Nitrogen Shortage in a Tomato Crop; Scaling up from Effects on Electron-Transport Rate to Plant Productivity

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Optimization of nitrogen supply in a tomato plant is stepwise assessed, from chloroplast characteristics to whole crop performance. Experiments are reported in which important key processes are quantified in relation to the nitrogen content of leaves. Interactions of N effects with leaf aging and canopy light distribution are analyzed.

A simulation model that integrates this knowledge is constructed. The results of three nitrogen partitioning scenarios for Rubisco suggest that optimization of the distribution of Rubisco between leaf layers is less important for plant productivity than within-leaf optimization of the photosynthetic apparatus.

Introduction

Changes of the plant's architecture, developmental state and environment require continuous adaptation of its physiological systems. In part, these modifications of physiological processes are thought necessary to optimize the performance of the plant in response to changing conditions. Optimization is a broad concept which as used here will mean the maximization of flux through the photosynthetic pathway in vivo (within genetically determined limits) per unit investment into the pathway. Within photosynthesis, the balance between the electron transport and metabolic machinery on the one hand, and light-harvesting systems on the other seems to play a major role in optimization. Another important aspect of photosynthetic optimization in vivo is the total amount of resources available to the plant that are allocated to photosynthesis as a whole: allocate too many resources to photosynthesis and photosynthesis will only end up being limited by the low demand of other starved processes. Lastly, the environment of a plant changes, sometimes very rapidly, and this affects optimization in two ways. First, the costs of optimization must be considered as well as the benefits, and second the process of optimization may be too slow to track the environment even if the cost/benefit ratio were favourable. So, even if photosynthesis is optimized for average conditions, at any instant the photosynthetic machinery could be suboptimal, and thus more vulnerable to stress-related damage.

Nitrogen is a basic component of the physiological machinery as a whole and is frequently a limiting nutrient. Consequently, its allocation to the photosynthetic machinery, and its subsequent distribution within that machinery, plays a major role in the optimization of photosynthesis (Evans, 1987). When irradiance is low, an investment of nitrogen into Rubisco and other enzymes and cofactors of the electron transport and metabolic pathways will not be beneficial to growth. Allocation to light absorbing complexes, or an increase of leaf expansion rate in order to intercept more light, will be more beneficial. Conversely, under high light conditions a greater investment in the metabolic and electron transport pathways to increase their capacity will be more beneficial than an increase in light-trapping. An optimum nitrogen profile in a canopy should, therefore, reflect the requirements of the most responsive processes under the given environmental conditions (Evans, 1989; Hirose and Werger, 1987).

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The photosynthetic light-harvesting and electron-transport systems are sources of active oxygen species and vulnerable to these species (Foyer and Harbinson, 1994), and the reaction centres of both photosystems are vulnerable to photoinhibition (Baker, 1996). Though these injurious processes occur under all conditions of irradiance, they are greater in leaves that are sub-optimally adapted to their environment, for example due to the dynamic nature of the environment or as a result of other environmentally or genetically determined limits placed on the operation of photosynthesis. Protection against damage caused by, or occurring within, the photosynthetic machinery requires mechanisms that can accommodate the imbalance between energy input and energy demand (Genty *et al.,* 1989). There are a number of dissipative mechanisms for excess absorbed photons such as the xanthophyll cycle, photorespiration and other sinks for electron flow (for review see Baker, 1996). Nitrogen plays an important role in the dissipating mechanisms (Hikosaka and Terashima, 1995).

Of the total leaf nitrogen, 50 to 80% is allocated to photosynthetic machinery, so the short term regulation and long term acclimation of photosynthesis, with respect to nitrogen costs, are major subjects of ecophysiological studies. Within a canopy the situation is more complex than in a single leaf. Increasing depth within the canopy, which parallels leaf age, results in the leaf becoming more light-limited. Consequently it might be expected that nitrogen will be redistributed within the canopy to reflect the requirements of the most responsive processes under the given environmental conditions (Evans, 1989; Hirose and Werger, 1987). In the present case study we will show the effect of nitrogen on photosynthetic regulation considering both the effects of the role of light and nitrogen distribution within the canopy. A crop growth model was developed to extrapolate the responses measured at the level of leaf process to whole crop productivity. With this model, the consequences of manipulation of vertical leaf N distribution on yield of the tomato crop can be predicted.

Material and Methods

Growth conditions

Single tomato plants (Lycopersicon esculentum) were grown in a climate room in containers with 40 liters aerated nutrient solution in which nitrate was replaced by chloride. The temperature was 22.0 °C and the relative humidity 70%. PPFD at the top of the plants was 250 $\mu mol\ m^{-2}\ s^{-1}$ for a 12 hour photoperiod followed by half an hour low intensity incandescent light. The pH of the nutrient solution was daily adjusted to a value between 5 and 6. Nitrogen was supplied at a relative addition rate, to give a constant organic N-content of the leaves (Ingestad, 1982; Agren and Ingestad, 1987; Schapendonk *et al.,* 1990). Nitrogen content in the leaves was 4.6% for the optimal plants and 2.3% for the suboptimal plants.

Chlorophyll content and absorptance

Chlorophyll contents of the upper leaves were determined spectrophotometrically after extraction with dimethylformamide (Inskeep and Bloom, 1985) or by using a hand-held automated chlorophyll meter, SPAD 502 (Minolta). Absorption characteristics of leaves between 400 and 700 nm were measured with a LICOR (LI-1800) spectroradiometer equipped with an integrating sphere.

Gas exchange measurements

Two open gas analysis systems were employed. When CO_2 fixation was measured in parallel with chlorophyll fluorescence and light-induced absorbance changes, the analysis system was that described by Kingston-Smith *et al.* (1999). When CO_2 fixation was measured in parallel with transpiration another system was used in which the air velocity over the leaf was 1.5 $m\ s^{-1}$ resulting in a boundary layer conductance to H_2O of 0.7 $mol\ m^{-2}\ s^{-1}$. Photosynthetic and transpiration rates were calculated from the flow rates and the measured concentrations of CO_2 and water vapour in the ingoing and outgoing air streams (von Caemmerer and Farquhar, 1981).

Gas exchange measurements were made in four replicates on the third leaf from the top. Plants were acclimated prior to the measurements in a growth cabinet at a photon flux density (PPFD) of 250 ($\mu mol\ m^{-2}$ and 22 °C.

Chlorophyll fluorescence and light induced absorbance changes around 820 nm

Estimates of the quantum yields for electron transport by photosystems I and II under steadystate irradiances were made from 820 nm absorbance change and chlorophyll fluorescence measurements, respectively, using the methods described by Kingston-Smith *et al.* (1999).

Modelling

A crop growth model was developed to extrapolate the responses of leaves to whole crop productivity. Our model uses the same method of deriving seasonal crop growth patterns from short-term leaf level processes as an earlier published crop growth model, SUCROS (Goudriaan and Van Laar, 1994). Seasonal growth patterns are derived by iterative calculation of daily growth processes. The model calculates daily canopy photosynthetic rate, corrects it for respiration, and distributes the resulting net biomass increase over the different organs of the crop according to thermal-time dependent allocation functions. Daily photosynthetic rates are found for each day of the growing season by integrating the diurnal course of instantaneous canopy photosynthetic rate. Instantaneous canopy photosynthetic rates are calculated in three steps: (1) calculation of the light profile in the canopy; (2) calculation of photosynthetic rates for the different leaf layers; (3) summation of photosynthetic rates of leaves to arrive at the value for the whole canopy. In all these respects the model equals other SUCROS-based tomato crop growth models (e.g. Heuvelink, 1995). However, our model differs in two respects. First, we introduced the Farquhar equations (Farquhar *et al.,* 1980) to calculate leaf photosynthetic rate, and made the maximum rates of electron transport (J_{max}) and carboxylation ($V_{c,max}$) proportional to the leaf contents of chlorophyll and Rubisco, respectively. Secondly, our model allows for the possibility of differences in photosynthetic capacity (chlorophyll and Rubisco contents) between leaf layers. Because of these changes, the leaf level measurements reported in this paper can be used for model parameterization, and, vice versa, the model can be used to test the importance of leaf level traits and processes for seasonal crop growth and yield.

Results and Discussion

Light absorptance

Visible effects of low nitrogen supply, such as thicker chlorotic leaves, had less effect on the absorptance than would intuitively be expected. We found that a decline of the chlorophyll content from 0.55 mmol m^{-2} (optimal N) to 0.24 mmol m^{-2} (sub-optimal N) only decreased light absorptance from 87% to 79% i.e. approximately a 10% decrease for half of the chlorophyll content. This limited effect of deceased chlorophyll concentration on light absorptance has two causes. First, light absorbance, not absorptance, is linearly related to the amount of chlorophyll, and as percentage absorptance equals $100*(1-10^{-(\text{absorbance})})$ a relative insensitivity of absorptance to chlorophyll concentration is not surprising given the high absorbance of leaves (approx. 0.7). Second, light absorption of a leaf is strongly affected by reflections within the tissue and its scattering properties as well as by the chlorophyll content itself (Terashima and Saeki, 1985).

The ratio between chlorophyll *a* and chlorophyll *b* declined with decreasing nitrogen content. Chlorophyll *b* is bound to the light-harvesting class of chlorophyll binding proteins, of which more are associated with photosystem II than I. So, a decrease in the ratio of chorophylls *a* and *b* implies an increase in light-harvesting capacity, and in the absence of large changes in the ratios of the reaction centres of photosystems I and II, implies that the balance of allocation between reaction centres and antennae pigments shifted in favour of light-harvesting and away from electron transport (as represented by the reaction centres). Leaves adapted to low irradiances also have low nitrogen levels and contain relatively more antennae complexes, i.e. a low chlorophyll *a/b* ratio.

Carbon and nitrogen in the photosynthetic machinery

Optimization of photosynthetic nitrogen utilization requires that the plant invests relatively more in electron-transport carriers and in photosynthetic enzymes for leaves in high light (Evans, 1989). Increasing evidence shows that maximum electron transport rates and maximum carboxylating rates are often co-limiting. Various components of the thylakoid, like the cytochrome b_6/f complex and, to a lesser extent, the reaction centre of photosystem II, are linearly related to light-saturated potential photosynthetic rates (see articles in Baker, 1996), as is the amount of Rubisco. A review of these relationships and a set of valuable data have been published recently by Hikosaka and Terashima (1995). These authors continued their analysis with a general model for leaf photosynthesis of C_3 species, with which they calculated which within-leaf partitioning of nitrogen among photosynthetic compounds maximizes leaf photosynthetic rate (Fig. 1). The calculated optimal partitioning varied with N-supply and light intensity (Fig. 1), but generally correlated closely with observed partitioning (Evans, 1989; Hikosaka and Terashima, 1995).

Leaf position within a canopy

In a canopy, an increase in leaf age will be accompanied by a change in the level and the quality of the irradiance. Leaves of different age and, consequently, different position within a canopy, differ in photosynthetic properties. Both leaf senescence and the light level will affect photosynthetic properties and the allocation of nitrogen. We assessed photosynthetic traits of tomato leaves by measuring the 2nd leaf of tomato plants over a period of three weeks. During this time period, the position of the 2nd leaf changed from being the top fully expanded leaf on the plant to a position where 4 fully expanded leaves were present above the 2nd leaf. During the period, the light saturated rate of CO_2-fixation decreased by more than 50% (Fig. 2A). Over the same period of time, the decrease of the quantum yields of electron transport by photosystems I and II (Φ_{PSI}, Φ_{PSII}) induced by increasing irradiance became greater (results not shown). The product of Φ_{PSI} and irradiance and the product of Φ_{PSII} and irradiance gives an index of the rate of electron transport by photosystems I and II, respectively. Interestingly, while the rate of CO_2-fixation dropped strongly, the relationship between CO_2-fixation rate and the indices for electron transport was largely unaffected by the time dependent change in leaf position within the canopy (Figs 2B, 2C). The small changes that did occur may be due to changes in leaf absorption for PAR that occurred at the same time.

Energy dissipation

The irradiance dependency of the quantum yield of electron transport (or photochemistry) by PSII for control and severely N-starved leaves (Fig. 3A) showed two important features. First, following N-starvation, the maximum efficiency of PSII decreased indicating that some photoinhibition had occurred. Second, the efficiency of PSII in the

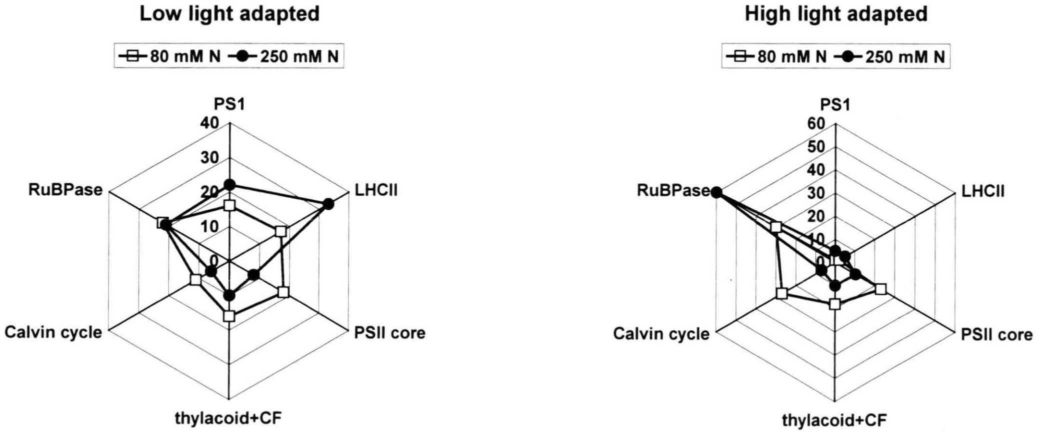

Fig. 1. Optimal partitioning of nitrogen across photosynthetic machinery for leaves adapted to low light (125 (μmol m^{-2} s^{-1}; Fig. 1A) and to high light (2000 (μmol m^{-2} s^{-1}; Fig. 1B) at two leaf nitrogen contents, 250 mmol m^{-2} and 80 mmol m^{-2}. The graphs summarize data that were calculated by Hikosaka and Terashima (1995) with a leaf photosynthesis model. The model calculated how a given amount of nitrogen should be allocated to six different groups of N-containing photosynthetic compounds to maximize photosynthesis. The graphs show the percentages of N in, clockwise from the top: Core complex of PSI and light-harvesting complex I, Light harvesting complex II, Core complex of PSII, Thylacoid electron carriers and coupling factor, Calvin cycle enzymes except Rubisco (RuBPase), and Rubisco itself.

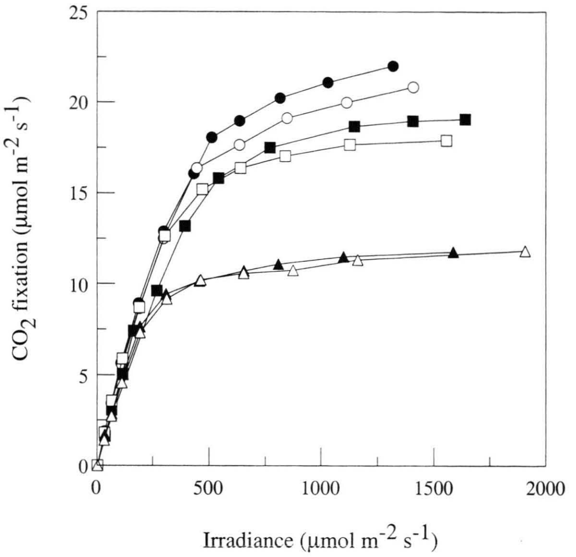

Fig. 2A. Relationship between CO_2-fixation rate and irradiance for the 2nd leaf of tomato. ●: 23, ○: 27, ■: 30, □: 34, ▲: 37 and △: 41 days after sowing. From 23 to 41 days after sowing, the number of leaves overshadowing the 2nd leaf increased from zero to four.

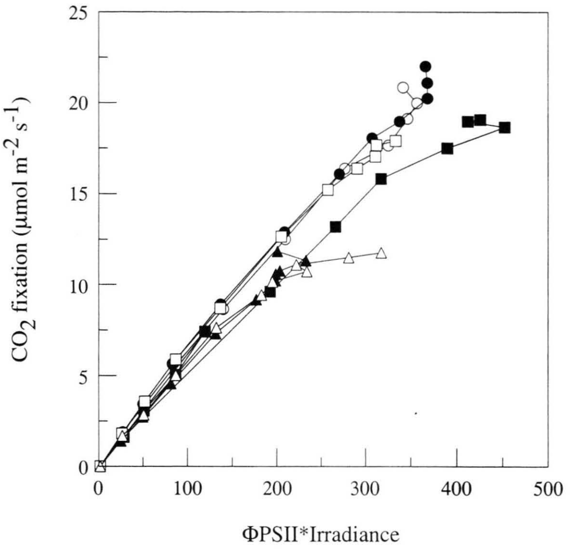

Fig. 2B. Relationship between CO_2-fixation rate and the index of electron transport by PSII.

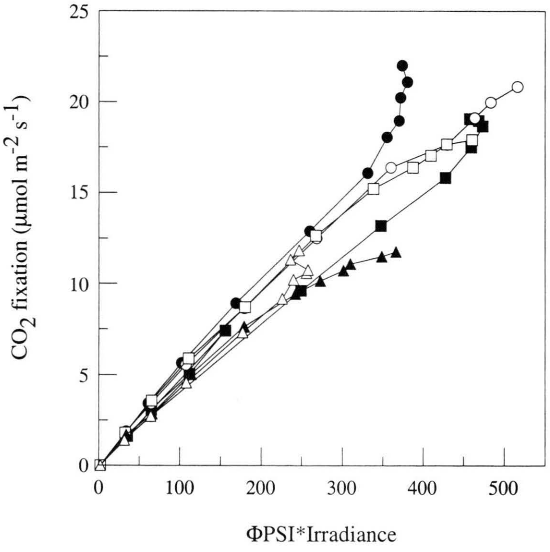

Fig. 2C. Relationship between CO_2-fixation rate and the index of electron transport by PSI.

starved leaf declined much more with increasing irradiance than in the control. This greater light-induced decrease of PSII efficiency in the starved leaf implies that more absorbed quanta must have been dissipated by non-photochemical pathways. The inducible non-photochemical quenching pathway, which is associated with zeaxanthin synthesis, reduced Fv′/Fm′, the quantum yield for electron transport by open PSII reaction centres. With increasing irradiance Fv′/Fm′ also decreased, but clearly much more for the N-starved plants than for the control plants (Fig 3A). Notably, however, when the losses of the quantum yields for PSII photochemistry and Fv′/Fm′ produced by increas-

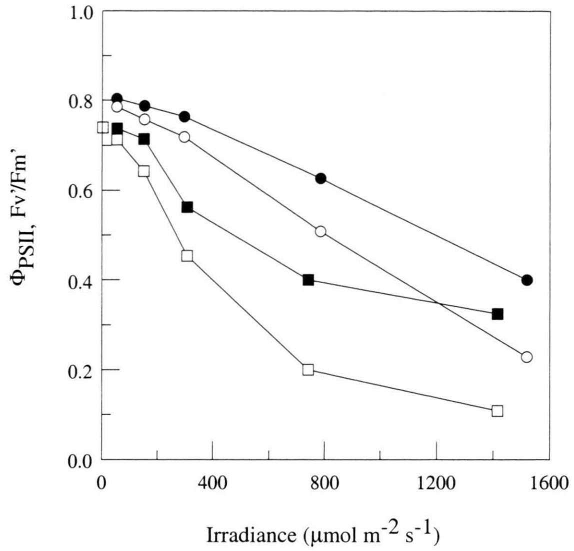

Fig. 3A. Irradiance dependency of the quantum yield of electron transport by photosystem II (Φ_{PSII}) and the quantum yield of electron transport by open photosystem II centres (Fv′/Fm′) for the second leaf of tomato plants at high N (grown hydroponically on 12 mM nitrate) and low N (plants grown on 12 mM nitrate followed by 7 days of N depletion at 0 mM nitrate).

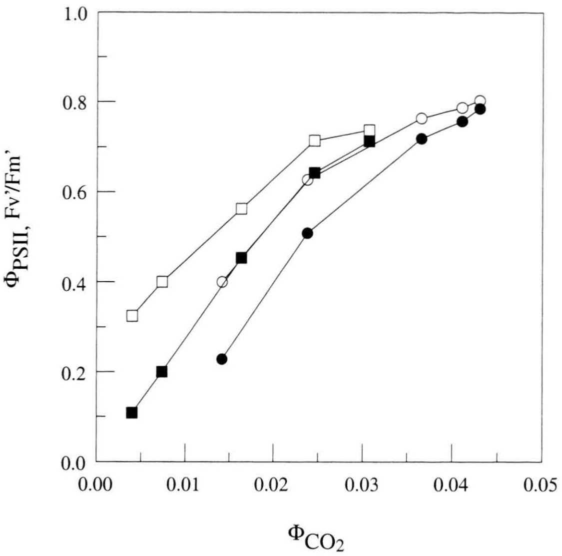

Fig. 3B. (Φ_{PSII} and Fv′/Fm′ as a function of the quantum yield of CO_2 fixation (Φ_{CO2}) for the second leaf of tomato plants at high N and low N. ● ■: Φ_{PSII}, ○ □: Fv′/Fm′, ● ○: high N, ■ □: low N.

ing irradiance are compared to the losses of the quantum yields for CO_2 fixation (Fig 3B), it is clear that the relationships are very similar for N-starved and control plants. The major difference between the N-starved and control leaves is that relative to the relationships obtained from control leaf, those of the N-starved leaf have been shifted to lower quantum yields of CO_2 fixation. This shift is probably due to decreased leaf absorbance in the N-starved plant, and possibly combined with increased photorespiration.

Stomatal regulation

Stomatal conductance regulates both photosynthesis and the hydrological status of the plant. It has been argued that nitrogen deficiency increases the hydraulic resistance of the root and consequently a reduced shoot water potential will limit leaf expansion either directly by a decrease of leaf turgor or indirectly by a decline of cell wall extensibility (Chapin *et al.,* 1988). This has a negative effect on growth processes and sink activity. The nitrogen effects on sink activity are mediated faster than on photosynthetic machinery and therefore photosynthesis must be down-regulated and stomatas will close. In our experiments, nitrogen limitation indeed reduced stomatal conductance (Fig. 4). However, response curves of photosynthesis to internal CO_2 concentration (A/C_i curves) over a wide range of leaf nitrogen contents revealed a predominant role for the mesophyll: at any given C_i, photosynthesis was lower at low nitrogen contents. Under non-photorespiratory conditions, N-deficiency decreased stomatal conductance as a function of C_i by on average 40% (Fig. 4), which was of similar magnitude as the decrease in CO_2 assimilation. Therefore, in our experiments tomato stomatal conductance followed changes in photosynthesis, and not vice versa. Note, however, that for other plant species and N levels, stomatal limitation has been reported: in Phaseolus vulgaris a transition from mesophyll to stomatal limitation occurred when N-deficiency increased (von Caemmerer and Farquhar, 1981).

As nitrogen content did not affect tomato stomatal conductance directly, but only through changes in photosynthesis, the differences in pho-

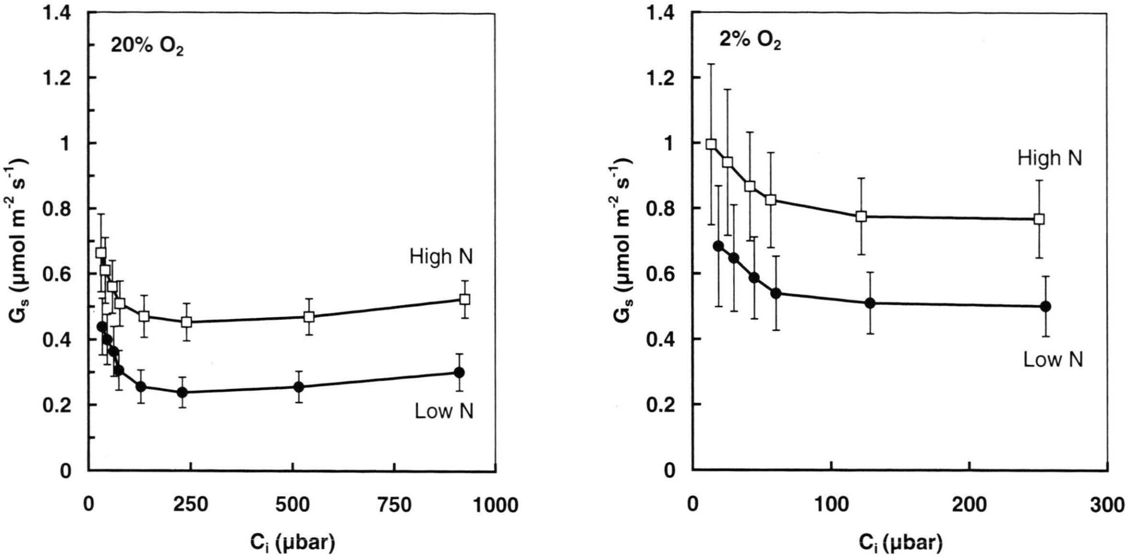

Fig. 4. Stomatal conductance as a function of intercellular CO_2 concentration (Ci) at high nitrogen (open symbols) and low nitrogen content (closed symbols) at 20% O_2 (Fig. 4A) and 2% O_2 (Fig. 4B).

torespiration between the high and the low N-treatments (Fig. 5) could not be attributed to decreased CO_2 uptake through stomata. We therefore argue that the CO_2 concentration at the carboxylation site is lower under low nitrogen, possibly due to a high diffusion resistance in the intercellular space within the leaves. Evans *et al.* (1986) already suggested this in 1986 but no data to support this had emerged since.

Integration of results

The preceding paragraphs showed how nitrogen supply, light intensity and leaf age affect nitrogen

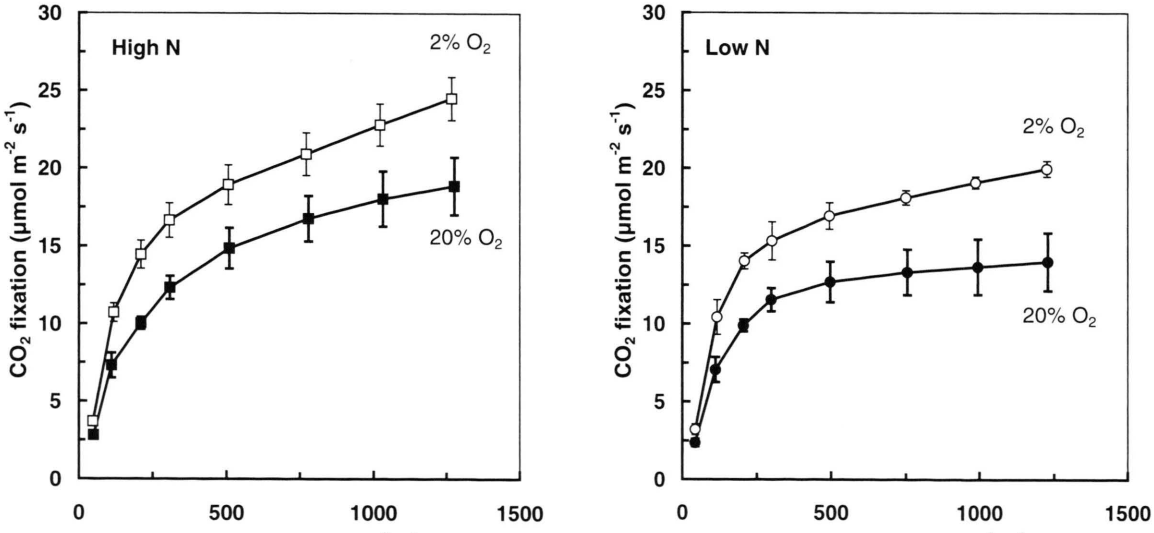

Fig. 5. Photosynthetic rate as a function of irradiance of single leaves at high (Fig. 5A) and low (Fig. 5B) nitrogen under photorespiratory (20% O_2; closed symbols) and non-photorespiratory conditions (2% O_2; open symbols).

partitioning within leaves (to different parts of the photosynthetic apparatus) and between leaves (whereby older leaves lose photosynthetic capacity). We combined this information in our crop growth model to evaluate the effects of nitrogen partitioning on crop productivity. The analysis of leaves at different positions within the canopy (Fig. 2) revealed that light-saturated photosynthetic capacity varied more with leaf position than electron transport capacity did. This suggests a major role for leaf position dependent variation in Rubisco content, and we used the model to determine to what extent different Rubisco profiles in the canopy would affect seasonal crop growth and yield. We ran simulations for seasons with high or low light intensity, and high or low nitrogen supply. In all simulation runs the average Rubisco content of leaves was kept at the measured values ("high N simulations") or at half those values ("low N" simulations). Crop growth and yield was calculated for three types of Rubisco profile within the canopy: "uniform" (same Rubisco level for all leaf layers), "age-dependent" (Rubisco level decreases with leaf age proportional to observed reductions in light-saturated photosynthetic rates) and "light-dependent" (Rubisco levels decreases exponentially from top to bottom leaves conform the canopy light profile). The results are shown in Table I. Both age- and light-dependent Rubisco profiles are superior to the uniform profile: yields are increased by 1.3–7.8% depending on light intensity and N-supply. Light-dependent profiles are best, but real profiles may follow the age-dependent profile instead because that involves less remobilization of structural nitrogen for redistribution to higher leaf layers.

The calculated yields for the three types of Rubisco profile differed relatively little. We conclude that the various leaf level adaptation processes discussed in the first part of this paper may be more important for tomato plant performance at different levels of nitrogen supply than optimization of canopy Rubisco profiles.

Table I. Simulations of the effects of Rubisco distribution between leaf layers on fruit dry matter yield after 20 weeks of growth, at two levels of light intensity (normal, i.e. an average of 725 μmol PAR m^{-2} s^{-1}, and low, i.e. 80% of normal) and nitrogen supply (optimal and 50% of optimal). Leaf Rubisco contents were assumed: (1) equal for all leaf layers ("Uniform distribution"); (2) decreasing with leaf age ("Age-dependent distribution"); (3) proportional to the canopy light profile ("Light-dependent distribution").

Light intensity	N supply	Fruit yield			
		Uniform Rubisco distribution		Age-dependent Rubisco distribution	Light-dependent Rubisco distribution
		(g m^{-2})	(%)	(% of Uniform)	(% of Uniform)
High	High	585	100.0	104.3	105.3
	Low	235	100.0	103.4	107.8
Low	High	515	100.0	102.0	105.0
	Low	210	100.0	101.3	106.8

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