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Rubisco activation state decreases with increasing nitrogen content in apple leaves

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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 (C_i) in apple, it is hypothesized that Rubisco activation state decreases with increasing leaf N content and this decreased activation state accounts for the curvilinear relationship between leaf N and CO_2 assimilation. A range of leaf N content (1.0–5.0 g m^{-2}) was achieved by fertilizing bench-grafted Fuji/M.26 apple (*Malus domestica* Borkh.) trees for 45 d with different N concentrations, using a modified Hoagland's solution. Analysis of A/C_i curves under saturating light indicated that CO_2 assimilation at ambient CO_2 fell within the Rubisco limitation region of the A/C_i 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/C_i 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/C_i 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.

Key words: Apple, CO_2 assimilation, leaf N content, *Malus domestica*, Rubisco activation state.

Introduction

Whenever a sufficiently wide range of leaf N content has been examined, the relationship between leaf N and light-saturated CO_2 assimilation is 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, 1994*a*, *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 (C_i) was curvilinearly related to total Rubisco activity. This curvature was attributed to CO_2 transfer resistance from the intercellular air spaces to the carboxylation sites in chloroplasts. Based on the C_3 photosynthesis model (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 non-linear relationship between V_{cmax} and the initial slope of the A/C_i curves is expected (von Caemmerer and Evans, 1991; see also the Appendix).

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Abbreviations: A, CO_2 assimilation; C_i, intercellular CO_2 concentration; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; V_{cmax}, maximum activity of Rubisco.

von Caemmerer and Evans found that CO_2 transfer conductance increased with increasing N content in wheat leaves (von Caemmerer and Evans, 1991). As leaf N increases, the ratio of CO_2 transfer conductance to Rubisco activity may or may not remain constant. However, when Rubisco is fully activated *in vivo*, a curvilinear relationship between total Rubisco activity and the initial slope of the A/C_i curves does not necessarily translate into a curvilinear relationship between leaf N and the initial slope, if the ratio of Rubisco to leaf N increases with increasing leaf N. In rice (*Oryza sativa*) leaves, CO_2-limited photosynthesis (at $C_i = 20$ Pa) was curvilinearly related to total Rubisco content, but linearly related to leaf N (Makino *et al*., 1994*a*). 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*., 1994*a*). 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 (Evans, 1983; Makino *et al*., 1992), but it increased with increasing leaf N content in rice (Makino *et al*., 1992, 1994*a*, 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 catalyse the carboxylation and oxygenation reactions. Activation of Rubisco involves the reversible reaction of a CO_2 molecule with a lysine residue within the active site to form a carbamate, followed by the rapid binding of a magnesium ion to create an active ternary structure. This activation process *in vivo* is regulated by Rubisco activase (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 CO_2 assimilation will be curvilinear. Evans and Terashima showed that there was no apparent deactivation of Rubisco in spinach leaves under a high N supply (Evans and Terashima, 1988). In contrast, it was found that Rubisco activation state in wheat leaves decreased as the N supply increased (Mächler *et al*., 1988). However, plants were grown at a relatively low photon flux density (PFD of 540 μmol m^{-2} s^{-1}), and CO_2 assimilation and leaf N were not measured. In addition, it was shown 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} (Lawlor *et al*., 1987). 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/C_i curves showed curvilinear relationships with N content in leaves of apple (*Malus domestica* Borkh.) plants grown under full sunlight (Cheng and Fuchigami, 2000). The objective of this study is to test the hypothesis 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.

Materials and methods

Plant material

'Fuji' apple (*Malus domestica* Borkh.) trees on M.26 rootstocks were used. Bench-grafting was done in late March, 1996 and each grafted tree was planted immediately into a 3.8 l pot containing a mixture of peat moss, pumice and sandy loam soil (1:2:1 by vol.). The plants were grown in a lathhouse until early June. During this period, beginning from budbreak in early May, they were fertilized every 2 weeks with 10.7 mM N, using Plantex® 20N-10P_2O_5-20K_2O water-soluble fertilizer with micronutrients (Plantex Corporation, Ontario, Canada). When the scion shoots were approximately 15 cm tall, plants were selected for uniformity, and moved to full sunlight. Thereafter, they were fertilised weekly with Plantex® for 3 weeks. Beginning on 30 June, plants were fertilized twice weekly with one of seven N concentrations (0, 2.5, 5.0, 7.5, 10.0, 15.0 or 20.0 mM N from NH_4NO_3) 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) so that N was supplied solely from NH_4NO_3. There were four replications for each N treatment in a completely randomized design. Irrigation was provided from a saucer placed at the bottom of each pot to ensure adequate water supply. After 45 d, recent fully expanded leaves at the same developmental stage across the treatments were selected for gas exchange and Rubisco activity measurements.

Gas exchange measurements

Measurements were made using a CIRAS-1 portable photosynthesis system (PP Systems, Hitchin, Herts., UK). For all measurements, leaf temperature and ambient water vapour pressure were kept at 26.2 ± 1.0 °C, and 1.30 ± 0.15 kPa, respectively. Responses of CO_2 assimilation to PFD were measured first to determine the PFD that saturates CO_2 assimilation for all the leaves over the range of leaf N content used in this study (1.0–5.0 g m^{-2}). The results were very similar to those reported previously (Cheng and Fuchigami, 2000) and the light saturation point for CO_2 assimilation was below 1400 μmol $m^{-2}s^{-1}$ in leaves with the highest N content (data not shown). A PFD of 1700 ± 40 μmol m^{-2} s^{-1}, a level well above the light saturation point for all the subsequent measurements, was chosen for the study. Light-saturated CO_2 assimilation was measured at an ambient CO_2 concentration of 350 ± 2 μmol mol^{-1}. The initial slope of the A/C_i curve was estimated as the slope of the linear regression between intercellular CO_2 (C_i) and CO_2 assimilation (A), from at least three points measured at C_i below 200 μmol mol^{-1}. Responses of A to C_i were determined in ascending order at air CO_2 concentrations of 110, 190, 270, 350, 450, 600, 800, 1000, 1200 or 1500 μmol mol^{-1}, until C_i reached approximately 1000 μmol mol^{-1}. At each concentration, CO_2 assimilation and stomatal conductance were first measured at 21% O_2, after 10 min for them to reach steady state. The O_2 supply was then switched to 2% and measurements were made after re-equilibration.

Rubisco activity measurements

Rubisco extraction and activity measurements were modified from previous methods (Tissue *et al.*, 1993; Sharkey *et al.*, 1991). Briefly, two leaf discs (total of 2 cm^2) were taken from each leaf under full sun (PFD of 1700 µmol m^{-2} s^{-1}) at noon (11.00–12.30 h), cut into small pieces, and placed in 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 25–30 s with a Tissuemizer (Tekmar Company, Cincinnati, Ohio, USA) at 18 000 rpm. The extract was then centrifuged at 13000 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 (ribulose 1,5-bisphosphate) carboxylation to NADH oxidation (Lilley and Walker, 1974). NADH oxidation was monitored at 340 nm in a Shimadzu UV-160 Spectrophotometer (Shimadzu Corp., Kyoto, Japan). For initial Rubisco activity, a 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 of 0.5 mol m^{-3} RuBP was added 15 min later, after a sample extract was combined with the assay solution to activate all the Rubisco fully. 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-phosphoglyceric 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.

Leaf N content

The same leaves used for gas exchange and Rubisco activity measurements were frozen in liquid nitrogen and stored at –80 °C until freeze-dried. Leaf N content was determined by the Kjeldahl procedure (Schuman *et al.*, 1973).

Results

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/C_i curves increased linearly with increasing leaf N at first, then levelled off at a leaf N content of approximately 3 g m^{-2} (Fig. 1A, B). Photosynthetic N use efficiency decreased linearly as leaf N increased (Fig. 1C).

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

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

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

To determine which process limited light-saturated CO_2 assimilation at ambient CO_2, A/C_i curves were constructed under both 21% and 2% O_2 conditions. Regardless of the leaf N status, light-saturated CO_2 assimilation at ambient CO_2 fell within the linear region of the A/C_i curves (Fig. 6).

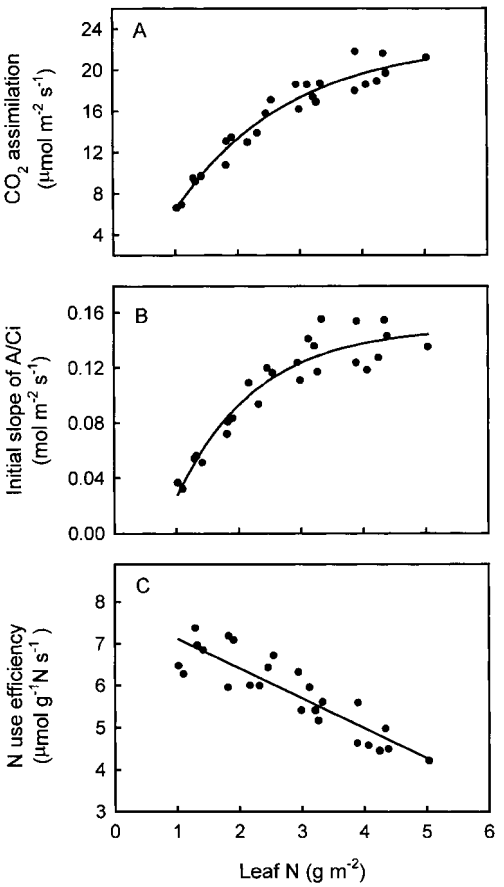

Fig. 1. Light-saturated CO_2 assimilation at ambient CO_2 (A), the initial slope of the A/C_i curves (B), and photosynthetic N use efficiency (C) in relation to N content in apple leaves. Regression equations: (A) $y=28.31\times(1-e^{-0.548x})-5.53$ ($R^2=0.946$, $P=0.0001$); (B) $y=0.2697(1-e^{-0.782x})-0.1204$ ($R^2=0.904$, $P=0.0001$); (C) $y=7.83–0.71x$ ($R^2=0.782$, $P=0.0001$).

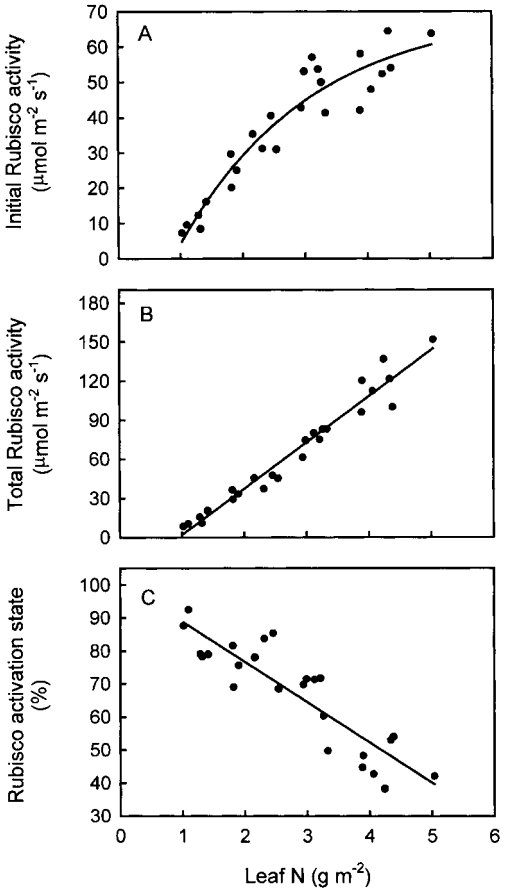

Fig. 2. Initial Rubisco activity (A), total Rubisco activity (B), and Rubisco activation state (C) in response to N content in apple leaves. Regression equations: (A) $y=106.5(1-e^{-0.476x})-36.2$ ($R^2=0.902$, $P=0.0001$); (B) $y=-33.68+35.41x$ ($R^2=0.958$, $P=0.0001$); (C) $y=101.1-12.2x$ ($R^2=0.789$,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

Discussion

Total Rubisco activity increased linearly with increasing leaf N, whereas initial activity showed a curvilinear response. This resulted in decreased Rubisco activation state with increasing N content (Fig. 2). Both light-saturated CO_2 assimilation at ambient CO_2 and the initial slope of the A/C_i curves were linearly related to initial Rubisco activity, but curvilinearly related to total activity (Fig. 3). These results are consistent with the 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 (Lawlor *et al.*, 1987) and demonstrated (Mächler *et al.*, 1988) in wheat leaves. However, because the plants were grown under relatively low PFD ($540\sim550$ μmol m^{-2} s^{-1}) conditions in both experiments, the possibility that deactivation of Rubisco was caused by low electron transport capacity cannot be ruled out. In this experiment, apple plants were grown under full sunlight, and measurements were made at a PFD well above the light saturation point (Cheng and Fuchigami, 2000). It was found that Rubisco activation state decreased with increasing N content in apple leaves. This contrasts with earlier findings (Evans and Terashima, 1988), where no apparent deactivation of Rubisco in spinach was found under a high N supply. Although CO_2 transfer conductance was not measured in this experiment, decreased Rubisco activation state with increasing leaf N alone would result in curvilinear relationships between total Rubisco activity and the

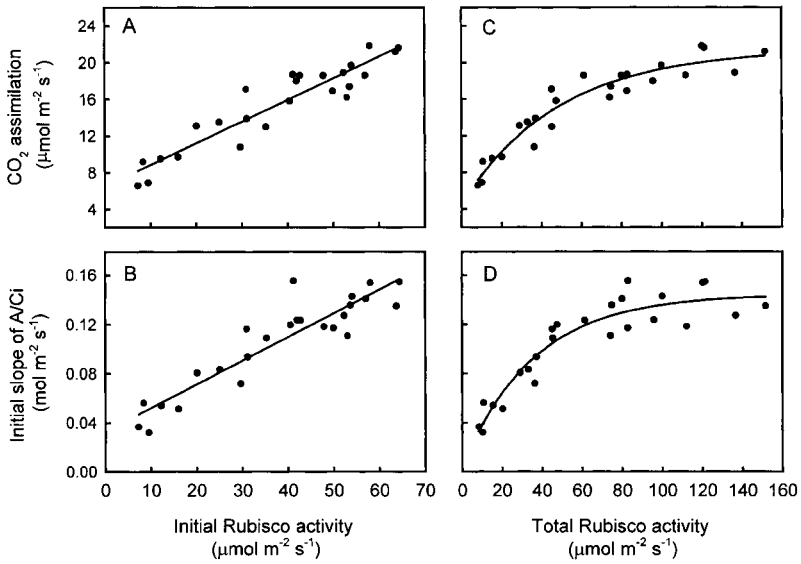

Fig. 3. Light-saturated CO_2 assimilation at ambient CO_2 and the initial slope of the A/C_i curves in relation to initial Rubisco activity (A, B) and total Rubisco activity (C, D) in apple leaves. Regression equations: (A) $y=6.50+0.235x$ ($R^2=0.871$, $P=0.0001$); (B) $y=0.033+0.0019x$ ($R^2=0.847$, $P=0.0001$); (C) $y=16.92(1-e^{-0.0205x})+4.53$ ($R^2=0.918$, $P=0.0001$); (D) $y=0.1405(1-e^{-0.0281x})+0.0038$ ($R^2=0.887$, $P=0.0001$).

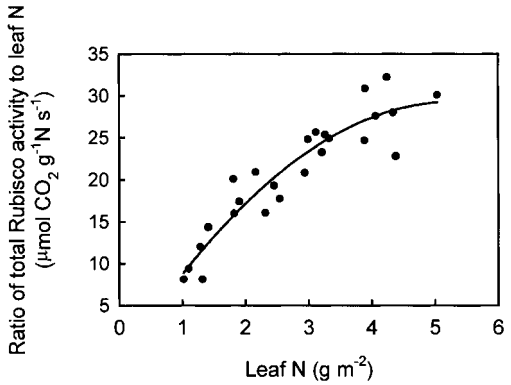

Fig. 4. The ratio of total Rubisco activity to leaf N, in response to N content in apple leaves. Regression equation: $y=-1.95+11.76x-1.11x^2$ ($R^2=0.874$, $P=0.0001$).

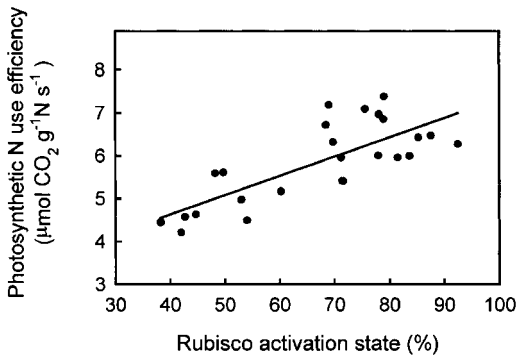

Fig. 5. Photosynthetic N use efficiency in relation to Rubisco activation state in apple leaves. Regression equation: $y=2.83+0.045x$ ($R^2=0.591$, $P=0.0001$).

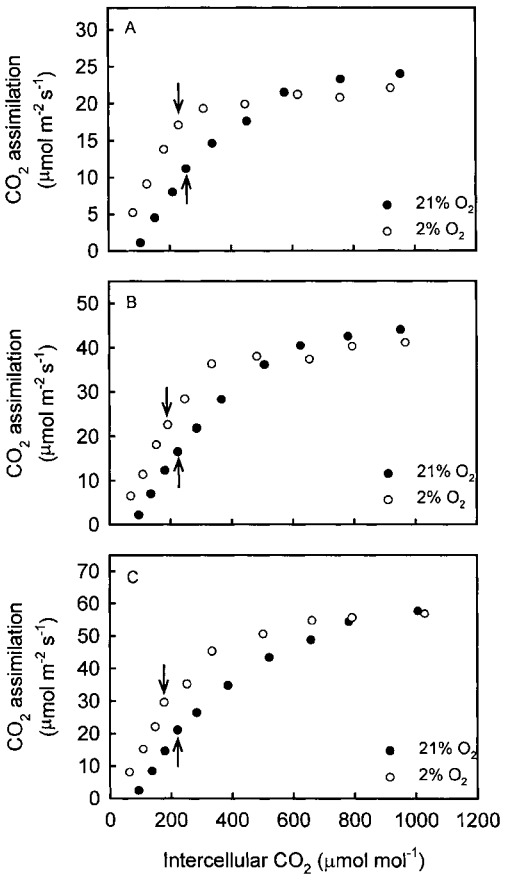

Fig. 6. Responses of CO_2 assimilation to intercellular CO_2 concentration in apple leaves at 21% and 2% O_2. Leaf N content (g m^{-2}) is 1.52 ± 0.07 (A), 2.54 ± 0.09 (B), and 4.06 ± 0.10 (C). Each data point represents the average of three replications. The arrows indicate the intercellular CO_2 concentration corresponding to the 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 vapour pressure of 1.28 ± 0.05 kPa.

initial slope of the A/C_i curves, and between leaf N and the initial slope, even if the ratio of CO_2 transfer conductance to the effective V_{cmax} remained constant (see Appendix). The relationship between total Rubisco activity and the initial slope of the A/C_i curves was more curvilinear than that between leaf N and the initial slope because the ratio of total Rubisco to leaf N increased with increasing leaf N (Fig. 4).

Rubisco activation state in apple leaves is higher at low rather than high leaf N levels (Fig. 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 present. As total 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/C_i curve (Fig. 6), which is characteristic of Rubisco limitation based on the C_3 photosynthesis model of Farquhar *et al.* (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/C_i curves, and the curvilinear relationship between total Rubisco activity and the initial slope found in this experiment indicate that the initial slope reflects the amount of Rubisco that is active *in vivo*, not the total amount of Rubisco. The total amount is reflected only if all the Rubisco is active *in vivo*. In spinach leaves the initial slope of the A/C_i 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 rates 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. It may also explain why photosynthetic N use efficiency decreases with increasing leaf N. However, the exact mechanism that causes deactivation of Rubisco in high N leaves is unclear. Rubisco activity is regulated by the counteraction of tight binding inhibitors and Rubisco activase that removes these inhibitors (Portis, 1992; Salvucci and Ogren, 1996). There are several tight binding inhibitors, including RuBP, and in some plants, 2-carboxyarabinitol 1-phosphate (CA1P), a naturally occurring nocturnal inhibitor. Recently, some unidentified sugar phosphates were shown to reduce Rubisco activity in the light (Keys *et al*., 1995; Parry *et al*., 1997). It appears that CA1P does not play any significant role in the light regulation of Rubisco activity in apple leaves because no difference in total Rubisco activity was detected between measurements made at noon under full sunlight and at night (L Cheng and LH Fuchigami, unpublished data). In addition, Rubisco activities were measured at noon under full sunlight in this experiment when CA1P concentration would have been minimal even if apple leaves had considerable amounts of CA1P at night. Therefore, the observed decrease in Rubisco activation state in relation to leaf N may have nothing to do with leaf CA1P level at night. However, it is not known whether N supply would alter the levels of other tight binding inhibitors. One possibility is that 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 does not seem to be the case 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). Since ATP hydrolysis is required for Rubisco activase to remove the inhibitors from Rubisco while ADP inhibits this reaction, the activity of Rubisco activase can be regulated by ATP/ADP ratio *in vivo* (Portis, 1990). In addition, Rubisco activase may be also subject to redox regulation at least in some species (Zhang and Portis, 1999). Therefore, alteration of Rubisco activation state may occur if N supply affects ATP/ADP ratio and/or redox potential. It was shown that an increased Rubisco activation state was associated with an increased ATP/ADP ratio in transgenic tobacco plants with an antisense gene directed against Rubisco mRNA (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). This ratio was higher in wheat 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 N content in apple leaves.

The finding that Rubisco activation state decreases with increasing N content in apple leaves under saturating light conditions supports 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 longstanding, but has been controversial. Considering that Rubisco is so expensive in terms of N investment, it has been argued that N resources would be wasted if Rubisco were present in great excess. This argument is generally valid in most cases. Indeed, 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/C_i 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/C_i curve, factoring in CO_2 transfer resistance has accounted for the apparent deviation from the expected behaviour of Rubisco (Evans, 1983; Evans and Seemann, 1984; Evans and Terashima, 1988; von Caemmerer and Evans, 1991; Makino *et al*., 1994*a*). 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 serves 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).

In conclusion, Rubisco activation state decreases with increasing N content in apple leaves and Rubisco may serve as a storage protein in leaves with a high N content. The mechanism of deactivation of Rubisco in high N leaves under saturating light conditions deserves further research.

Appendix

The initial slope of the A/C_i 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 (C_i) is given as (von Caemmerer and Evans, 1991):

$$\frac{dA}{dC_i}=\frac{g_w(V_{cmax}-A-R_d)}{g_w(C_i+K_c(1+O/K_o))+V_{cmax}-R_d-2A} \quad (1)$$

where g_w is the CO_2 transfer conductance from the intercellular air spaces to the carboxylation sites 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 *et al.*, 1980).

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

$$A=-R_d, \quad (2)$$

and

$$C_i=A/g_w+\Gamma^* \quad (3)$$

Substituting for A and C_i, equation (1) simplifies to (without assuming $R_d \ll V_{cmax}$, as in von Caemmerer and Evans, 1991):

$$\frac{dA}{dC_i}(\Gamma^*)=\frac{g_w V_{cmax}}{V_{cmax}+g_w(\Gamma^*+K_c(1+O/K_o))} \quad (4)$$

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* under saturating light, V_{cmax} is replaced by the effective $V_{cmax}(V'_{cmax})$

$$\frac{dA}{dC_i}(\Gamma^*)=\frac{g_w V'_{cmax}}{V'_{cmax}+g_w(\Gamma^*+K_c(1+O/K_o))} \quad (5)$$

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

$$V_{cmax}=\alpha N+\beta \quad (6)$$

and

$$\frac{V'_{cmax}}{V_{cmax}}=\alpha N+\beta \quad (7)$$

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

$$V'_{cmax}=\frac{a}{\alpha}V_{cmax}^2+\left(b-\frac{a\beta}{\alpha}\right)V_{cmax} \quad (8)$$

and

$$V'_{cmax}+a\alpha N^2+(a\beta+b\alpha)N+b\beta \quad (9)$$

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

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References

Brooks A. 1986. Effects of phosphorus nutrition on ribulose-1,5-bisphosphate carboxylase activation, photosynthetic quantum yield and amounts of some Calvin-cycle metabolites in spinach leaves. *Australian Journal of Plant Physiology* **13,** 231–237.

Cheng L, Fuchigami LH. 2000. CO_2 assimilation in relation to nitrogen in apple leaves. *Journal of Horticultural Science and Biotechnology* **75,** 383–387.

DeJong TM, Doyle JF. 1985. Seasonal relationships between leaf nitrogen content (photosynthetic capacity) and leaf canopy light exposure in peach (*Prunus persica*). *Plant Cell and Environment* **8,** 701–706.

Evans JR. 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiology* **72,** 297–302.

Evans JR. 1986. The relationship between carbon-dioxide-limited photosynthetic rate and ribulose-1,5-bisphosphate-carboxylase content in two nuclear-cytoplasm substitution lines of wheat, and the co-ordination of ribulose-bisphosphate-carboxylation and electron-transport capacities. *Planta* **167,** 351–358.

Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* **78,** 9–19.

Evans JR, Seemann JR. 1984. Differences between wheat genotypes in specific activity of ribulose-1,5-bisphosphate carboxylase and the relationship to photosynthesis. *Plant Physiology* **74,** 759–765.

Evans JR, Terashima I. 1988. Photosynthetic characteristics of spinach leaves grown with different nitrogen treatments. *Plant Cell Physiology* **29,** 157–165.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149,** 78–90.

Hoagland DR, Arnon DI. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular*, **347**.

Hudson GS, Evans JR, von Caemmerer S, Arvidsson YBC, Andrews TJ. 1992. Reduction of ribulose-1,5-bisphosphate

carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco plants. *Plant Physiology* **98,** 294–302.

Keys AJ, Major I, Parry MAJ. 1995. Is there another player in the game of Rubisco regulation? *Journal of Experimental Botany* **46,** 1245–1251.

Lawlor DW, Boyle FA, Young AT, Keys AJ, Kendall AC. 1987. Nitrate nutrition and temperature effects on wheat: photosynthesis and photorespiration of leaves. *Journal of Experimental Botany* **38,** 393–408.

Lee HJ, Titus JS. 1992. Nitrogen accumulation and nitrate reductase activity in MM. 106 apple trees as affected by nitrate supply. *Journal of Horticultural Science* **67,** 273–281.

Lilley RM, Walker DA. 1974. An improved spectrophotometric assay of ribulose-bisphosphate carboxylase. *Biochimica et Biophysica Acta* **358,** 226–229.

Mächler F, Oberson A, Grub A, Nösberger J. 1988. Regulation of photosynthesis in nitrogen-deficient wheat seedlings. *Plant Physiology* **87,** 46–49.

Makino A, Osmond B. 1991. Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. *Plant Physiology* **96,** 355–362.

Makino A, Sakashita H, Hidema J, Mae T, Ojima K, Osmond B. 1992. Distinctive responses of ribulose-1,5-bisphosphate carboxylase and carbonic anhydrase in wheat leaves to nitrogen nutrition and their possible relationships to CO_2-transfer resistance. *Plant Physiology* **100,** 1737–1743.

Makino A, Nakano H, Mae T. 1994*a*. Responses of ribulose-1,5-phosphate carboxylase, cytochrome *f*, and sucrose synthesis enzymes in rice leaves to leaf nitrogen and their relationships to photosynthesis. *Plant Physiology* **105,** 173–179.

Makino A, Nakano H, Mae T. 1994*b*. Effects of growth temperature on the response of ribulose-1,5-bisphosphate carboxylase, electron transport components and sucrose synthesis enzymes to leaf nitrogen in rice, and their relationships to photosynthesis. *Plant Physiology* **105,** 1231–1238.

Makino A, Sato T, Nakano H, Mae T. 1997. Leaf photosynthesis, plant growth and nitrogen allocation in rice under different irradiances. *Planta* **203,** 390–398.

Mate CJ, von Caemmerer S, Evans JR, Hudson GS, Andrews TJ. 1996. The relationship between CO_2-assimilation rate, Rubisco carbamylation and Rubisco activase content in activase-deficient transgenic tobacco suggests a simple model of activase action. *Planta* **198,** 604–613.

Nakano H, Makino A, Mae T. 1997. The effect of elevated partial pressure of CO_2 on the relationship between photosynthetic capacity and N content in rice leaves. *Plant Physiology* **115,** 191–198.

Parry MAJ, Andralojc PJ, Parmar S, Keys AJ, Habash D, Paul MJ, Alred R, Quick WP, Servaites JC. 1997. Regulation of Rubisco by inhibitors in the light. *Plant, Cell and Environment* **20,** 528–534.

Portis Jr AR. 1990. Rubisco activase. *Biochimica et Biophysica Acta* **1015,** 15–28.

Portis Jr AR. 1992. Regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity. *Annual Review of Plant Physiology and Plant Molecular Biology* **43,** 415–437.

Quick WP, Schurr U, Scheibe R, Schulze E-D, Rodermel SR, Bogorad L, Stitt M. 1991. Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' rbcS. I. Impact on photosynthesis in ambient growth conditions. *Planta* **183,** 542–554.

Sage RF. 1990. A model describing the regulation of ribulose-1,5-bisphosphate carboxylase, electron transport and triose phosphate use in response to light intensity and CO_2 in C_3 plants. *Plant Physiology* **94,** 1728–1734.

Sage RF, Pearcy RW, Seemann JR. 1987. The nitrogen use efficiency of C_3 and C_4 plants. III. Leaf nitrogen effects on the activity of carboxylating enzymes in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiology* **85,** 355–359.

Sage RF, Reid CD, Moore BD, Seemann JR. 1993. Long-term kinetics of the light-dependent regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity in plants with and without 2-carboxyarabinitol 1-phosphate. *Planta* **191,** 222–230.

Sage RF, Sharkey TD, Seemann JR. 1988. The *in vivo* response of the ribulose-1,5-bisphosphate carboxylase activation state and the pool sizes of photosynthetic metabolites to elevated CO_2 in *Phaseolus vulgaris* L. *Planta* **174,** 407–416.

Sage RF, Sharkey TD, Seemann JR. 1990. Regulation of ribulose-1,5-bisphosphate carboxylase activity in response to light intensity and CO_2 in the C_3 annuals *Chenopodium album* L. and *Phaseolus vulgaris* L. *Plant Physiology* **94,** 1735–1742.

Salvucci ME, Ogren WL. 1996. The mechanism of Rubisco activase: insights from studies of the properties and structure of the enzyme. *Photosynthesis Research* **47,** 1–11.

Schuman GE, Stanley AM, Knudsen D. 1973. Automated total nitrogen analysis of soil and plant samples. *Proceedings of the Soil Science Society of America* **37,** 480–481.

Sharkey TD. 1990. Feedback limitation of photosynthesis and the physiological role of ribulose bisphosphate carboxylase carbamylation. *Botanical Magazine, Tokyo Special Issue* **2,** 87–105.

Sharkey TD, Savitch LV, Butz ND. 1991. Photometric method for routine determination of K_{cat} and carbamylation of Rubisco. *Photosynthesis Research* **28,** 41–48.

Sinclair TR, Horie T. 1989. Leaf nitrogen, photosynthesis and crop radiation use efficiency: a review. *Crop Science* **29,** 90–98.

Tissue DT, Thomas RB, Strain BR. 1993. Long-term effects of elevated CO_2 and nutrients on photosynthesis and Rubisco in loblolly pine seedlings. *Plant, Cell and Environment* **16,** 859–865.

von Caemmerer S, Edmondson DL. 1986. Relationship between steady-state gas exchange, *in vivo* ribulose bisphosphate carboxylase activity and some carbon reduction cycle intermediates in *Raphanus sativus*. *Australian Journal of Plant Physiology* **13,** 669–688.

von Caemmerer S, Evans JR. 1991. Determination of the average partial pressure of CO_2 in chloroplasts from leaves of several C_3 species. *Australian Journal of Plant Physiology* **18,** 287–305.

von Caemmerer S, Farquhar GD. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153,** 376–387.

von Caemmerer S, Evans JR, Hudson GS, Andrews TJ. 1994. The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase *in vivo* inferred from measurements of photosynthesis in leaves of transgenic tobacco. *Planta* **195,** 88–97.

Zhang N, Portis Jr AR. 1999. Mechanism of light regulation of Rubisco: a specific role for the larger Rubisco activase isoform involving reductive activation by thioredoxin-*f*. *Proceedings of National Academy of Sciences, USA* **96,** 9438–9443.