and the initial slope of the A/Ci curves showed curvilinear relationships with N content in leaves of apple (*Malus domestica* Borkh.) plants grown under full sunlight (Chapter 3). We hypothesized that rubisco activation state decreases with increasing leaf N content, and that this decreased activation state accounts for the curvilinear relationship between leaf N and CO_2 assimilation.

4.3 Materials and Methods

4.3.1 *Plant material*

'Fuji' apple (*Malus domestica* Borkh.) trees on M_{26} rootstocks were used. Bench-grafting was done in late March, and each grafted tree was immediately put into a 3.8-L pot containing a mixture of peat moss, pumice and sandy loam soil (1:2:1 by volume). The plants were grown in a lathhouse until early June. During this period, they were fertigated with 150 ppm N using Plantex® 20-10-20 with micronutrients (Plantex Corp., Ontario, Canada) every two weeks, beginning from budbreak in early May. When new shoots were approximately 15 cm tall, plants were selected for uniformity, and moved out to full sunlight. Thereafter, they were fertigated weekly with Plantex® for three weeks. Beginning on June 30, plants were fertigated twice weekly with one of seven N concentrations (0, 2.5, 5, 7.5, 10, 15, or 20 mM N from NH_4NO_3) by applying 300 ml of a modified Hoagland's solution to each pot (Chapter 3). After 45 days, recent fully expanded leaves were chosen for gas exchange and rubisco activity measurements.

4.3.2 *Gas exchange measurements*

Measurements were made using a CIRAS-1 portable photosynthesis system (PP Systems, Herts., UK). For all measurements, PFD, leaf temperature and ambient water vapor pressure were kept at 1700 ± 40 μmol m^{-2} s^{-1}, 26.2 ± 1.0 °C, and 1.30± 0.15 kPa, respectively. Light-saturated photosynthesis was measured at an ambient CO_2 concentration of 350 ± 2 ppm. The initial slope of the A/Ci curve was estimated as the slope of the linear regression between intercellular CO_2 (Ci) and CO_2 assimilation (A) from at least three points measured at Ci below 200 ppm. Responses of CO_2 assimilation to intercellular CO_2 at 21% and 2% O_2 were determined in ascending order at air CO_2 concentrations of 110, 190, 270, 350, 450, 600, 800, 1000, 1200, or 1500 ppm, until Ci reached approximately 1000 ppm. At each concentration, CO_2 assimilation and stomatal conductance were first measured at 21% O_2, after allowing enough time for them to reach steady state. The O_2 supply was then switched from 21% to 2% and measurements were made after re-equilibration.

4.3.3 *Rubisco activity measurements*

Rubisco extraction and activity measurements were modified from the methods of Tissue et al. (1993) and Sharkey et al. (1991). Briefly, two leaf discs (total of 2 cm^2) were taken from each leaf under sunlight, cut into small pieces, and placed into an ice-cold test tube with 3 ml extraction buffer [100 mol m^{-3} Bicine (pH 7.8 at 25 °C), 5 mol m^{-3} EDTA, 0.75% (w/v) polyethylene glycol 20,000, 14 mol m^{-3} β-mercaptoethanol, 1% (v/v) Tween 80, and 1.5% (w/v) insoluble polyvinylpyrrolidone]. The leaf tissue was homogenized for 25 to 30 s with a Tissuemizer (Tekmar Company, Cincinnati, Ohio,

USA) at 18,000 rpm. The extract was then centrifuged at 13,000 g for 40 s in an Eppendorf microcentrifuge, and the supernatant was used immediately in the rubisco activity assay.

Rubisco activity was measured at 25 °C by enzymatically coupling RuBP (ribulose1,5-bisphosphate) carboxylation to NADH oxidation (Lilley and Walker 1974). NADH oxidation was monitored at 340 nm by using a Shimadzu UV-160 Spectrophotometer (Shimadzu Corp., Kyoto, Japan). For initial rubisco activity, 50 µl sample extract was added to a semi-microcuvette containing 900 µl of an assay solution, immediately followed by adding 50 µl 0.5 mol m^{-3} RuBP, then mixing well. The change of absorbance at 340 nm was monitored for 40 s. For total rubisco activity, 50 µl 0.5 mol m^{-3} RuBP was added 15 min later after a sample extract was combined with the assay solution to fully activate all the rubisco. The assay solution for both initial and total activity measurements contained 100 mol m^{-3} Bicine (pH 8.0 at 25 °C), 25 mol m^{-3} $KHCO_3$, 20 mol m^{-3} $MgCl_2$, 3.5 mol m^{-3} ATP, 5 mol m^{-3} phosphocreatine, 80 nkat glyceraldehyde-3-phosphate dehydrogenase, 80 nkat 3-phospoglyceric phosphokinase, 80 nkat creatine phosphokinase, and 0.25 mol m^{-3} NADH. Rubisco activation state was calculated as the ratio of initial activity to total activity.

4.3.4 *Leaf N content*

Leaves were frozen at -80 °C and freeze-dried. N content was determined by following the Kjeldahl procedure (Horneck et al., 1989).

4.4 Results

4.4.1 *Relationships between leaf N, rubisco activities and CO_2 assimilation*

Light-saturated CO_2 assimilation at ambient CO_2 and the initial slope of the A/Ci curves increased linearly with increasing leaf N first, then leveled off at a leaf N content of approximately 3 g m^{-2} (Figure 4-1A, B). Photosynthetic N use efficiency decreased as leaf N increased (Figure 4-1C).

Initial rubisco activity showed a curvilinear response to leaf N (Figure 4-2A). Initial rubisco activity increased almost linearly with increasing leaf N first, then began to level off when leaf N exceded 3 g m^{-2}. In contrast, total rubisco activity increased linearly throughout the leaf N range (Figure 4-2B). As a result, rubisco activation state decreased with increasing leaf N (Figure 4-2C).

Both light-saturated CO_2 assimilation at ambient CO_2 and the initial slope of the A/Ci curves were linearly correlated with initial rubisco activity (Figure 4-3A, B), but were curvilinearly related to total rubisco activity (Figure 4-4A, B). The curvatures in the relationships of both light-saturated CO_2 assimilation at ambient CO_2 and the initial slope of the A/Ci curve with total rubisco activity were more pronounced than in their relationships with leaf N. As leaf N increased, the ratio of total rubisco activity to leaf N increased (Figure 4-5). Photosynthetic N use efficiency decreased as rubisco activation state decreased with increasing leaf N (Figure 4-6).

4.4.2 *Responses of CO_2 assimilation to intercellular CO_2 concentration at 21 and 2% O_2*

To determine which process limits light-saturated CO_2 assimilation at ambient CO_2, we constructed A/Ci curves under both 21% and 2% O_2 conditions. Regardless of the N status of the leaves, light-saturated CO_2 assimilation at ambient CO_2 fell within the linear region of the A/Ci curves (Figure 4-7; notice the difference in scale on the y-axes).

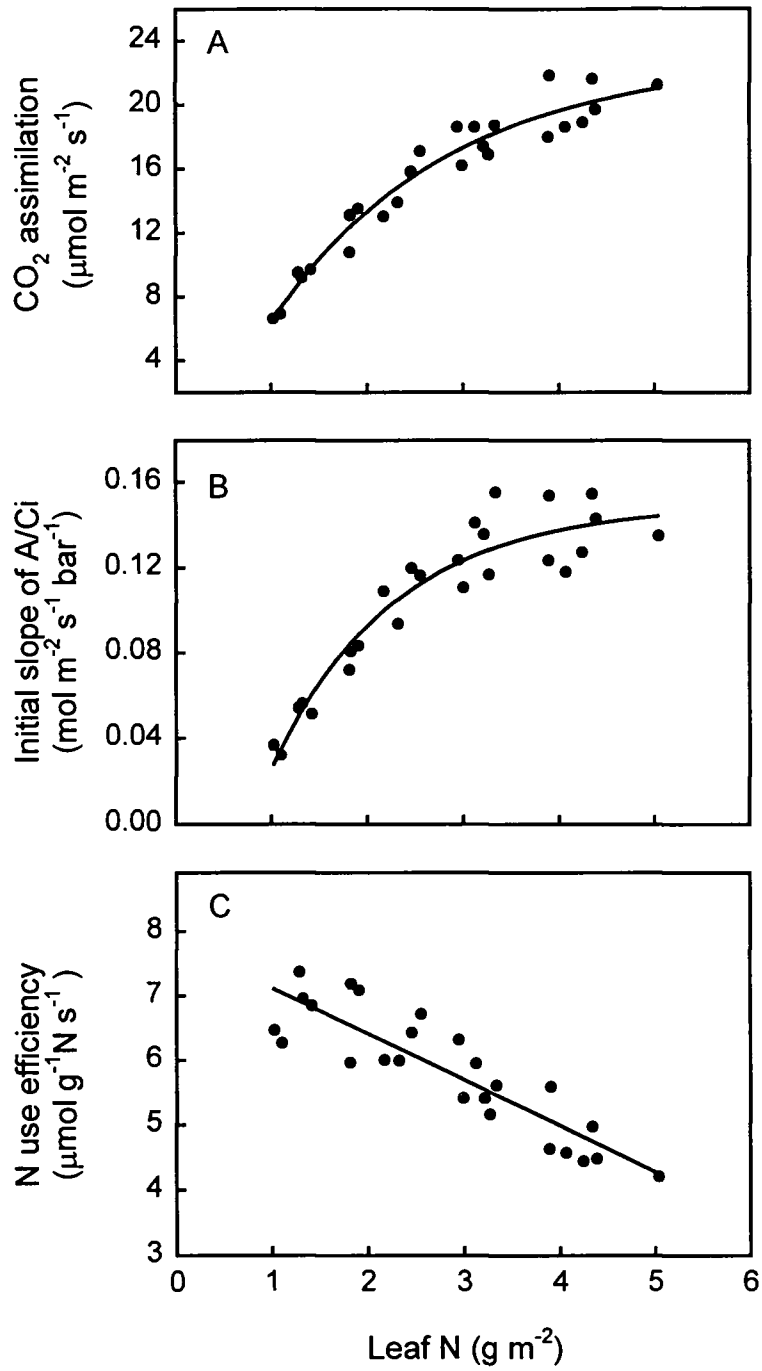

Figure 4-1. Light-saturated CO_2 assimilation at ambient CO_2 (A), the initial slope of the A/Ci curves (B), and photosynthetic N use efficiency (C) in relation to N content in apple leaves. Regression equations: (A) $Y = 28.31(1 - e^{-0.548X}) - 5.53$ ($R^2 = 0.946$, $p = 0.0001$); (B) $Y = 0.2697(1 - e^{-0.782X}) - 0.1204$ ($R^2 = 0.904$, $p = 0.0001$); (C) $Y = 7.83 - 0.71X$ ($R^2 = 0.782$, $p = 0.0001$).

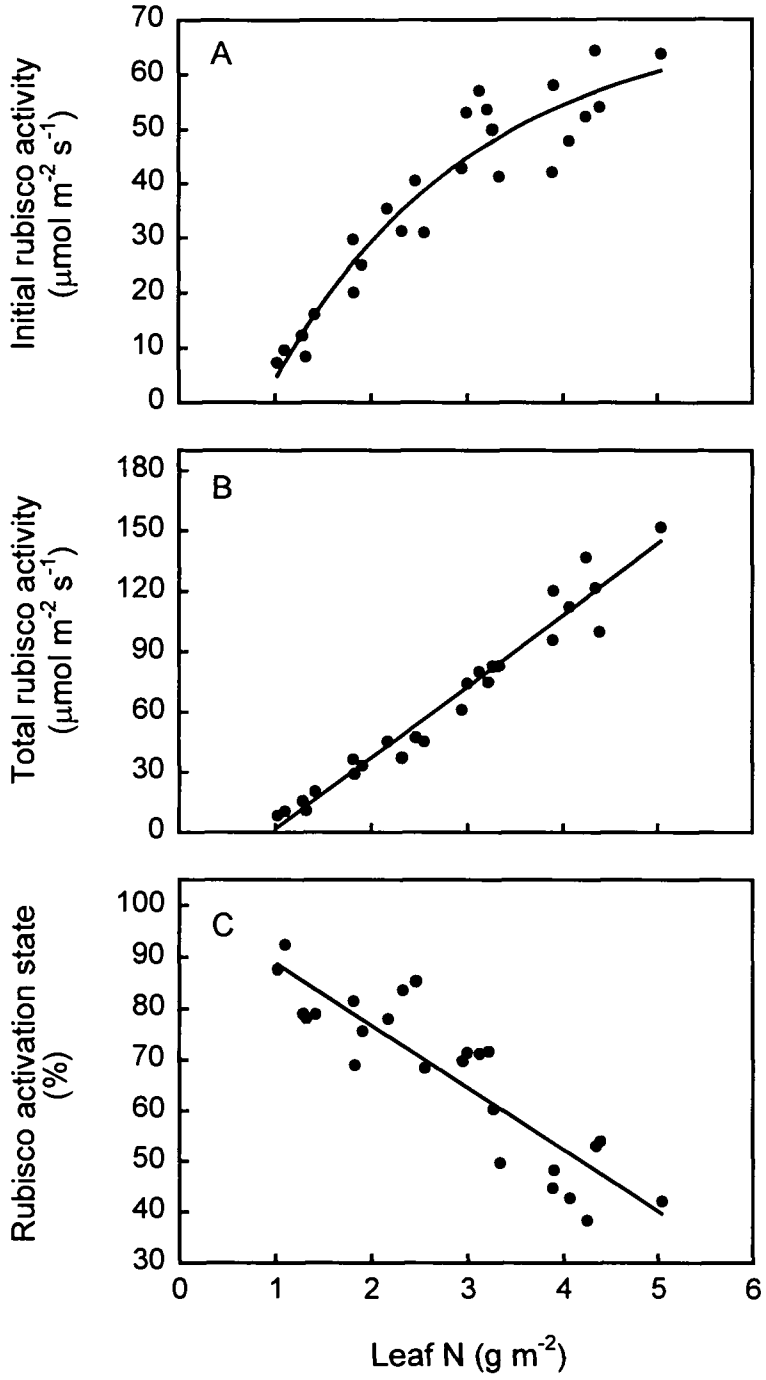

Figure 4-2. Initial rubisco activity (A), total rubisco activity (B), and rubisco activation state (C) in response to N content in apple leaves. Regression equations: (A) $Y = 106.5(1 - e^{-0.476X}) - 36.2$ ($R^2 = 0.902$, $p = 0.0001$); (B) $Y = -33.68 + 35.41X$ ($R^2 = 0.958$, $p = 0.0001$); (C) $Y = 101.1 - 12.2X$ ($R^2 = 0.789$, $p = 0.0001$).

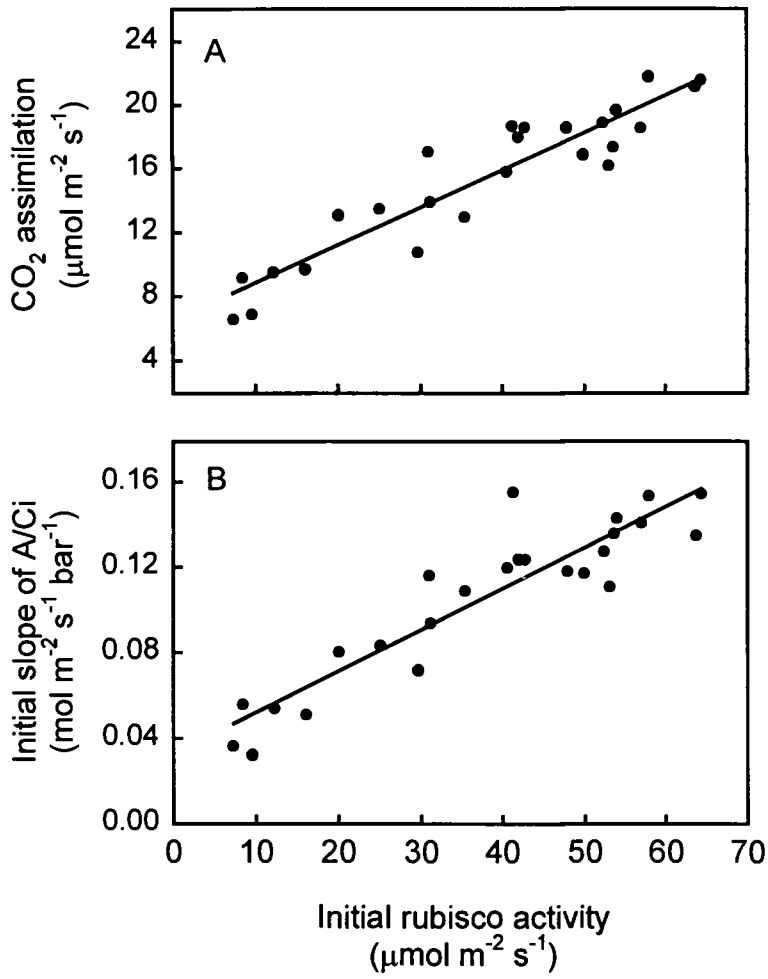

Figure 4-3. Light-saturated CO_2 assimilation at ambient CO_2 (A) and the initial slope of the A/Ci curves in relation to initial rubisco activity in apple leaves. Regression equations: (A) $Y = 6.50 + 0.235X$ ($R^2 = 0.871$, $p = 0.0001$); (B) $Y = 0.033 + 0.0019X$ ($R^2 = 0.847$, $p = 0.0001$).

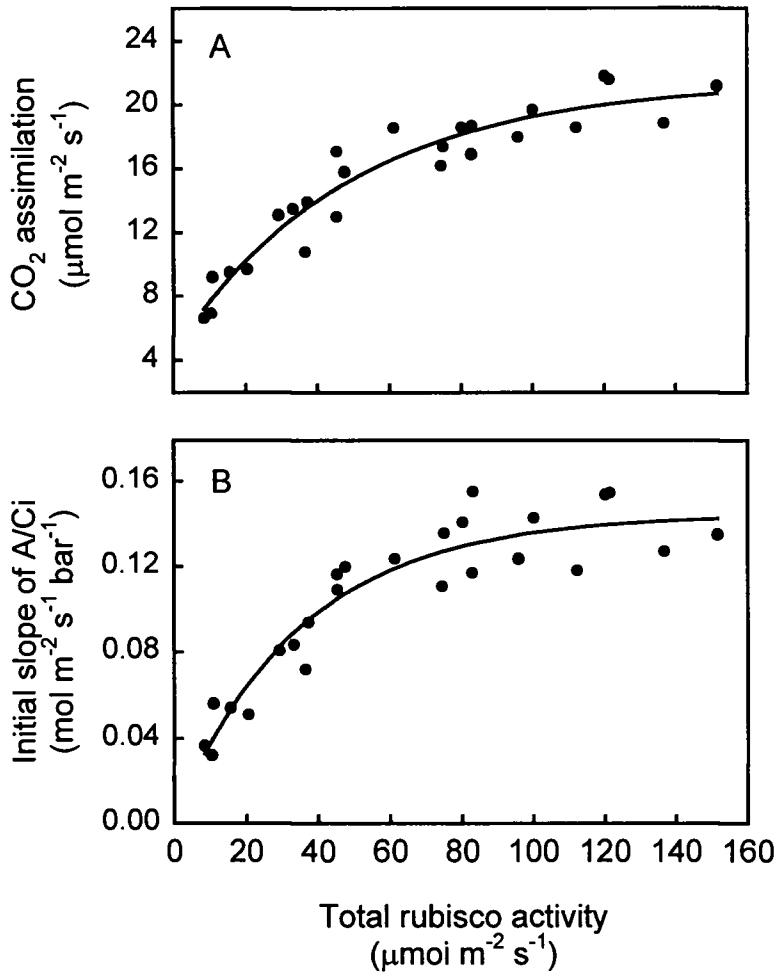

Figure 4-4. Light-saturated CO_2 assimilation at ambient CO_2 (A) and the initial slope of the A/Ci curves in relation to total rubisco activity in apple leaves. Regression equations: (A) $Y = 16.92(1 - e^{-0.0205X}) + 4.53$ ($R^2 = 0.918$, $p = 0.0001$); (B) $Y = 0.1405(1 - e^{-0.0281X}) + 0.0038$ ($R^2 = 0.887$, $p = 0.0001$).

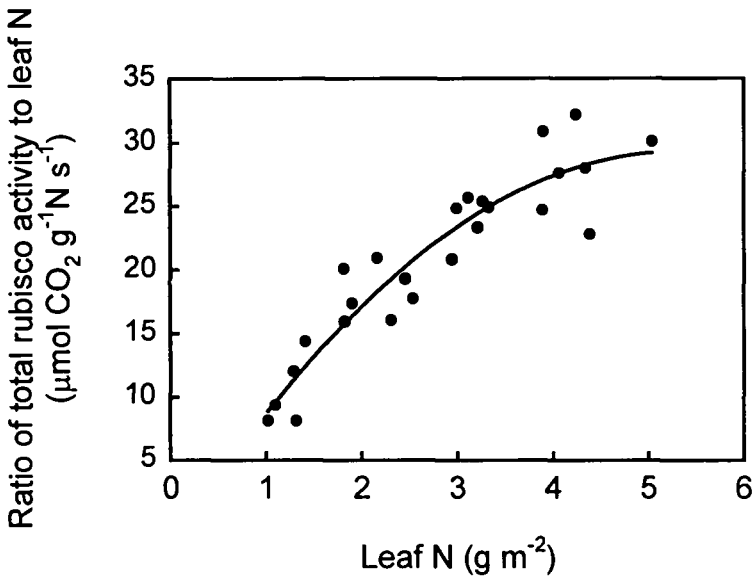

Figure 4-5. The ratio of total rubisco activity to leaf N in response to N content in apple leaves. Regression equation: $Y = -1.95 + 11.76X - 1.11X^2$ ($R^2 = 0.874$, $p = 0.0001$).

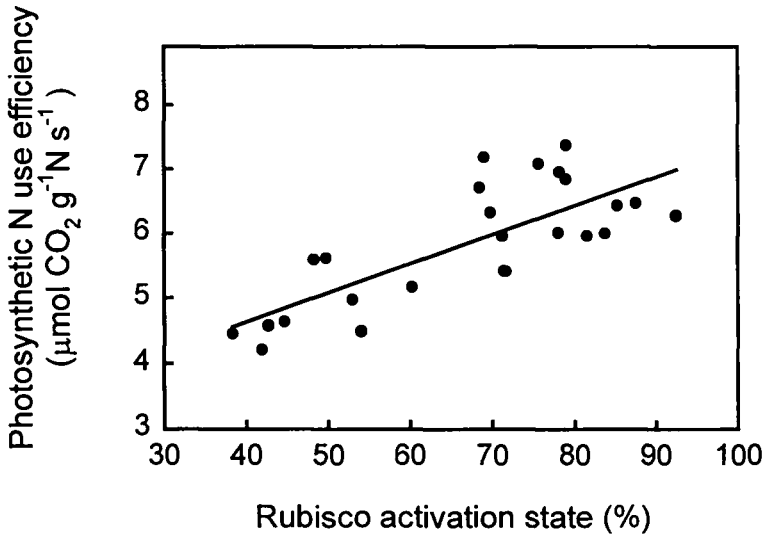

Figure 4-6. Photosynthetic N use efficiency in relation to rubisco activation state in apple leaves. Regression equation: $Y = 2.83 + 0.045X$ ($R^2 = 0.591$, $p = 0.0001$).

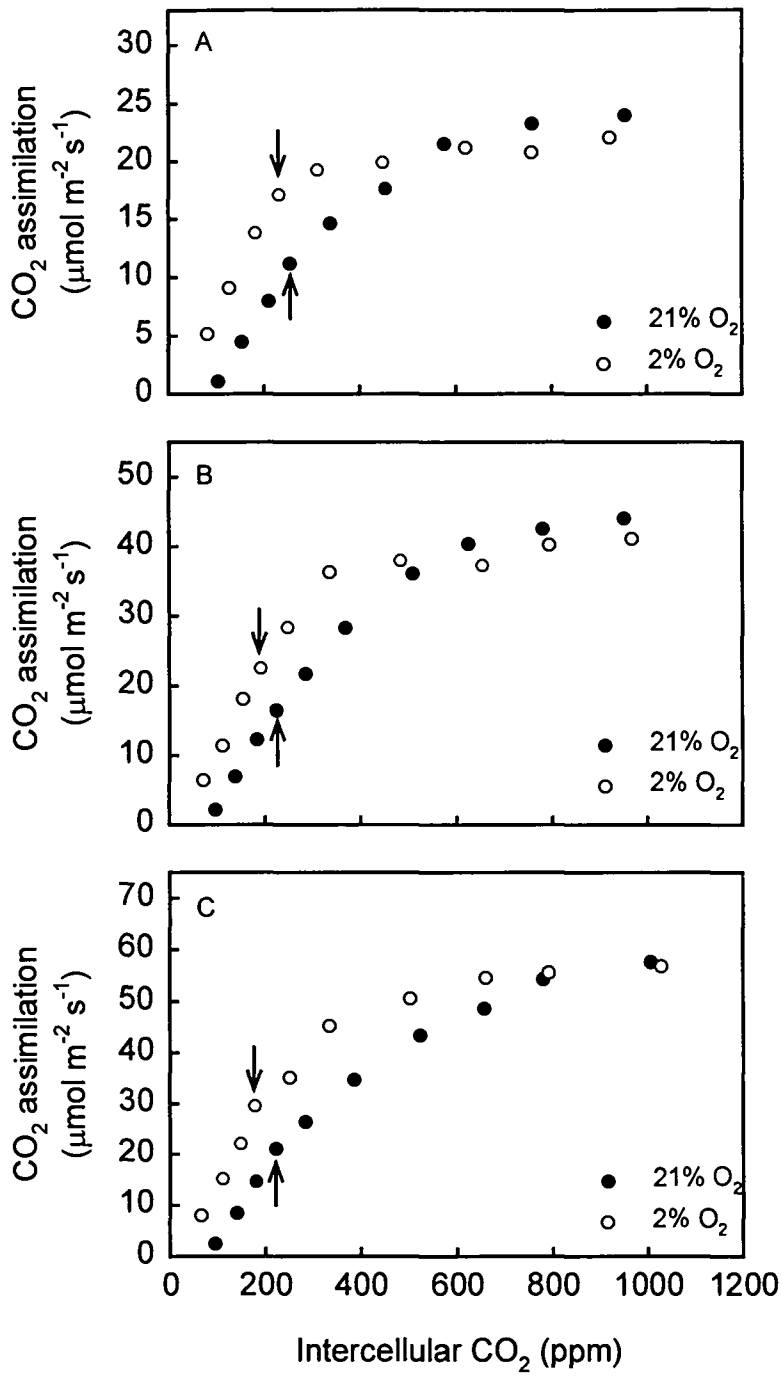

Figure 4-7. Responses of CO_2 assimilation to intercellular CO_2 concentration in apple leaves at 21 and 2% O_2. Leaf N content (g m^{-2}) is 1.52 (A), 2.54 (B), and 4.06 (C). The arrows indicate the intercellular CO_2 concentration corresponding to ambient CO_2 concentration. Measurements were made at a PFD of 1700 ± 20 μmol m^{-2} s^{-1}, a leaf temperature of 27.0 ± 1.0 °C, and an ambient water vapor pressure of 1.28 ± 0.05 kPa.

4.5 Discussion

Total rubisco activity increased linearly with increasing leaf N whereas initial rubisco activity showed a curvilinear response to leaf N. This resulted in decreased rubisco activation state with increasing N content in apple leaves (Figure 4-2). Both light-saturated CO_2 assimilation at ambient CO_2 and the initial slope of the A/Ci curves were linearly related to initial rubisco activity, but curvilinearly related to total rubisco activity (Figure 4-3, 4-4). These results are consistent with our hypothesis that rubisco activation state decreases with increasing leaf N, and this decreased rubisco activation state accounts for the curvilinear relationship between leaf N and CO_2 assimilation.

Decreased rubisco activation state in response to an increasing N supply was suggested by Lawlor et al. (1987) and demonstrated by Mächler et al. (1988) in wheat leaves. However, because the plants were grown under relatively low light (540~550 $\mu mol\ m^{-2}\ s^{-1}$) in both experiments, the possibility that deactivation of rubisco was caused by low electron transport capacity can not be ruled out. In our experiment, apple plants were grown under full sunlight, and measurements were made at a PFD above the light saturation point (Chapter 3). We found that rubisco activation state decreased with increasing N content in apple leaves. This contrasts with the finding by Evans and Terashima (1988), who did not observe any apparent deactivation of rubisco in spinach under a high N supply. Although we did not measure CO_2 transfer conductance, decreased rubisco activation state with increasing leaf N alone would result in curvilinear relationships between total rubisco activity and the initial slope of the A/Ci curves, and between leaf N and the initial slope of the A/Ci curves, even if the ratio of CO_2 transfer

conductance to the effective V_{cmax} remained constant (see the appendix). The relationship between total rubisco activity and the initial slope of the A/Ci curves was more curvilinear than that between leaf N and the initial slope of the A/Ci curves because the ratio of total rubisco to leaf N increased with increasing leaf N (Figure 4-5).

The curvature in the relationship between leaf N and light-saturated CO_2 assimilation at ambient CO_2 was similar to that in the relationship between leaf N and the initial slope of the A/Ci curves (Figure 4-4). When a more noticeable curvature was found (Evans, 1983; DeJong and Doyle, 1985), other factors may have contributed to the curvilinear relationship between leaf N and light-saturated CO_2 assimilation at ambient CO_2. In peach (*Prunus persica*) leaves, the more curvilinear relationship between leaf N and photosynthesis was related to the relatively larger stomatal limitation in high N leaves (DeJong and Doyle, 1985).

Rubisco activation state in apple leaves is higher at low rather than high leaf N levels (Figure 4-2C). High rubisco activation state in low N leaves indicates that light-saturated CO_2 assimilation at ambient CO_2 is limited mainly by the amount of rubisco in these leaves. As total rubisco activity increases with increasing leaf N, a lower proportion of the rubisco is active. The amount of rubisco apparently does not limit CO_2 assimilation of apple leaves under saturating light when the N supply is in excess. However, even in high N leaves, CO_2 assimilation at ambient CO_2 still fell within the linear region of the A/Ci curve, which is characteristic of rubisco limitation based on the C_3 photosynthesis model of Farquhar et al. (1980). Either rubisco activation state limits photosynthesis in high N leaves, or decreased rubisco activation state reflects a regulatory response to an excess N supply to balance a limitation elsewhere in the photosynthetic

system (Sage et al., 1988; Sage, 1990). The nature of the response of rubisco activation state to leaf N obviously deserves further study.

Regardless of the nature of rubisco regulation, when rubisco is not fully activated *in vivo*, it is the amount of activated rubisco that determines the rate of CO_2 assimilation, rather than the total amount of rubisco. Both the linear relationship between initial rubisco activity and the initial slope of the A/Ci curves, and the curvilinear relationship between total rubisco activity and the initial slope of the A/Ci curves that we found indicate that the initial slope of the A/Ci curves reflects the amount of rubisco that is active *in vivo*, not the total amount of rubisco. It reflects the total amount of rubisco only if all the rubisco is active *in vivo*. In spinach leaves the initial slope of the A/Ci curve was also highly correlated with initial rubisco activity when the phosphorus supply was altered (Brooks, 1986). When light is the only source of variation, *in vitro* initial rubisco activity has been closely correlated with the calculated effective rubisco activity, which represents the CO_2- and RuBP-saturated rate achieved by the active sites of rubisco (von Caemmerer and Edmondson, 1986). In transgenic tobacco plants with an antisense gene directed against rubisco activase, CO_2 assimilation was determined by the number of carbamylated rubisco sites, not the total rubisco content (Mate et al., 1996). When rubisco is not fully activated *in vivo* under ambient CO_2 and saturating light conditions, the current C_3 photosynthesis model must be modified to include the rubisco activation state factor. If, under saturating light conditions, rubisco activation state at a CO_2 partial pressure below ambient is the same as that under ambient CO_2 conditions, the only modification to the current model is to replace V_{cmax} with the effective V_{cmax}. This can be

estimated from the response of CO_2 assimilation to intercellular CO_2 concentrations at low CO_2 concentrations, based on the kinetic properties of rubisco.

Decreased rubisco activation state with increasing leaf N accounts for the curvilinear relationship between leaf N and light-saturated CO_2 assimilation in apple leaves. It may also explain why photosynthetic N use efficiency decreases with increasing leaf N. However, the exact mechanism by which rubisco is down-regulated in high N leaves is unclear. Activation of rubisco *in vivo* is catalyzed by rubisco activase. Catalysis of rubisco activase requires ATP and is inhibited by ADP (Portis, 1990). In transgenic tobacco plants with an antisense gene directed against rubisco mRNA, increased rubisco activation state was associated with an increased ATP/ADP ratio when plants were grown under low light conditions (Quick et al., 1991). When photosynthesis was limited by triose phosphate utilization in sucrose and starch synthesis, deactivation of rubisco was also associated with a decreased ATP/ADP ratio (Sharkey, 1990). The ATP/ADP ratio was higher in spinach leaves under low rather than high N supplies (Mächler et al., 1988). This may provide a mechanism to explain how rubisco activation state responds to leaf N. Alternatively, the amount of rubisco activase may not keep pace with the increase in the amount of rubisco as leaf N increases, resulting in decreased rubisco activation state. This seems unlikely because a 95% reduction in rubisco activase activity by antisense inhibition of rubisco activase gene expression was required before rubisco deactivation and decreased CO_2 assimilation were observed in tobacco transgenic plants (Mate et al., 1996).

Our finding that rubisco activation state decreases with increasing N content in apple leaves under saturating light conditions is consistent with the idea that rubisco can

serve as a storage protein when the N supply is in excess. The notion of rubisco as a form of storage protein is long-standing, but has been controversial. Considering that rubisco is so expensive in terms of N investment, it has been argued that it would represent a waste of N resources if rubisco were present in great excess. This argument is generally valid under a normal N supply. Indeed, rubisco activity measurements have shown that rubisco is fully activated or nearly fully activated at ambient to low CO_2 under light saturating conditions (von Caemmerer and Edmondson, 1986; Evans and Terashima, 1988; Sage et al., 1990, 1993). In addition, the initial slope of the A/Ci curve is linearly correlated with total rubisco activity (von Caemmerer and Farquhar, 1981; Hudson et al., 1992; von Caemmerer et al., 1994). When curvilinear relationships have been observed between total rubisco activity and the initial slope of the A/Ci curve, factoring in CO_2 transfer resistance has accounted for the apparent deviation from the expected behavior of rubisco (Evans, 1983; Evans and Seemann, 1984; Evans and Terashima, 1988; von Caemmerer and Evans, 1991, Makino et al., 1994a). However, under an excess N supply, accumulating surplus rubisco may benefit plants in terms of N acquisition and reutilization because N is such an important resource for plant growth and development. Especially for plants such as apple, which have very low nitrate concentrations in leaves even under a high nitrate supply (Lee and Titus, 1992), rubisco apparently does serve as a storage protein. Compared with a strict storage protein, an excessive amount of rubisco in high N leaves may also offer advantages such as slightly higher steady state CO_2 assimilation and higher water use efficiency. This has been seen in comparisons between wild tobacco plants and antisense rubisco plants (Quick et al., 1991).

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CHAPTER 5

LIGHT ABSORPTION AND PARTITIONING IN RELATION TO NITROGEN CONTENT IN APPLE LEAVES

5.1 Abstract

Bench-grafted Fuji/M_{26} apple (*Malus domestica* Borkh) trees were fertigated for 6 weeks with different concentrations of nitrogen, using a modified Hoagland's solution. 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 CO_2 assimilation expressed on the basis of absorbed light increased linearly with increasing leaf N first, then reached a plateau at a leaf N content of approximately 3 g m^{-2}. Under high light conditions (1500 μmol m^{-2} s^{-1}), the amount of absorbed light in excess of that required to saturate CO_2 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 drop at the lower end. As leaf N increased, non-photochemical quenching under high light declined. There was a corresponding 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 CO_2 assimilation. The fraction of absorbed light potentially going into singlet oxygen formation was estimated to be about 10% regardless of leaf N status. In conclusion, there is more excess absorbed light in low N leaves than in high N leaves under high light conditions. Non-photochemical

quenching is enhanced with decreasing leaf N to reduce PSII efficiency and the probability of photo-damage by excess absorbed light.

5.2 Introduction

CO_2 assimilation is closely related to leaf nitrogen content because the large number of enzymes or proteins involved in the process of photosynthesis accounts for the majority of the leaf N. A curvilinear relationship has been found between leaf N and light-saturated CO_2 assimilation in apple leaves (Chapter 3), and the decreased activation state of ribulose1,5-bisphosphate carboxylase/oxygenase (rubisco) with increasing leaf N accounts for this curvilinearity (Chapter 4). Low total rubisco activity and high rubisco activation state in low N leaves indicate that light-saturated CO_2 assimilation is mainly limited by the amount of rubisco (Chapter 4). However, low N leaves also have low chlorophyll content. Thus, it is unclear whether light absorption limits CO_2 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 utilized in photosynthetic electron transport (Horton et al., 1996). C_3 photosynthesis has the structure of a convergent metabolic pathway. Therefore, at steady state photosynthesis, light input flux is coordinated with CO_2 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 (Woodrow and Berry, 1988; Sharkey, 1990). When photon flux density (PFD) 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 linearly correlated to leaf N, indicating that light and dark reactions are equally altered by leaf N (Evans, 1996). However, the input of PFD fluctuates dramatically during a clear day, with the maximum PFD occurring at midday under natural conditions. Chlorophyll content is linearly correlated with N content in apple leaves (Chapter 3). Decreased light absorption by antenna pigments in low N leaves, however, may not be sufficient to deal with the large fluctuation of PFD under natural conditions. Quantum efficiency must be maximized under low PFD, yet leaves must be protected from excessive light damage under high PFD. 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 PFD conditions.

When plants are exposed to PFDs in excess of those that can be utilized in photosynthetic electron transport, excess excitation energy can be dissipated as heat in the antenna pigments of PSII. This involves the xanthophyll cycle and low thylakoid pH (Demmig-Adams et al., 1997; Gilmore, 1997). Analyzing changes of chlorophyll fluorescence emission via pulse-modulated fluorescence monitoring system (Schreiber et al., 1994) has been increasingly used to estimate the activity of thermal dissipation process as well as the photosynthetic electron transport in leaves (Genty et al., 1989; Demmig-Adams et al., 1996). 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 (Gilmore and Björkman, 1994a, b; Adams and Demmig-Adams, 1995; Adams et

al., 1995a, 1995b; Verhoeven et al., 1996). However, information is very limited about thermal dissipation of excitation energy in response to leaf N status (Khamis et al., 1990; Verhoeven et al., 1997).

The objectives of this study were, therefore, to determine under high PFD conditions 1) if there is more excess absorbed PFD in low N leaves than in high N leaves; and 2) partitioning of absorbed PFD into photochemical and non-photochemical processes in response to N content in apple leaves.

5.3 Materials and Methods

5.3.1 *Plant material*

'Fuji' apple (*Malus domestica* Borkh.) trees on M_{26} rootstocks were used. Bench-grafting was done in late March, and each grafted tree was immediately put into a 3.8-L pot containing a mixture of peat moss, pumice and sandy loam soil (1:2:1 by volume). The plants were grown in a lathhouse until early June. During this period, they were fertigated with 150 ppm N using Plantex® 20-10-20 with micronutrients (Plantex Corp., Ontario, Canada) every two weeks, beginning from budbreak in early May. When new shoots were approximately 15 cm tall, plants were selected for uniformity, and moved out to full sunlight. Thereafter, they were fertigated weekly with Plantex® for three weeks. Beginning on June 30, plants were fertigated twice weekly with one of seven levels of N concentrations (0, 2.5, 5.0, 7.5, 10.0, 15, 20 mM N from NH_4NO_3) by applying 300 ml of a modified Hoagland's solution to each pot (Chapter 3). Plants were sub-irrigated with a saucer placed at the bottom of each pot. After six weeks, recent fully expanded leaves were chosen for gas exchange and chlorophyll fluorescence measurements.

5.3.2 *Measurements of leaf absorptance, gas exchange and chlorophyll fluorescence*

An LI-1800 Spectroradiometer with the 1800-12S Integrating Sphere attachment (Li-Cor Inc., Lincoln, NE) 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.

CO_2 assimilation and PSII efficiency were monitored concurrently with a system that combined a CIRAS-1 gas exchange system (PP Systems, Herts., UK) and an FMS-1 pulse-modulated fluorometer (Hansatech Instruments Ltd., Norfolk, UK). 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 PFD distribution at the leaf surface, yet it allowed delivery of a saturation pulse of actinic light and detection of fluorescence signals. Measurements of CO_2 assimilation and PSII efficiency in response to PFD were made at incident PFD of 1500, 1150, 850, 600, 400, 250, 150, 75, and 0 μmol m^{-2} s^{-1}, from high to low density at ambient CO_2 (350 ppm) and O_2 (21%). Because the two light sensors in the cuvette were not located exactly at the leaf surface, the actual corresponding incident PFDs 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 LI-190SA quantum sensor. At each PFD, CO_2 assimilation and actual PSII efficiency were recorded after reaching steady state. Actual PSII efficiency was measured in essentially the same way as under natural PFD

conditions. Total electron transport was calculated as: Incident PFD × Absorptance × 0.5 × PSII (Krall and Edwards, 1992). Responses of CO_2 assimilation and actual PSII efficiency to PFD were measured on 20 leaves with different N contents. Each photosynthetic light response curve was fitted to the following negative exponential model by non-linear regression analysis using the SAS procedure NLIN with the "Marquardt" option (Hampson et al., 1996):

$$A = Rd + S(1-e^{-G*PFD}),$$

where A is net CO_2 assimilation, Rd 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 PFD is photosynthetic photon flux density. Quantum yield corresponded to the initial slope of the curve, which was calculated as GS. The light saturation point for CO_2 assimilation was estimated as the PFD at which 95% of the light-saturated assimilation rate was attained.

The relationship between leaf N and chlorophyll fluorescence parameters was studied either pre-dawn or at a PFD of 1500 ± 50 μmol m^{-2} s^{-1} at noon, under natural conditions, using the FMS-1 fluorometer. Maximal fluorescence (Fm) and minimal fluorescence (Fo) of dark-adapted leaves were measured pre-dawn. For leaves exposed to natural sunlight, steady state fluorescence (Fs) was monitored to ensure it was stable before a reading was taken. Maximal fluorescence (Fm') under natural light exposure was obtained by imposing a 1 second saturating flash of approximately 18,000 μmol m^{-2} s^{-1} PFD at the end of the fiber optic to the leaf in order to reduce all the PSII centers. To determine the minimal fluorescence (Fo') under natural light exposure, a black envelope was placed around the leaf and leaf-clip while a far-red light was switched on to rapidly

oxidize PSII by drawing electrons from PSII to PSI. The maximum PSII efficiency of dark-adapted leaves was calculated as Fv/Fm = (Fm-Fo)/Fm (for fluorescence nomenclature see van Kooten and Snel, 1990). For leaves exposed to natural sunlight, thermal dissipation was estimated from non-photochemical quenching as Fm/Fm'-1 (Stern-Volmer quenching or NPQ; see Bilger and Björkman, 1990) or non-photochemical quenching coefficient qN = 1-(Fm'-Fo')/(Fm-Fo). The efficiency of open PSII centers under natural light exposure (Fv'/Fm') was calculated as (Fm'-Fo')/Fm'. 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 qP was (Fm'-Fs)/(Fm'-Fo'). The degree of closure of PSII reaction centers was estimated as (1-qP). Actual PSII efficiency was (Fv'/Fm')qP = (Fm'-Fs)/Fm' (Genty et al., 1989. The percentage of absorbed light potentially going into singlet oxygen formation was estimated as (Fv'/Fm')(1-qP) (Demmig-Adams et al., 1996).

5.3.3 *Leaf N and chlorophyll analysis*

After all the above measurements, leaf area was determined with the LI-3000 Area Meter. Leaves were frozen at –80 °C and freeze-dried. N content was determined by following the Kjeldahl procedure (Horneck et al., 1989). Leaf chlorophyll content was measured according to Arnon (1949)

5.4 Results

5.4.1 *Leaf absorptance in response to leaf N*

A curvilinear relationship was found between leaf chlorophyll content and leaf absorptance (Figure 5-1A). Leaf absorptance increased almost linearly with increasing

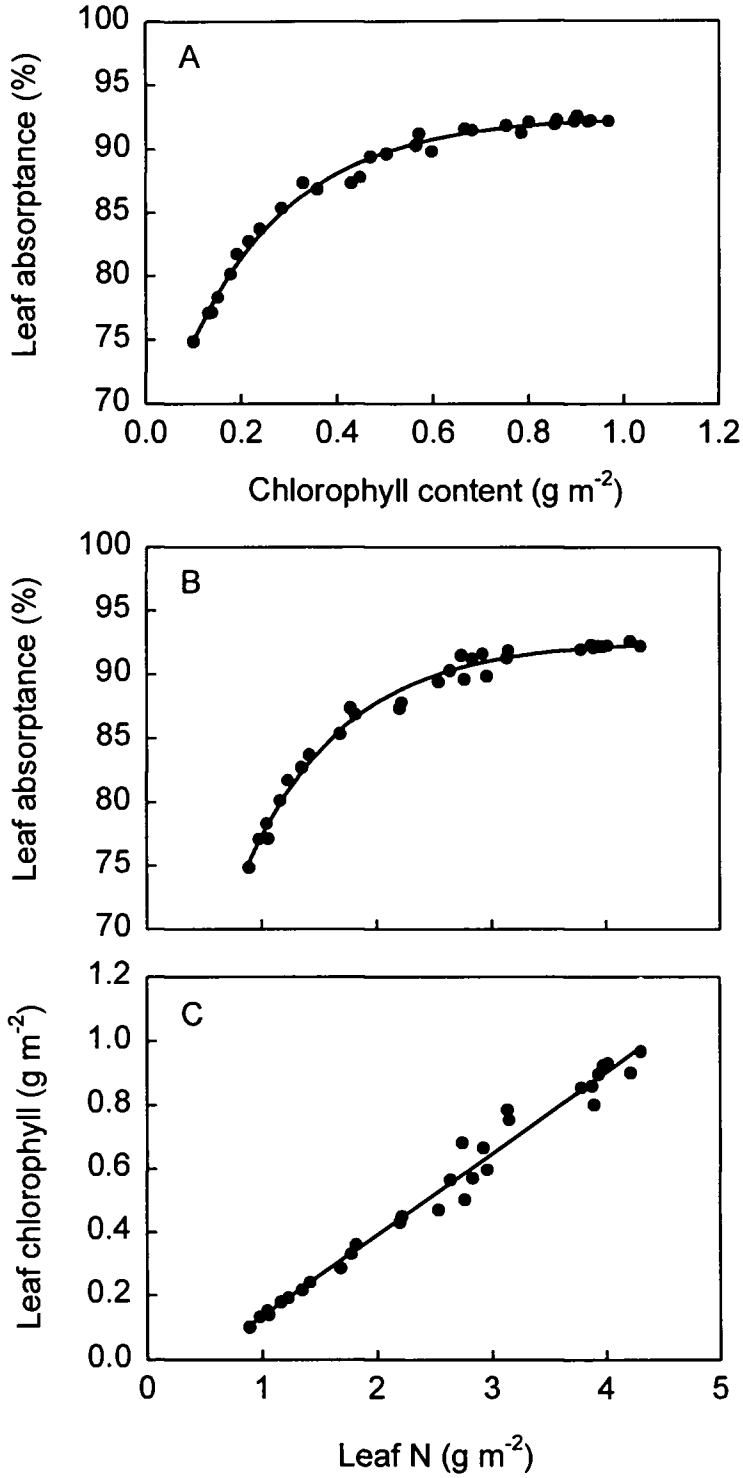


Figure 5-1. Leaf absorptance in relation to chlorophyll content (A) and nitrogen content (B), and the relationship between leaf N and chlorophyll content (C) in apple leaves. Regression equations: (A) $Y = 27.94(1 - e^{-4.594X}) + 64.58$ ($R^2 = 0.99$, $p = 0.0001$); (B) $Y = 48.39(1 - e^{-1.153X}) + 44.18$ ($R^2 = 0.99$, $p = 0.0001$); (C) $Y = -0.122 + 0.256X$ ($R^2 = 0.98$, $p = 0.0001$).

leaf chlorophyll content up to about 0.3 g m^{-2}, then began to level off with a further rise in leaf chlorophyll. Leaf absorptance also showed a curvilinear response to leaf N (Figure 5-1B) because chlorophyll content was linearly correlated with leaf N (Figure5-1C). Although leaf N content varied from 0.9 to 4.3 g m^{-2}, leaf absorptance only increased from 74.8 to 92.5%.

5.4.2 *Responses of CO_2 assimilation and electron transport to PFD, and excess absorbed PFD in relation to leaf N*

Figure 5-2 shows the response of CO_2 assimilation, actual PSII efficiency, and calculated total electron transport to incident PFD in relation to leaf N content. For all leaves, CO_2 assimilation increased with increasing PFD up to a saturation point, then showed little response to any further rise in PFD (Figure 5-2A). Actual PSII efficiency decreased with increasing PFD (Figure 5-2B). Calculated total electron transport showed a response to PFD similar to that of CO_2 assimilation (Figure 5-2C). At each given incident PFD, low N leaves had lower CO_2 assimilation, actual PSII efficiency, and total electron transport than did high N leaves. A linear relationship was found between actual PSII efficiency and CO_2 assimilation under light-saturated conditions (Figure 5-3A), and also between total electron transport and CO_2 assimilation (Figure 5-3B).

After accounting for leaf absorptance, CO_2 assimilation in response to absorbed PFD was still dependent on leaf N (Figure 5-4). Quantum yield for CO_2 assimilation remained relatively unchanged except for a decrease at the lower end of the leaf N range (Figure 5-5). The light saturation point, expressed as absorbed PFD, increased linearly with increasing leaf N first, then reached a plateau at a leaf N content of approximately 3 g m^{-2} (Figure 5-6A). At an incident PFD of 1500 μmol m^{-2} s^{-1}, the amount of excess

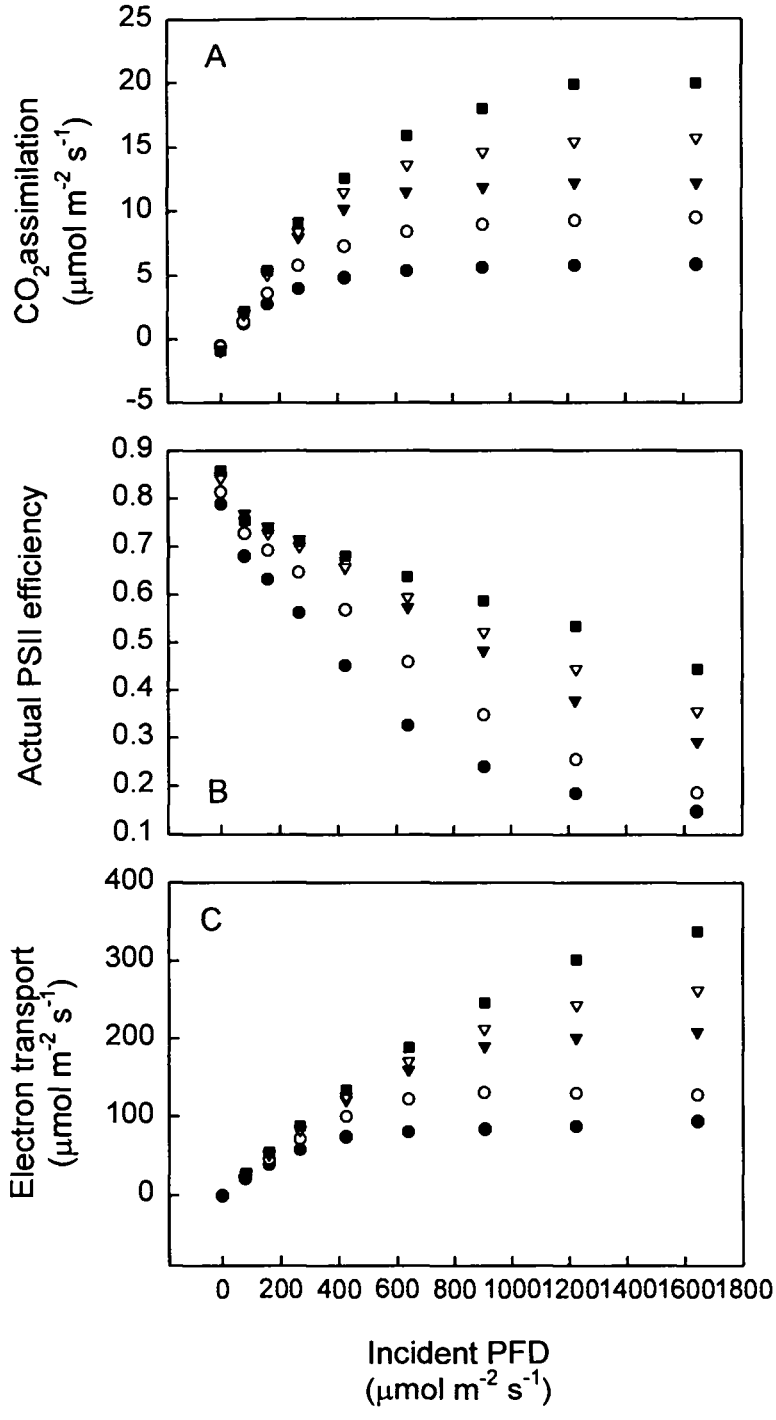


Figure 5-2. CO_2 assimilation (A), actual PSII efficiency (B), and electron transport (C) of apple leaves in response to incident photon flux density (PFD). Measurements were made at a CO_2 concentration of 350 ± 2 ppm, a leaf temperature of 25 ± 0.2 °C, and an ambient water vapor pressure of 1.55 ± 0.2 kPa. Leaf N content (g m^{-2}) is: 1.06 (●), 1.35 (○), 1.82 (▼), 2.77(▽), and 4.02 (■).

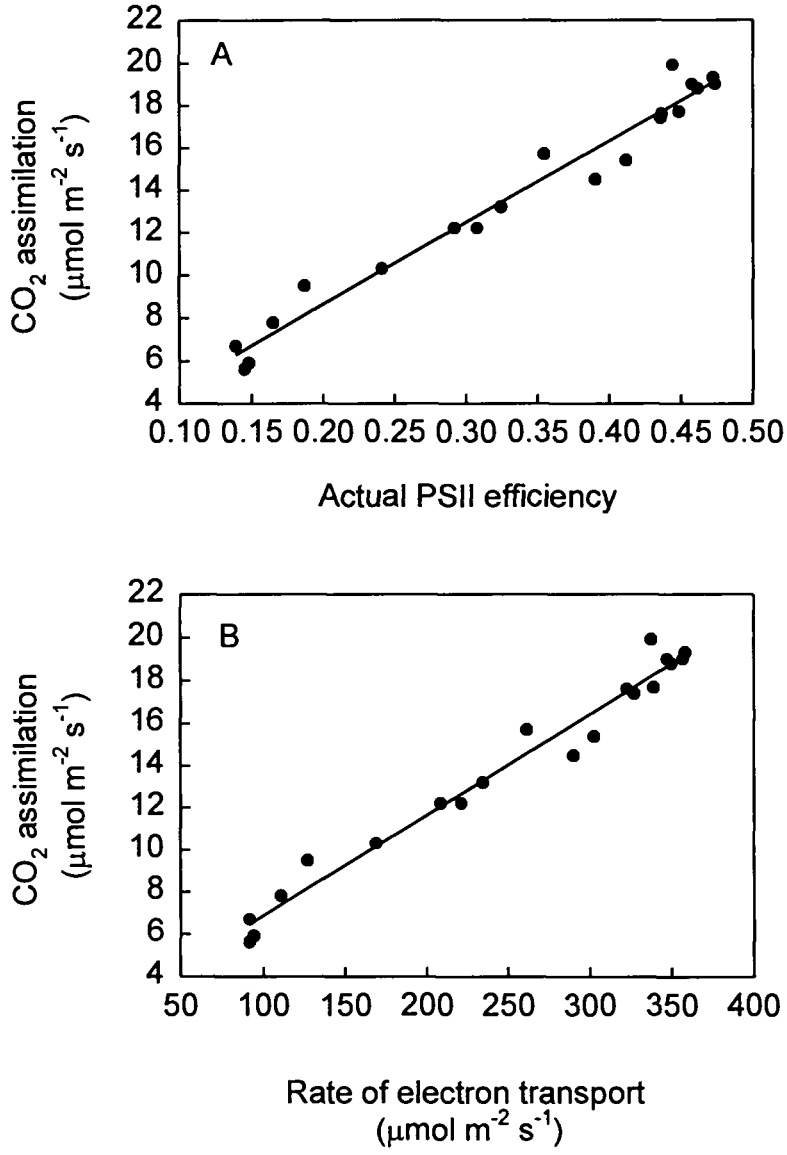


Figure 5-3. CO_2 assimilation in relation to actual PSII efficiency (A) and total electron transport (B) in apple leaves at an incident PFD of 1645 μmol m^{-2} s^{-1}. Regression equations: (A) Y = 0.98 + 38.28X (R^2 = 0.97, p = 0.0001); (B) Y = 2.11 + 0.0476X (R^2 = 0.97, p = 0.0001). Measurement conditions were the same as in Figure 5-2.

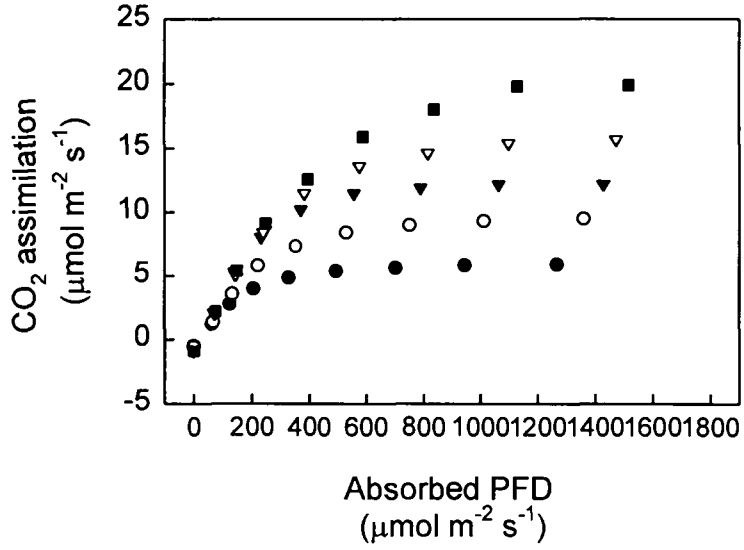


Figure 5-4. CO_2 assimilation of apple leaves in response to absorbed PFD. Leaf N content (g m^{-2}) is: 1.06 (●), 1.35 (○), 1.82 (▼), 2.77(▽), and 4.02 (■). Measurement conditions were the same as in Figure 5-2.

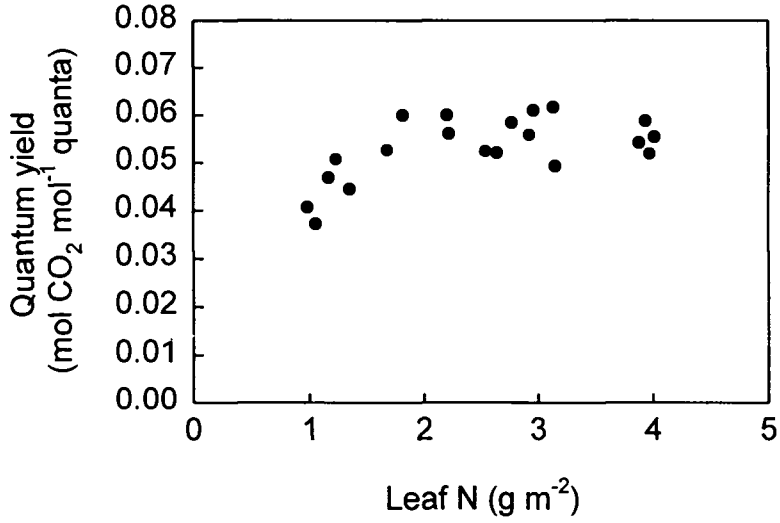


Figure 5-5. True quantum yield for CO_2 assimilation in relation to N content in apple leaves. Measurement conditions were the same as in Figure 5-2.

absorbed light (calculated as the difference between the actual absorbed PFD and the light saturation point expressed as absorbed PFD) decreased with increasing leaf N (Figure 5-6B).

5.4.3 *Chlorophyll fluorescence parameters in relation to leaf N*

Maximum PSII efficiency (Fv/Fm) of dark-adapted leaves remained relatively unchanged except for a slight drop at the lower end of the leaf N range (Figure 5-7A). Thermal dissipation of excitation energy, indicated by both non-photochemical quenching (NPQ) and the non-photochemical quenching coefficient (qN) decreased with increasing leaf N (Figure 5-7B, C). Correspondingly, the efficiency of excitation capture (Fv'/Fm') by open PSII reaction centers increased almost linearly with increasing leaf N up to nearly 3 g m^{-2}, then leveled off with a further rise in leaf N (Figure 5-7D). Photochemical quenching (qP) remained high except for a drop at the lower end of the leaf N range (Figure 5-7E). This corresponded with a low degree of closure of PSII (1-qP) over the leaf N range, except in extremely low N leaves (Figure 5-7G). Actual PSII efficiency increased linearly with increasing leaf N first, then leveled off with further increases in leaf N (Figure 5-7F). The fraction of absorbed light potentially used for singlet oxygen formation remained at approximately 10% over the leaf N range examined (Figure 5-7H).

5.5 Discussion

Light absorption is less in low N leaves, but those leaves still have more excess absorbed light than do high N leaves under high light conditions (Figure 5-6B). This is because of their low rate of electron transport. Non-photochemical quenching is

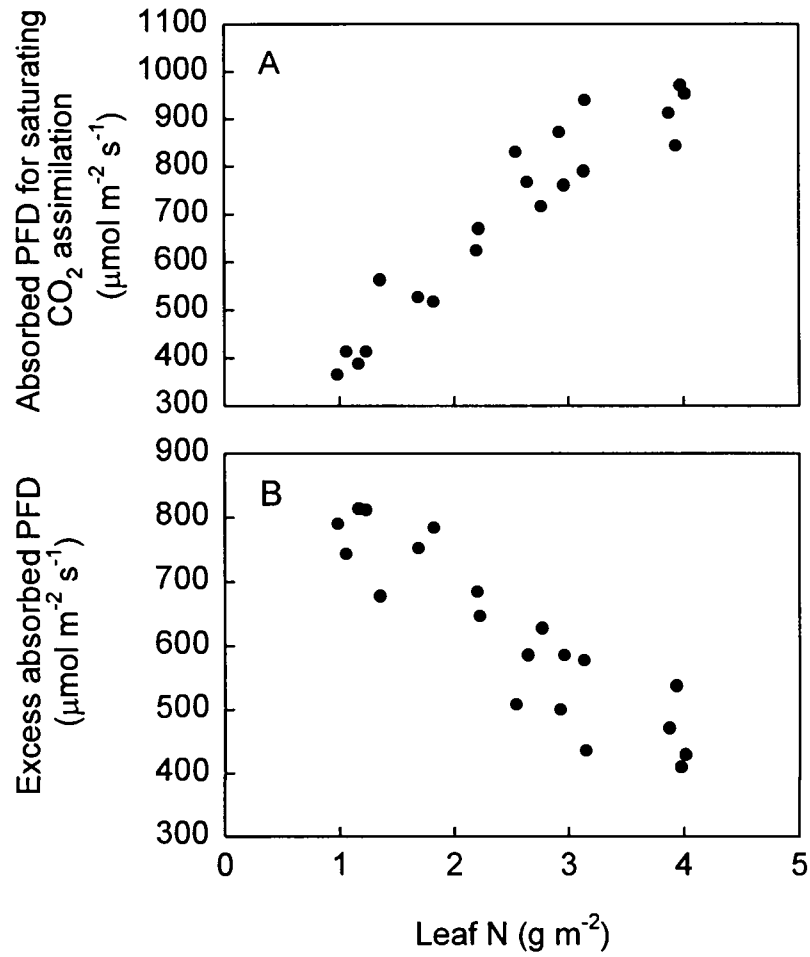
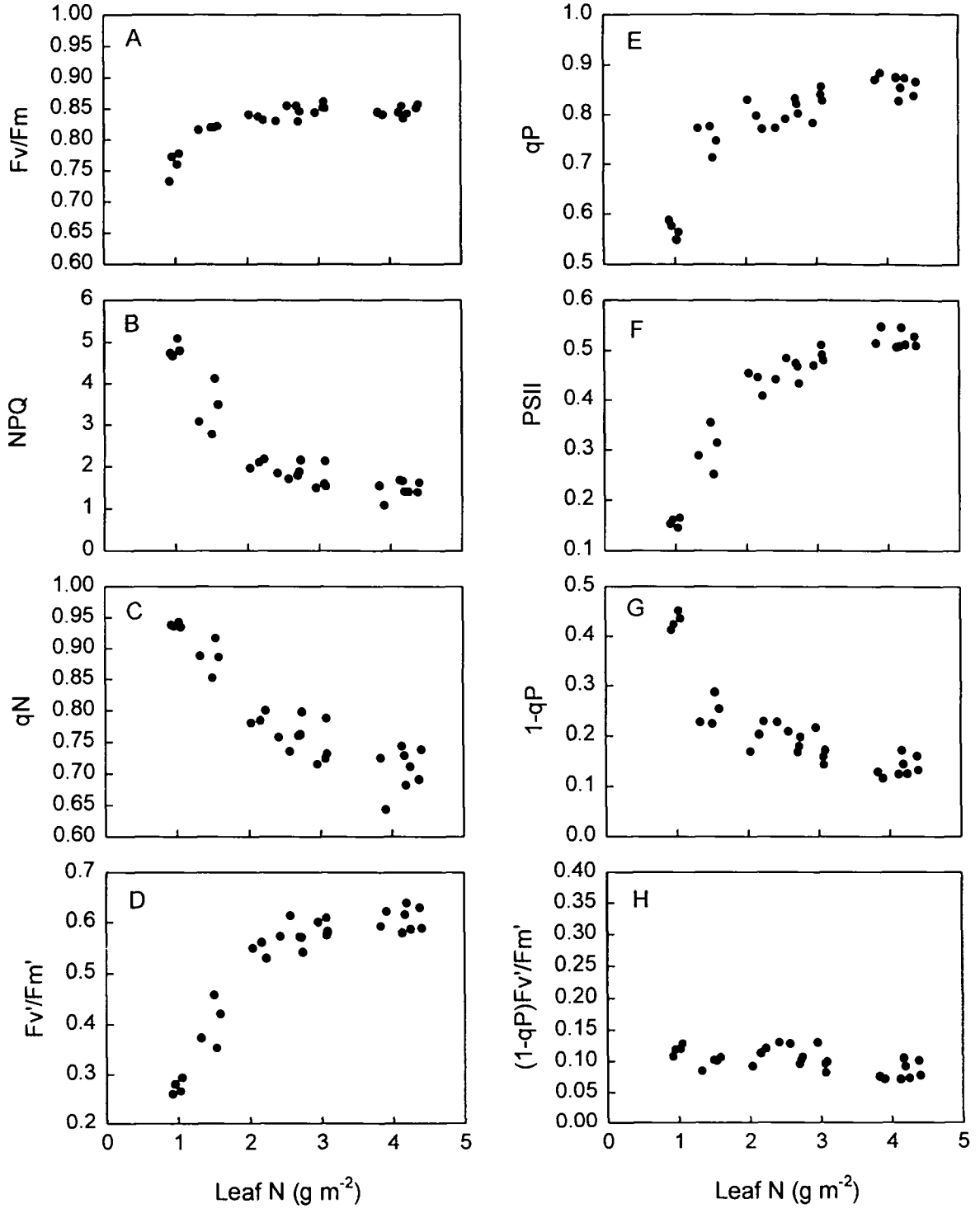


Figure 5-6. The relationship between leaf N and the light saturation point (A) expressed as absorbed PFD, and excess absorbed PFD (B) at an incident PFD of 1500 μmol m^{-2} s^{-1}. Excess absorbed PFD was calculated as the difference between the actual absorbed PFD and the light saturation point expressed as absorbed PFD. Measurement conditions were the same as in Figure 5-2

Figure 5-7. Chlorophyll fluorescence parameters in relation to N content in apple leaves. Maximum PSII efficiency (Fv/Fm) of dark-adapted leaves was measured pre-dawn on August 11, 1997. The remaining parameters were measured at PFD of 1500 ± 50 μmol m^{-2} s^{-1} and air temperature of 29 ± 1 °C at noon. NPQ: non-photochemical quenching (Fm/Fm'-1); qN: non-photochemical quenching coefficient (1-(Fm'-Fo')/(Fm-Fo)); Fv'/Fm': efficiency of excitation capture by open PSII centers ((Fm'-Fo)/Fm'); qP: photochemical quenching coefficient ((Fm'-Fs)/(Fm'-Fo')); PSII: actual PSII efficiency ((Fm'-Fs)/Fm'); 1-qP: degree of closure of PSII centers; (Fv'/Fm')(1-qP): the fraction of absorbed PFD potentially going into singlet oxygen formation.


A
Fv/Fm
B
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Leaf N (g m-2)

enhanced in low N leaves to reduce PSII efficiency (Figure 5-7B,C). This is consistent with the hypothesis that extra thermal dissipation reduced the probability of photo-oxidation by excess absorbed light in low N leaves under high light conditions.

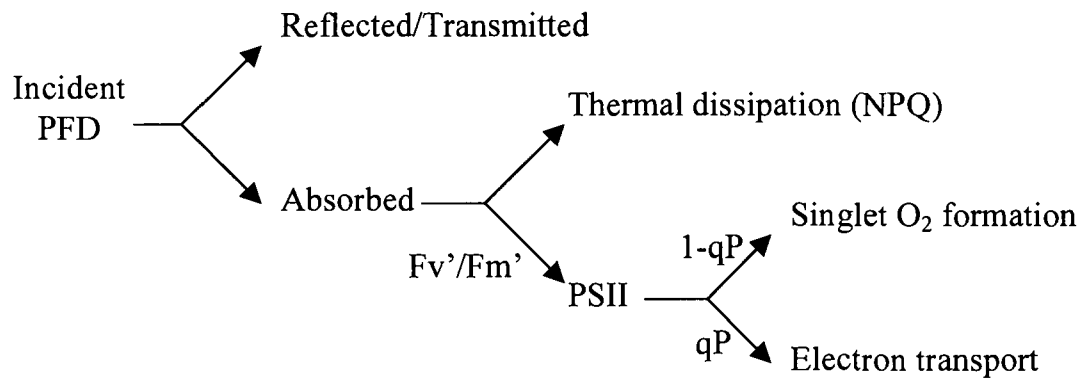

Figure 5-8. Light absorption and partitioning in apple leaves. Thermal dissipation of excitation energy is indicated by NPQ (non-photochemical quenching of chlorophyll fluorescence). Fv'/Fm' is the fraction of absorbed PFD that is delivered to open PSII centers. Of the PFD delivered to PSII, the proportion that is 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 PFD used in electron transport is the actual PSII efficiency (Fv'/Fm')qP (Genty et al., 1989). The percentage of absorbed light potentially going into singlet oxygen formation is estimated as (Fv'/Fm')(1-qP) (Demmig-Adams et al., 1996).

Light absorption and partitioning into different pathways in apple leaves can be depicted as in Figure 5-8. 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 PFD, either most or only a proportion of it is delivered to open PSII centers. At low PFDs, most of the absorbed light is utilized in photosynthetic electron transport; any

dissipation of excitation energy via an alternative pathway would decrease the efficiency of PSII. At high PFDs, however, not all the absorbed light can be used in photosynthetic electron transport, and the excess excitation energy has to be dissipated either through a harmless thermal process or by formation of toxic activated oxygen species (Demmig-Adams et al., 1996, 1997).

Apple plants 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). Decreased light absorption by reducing chlorophyll content is the first line of defense for protecting leaves from photo-oxidation in response to low N availability. This was suggested in studies with maize (Khamis et al., 1990) and spinach (Verhoeven et al., 1997), although leaf absorptance was not measured in either experiment. A decrease in chlorophyll content provides a coarse regulation mechanism for reducing light absorption at each given incident PFD. Decreased light absorption alone is not enough to cope with high light for low N leaves because light absorption is not proportionally reduced when leaf N decreases (Figure 5-1B). In our experiment, under high light conditions, low N leaves had more excess absorbed light than did high N leaves (Figure 5-6B) because photosynthetic electron transport used only a small percentage of the absorbed light.

Actual PSII efficiency is determined by both the photochemical quenching (qP) and the efficiency of excitation capture (Fv'/Fm') 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 (Genty et al., 1990; Demmig-Adams et al., 1995, 1996). 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, leading to increased formation of triplet excited chlorophyll (^{3}P680*), which has the potential to react with oxygen to form toxic singlet oxygen (Asada, 1996).

Thermal dissipation can safely remove excess excitation energy before it reaches PSII reaction centers, thus protecting PSII reaction 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 non-photochemical quenching, was enhanced in low N leaves in response to more excess absorbed light, compared with high N leaves under high light (Fig. 5-7B,C). This increase in thermal dissipation was so effective in decreasing the efficiency for delivering excitation energy to open PSII centers (Figure 5-7D) that the fraction of absorbed light potentially going into singlet oxygen formation remained at approximately 10% across the leaf N range (Figure 5-7H). 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-deficient plants than in N-replete controls (Khamis et al., 1990).

Under slowly developed water stress, non-photochemical 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 PFD, whether this results from increasing PFD 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 PFD and protect leaves from photo-oxidation (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, Fv/Fm of dark-adapted leaves remained relatively unchanged across the leaf N range (Figure 5-7A). This is consistent with the result of quantum yield of CO_2 assimilation in response to leaf N (Figure 5-5). This decrease in Fv/Fm 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, b; Verhoeven et al., 1996, 1997). When plants growing in natural gaps of a tropical forest were exposed to direct sunlight for 1-2 hours at midday, the decline in Fv/Fm 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 Fv/Fm of dark-adapted leaves after midday direct sun exposure may also result from sustained xanthophyll cycle activity (Thiele et al., 1998).

Fv/Fm of dark-adapted apple leaves was not affected by water stress, but CO_2 assimilation decreased (Massacci and Jones, 1990). Measuring Fv/Fm of dark-adapted leaves alone may not provide enough information on the CO_2 assimilation side of the photosynthesis process. The linear relationship between actual PSII efficiency ((Fm'-Fs)/Fm') and CO_2 assimilation suggests that actual PSII efficiency may better indicate the

rate of CO_2 assimilation at a fixed PFD 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 PFD conditions, diepoxide violaxanthin (V) is de-epoxidized via the monoepoxide antheraxanthin (A) to the epoxide-free form zeaxanthin (Z). When PFD decreases, epoxidation of Z occurs in the reverse direction to form V via A. Non-photochemical energy dissipation is closely correlated 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 de-epoxidase 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 et al., 1995; Gilmore, 1997).

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 Fv/Fm of dark-adapted leaves, the pool size and de-epoxidation state of the xanthophyll cycle at midday in *Clematis vitalba* grown at several PFDs (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 research attention.

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CHAPTER 6

THE RELATIONSHIP BETWEEN ACTUAL PHOTOSYSTEM II EFFICIENCY AND QUANTUM YIELD FOR CO_2 ASSIMILATION IS NOT AFFECTED BY NITROGEN CONTENT IN APPLE LEAVES

6.1 Abstract

Bench-grafted Fuji/M_{26} apple (*Malus domestica* Borkh) trees were fertigated with different concentrations of nitrogen by using a modified Hoagland's solution for 45 days. CO_2 assimilation and actual photosystem II (PSII) efficiency in response to incident photon flux density (PFD) were measured simultaneously in recent fully expanded leaves under low O_2 (2%) and saturated CO_2 (1300 ppm) conditions. A single curvilinear relationship was found between true quantum yield for CO_2 assimilation and actual PSII efficiency for leaves with a wide range of leaf N content. The relationship was linear up to a quantum yield of approximately 0.05 mol CO_2 mol^{-1} quanta, then became curvilinear with a further rise in quantum yield in response to decreasing PFD. This relationship was subsequently used as a calibration curve to assess the rate of linear electron transport associated with rubisco and partitioning of electron flow between CO_2 assimilation and photorespiration in different N leaves in response to intercellular CO_2 concentration (Ci) under normal O_2 conditions. Both the rate of linear electron flow, and the rate to CO_2 or O_2 increased with increasing leaf N at any given Ci, but the percentage of linear electron flow to CO_2 assimilation remained the same regardless of leaf N content. As Ci increased, the percentage of linear electron flow to CO_2 assimilation increased. In conclusion, the relationship between actual PSII efficiency and quantum yield for CO_2

assimilation and the partitioning of electron flow between CO_2 assimilation and photorespiration are not affected by N content in apple leaves.

6.2 Introduction

Light-driven photosynthetic electron transport provides reducing power for photosynthetic carbon reduction and photosynthetic carbon oxidation. Both these processes are associated with the activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco). Electron transport may also provide a source for alternative electron sinks such as nitrate reduction and direct reduction of O_2 in the Mehler reaction. Genty et al. (1989) first found a linear relationship between quantum yield for CO_2 assimilation and the product of photochemical quenching (qP) and the efficiency of excitation capture (Fv'/Fm') by open photosystem II (PSII) centers under non-photorespiratory conditions. This provides the basis for using qP×Fv'/Fm' (actual PSII efficiency) to monitor changes in quantum yield of linear electron transport *in vivo*. This relationship also indicates that CO_2 assimilation is the major sink for electron transport, and is closely related to PSII activity under non-photorespiratory conditions. The quantitative relationship between actual PSII efficiency and the quantum yield for CO_2 assimilation, developed under non-photorespiratory conditions, has subsequently been used as a calibration curve to estimate the rate of linear electron transport associated with rubisco and partitioning of electron flow between CO_2 assimilation and photorespiration under photorespiratory conditions (Cornic and Briantais, 1991; Cornic and Ghashghaie, 1991; Ghashghaie and Cornic, 1994; Habash et al., 1995).

The relationship between quantum yield for CO_2 assimilation and PSII efficiency has been studied extensively. Linear relationships have been reported in many species under non-photorespiratory conditions (Genty et al., 1989; Harbinson et al., 1990; Keiller and Walker, 1990; Krall and Edwards, 1990, 1991; Cornic and Briantais, 1991; Cornic and Ghashghaie, 1991; Krall et al., 1991; Ghashghaie and Cornic, 1994). The relationship between quantum yield for O_2 evolution and actual PSII efficiency was linear when gross O_2 evolution was measured using $^{18}O_2$ under photorespiratory conditions (Genty et al., 1992; Maxwell et al., 1998). However, the relationship between PSII efficiency and quantum yield for CO_2 assimilation or O_2 evolution became non-linear in the high quantum yield region or at low measuring PFD under non-photorespiratory conditions (Seaton and Walker, 1990; Öquist and Chow, 1992). Seaton and Walker (1990) reported a single curvilinear relationship between the apparent quantum yield for O_2 evolution and actual PSII efficiency at saturated CO_2 for 16 species including C_3, C_4 and CAM plants. Water stress or elevated CO_2 treatment did not change the relationship between PSII efficiency and quantum yield for CO_2 assimilation (Cornic and Ghashghaie, 1991; Habash et al., 1995). These findings suggest that the difference among species or caused by water stress or elevated CO_2 is small in the partitioning of electron flow to rubisco-associated CO_2 assimilation relative to alternative electron sinks.

Although considerable effort has been made to understand the relationship between quantum yield for CO_2 assimilation and actual PSII efficiency, very little is known about how leaf N affects this relationship. Khamis et al. (1990) found that N deficiency resulted in a small decrease in quantum yield for CO_2 assimilation at any given PSII efficiency. The decrease in light absorption caused by N limitation, however,

may not have been considered. Both *in vivo* rubisco activity and actual PSII efficiency are closely coordinated in apple (*Malus domestica* Borkh.) leaves in response to nitrogen. Although total rubisco activity increased with increasing leaf N, rubisco activation state decreased so that only a proportion of the total rubisco was engaged in photosynthesis in high N leaves (Chapter 4). Under high PFD conditions, the actual PSII efficiency of apple leaves was down-regulated by increased thermal dissipation in response to decreasing leaf N, to match rubisco activity (Chapter 5). Both CO_2 assimilation and actual PSII efficiency were curvilinearly related to leaf N, with a linear relationship between actual PSII efficiency and CO_2 assimilation in response to leaf N (Chapter 5). This suggests that the *in vivo* balance between electron transport and rubisco activity, and the ratio between CO_2 assimilation and photorespiration remain unchanged.

The objectives of this study were to (1) determine the relationship between actual PSII efficiency and quantum yield for CO_2 assimilation in response to leaf N under non-photorespiratory conditions; and (2) assess the partitioning of linear electron flow between CO_2 assimilation and photorespiration in relation to N in apple leaves.

6.3 Materials and Methods

6.3.1 *Plant material*

'Fuji' apple (*Malus domestica* Borkh.) trees on M_{26} rootstocks were used. Bench-grafting was done in late March, and each grafted tree was immediately put into a 3.8-L pot containing a mixture of peat moss, pumice and sandy loam soil (1:2:1 by volume). The plants were grown in a lathhouse until early June. During this period, they were fertigated with 150 ppm N using Plantex® 20-10-20 with micronutrients (Plantex Corp.,

Ontario, Canada) every two weeks, beginning from budbreak in early May. When new shoots were approximately 15 cm long, plants were selected for uniformity, and moved out to full sunlight. Thereafter, they were fertigated weekly with Plantex® for three weeks. Beginning June 30, plants were fertigated twice weekly with one of seven levels of N concentrations (0, 2.5, 5.0, 7.5, 10.0, 15, or 20 mM N from NH_4NO_3) by applying 300 ml of a modified Hoagland's solution to each pot (Chapter 3). Irrigation was provided from a saucer placed at the bottom of each pot. After 45 days, recent fully expanded leaves were chosen for gas exchange and chlorophyll fluorescence measurements.

6.3.2 *Measurements of gas exchange and chlorophyll fluorescence*

CO_2 assimilation and chlorophyll fluorescence were measured simultaneously using a system that combined a CIRAS-1 gas exchange system (PP Systems, Herts., UK) and an FMS-1 pulse-modulated fluorometer (Hansatech Instruments Ltd., Norfolk, UK). The light- and temperature-controlled cuvette of the CIRAS-1 system was modified as follows: the fiber optic of the FMS-1 was inserted into the cuvette at a 60° angle without significantly interfering with PFD distribution at the leaf surface, yet it allowed for delivery of a saturation pulse of actinic light and detection of fluorescence signals. All gas exchange measurements were made at a leaf temperature of 25 ± 0.2 °C.

The relationship between the quantum yield for CO_2 assimilation and actual PSII efficiency was studied by altering incident PFD. Measurements of CO_2 assimilation and chlorophyll fluorescence parameters in response to PFD were made in the following order, at incident PFD of 1800, 1500, 1200, 900, 600, 400, 250, 150, or 75 μmol m^{-2} s^{-1} at low O_2 (2%) and saturated CO_2 (1300 ppm). Because the two light sensors in the cuvette

were not located exactly at the leaf surface, the actual corresponding incident PFDs at the leaf surface level were 1990, 1614, 1242, 891, 593, 398, 255, 160, or 87 μmol m^{-2} s^{-1}, as measured with a recently calibrated LI-190SA quantum sensor (Li-Cor Inc., Lincoln, Nebraska, USA). At each PFD, CO_2 assimilation, stomatal conductance and steady state fluorescence (Fs) were monitored to ensure that they reached a steady state before a reading was taken. Maximal fluorescence under light exposure (Fm') was obtained by imposing a 1 second saturating flash of approximately 18,000 μmol m^{-2} s^{-1} PFD at the end of the fiber optic to the leaf in order to reduce all the PSII centers. Minimal fluorescence (Fo') under light exposure was determined by covering the cuvette with a black cloth while a far-red light was switched on to rapidly oxidize PSII by drawing electrons from PSII to PSI. Maximal fluorescence (Fm) and minimal fluorescence (Fo) of dark-adapted leaves were measured pre-dawn.

Non-photochemical quenching (NPQ) was expressed as Fm/Fm'-1 (Stern-Volmer quenching, see Bilger and Björkman, 1990). The efficiency of excitation capture by open PSII centers (Fv'/Fm') was calculated as (Fm'-Fo')/Fm', which is also referred to as the efficiency with which excitation energy is delivered to open PSII centers. The photochemical quenching coefficient qP was defined as (Fm'-Fs)/(Fm'-Fo'). Actual PSII efficiency was (Fv'/Fm')qP = (Fm'-Fs)/Fm' (Genty et al., 1989). Quantum yield for CO_2 assimilation was calculated as gross CO_2 assimilation (A+Rd) divided by either incident PFD or absorbed PFD. Rd was day respiration under light from processes other than photorespiration, which was approximated as dark respiration for this experiment. Leaf absorptance was measured with an LI-1800 Spectrophotometer with a 1800-12S Integrating Sphere (Chapter 5).

Partitioning of linear electron flow between CO_2 assimilation and photorespiration in relation to leaf N was studied by looking at responses of CO_2 assimilation and actual PSII efficiency to intercellular CO_2 concentrations (Ci) at normal O_2 (21%) conditions. Response curves of CO_2 assimilation and actual PSII efficiency to Ci were constructed at an incident PFD of 1000 μmol m^{-2} s^{-1} by altering air CO_2 concentrations (Ca) from 50 to 1200 ~ 1400 ppm in 9 or 10 steps, until the highest Ci reached approximately 1000 ppm. At each Ca, readings of CO_2 assimilation, stomatal conductance, and steady state fluorescence were taken after they all reached steady state. Actual PSII efficiency was measured as described above.

Rate of linear electron transport and its partitioning to CO_2 assimilation and photorespiration were calculated according to Ghashghaie and Cornic (1994). Briefly, the quantitative relationship between quantum yield for CO_2 assimilation and actual PSII efficiency, developed under non-photorespiratory conditions, was used as a calibration curve. Quantum yield for CO_2 assimilation (Φ_{CO2}) obtained from the calibration curve corresponding to the actual PSII efficiency measured under photorespiratory conditions allows the rate of linear electron flow (J_L) to be calculated as $J_L=\Phi_{CO2}\times PFD\times 4$. The rate of linear electron flow to CO_2 assimilation (J_A) and to photorespiration (Jo) were calculated as $J_A=4(A+Rd)$, and $Jo= J_L - J_A$, respectively.

6.3.3 *Leaf N content*

After all the above measurements, leaf area was determined with LI-3000 Area Meter. Leaves were frozen at –80 °C, then freeze-dried. N content was determined via the Kjeldahl method (Horneck et al., 1989).

6.4 Results

6.4.1 *The relationship between quantum yield for CO_2 assimilation and actual PSII efficiency under non-photorespiratory conditions*

Figure 6-1 shows CO_2 assimilation, apparent quantum yield for CO_2 assimilation and actual PSII efficiency in response to incident PFD. As PFD increased, CO_2 assimilation increased almost linearly first, then reached a saturation point (Figure 6-1A), beyond which CO_2 assimilation showed little response to increasing PFD. Both apparent quantum yield for CO_2 assimilation (Figure 6-1B) and actual PSII efficiency (Figure 6-1C) decreased with increasing PFD. At each given PFD, CO_2 assimilation, apparent quantum yield for CO_2 assimilation, and actual PSII efficiency all decreased as leaf N decreased (Figure 6-1).

Chlorophyll fluorescence quenching parameters in response to incident PFD are shown in Figure 6-2. As PFD increased, non-photochemical quenching (NPQ) increased, then tended to level off with further increases in PFD (Figure 6-2A). Correspondingly, the efficiency of excitation capture by open PSII centers (Fv'/Fm') decreased with increasing PFD (Figure 6-2B). The photochemical quenching coefficient (qP) also decreased with increasing PFD (Figure 6-2C). At each given PFD, NPQ increased with decreasing leaf N (Figure 6-2A), resulting in a corresponding decline in Fv'/Fm' (Figure 6-2B); qP also decreased with decreasing leaf N content (Figure 6-2C). The decline in both Fv'/Fm' and qP in response to increasing PFD led to the fall in actual PSII efficiency shown in Figure 6-1C. At each given PFD, the decrease in both Fv'/Fm' and qP contributed to a decline in actual PSII efficiency with decreasing leaf N content (Figure 6-1C).

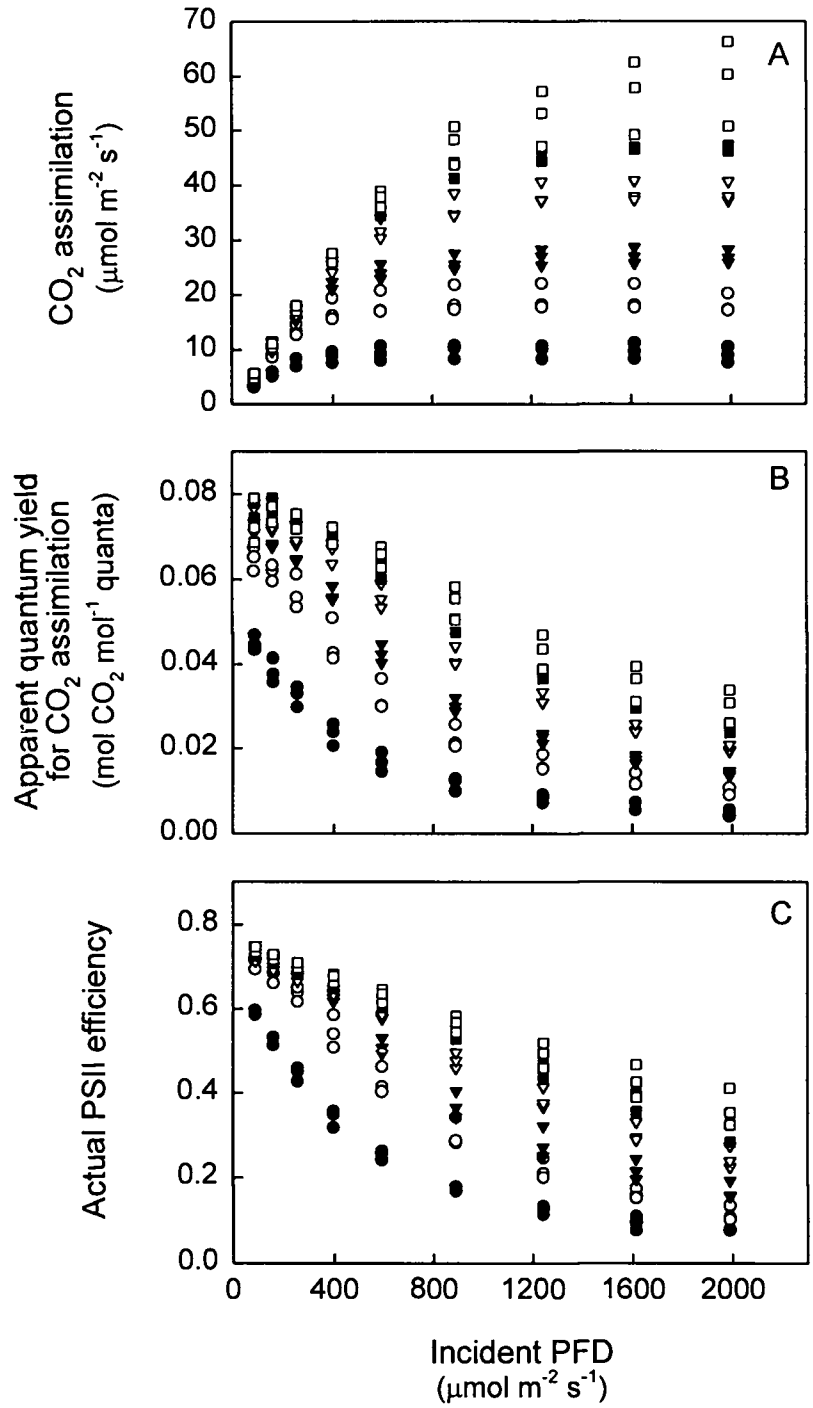

Figure 6-1. CO_2 assimilation (A), apparent quantum yield for CO_2 assimilation (B), and actual PSII efficiency (C) of apple leaves in response to incident photon flux density (PFD) under low O_2 (2%) and saturated CO_2 (1300 ppm) conditions. Measurements were made at a leaf temperature of 25 ± 0.2 °C and an ambient water vapor pressure of 1.37 ± 0.15 kPa. Leaf N content (g m^{-2}) is: 1.022 ± 0.077 (●); 1.566 ± 0.065 (○); 1.802 ±0.10 (▼); 2.361 ± 0.031 (▽); 3.227 ± 0.011 (■); and 4.044 ± 0.207 (□).

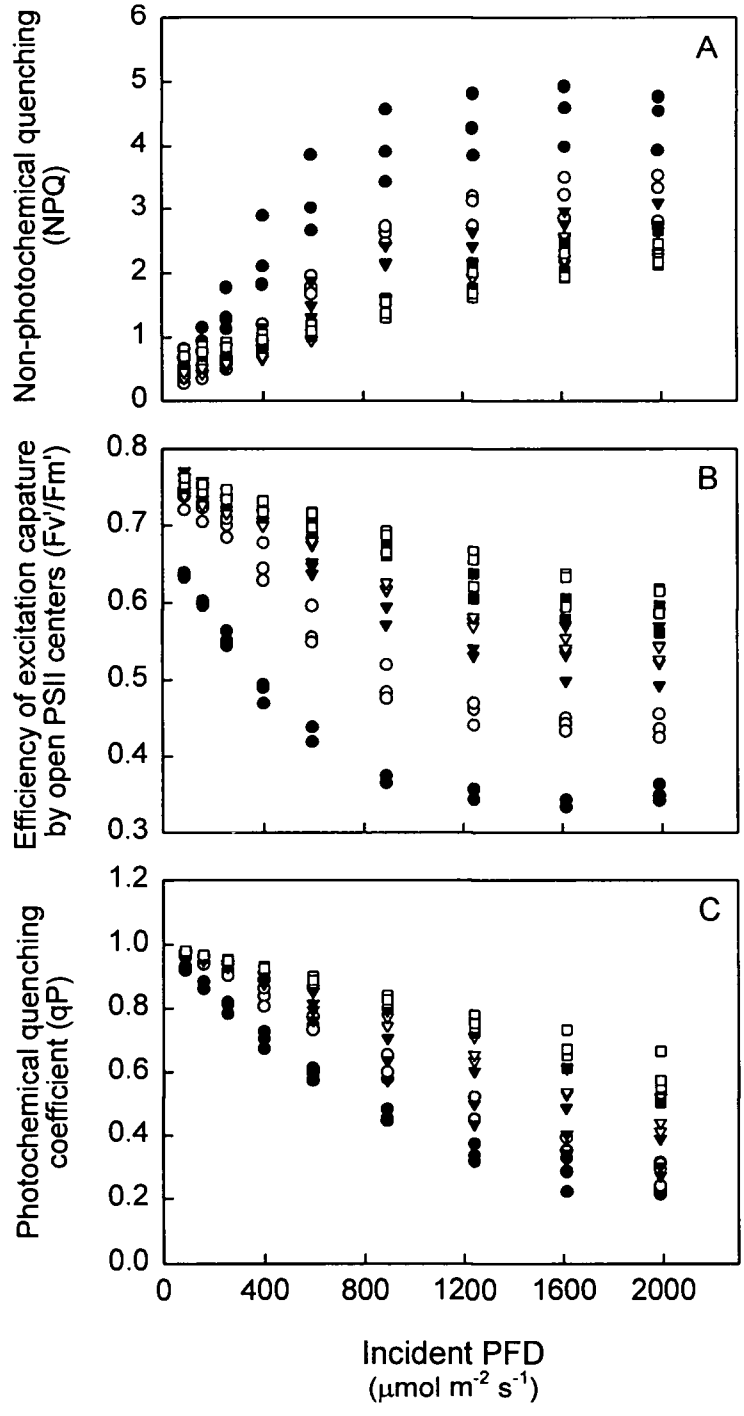

Figure 6-2. Non-photochemical quenching (A), efficiency of excitation capture by open PSII centers (B), and photochemical quenching (C) of apple leaves in response to incident photon flux density (PFD) under low O_2 (2%) and saturated CO_2 (1300 ppm) conditions. Measurement conditions and symbols were the same as in Figure 6-1.

The variation in each fluorescence parameter (NPQ, Fv'/Fm', and qP) caused by leaf N (Figure 6-2) was considerably reduced when each was plotted against the apparent quantum yield for CO_2 assimilation (Figure 6-3). Curvilinear relationships to apparent quantum yield for CO_2 assimilation were seen for NPQ (Figure 6-3A), Fv'/Fm' (Figure 6-3B), and qP (Figure 6-3C). In response to rising PFD or decreasing leaf N, NPQ increased. This resulted in a decrease in Fv'/Fm'. A decline in qP also contributed to the lowering of quantum yield for CO_2 assimilation in response to rising PFD or decreasing leaf N.

A close relationship was found between quantum yield for CO_2 assimilation and actual PSII efficiency (Figure 6-4). At any given PSII efficiency, low N leaves had a slightly lower apparent quantum yield for CO_2 assimilation (Figure 6-4A). However, when quantum yield was expressed on an absorbed PFD basis (i.e., true quantum yield), all the data points from leaves with different N contents nearly fell into a single line (Figure 6-4B). The relationship was linear up to a true quantum yield of approximately 0.05 mol CO2 mol^{-1} quanta, and then became curvilinear with a further rise in quantum yield in response to decreasing PFD.

6.4.2 *Partitioning of electron flow to CO_2 assimilation and photorespiration in response to intercellular CO_2 concentration (Ci)*

As Ci increased, CO_2 assimilation increased linearly first, then reached a plateau (Figure 6-5A). Both the initial slope and the maximum CO_2 assimilation increased with increasing leaf N. Actual PSII efficiency showed a response to Ci similar to that of CO_2 assimilation, but reached a plateau at a lower Ci (Figure 6-5B). This was most obvious for high N leaves. Using the curve in Figure 6-4B, it is possible to estimate the rate of

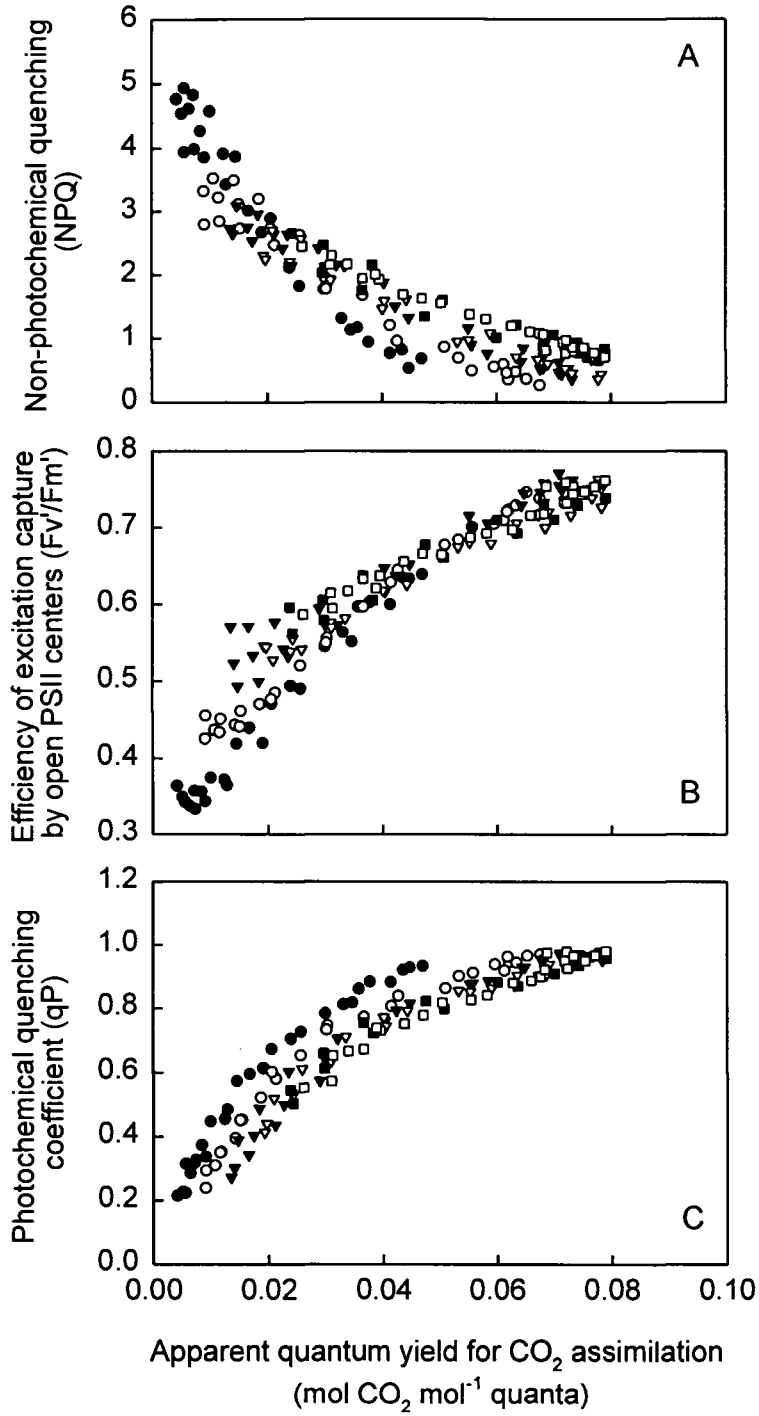

Figure 6-3. Apparent quantum yield for CO_2 assimilation in relation to chlorophyll fluorescence quenching parameters in apple leaves under low O_2 (2%) and saturated CO_2 (1300 ppm) conditions. A: Non-photochemical quenching (NPQ); B: Efficiency of excitation capture by open PSII centers (Fv'/Fm'); and C: Photochemical quenching coefficient (qP). Measurement conditions and symbols were the same as in Figure 6-1.

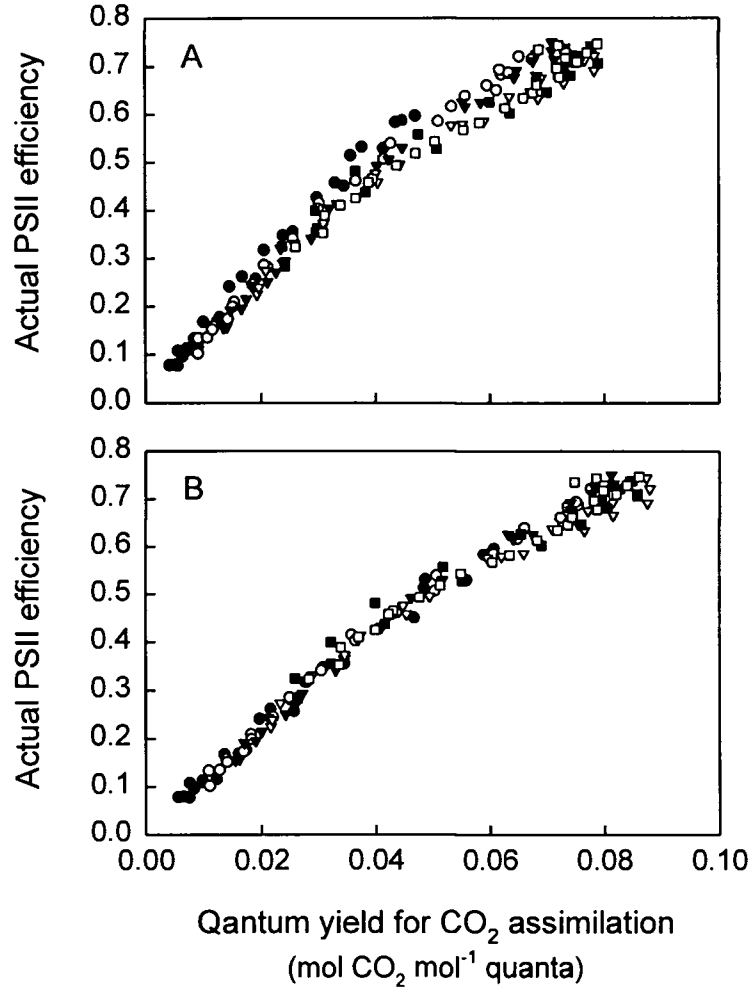

Figure 6-4. Actual PSII efficiency in relation to apparent quantum yield (A) and true quantum yield (B) for CO_2 assimilation in apple leaves under low O_2 (2%) and saturated CO_2 (1300 ppm) conditions. Measurement conditions and symbols were the same as in Figure 6-1.

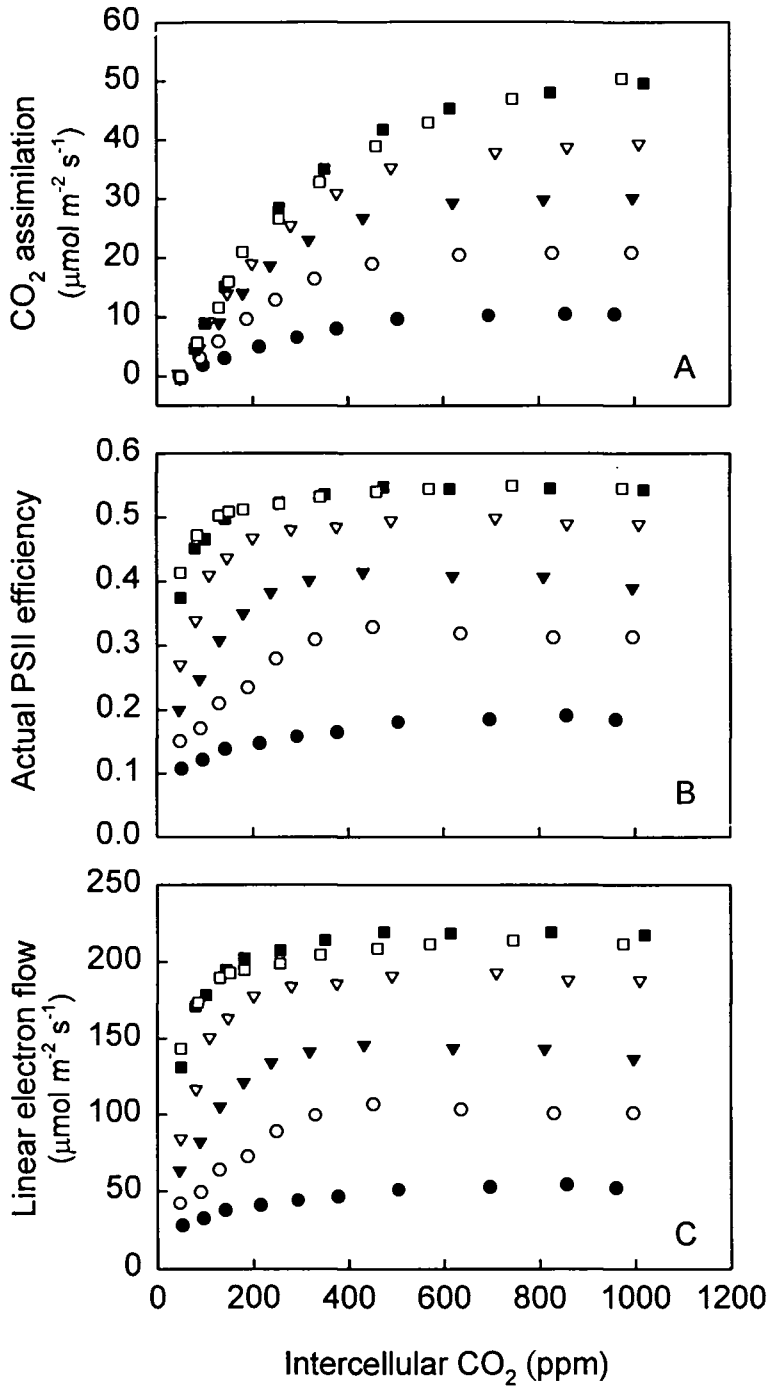

Figure 6-5. CO_2 assimilation (A), actual PSII efficiency (B), and total linear electron transport (C) of apple leaves in response to intercellular CO_2 concentration at 21% O_2. Measurements were made at an incident PFD of 1000 μmol m^{-2} s^{-1}, a leaf temperature of 25± 0.2 °C, and an ambient water vapor pressure of 1.45±0.1 kPa. Leaf N content (g m^{-2}) is: 1.10 (●); 1.53 (○); 1.99 (▼); 2.32 (▽); 3.22 (■); and 3.81 (□).

linear electron transport associated with rubisco under photorespiratory conditions. Linear electron transport followed almost the same pattern as actual PSII efficiency in response to Ci. At any given Ci, linear electron transport increased with increasing leaf N (Figure 6-5C).

Linear electron flow to CO_2 showed the same response to Ci as had CO_2 assimilation (Figure 6-6A). As Ci increased, electron flow to CO_2 increased almost linearly first, then leveled off with a further rise in Ci. At any given Ci, electron flow to CO_2 was higher in high N leaves than in low N leaves, although the difference was smaller at low rather than high Ci (Figure 6-6A). Linear electron flow to O_2 generally decreased as Ci increased (Figure 6-6B). The difference in electron flow to O_2 at different N contents was larger at lower than at higher Ci. When Ci dropped below about 100 ppm, a decrease in linear electron flow to O_2 was noticed in medium to high N leaves, but not in low N leaves (Figure 6-6B). When the electron flow to CO_2 was expressed as a percentage of linear electron flow, all data for leaves with different N contents nearly fell on one curve (Figure 6-6C). As Ci increased, an increasing proportion of the linear electron flow was partitioned to CO_2 assimilation.

6.5 Discussion

Photosynthetic electron transport drives both rubisco-associated CO_2 assimilation and photorespiration, and also supplies electrons to other alternative electron sinks. We found a single curvilinear relationship between true quantum yield for CO_2 assimilation and actual PSII efficiency in apple leaves with different N contents under non-photorespiratory conditions (Figure 6-4B). Based on this relationship, we looked at

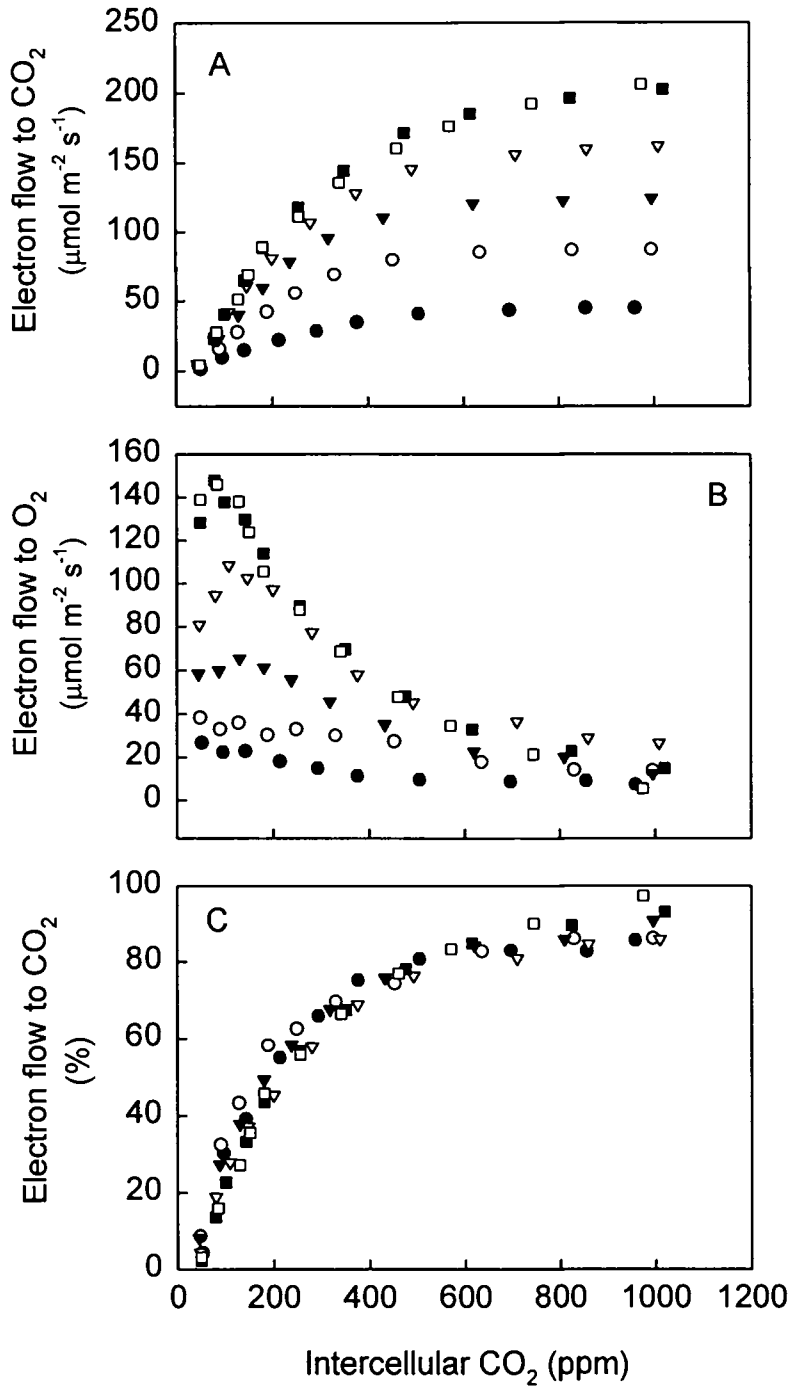

Figure 6-6. Partitioning of total linear electron flow to CO_2 assimilation and photorespiration of apple leaves in response to intercellular CO_2 concentration at 21% O_2. A: Electron flow to CO_2; B: Electron flow to O_2; C: Percentage of electron flow to CO_2. Measurement conditions and symbols were the same as in Figure 6-5.

partitioning of linear electron flow between CO_2 assimilation and photorespiration in response to Ci, as affected by leaf N. At any given Ci, the percentage of total linear electron flow to CO_2 assimilation remained unchanged regardless of leaf N content (Figure 6-6C). The relationship between actual PSII efficiency and quantum yield for CO_2 assimilation under non-photorespiratory conditions, and the partitioning of linear electron flow between CO_2 assimilation and photorespiration at normal O_2 conditions, therefore, are not affected by N content in apple leaves.

The slope of the relationship between quantum yield for CO_2 assimilation and actual PSII efficiency depends on the partitioning of linear electron flow between rubisco-associated CO_2 assimilation and other alternative electron sinks under non-photorespiratory conditions. At any given PSII efficiency, if other electron sinks use more reducing power, the quantum yield for CO_2 assimilation would be reduced.

Among the alternative electron sinks, nitrate reduction can consume up to 8% of the total linear electron flow (Evans, 1987). In addition to serving as a substrate in photorespiration, O_2 also functions as an electron acceptor in the Mehler reaction (Badger, 1985). Although nitrate reduction primarily occurs in the root system of apple trees, nitrate reductase activity has been detected in leaves as the nitrate supply increases (Lee and Titus, 1992). This altered proportion of nitrate reduction in apple leaves in response to N supply, or a possible increase of the Mehler reaction in low N leaves under high light, could change the slope of the relationship. However, we found a single curvilinear relationship between actual PSII efficiency and true quantum yield for CO_2 assimilation in apple leaves with different N contents. This indicates that leaf N content does not change the partitioning of linear electron flow to rubisco-associated CO_2

assimilation relative to alternative electron sinks under non-photorespiratory conditions. The observed decrease in apparent quantum yield for CO_2 assimilation at any given PSII efficiency was mainly caused by failing to account for the decrease in light absorption in low N leaves.

The curvilinear relationship we found between quantum yield for CO_2 assimilation and actual PSII efficiency is similar to that noted by Seaton and Walker (1990) and Öquist and Chow (1992). Although the exact cause for this nonlinearity is unclear, several factors may contribute. They can be divided into two categories: (1) physiological factors associated with PSII centers and electron transport, and (2) those related to measurement techniques.

By using isolated chloroplasts, Hormann et al. (1994) demonstrated that a fraction of PSII centers contributed substantially to quantum yield at low PFD. The closure of these centers with increasing PFD corresponded to only a small decrease in PSII efficiency. This suggests that PSII heterogeneity may be responsible for the observed non-linearity (Hormann et al.,1994; Schreiber et al., 1995). Changes in electron cycling around PSII, and partitioning of linear electron flow to processes other than CO_2 assimilation, could possibly cause non-linearity (Genty et al., 1989). Because accurate measurement of day respiration under light is difficult (Brooks and Farquhar, 1985), dark respiration has often been taken as day respiration to develop the relationship between PSII efficiency and quantum yield for CO_2 assimilation. This could potentially cause non-linearity, as shown by Oberhuber et al. (1993). In addition, non-linearity could occur if chlorophyll fluorescence and gas exchange measurements were monitoring different populations of leaf cells.

We clearly saw the effects of non-photochemical and photochemical quenching on quantum yield for CO_2 assimilation. Increased NPQ in response to increasing incident PFD or decreasing leaf N caused a decline in quantum yield for CO_2 assimilation by decreasing the efficiency with which excitation energy was delivered to open PSII centers (Figure 6-2A, B, 6-3A, B). At high PFD, especially in low N leaves when NPQ reached its maximum activity (Figure 6-2A), Fv'/Fm' decreased to its minimum and no longer responded to increasing PFD (Figure 6-2B). As qP decreased in response to increasing PFD or decreasing leaf N (Figure 6-2C), actual PSII efficiency was reduced (Figure 6-1C). Therefore, it is both non-photochemical and photochemical quenching that determine actual PS II efficiency (Genty et al., 1989).

The quantitative relationship between PSII efficiency and CO_2 assimilation that developed under non-photorespiratory conditions served as a calibration curve in our experiment. This curve was used to estimate the rate of linear electron transport associated with rubisco and the partitioning of linear electron flow between CO_2 assimilation and photorespiration under photorespiratory conditions, assuming that alternative electron sinks accounted for the same proportion of total linear electron transport under both photorespiratory and non-photorespiratory conditions. Both the rate of linear electron flow associated with rubisco and the rate of electron flow to CO_2 and to O_2 increased with increasing leaf N at any given Ci. However, the percentage of linear electron flow to CO_2 assimilation remained the same regardless of leaf N content. This indicates that electron partitioning between CO_2 assimilation and photorespiration is not affected by leaf N content. For a given Ci, apple leaves with different N contents may be operating at a similar CO_2 concentration at the carboxylation site of rubisco. This is

because the ratio of carboxylation to oxygenation is mainly determined by the actual concentrations of CO_2 and O_2 within the chloroplasts at a given temperature and atmospheric pressure (Brooks and Farquhar, 1985; Jordan and Ogren, 1984). Consequently, the ratio of CO_2 assimilation to CO_2 transfer conductance from the intercellular air space to the carboxylation site within chloroplasts is expected to remain the same in apple leaves with different N contents at any given Ci (Evans and von Caemmerer, 1996).

The partitioning of linear electron flow between CO_2 assimilation and photorespiration in response to Ci in apple leaves found in our experiment is consistent with the result obtained by measuring $^{18}O_2$ uptake and net $^{16}O_2$ evolution in leaves of C_3 plants (Badger, 1985; Canvin et al., 1980; Gerbaud and Andre, 1980). In these studies, a decrease in O_2 uptake at low CO_2 concentrations was reported. It is interesting that we found a decrease in electron flow to O_2 when Ci dropped below about 100 ppm in medium to high N leaves, but not in low N leaves in this experiment. This decrease could be caused by deactivation of rubisco at low CO_2 concentrations (Badger, 1985). Indeed, rubisco activation state falls substantially when Ci drops below 100 ppm (von Caemmerer and Edmondson, 1986; Sage et al., 1990). The differential response of linear electron flow to O_2 at low Ci in apple leaves with different N contents in this experiment could be caused by the difference in the amount of active rubsico. In low N leaves, there is a very limited amount of rubisco and all the rubisco is active at ambient CO_2 (Chapter 4). When Ci decreases to below 100 ppm, there may still be enough CO_2 to keep all the rubisco activated in low N leaves. In contrast, the same low Ci may activate only a percentage of the rubisco in medium to high N leaves.

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CHAPTER 7

DISSERTATION SUMMARY

This research was initiated to help understand the relationship between N content and photosynthesis in apple leaves. Bench-grafted 'Fuji/M_{26}' trees were grown in full sun and leaf N content was altered by fertigating with different N concentrations using a modified Hoagland's solution. Four aspects of the relationship between leaf N content and photosynthesis were studied: 1) gas exchange; 2) rubisco activities and activation state; 3) light absorption and partitioning; and 4) the relationship between quantum yield for CO_2 assimilation and actual PSII efficiency.

CO_2 assimilation capacity was shown to be closely related to N content in apple leaves. Curvilinear relationships were found between leaf N and 1) light-saturated CO_2 assimilation at ambient CO_2, 2) the initial slope of the response of CO_2 assimilation (A) to intercellular CO_2 concentration (Ci), and 3) CO_2-saturated photosynthesis. All three initially increased linearly with increasing leaf N, then reached a plateau at a leaf N content of approximately 3 g m^{-2}. The curvilinear relationship between leaf N and CO_2 assimilation indicates that photosynthesis is less limited by N with increasing leaf N content. Analysis of A/Ci curves obtained under saturating light demonstrated that CO_2 assimilation at ambient CO_2 fell into the linear region of the A/Ci curves regardless of leaf N status.

Rubisco initial activity, total activity and activation state were influenced by leaf N content. Total rubisco activity increased linearly with increasing leaf N. Initial rubisco activity, however, showed a curvilinear response to leaf N. Rubisco activation state decreased with increasing leaf N. Both light-saturated CO_2 assimilation at ambient CO_2

and the initial slope of the A/Ci curves were linearly related to initial rubisco activity, but were curvilinearly related to total rubisco activity. Decreased rubisco activation state with increasing leaf N, therefore, accounted for the curvilinear relationship between leaf N and CO_2 assimilation. As leaf N increased, the percentage of rubisco that was not involved in photosynthesis increased. This resulted in a decrease in photosynthetic N use efficiency. The surplus rubisco that accumulated in apple leaves under a high N supply may serve as a storage protein.

Light absorption and partitioning between photochemical and non-photochemical processes were altered by leaf N content. Leaf chlorophyll content declined linearly with decreasing leaf N, but the reduction in leaf absorptance was not proportional to the decrease in leaf chlorophyll. Under high light conditions, the amount of absorbed light in excess of that required to saturate CO_2 assimilation increased with decreasing leaf N. This was because leaves with low N content have low rate of electron transport. As leaf N content decreased, thermal dissipation of excitation energy was enhanced. This reduced the efficiency with which excitation energy was delivered to open PSII reaction centers, thereby protecting leaves from photo-oxidation by excess absorbed light.

A single curvilinear relationship was found between true quantum yield for CO_2 assimilation and actual PSII efficiency for leaves with a wide range of leaf N content under non-photorespiratory conditions. The relationship was linear up to a quantum yield of approximately 0.05 mol CO_2 mol^{-1} quanta, then it became curvilinear with a further rise in quantum yield in response to decreasing PFD. Both the rate of linear electron flow and the rate to CO_2 or O_2 increased with increasing leaf N at any given Ci under photorespiratory conditions. The percentage of linear electron flow to CO_2 assimilation,

however, remained the same regardless of leaf N content. Therefore, the relationship between quantum yield for CO_2 assimilation and actual PSII efficiency, and the partitioning of linear electron flow between CO_2 assimilation and photorespiration were not affected by leaf N content.

To conclude, this study has shown that there is a close relationship between leaf N content and CO_2 assimilation capacity in apple leaves. N-deficient leaves have low CO_2 assimilation capacity because of limited amount of rubisco. However, almost all the rubisco is involved in photosynthesis in these leaves. This results in a high photosynthetic N use efficiency. Because photosynthetic electron transport uses only a small proportion of the absorbed light in N-deficient leaves, there is more excess absorbed light in these leaves than in high N leaves. Thermal dissipation of excitation energy is enhanced in N-deficient leaves to reduce PSII efficiency and the probability of photo-damage by excess absorbed light. Leaves with high N content have higher CO_2 assimilation capacity than N-deficient leaves, but CO_2 assimilation capacity is not proportional to leaf N content. In contrast to N-deficient leaves, high N leaves accumulate large amounts of rubisco, but only a proportion of the rubisco is involved in photosynthesis. This explains why high N leaves have a low photosynthetic N use efficiency. The surplus rubisco may serve as a storage protein in these leaves. Leaf N content does not alter the relationship between quantum yield for CO_2 assimilation and actual PSII efficiency, nor influence the partitioning of linear electron flow between CO_2 assimilation and photorespiration.

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APPENDIX

The initial slope of the A/Ci curve in relation to rubisco activities

When rubisco is fully activated *in vivo* at low CO_2 under saturating light, the initial slope of the response of CO_2 assimilation (A) to intercellular CO_2 concentrations (Ci) is given by von Caemmerer and Evans (1991) as:

$$\frac{dA}{dC_i} = \frac{g_w(V_{c\max} - A - R_d)}{g_w(C_i + K_c(1 + O/K_o)) + V_{c\max} - R_d - 2A} \qquad [E1]$$

where g_w is the CO_2 transfer conductance from the intercellular air space to the carboxylation site within chloroplasts; V_{cmax} is the maximum rubisco activity; K_c and K_o are the Michaelis constants for CO_2 and O_2 respectively; O is the partial pressure of O_2 in the chloroplasts; and R_d is the respiration under light other than that associated with photorespiration (Farquhar and Caemmerer 1982).

At the photocompensation point of the CO_2 partial pressure in the chloroplast, Γ^*, where photorespiratory CO_2 evolution equals the rate of carboxylation.

$$A = -R_d \text{, and} \qquad [E2]$$

$$Ci = A/g_w + \Gamma^*. \qquad [E3]$$

Substituting for A and Ci, Eq.1 simplifies to (without assuming $R_d << V_{cmax}$, as in von Caemmerer and Evans 1991):

$$\frac{dA}{dC_i}(\Gamma^*) = \frac{g_w V_{c\max}}{V_{c\max} + g_w(\Gamma^* + K_c(1 + O/K_o))} \qquad [E4]$$

So, when g_w/V_{cmax} remains constant, dA/dC_i is linearly related to V_{cmax}.

When rubisco is not fully activated *in vivo*, V_{cmax} is replaced by the effective V_{cmax} (V'_{cmax}).

$$\frac{dA}{dC_i}(\Gamma^*) = \frac{g_w V'_{c\max}}{V'_{c\max} + g_w(\Gamma^* + K_c(1 + O/K_o))} \qquad [E5]$$

When V_{cmax} increases linearly with leaf N, and the rubisco activation state decreases with increasing leaf N as:

$$V_{c\max} = \alpha N + \beta \text{ and} \qquad \text{[E6]}$$

$$\frac{V'_{c\max}}{V_{c\max}} = aN + b. \qquad \text{[E7]}$$

Substituting for N, or V'_{cmax}, and rearranging:

$$V'_{c\max} = \frac{a}{\alpha} V^2_{c\max} + (b - \frac{a\beta}{\alpha}) V_{c\max} \text{ and} \qquad \text{(E8)}$$

$$V'_{c\max} = a\alpha N^2 + (a\beta + b\alpha)N + b\beta. \qquad \text{(E9)}$$

So, if g_w/V'_{cmax} remains constant as leaf N increases, it is obvious from Eqs. 5, 8 and 9 that deactivation of rubisco alone will cause curvilinear relationships between V_{cmax} and the initial slope of the A/Ci curves, and between leaf N and the initial slope of the A/Ci curves.