

Nitrate Nutrition and Temperature Effects on Wheat: Photosynthesis and Photorespiration of Leaves

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ABSTRACT

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Photosynthetic and photorespiratory carbon dioxide exchange by the third leaf of spring wheat (*Triticum aestivum* cv. Kolibri), was analysed for plants grown at 13/10 °C (day/night temperature) and 23/18 °C with two rates of nitrate fertilization (a basal rate, –N, and a 4-fold larger rate, +N) and, in some experiments, with two photon fluxes. Net photosynthesis was greatest at the time of maximum lamina expansion, and for leaves grown with additional nitrate. Maximum rate of photosynthesis, carboxylation efficiency and photochemical efficiency at maturity were slightly decreased by nitrate deficiency but photosystem activity was similar under all conditions. As leaves aged, photosynthesis and photochemical efficiency decreased; carboxylation efficiency decreased more than photochemical efficiency particularly with basal nitrate. Low oxygen increased the carboxylation and photochemical efficiencies, and increased the maximum rate of assimilation by a constant proportion in all treatments. Photorespiration, measured by CO₂ efflux to CO₂-free air, by ¹⁴CO₂ uptake, and from compensation concentration, was proportional to assimilation in all treatments. It was greater, and formed a larger proportion of net photosynthesis, when measured in warm than in cold conditions but was independent of growth conditions. Assimilation was related to RuBPC-o activity in the tissue. Relationships between photosynthesis, photorespiration and enzyme complement are discussed.

Key words—Wheat, leaves, nitrate nutrition, temperature effect, photosynthesis, photorespiration.

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INTRODUCTION

Nitrate fertilization of crops influences many stages in the production of dry matter, e.g. by increasing leaf area and light absorption (Bowes, Ogren, and Hageman, 1972), and by increasing both the rate and the efficiency of photosynthesis (Evans, 1983). The efficiency of carbon dioxide assimilation is determined by the rate of gross photosynthesis and by the rate of CO₂ loss in photorespiration, both of which are related to the amount of ribulose bisphosphate carboxylase–oxygenase enzyme (RuBPC-o) and its characteristics, and by dark respiration (Farquhar and von Caemmerer, 1982).

In a preceding paper (Lawlor, Boyle, Kendall, and Keys, 1987a) we showed that activity (per unit leaf area) of some enzymes of the photosynthetic and photorespiratory carbon and nitrogen cycles, changed in proportion to soluble protein content when nitrate supply, temperature and age were varied. A constant ratio of chlorophyll to RuBPC-o was maintained, suggesting close linkage between light harvesting and the processes of carboxylation. The ratio of RuBPC to RuBPo activity, measured under constant conditions, was the same in protein from leaves grown under different nitrogen nutrition and temperature. A constant ratio of photorespiration to photosynthesis at a particular temperature would, therefore, be expected. However, Thomas (1976, 1977) presented evidence that abundant nitrate fertilization of wheat in the field increases the ratio of photorespiration to photosynthesis, suggesting modifications to photorespiratory metabolism. Nitrate reductase activity showed greater change with nitrate nutrition and temperature than did RuBPC-o activity or that of other photosynthetic enzymes, suggesting that the balance between nitrate reduction and assimilation might be varied by environmental conditions and provide a mechanism by which metabolism and cell composition respond to differences in nitrate supply and temperature (Lawlor *et al.*, 1987a).

If the efficiency of the photosynthetic system, including carbon and nitrogen assimilation, is to be improved, it is essential that we understand the factors regulating the amounts of components, efficiencies of light harvesting and of the enzymatic 'dark' processes of photosynthesis and photorespiration, and the balance between these various processes (Farquhar and von Caemmerer, 1982). Studies are often restricted to particular metabolic processes, yet the agriculturally important feature is the production of the whole plant. To analyse the mechanisms of plant response to nitrate nutrition and temperature we have measured photosynthesis, stomatal conductance, photorespiration and photosystem electron transport of the 3rd leaf of wheat; they are related to changes in leaf composition, reported by Lawlor *et al.* (1987a), to understand the integration of metabolic and physiological processes and the effects of environment on the plant responses.

MATERIALS AND METHODS

Growth conditions

Plants of spring wheat (*Triticum aestivum* var. Kolibri) were grown as described in Lawlor *et al.* (1987a) with low or high ($4 \times$ low) nitrate concentration ($-N$ and $+N$; 0.9 and 4.0 mmol NO_3^- weekly per pot respectively) supplied in Hoagland's nutrient, at day/night temperature of 13/10 °C (treatment C) and 23/18 °C (treatment W) at 550 μmol quanta photosynthetically active radiation (*PAR*) $\text{m}^{-2} \text{s}^{-1}$ or, in other experiments, at either 350 or 600 μmol quanta $\text{m}^{-2} \text{s}^{-1}$.

Leaves were sampled 13, 15, 17, 21 and 24 d after sowing in the warm conditions and after 22, 26, 29, 36 and 42 d in the cold. These times correspond to approximately equivalent physiological ages of the leaves, and to equivalent accumulated temperature above 0 °C. In the text the leaf growth stages are referred to as expanding, almost mature, mature, ageing and early senescent.

Measurement of net photosynthesis, photorespiration and CO₂ compensation concentration

Photosynthesis was measured in two gas exchange systems of different design. In one, net photosynthesis was measured on five detached leaf pieces (five pieces, 5.0 cm long, *c.* 10 cm²), standing with the cut ends in water, in an open flow gas stream without forced ventilation. The *PAR* flux was 1000 μmol quanta $\text{m}^{-2} \text{s}^{-1}$ from a tungsten-halogen lamp and air was supplied from commercial gas cylinders. Carbon dioxide exchange was measured in triplicate by infra-red gas analysis (Analytical Development Company, Hoddesdon, U.K.; model 225 Mk. II Analyser). Photorespiration was measured as CO₂ efflux into CO₂-free air. Temperature was regulated by circulating water from a water bath through a jacket surrounding the leaf chamber.

Photosynthesis was also measured in triplicate in an open gas exchange system, concomitant with water vapour exchange, on single attached leaves in a chamber with forced ventilation. Carbon

dioxide and oxygen concentrations in the chamber atmosphere were controlled by a gas blender (Signal Instrument Co., Croydon, U.K.) and measured using an infra-red gas analysis (ADC Mk. III Analyser) and a paramagnetic oxygen analyser (Hartman and Braun, West Germany) respectively. Photon flux density from mercury vapour lamps was varied by neutral wire-mesh screens. Leaf temperature was measured by a fine wire thermocouple pressed to the lower surface and was regulated by adjusting the temperature of the chamber water jacket. The water vapour content of the air was controlled by saturating with water vapour and cooling to required dew point before warming to the required temperature, and was measured by humidity sensors (Vaisala, U.K.) before and after passage over the leaf, vapour pressure deficit was maintained at 0.8 to 1.0 kPa. Leaf areas, for calculation of photosynthetic rates, were determined by an automatic planimeter (Platon Industries, Australia). Both photosynthesis rate and stomatal conductance were calculated according to Farquhar and Sharkey (1982).

The response of net photosynthesis to partial pressure of CO₂ ('CO₂ response curves') was measured at 1000 μmol quanta m⁻² s⁻¹, and at an O₂ partial pressure of either 2.0 or 21 kPa. The response to photon flux ('light response curves') was measured at 90 Pa CO₂ partial pressure in 2.0 or 21 kPa O₂. Carboxylation efficiency was derived from the initial slope of the CO₂ response curves, and the quantum yield (photochemical efficiency) was calculated from the initial slope of the light response curves, after correction for reflection and transmission of radiant energy by the leaves derived from an integrating sphere (Applied Photophysics, U.K.).

Carbon dioxide compensation concentration of leaves was determined in triplicate from the CO₂ response curves or from measurements made in a closed circuit system at constant temperature (20 °C) and light (1200 μmol quanta m⁻² s⁻¹). Equilibrium in the closed system was reached from initial conditions of zero CO₂ or from *c.* 40 Pa CO₂ and required 15 to 20 min; leakage of CO₂ into the closed system caused an increase in CO₂ partial pressure of less than 0.1 Pa CO₂ in 10 min.

Measurement of gross photosynthesis and photorespiration using ¹⁴CO₂

Assimilation of ¹⁴CO₂ was measured on attached leaves in the ventilated leaf chamber using ¹⁴CO₂ at constant specific activity, 14.3 kBq μmol⁻¹ CO₂, in air (21 kPa O₂, 79 kPa N₂) from a gas cylinder. Gross photosynthesis was calculated (Ludwig and Calvin, 1971) from measurements of ¹⁴CO₂ depletion made with a 20 cm³ ionisation chamber and electrometer (Varian Associates) with the switching system described by Lawlor, Mahon, and Fock (1977). Measurements were taken after 15 s exposure to ¹⁴CO₂ under steady-state CO₂ partial pressure and with a steady rate of net assimilation. After assimilation of ¹⁴CO₂, leaves were removed from the chamber and frozen in liquid nitrogen within 2 s.

Measurement of oxygen uptake and evolution

Oxygen exchange of cut sections of leaves grown in 350 or 600 μmol quanta PAR m⁻² s⁻¹ was measured by polarographic oxygen electrode (Hansatech Limited) at saturating CO₂ concentrations and 21–22 kPa O₂ in the atmosphere.

Photosystem assay

Activities of photosystems I and photosystems I + II were measured on thylakoid fragments prepared by the method of Powles and Critchley (1980) using a polarographic oxygen electrode at 20 °C with chlorophyll concentration of 300 to 400 μg cm⁻³ of solution. PS I was assayed by measuring the rate of O₂ consumption, with ascorbate/DCPIP as electron donor and methyl viologen (MV) as electron acceptor in an assay medium containing 30 mol m⁻³ orthophosphate buffer (pH 8.0), 10 mol m⁻³ MgCl₂, 1.0 mol m⁻³ ascorbate, 0.1 mol m⁻³ DCPIP, 0.1 mol m⁻³ MV and 2.5 mol m⁻³ NH₄Cl as uncoupler. Photosystem I plus II in combination was assayed by measuring O₂ evolution linked to potassium ferricyanide reduction in a reaction mixture containing 50 mol m⁻³ HEPES (pH 7.6), 1.0 mol m⁻³ MgCl₂, 1.0 mol m⁻³ EDTA and 1.5 mol m⁻³ K₃Fe(CN)₆. Actinic light, from a quartz-halogen lamp, was at saturating photon flux (1000 μmol quanta m⁻² s⁻¹). Two determinations were made on two occasions on groups of plants from each treatment at equivalent physiological stages of development.

Chlorophyll was measured by the method of Arnon (1949) in acetone extracts of leaves or homogenates from the photosystem assay. Measurements of RuBP carboxylase-oxygenase amount and activity and soluble protein were described by Lawlor *et al.* (1987a).

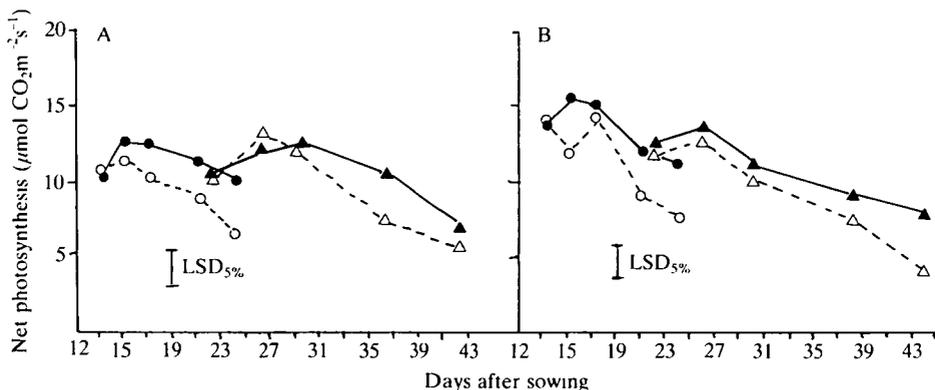


FIG. 1. Net photosynthesis related to age of the 3rd leaf of wheat grown at 13/10 °C (day/night temperatures) C, and 23/18 °C, W, and with low nitrate (–N) or high (4× the low amount of nitrate: +N). Measurements at 13 °C (Fig. 1a) and 23 °C (Fig. 1b); 33 Pa CO₂, 21 kPa O₂, 1000 μmol quanta (PAR) m⁻² s⁻¹ photon flux density. Bar represents least significant difference ($P = 5\%$).
C–N – – – Δ – – – , C+N —▲— , W–N – – – ○ – – – , W+N —●— .

RESULTS

Net photosynthesis and CO₂ response

Rates of P_{net} were measured in unstirred leaf chambers at two temperatures corresponding to the day-time growing temperature. Leaves grown under warmer conditions had higher P_{net} (Fig. 1a, b) than those grown in the cooler temperature when measured at 23 °C, but when measured at 13 °C there was little difference. Expanding leaves from all growth conditions had very similar rates of assimilation, and nitrogen supply had little effect. Mature leaves grown with additional nitrate had, on average, 15% higher rates of assimilation than those grown with low nitrate. At early senescence, P_{net} of leaves grown with extra nitrate decreased by about 25% in warm and 44% in the cool growth conditions respectively compared to the rate at maturity (and averaged over measurement temperature), and decreased without additional nitrogen by 47% and 67% in warm and cool growth conditions respectively. Attached leaves, in ventilated leaf chambers at 20 °C, had higher rates of assimilation when young (Fig. 2) than those measured in unstirred chambers at similar irradiance, but treatment effects were similar. P_{net} ranged from 26 to 18 μmol CO₂ m⁻² s⁻¹ at maximum leaf expansion in the W + N and C – N treatments respectively, but decreased to 11.0 and 8.0 μmol CO₂ m⁻² s⁻¹ in the same treatments at early senescence. Assimilation at saturating CO₂ (Fig. 2) was about 50% and 70% greater than in normal air (32 Pa CO₂) for leaves grown in warm and cool conditions respectively and was greater with additional nitrate, but independent of growth temperature.

Net photosynthesis was determined in the ventilated chamber at a range of CO₂ partial pressures and 1000 μmol quanta m⁻² s⁻¹ at 20 °C to analyse the response of assimilation to internal CO₂ pressure. In mature leaves grown with additional nitrate, P_n saturated at approximately 35 μmol CO₂ m⁻² s⁻¹ when the internal CO₂ pressure was *c.* 40 Pa, and there was little difference between the warm or cold grown plants. Leaves from warm-grown plants without additional N had a smaller maximum rate of assimilation than those grown with extra N (32 *cf.* 34 μmol m⁻² s⁻¹) but smaller (25 *cf.* 32 μmol m⁻² s⁻¹) when grown in the cold. The rate of assimilation per unit of internal CO₂ partial pressure and the maximum rate of photosynthesis decreased as the leaves aged in all treatments but remained greater in leaves from plants grown with extra nitrate. P_{max} decreased rapidly

particularly in warm conditions. By comparison net photosynthesis measured in 32 Pa CO₂ decreased less rapidly.

Carboxylation efficiency of young leaves grown at warm temperature (measured at 15 d) was greater than of plants grown in the cold (measured at 25 d) both with and without additional nitrate (W + N 1.62; W - N 1.50; C + N 1.55; C - N 1.38; (l.s.d. 0.105) $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$). As leaves aged the carboxylation efficiency decreased rapidly in all treatments; the rate of decrease per day was slightly greater for leaves grown in warm (measured at 25 d) than cool (measured at 40 d) conditions and without compared to with extra nitrate (W + N 0.80; W - N 0.79; C + N 0.40; C - N 0.45 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$).

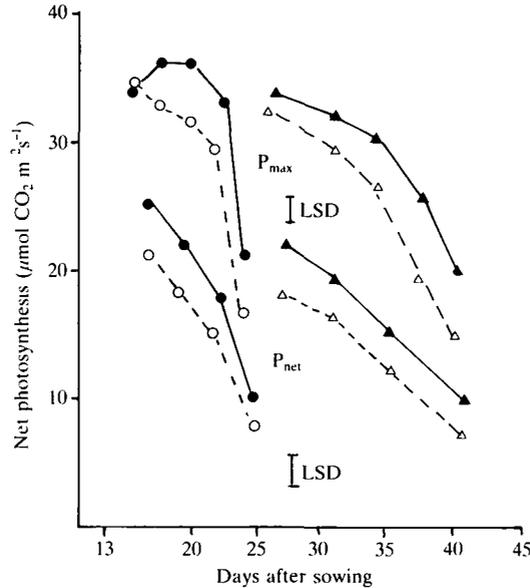


FIG. 2. Maximum photosynthetic rate (P_{max}) at saturating CO₂ (90 Pa), compared with the rate at 32 Pa CO₂ (P_n), and 1000 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ in 21 kPa O₂, 20 °C for leaves of wheat grown under C - N, C + N, W - N and W + N conditions. Bar represents least significant difference ($P = 5\%$).

Photosynthetic light response, photochemical efficiency and stomatal conductance

Assimilation by young leaves grown in warm conditions (Fig. 3a) increased with illumination from zero up to 1500 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ although the increase per unit of PAR was much less above $\sim 500 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. Maximum rate of assimilation was smaller with low nitrate, and with age P_{max} decreased, rather more with deficient nitrogen. Leaves of cold-grown plants (Fig. 3b) responded to increasing photon flux similar to warm-grown up to $\sim 1000 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ but with brighter light their assimilation rate decreased.

Stomatal conductance of young leaves as a function of photon flux (Fig. 4a) was little affected by growth conditions but (Fig. 4b) conductance of older, cold-grown leaves decreased markedly in both nitrate treatments. As leaves aged, conductance (Fig. 4b) measured at 1000 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (i.e. below the threshold for stomatal closure in cold-grown plants) decreased more rapidly in warm than in cold conditions. The absolute decrease in conductance was greatest with nitrate deficiency in cold conditions.

The photochemical efficiency (mol CO₂/mol quanta), calculated from the initial slope of

the light response curve, and measured in both warm and cool conditions, was similar in young leaves from warm (16-d-old) and cool (24-d-old) conditions (W + N 0.042; W - N 0.044; C + N 0.040; C - N 0.039; s.e.d. 0.005). It decreased with leaf age in all treatments, the decrease was somewhat greater with nitrate deficiency but differences were small and only significant in the case of the cold grown plants; at 25 d and 40 d in cool and warm conditions the values were W + N, 0.028; W - N, 0.028; C + N, 0.029 and C - N, 0.020.

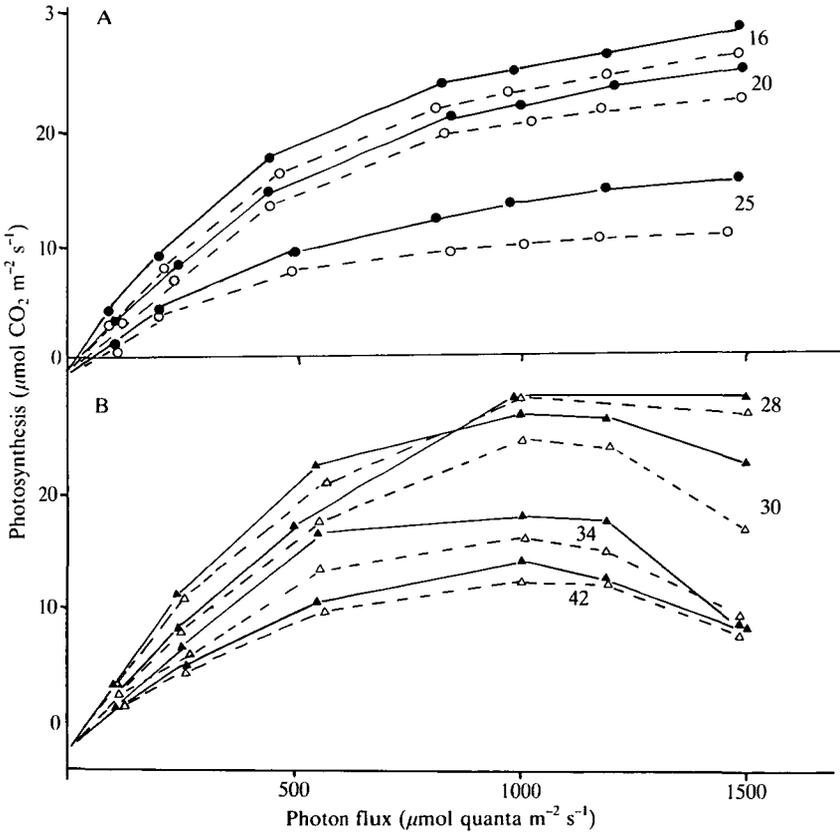


FIG. 3. Response of photosynthesis to photon flux, for the 3rd leaf of wheat grown under conditions described in legend to Fig. 1. Measurements at 32 Pa CO_2 , 21 kPa O_2 , 20 °C. (A) W + N, W - N plants, 16, 20 and 25-d-old. (B) C + N, C - N plants 28, 30 and 42-d-old. Typical responses for single leaves.

Effects of the oxygen partial pressure

Assimilation was compared in 2 and 21 kPa O_2 , at 23 °C and 13 °C (Table 1). Decreasing oxygen partial pressure increased photochemical efficiency by 32% when measured at 23 °C and 24% at 13 °C; photochemical efficiency was not significantly affected by nitrate supply and was greatest in warm grown plants measured at 23 °C in 2.0 kPa O_2 . The effect of decreasing O_2 concentration on CO_2 exchange was measured in the ventilated chamber at 1000 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ and 32 Pa CO_2 . Measured at 23 °C (Table 2) photosynthesis increased in low O_2 by between 28% and 35%; measured at 13 °C the increase in assimilation was between 12% and 28%. The smallest difference was in C - N grown plants and largest in the warm grown plants. However, there was no significant difference, averaged over all measurements, between plants grown with different nitrate supply or temperature.

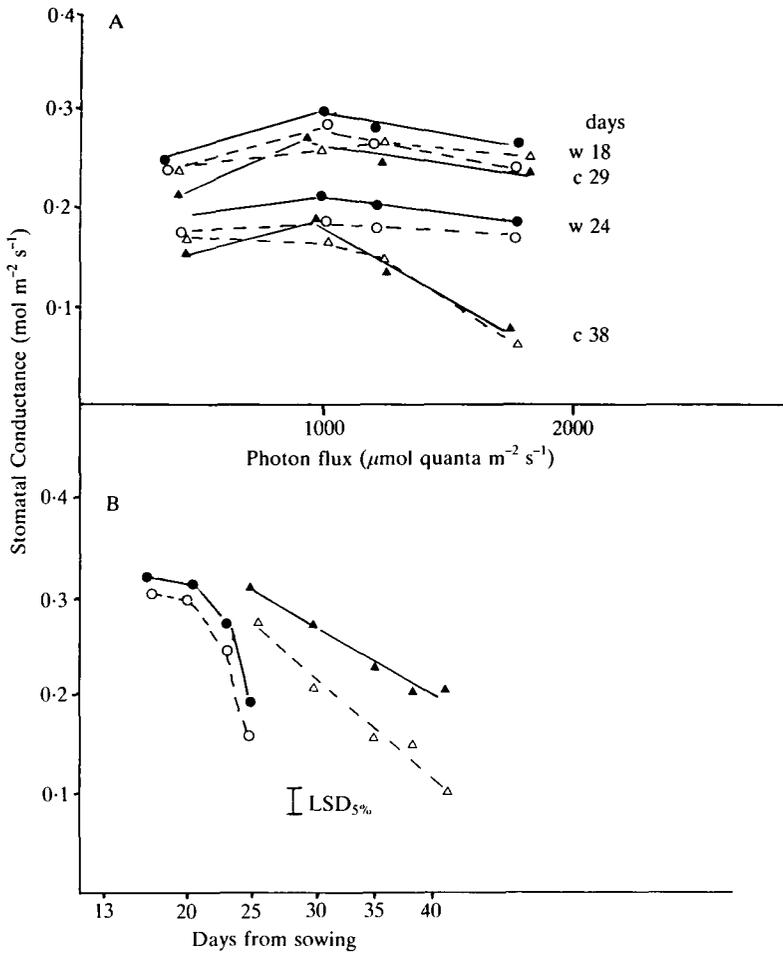


FIG. 4. Stomatal conductance of wheat leaves of (A) different ages (given on figure) in relation to photon flux and (B) stomatal conductance at 1000 μmol quanta m⁻² s⁻¹ with leaves of different ages; treatments described in legend to Fig. 1.

TABLE 1. Photochemical efficiency (mol CO₂ mol absorbed quanta⁻¹) for wheat leaves grown at two temperatures (13 °C and 23 °C) and with low or high nitrate supply and measured at 13 °C and 23 °C and in 2.0 and 21 kPa O₂; % increase in efficiency is (2.0 kPa/21 kPa) × 100

Measurement temperature	O ₂ (kPa)	Growth temperature				Standard error of difference
		13/10 °C		23/18 °C		
		N (24-28-d-old)	+N	N (16-18-d-old)	+N	
23 °C	2	0.047	0.052	0.058	0.058	0.007
	21	0.038	0.041	0.043	0.041	0.005
	% Increase	24	27	35	41	
13 °C	2	0.050	0.053	0.050	0.051	0.006
	21	0.037	0.041	0.044	0.042	0.005
	% Increase	35	29	14	21	

TABLE 2. Photosynthesis and photorespiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$) measured at $1000 \mu\text{mol quanta PAR m}^{-2} \text{s}^{-1}$, 32 Pa CO_2 and at $2.0 (P_2)$ and $21 \text{ kPa O}_2 (P_{21})$ and at two temperatures. Plants grown in two temperatures and with two nitrate concentrations. Photorespiration (PR) calculated as difference $P_2 - P_{21}$ and PR% as $100 (P_2 - P_{21}) / P_{21}$

Measurement temperature	O ₂ (kPa)	Growth temperature				Standard error of difference
		13/10 °C		23/10 °C		
		N	+N	N	+N	
23 °C	P ₂	23	28	23	28	3.19
	P ₂₁	18	21	17	21	2.90
	PR	5	7	6	7	1.56
	PR%	28	33	35	33	—
13 °C	P ₂	19	22	22	24	2.39
	P ₂₁	17	18	18	18	2.00
	PR	2	4	4	5	2.20
	PR%	12	22	22	28	—

Mean of 5 equivalent ages of plants.

Photorespiration and CO₂ compensation concentration

Photorespiration of leaves was measured in unstirred chambers as CO₂ efflux to CO₂-free air, at 13 °C and at 23 °C, with $1000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ and 21 kPa O₂. Photorespiration was greater at 23 °C than at 13 °C (Fig. 5) and in leaves grown with additional nitrate than without, particularly as the leaves aged. Cool growth temperature also decreased photorespiration compared to warm when measured at 23 °C. The ratio of photorespiration to net photosynthesis measured at 13 °C was approximately 14% and was similar in young

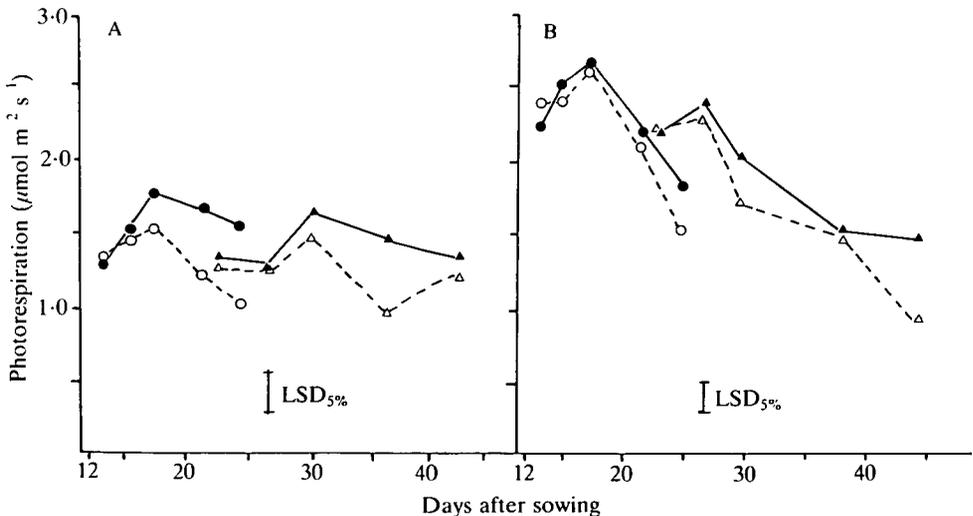


FIG. 5. Photorespiratory CO₂ evolution related to the age of wheat leaves grown under different temperature and nitrate supply, measured at (A) 13 °C and (B) 23 °C, by CO₂ efflux into CO₂-free air (21 kPa O₂, $1000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Bars represent least significant differences ($P = 5\%$).

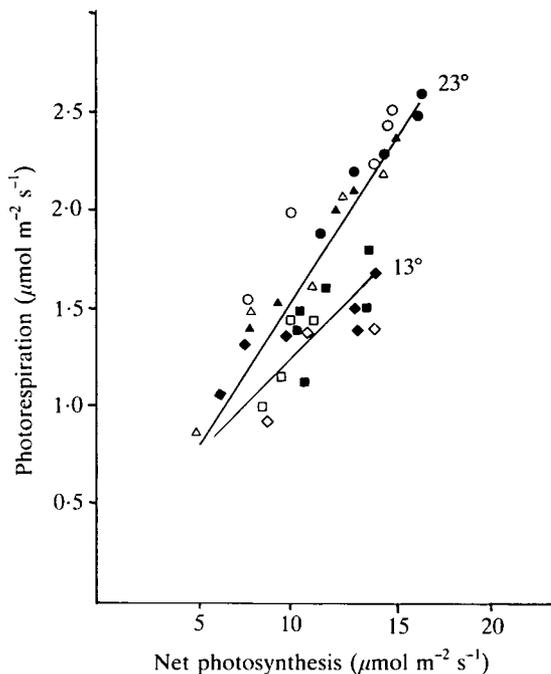


FIG. 6. Photorespiration in relation to net photosynthesis for the third leaf of wheat, grown under conditions described in legend to Fig. 1. Measurements made at 13 °C or 23 °C.

and old leaves on both +N and -N treatments. Photorespiration of leaves measured at 23 °C, was *c.* 17% of net photosynthesis in all treatments and did not change significantly with leaf age. Photorespiration measured at a particular temperature, was proportional to net photosynthesis for plants grown under all conditions (Fig. 6).

The compensation concentration of mature leaves (Table 3) was greater when measured at 23 °C than at 13 °C but did not differ significantly with growth conditions. Compensation concentration estimated from the CO₂ response curves was not significantly different between treatments, although, as the leaves aged, it increased in the low nitrate treatments more than in the high.

TABLE 3. Carbon dioxide compensation concentration (*P*_a) of detached wheat leaves measured at 850 μmol quanta m⁻² s⁻¹ and 21 kPa O₂ at two temperatures. Plants grown at two temperatures and at two levels of nitrate supply

Measurement temperature	Growth temperature				Standard error of difference
	13/10 °C		23/18 °C		
	N	+N	N	+N	
23 °C	3.90	3.73	4.06	4.01	0.12
13 °C	2.90	3.40	3.24	3.01	0.14

Average of 5 measurements.

The difference in photosynthesis between 21 and 2.0 kPa O₂ shows the potential rate of assimilation in the absence of photorespiration. Assimilation in 2.0 kPa O₂ was greater than that in air by approximately 32% (Table 2); this percentage increased slightly as leaves aged and was slightly (but not significantly) higher in low nitrate grown leaves.

Photorespiration was also estimated as the differences between net photosynthesis and calculated gross photosynthesis measured during steady-state photosynthesis 15 s after exposure to ¹⁴CO₂. The ratio of photorespiration to photosynthesis at 20 °C was 17% and 10% in C–N and C+N and 10% and 11% in W–N and W+N grown plants, but there was no significant difference between treatments. The mean values agreed with the estimates made by CO₂ efflux to CO₂-free air.

Oxygen evolution by leaves

Oxygen evolution was measured in saturating CO₂ at 20 °C as a function of photon flux on pieces of leaves grown in bright or dim light (600 and 350 μmol quanta m⁻² s⁻¹ respectively). In bright light (1500 μmol quanta m⁻² s⁻¹), O₂ evolution was stimulated in leaves grown in bright compared to dim light (27.2 cf. 23.3; l.s.d. 2.4 μmol O₂ m⁻² s⁻¹) averaged over other treatments. Cool growth conditions increased O₂ production (28.8 cf. 21.8 μmol O₂ m⁻² s⁻¹) with greater photochemical efficiency (0.048 cf. 0.038; l.s.d. 0.008 mol O₂ mol⁻¹ quanta). Additional nitrate supply increased O₂ evolution slightly in all growth conditions (26.8 cf. 24.5 μmol m⁻² s⁻¹), but by somewhat more in the warm (24 cf. 21 μmol O₂ m⁻² s⁻¹) than in the cold (30 cf. 27.5 μmol O₂ m⁻² s⁻¹). Efficiency did not increase significantly with additional nitrate, 0.050 cf. 0.045 in the cold and 0.039 cf. 0.037 in the warm.

Chlorophyll content and photosystem activity

Chlorophyll a and b content per unit leaf area was greatest (Table 4) in leaves grown at low temperature with additional N and in bright light. However, the chlorophyll a/b ratio was not significantly affected by illumination, temperature or additional nitrate. In other experiments the chl a/b ratio was 3.6 in plants from dim light and 3.2 from plants in

TABLE 4. Photosystem activity in wheat leaves grown under high and low nitrate supply, two temperatures and at two photon fluxes. *s.e.d.* is standard error of difference

Light	350 μmol quanta m ⁻² s ⁻¹				650 μmol quanta m ⁻² s ⁻¹				s.e.d.
	13/10 °C		23/18 °C		13/10 °C		23/18 °C		
Nitrate	N	+N	N	+N	N	+N	N	+N	
PSI (nmol O ₂ mg ⁻¹ chl s ⁻¹)	35.5	42.5	44.2	38.8	40.5	29.4	39.4	32.4	3.5
PSI (μmol O ₂ m ⁻² s ⁻¹)	17.4	23.5	16.8	18.4	21.0	18.5	14.9	16.2	4.0
PSI + II (nmol O ₂ mg ⁻¹ chl s ⁻¹)	12.4	10.9	12.9	12.0	17.8	15.1	11.1	13.3	1.1
PSI + II (μmol O ₂ m ⁻² s ⁻¹)	5.9	6.0	4.9	5.7	9.2	9.5	4.0	6.7	2.8
Chl a + b (g m ⁻²)	0.48	0.55	0.38	0.47	0.52	0.63	0.36	0.50	0.03
Chl a/b ratio	3.5	3.2	3.5	3.4	3.3	3.5	3.4	3.3	0.07

bright light. Photosystem I activity per unit of chlorophyll (Table 4), was greatest in leaves grown in low light W – N conditions; lowest activity was with bright light in C + N growth conditions. On a leaf area basis, PSI activity was slightly greater with additional N, in the cold and in dim light. Photosystem I+II activity per unit of chlorophyll, with ferricyanide as acceptor, was greatest with bright light in cold conditions particularly with low N (Table 4). PS I+II activity per unit leaf area was somewhat larger in plants grown in bright light particularly in cool growth conditions and additional nitrogen also increased it.

DISCUSSION

Assimilation rate, carboxylation efficiency and RuBPC-o characteristics

The methods of measuring gas exchange gave comparable results; in the ventilated leaf chamber, rates of net photosynthesis were greater than in the unventilated chamber under similar conditions, probably because of its larger boundary layer conductance. Net photosynthesis was greatest in leaves at maximum expansion and was slower when measured at 13 °C compared to 23 °C, probably due to slower rates of enzymatic processes.

Assimilation and photorespiration are related to the amounts of protein and pigments and to the activity of enzymes described previously (Lawlor *et al.*, 1987a). The rate of photosynthesis increased with increasing amount of soluble protein (Fig. 7a) up to *c.* 6.0 g m⁻² and with chlorophyll (Fig. 7b) up to 0.4 g m⁻², when averaged over different leaf ages and conditions. At greater amounts of protein, assimilation was nearly constant and, with large chlorophyll amount, even decreased. Assimilation is, therefore, not limited by the amount of bulk pigment or protein at high concentration. As the amount of RuBPC-o forms an almost constant proportion of soluble protein, assimilation remained almost constant with increasing amounts of RuBPC-o in excess of ~3.0 g m⁻², under all conditions of light, NO₃⁻ and temperature, so the rate of CO₂ assimilation per unit of RuBPC-o protein decreased from about 4.4 μmol CO₂ g⁻¹ protein s⁻¹ at 3.0 g RuBPC-o protein m⁻² to 2.7 μmol CO₂ g⁻¹ protein s⁻¹ at 6.0 g protein m⁻². Assimilation rate increased with increasing activity of RuBPC-o m⁻² leaf (both measured at 13 °C), but the relationship was not proportional. In nitrogen deficient leaves RuBPC-o activity m⁻² leaf was only slightly greater than measured *P_n* (activity range *c.* 11 to 22 μmol m⁻² s⁻¹; assimilation 9 to 14 μmol CO₂ m⁻² s⁻¹), but assimilation rate did not increase with RuBPC-o activity at higher protein content. If the activity of RuBPC-o at 23 °C (calculated from measured activity at 13 °C and corrected using a *Q*₁₀ for this temperature range of 2.4 from Jordan and Ogren (1984)) is compared to *P_n* measured at 23 °C, enzyme activity greatly exceeds the measured rate of assimilation. Thus, the assimilation rate is regulated by factors other than the amount or activity of RuBPC-o at larger protein contents. Photorespiration was a constant proportion of assimilation with different treatments and, therefore, not responsible for the restriction in assimilation.

The results suggest that some 50% of the RuBPC-o protein is not activated in leaves with high protein, or alternatively that only half the enzyme sites are functional. Poor correlation of RuBPC-o activity at large protein content, has been observed by Evans (1983) for wheat flag leaves grown in bright light and warm conditions with abundant nitrogen. Accumulation of RuBPC-o without apparent increase in enzyme activity may indicate a function for the protein as nitrogen store. However, Wittenbach, Franceschi, and Giaquinta (1984) rejected the suggestion that RuBPC-o protein is a specific storage protein although it is remobilized to other organs (Peoples, Beilharz, Waters, Simpson, and Dalling, 1980), often early in senescence and particularly with nitrogen deficiency as observed in the present

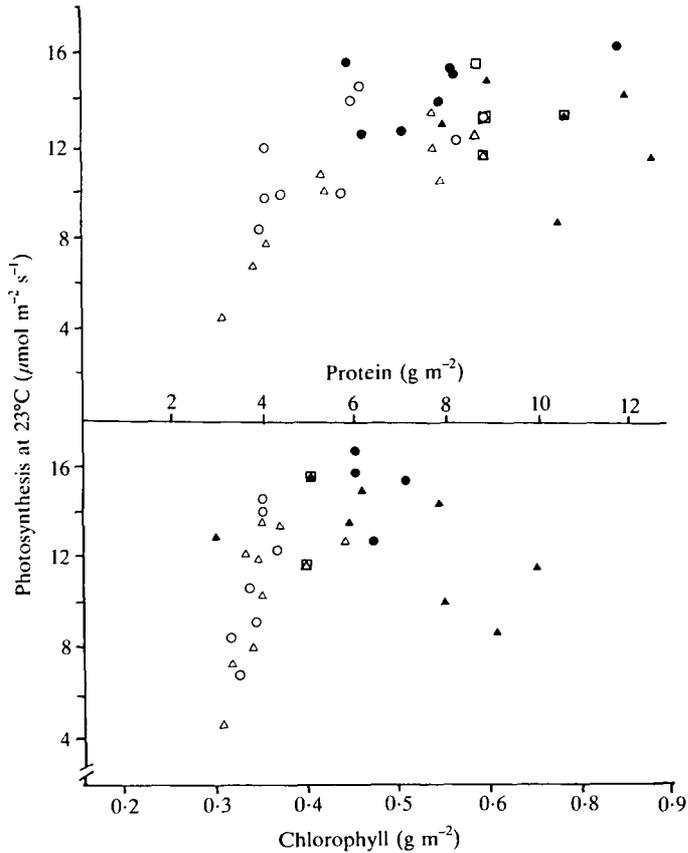


FIG. 7. Assimilation of wheat leaves of different ages, from different growth conditions, measured at 23 °C in relation to total soluble protein and chlorophyll content; data for leaves of different ages, grown under different nitrate fertilizer, temperature and light. Symbols as in Fig. 1 legend; symbols in boxes for plants grown in $600 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ P.A.R.

study. The enzyme therefore fulfils a dual rôle, primarily as the CO_2 fixing enzyme but also as a reserve of nitrogen.

RuBPC-o may have low catalytic activity *in vitro* due to inadequate CO_2 or magnesium for activation, or to incorrect pH (Boyle, 1983) or to decreased availability of inorganic phosphate and increased concentrations of non-reducing sugars, sugar phosphates etc. (Friedrich and Huffaker, 1980; Ku, Prickril, Reger, and Pallas, 1982; Schmidt, Cornelius, Burton, Parry, Millard, Keys, and Gutteridge, 1984) but the importance of these factors *in vivo* has not been assessed. Products of the PCR cycle might be expected to accumulate in chloroplasts with high rates of P_n , inhibiting assimilation. However, measurements (Lawlor, Boyle, Young, Kendall, and Keys, 1987b) showed that RuBP and 3PGA accumulated more with nitrate deficiency and in cool conditions, and ^{14}C -labelling indicated accumulation of phosphorylated intermediates under conditions where specific activity and $P_n/\text{RuBPC-o}$ ratio were generally large. If there is feed-back control of assimilation by the PCR cycle or related intermediates, faster growth in the warm and with additional nitrate should consume products, stimulate RuBPC-o activity and increase photosynthesis. However, no differences in RuBPC-o activity were apparent with temperature, and additional nitrate decreased activity.

Carboxylation efficiency in leaves at maturity was only slightly smaller with deficient nitrate than with ample, despite the large differences in protein and RuBPC-o carboxylase enzyme, suggesting that carboxylation efficiency is controlled by factors other than total amount of RuBPC-o. The model of Farquhar and von Caemmerer (1982) suggests that the amount of RuBPC-o determines the efficiency in saturating light, and Evans (1983) showed that carboxylation efficiency in wheat is strongly dependent on enzyme activity and, therefore, tissue nitrogen status. However, both carboxylation efficiency and the maximum rate of carbon reduction (Farquhar and von Caemmerer, 1982) may be controlled by other enzyme reactions. Thus fructose biphosphatase is a potential control step, but it decreased in our experiments in parallel to the RuBPC-o (Lawlor *et al.*, 1987a) as did several other enzymes associated with CO₂ assimilation.

The carbon dioxide supply to the active sites of RuBPC-o *in vivo* could, potentially, limit the rate of photosynthesis but the decrease in CO₂/O₂ ratio caused by differences in stomatal conductance would not have been sufficient to cause a large increase in photorespiration to photosynthesis ratio, and indeed none was observed with different growth conditions at any one measurement temperature. Evans (1983) calculated that with larger carboxylase concentration, assimilation rate would have been limited by the conductance of the pathway between intercellular spaces and the site of carboxylation, causing depleting CO₂ at the enzyme site and preventing P_n increasing. However, our observations that the enzyme, assayed directly from the tissue, had small specific activity in high protein leaves, conflicts with the interpretation and suggests either that RuBPC-o activity *in vivo* is controlled by unknown factors or other components of the assimilation system are limiting.

Photorespiration

The ratio of photorespiration to photosynthesis, measured at constant temperature by several gas exchange methods, was similar for leaves from different treatments, in agreement with the measurements on the RuBPC-o enzyme (Lawlor *et al.*, 1987a). Decreasing the O₂ content, from 21 to 2.0 kPa, increased assimilation as frequently observed. The O₂ response is consistent with the function of RuBPC-o (Jordan and Ogren, 1984); reducing oxygenation inhibits photorespiration and RuBP becomes available for CO₂ assimilation, so that the O₂ effect is larger than the measured rates of CO₂ release. The smaller proportion of photorespiration to photosynthesis observed at the cool compared to warm measurement temperatures in our experiments may be related to the higher solubility of CO₂ compared to O₂; low temperatures favour carboxylation over oxygenation. The evidence, from the O₂ effect, together with the similar compensation concentrations and similar rates of photorespiration estimated by ¹⁴CO₂, shows that photorespiration of wheat leaves is proportional to assimilation and is unaffected (within the errors of measurement) by growth temperature or nitrate supply, at least until early senescence.

This analysis does not support the conclusion reached by Thomas (1976, 1977) that high nitrate fertilization increases the proportion of photorespiration to net photosynthesis. However, Thomas studied mature wheat plants in the field, under hot and rather dry conditions, which may have induced stomatal closure, increasing the proportion (Lawlor, 1979). Our measurements were on young wheat under much cooler conditions and without water stress, so that variations in stomatal conductance and the CO₂/O₂ ratio at the active site would not have affected the ratio of RuBPC to RuBPo activity. The major effects of treatments on stomatal conductance were in bright light, in which cold grown plants had smaller stomatal conductance and smaller net photosynthesis, but it is not known if photorespiration increased as a proportion of photosynthesis or if the decreased

assimilation was due only to the decreased conductance; RuBPC activity may be inhibited under these conditions (Boyle, 1983).

Photosystems, photochemical efficiency and relation to carboxylation

Nitrogen deficiency had little effect on the photosystem activity, or photochemical efficiency, although it did decrease the chlorophyll content; cool conditions increased chlorophyll and the PSI and II activity in bright light. The chlorophyll/soluble protein ratio was similar in the different treatments, so it would be expected that the overall capacity for light harvesting and energy transduction would have been greater in leaves from cool conditions compared to warm, and slightly greater with additional nitrate than without. However, differences in photochemistry and chlorophyll were larger than those in assimilation.

Light reactions and electron transport lead to the formation of reductant (NADPH and reduced ferredoxin) and ATP, which are consumed in RuBP synthesis and also in nitrate reduction. There was more RuBP in nitrate deficient leaves and in cold conditions (Lawlor *et al.*, 1987b) suggesting that photochemical processes were slightly more efficient in those conditions. If RuBP is bound to the active sites of RuBPC-o and RuBP synthesis is not limiting, it might be expected that RuBP content would increase with protein, but this was not so. The complexity of the system for synthesis of RuBP, and the changing demand for it, makes interpretation difficult, particularly as differences in RuBP content were small.

Changes in assimilation and tissue composition with age

In our experiments assimilation decreased in proportion to protein and chlorophyll as leaves aged, as frequently observed (Čatský, Tichá, and Solárová, 1980; Hall, Keys, and Merrett, 1978), and the decrease was faster in warm than in cool conditions and with deficient nitrate supply, but not for those grown in the C+N treatment in which pigment increased and yet assimilation decreased. Loss of assimilation was earlier and greater with nitrate deficiency and in warm conditions, and occurred before the decrease in contents of chlorophyll or protein. Photochemical efficiency decreased by about 50% in the cold for both N treatments and 40% and 20% in W-N and W+N conditions respectively. Over the same period RuBP content (Lawlor *et al.*, 1987b) decreased by approximately 65% and 90% in C-N and C+N conditions and 88% and 81% in W-N and W+N conditions respectively. This suggests that RuBP synthesis is inhibited more than energy transduction with ageing, and is related to the more rapid decrease in carboxylation efficiency with age and deficient nitrogen; decreased assimilation capacity is probably related to changes in stromal enzymes rather than to light harvesting membranes. Nitrogen deficiency appears to stimulate the mechanisms leading to protein degradation, and remobilization of stromal proteins may be faster than those of the thylakoids. Camp, Huber, Broke, and Moreland (1982) observed that RuBPC activity decreased before photosynthetic electron transport, consistent with our data. However, we also observed that chlorophyll and soluble protein decreased together despite the relatively slower loss of photochemical efficiency. Possibly degradation of chlorophyll in thylakoid protein complexes occurs before energy transduction is affected. This is not consistent with the results of Jenkins, Baker, and Woolhouse (1982) and Jenkins and Woolhouse (1982) for *Phaseolus vulgaris*. Senescing leaves lost reaction centre function, suffered earlier loss of PSI compared to PSII and decreased electron transport, which probably preceded loss of antenna chlorophyll. Faster loss of RuBPC-o than of photochemical components suggests that the changes in the mechanism responsible for the decrease in assimilation of CO₂ involve differential loss of components from chloroplasts, rather than the destruction of complete chloroplasts which has been

described by Peoples *et al.* (1980) and Camp *et al.* (1982). How the structure and function of the carboxylation and photochemical mechanisms are regulated is unknown (Huffaker, 1983).

In conclusion the changes induced in the composition of young leaves and the rates of assimilation by growth temperature and nitrate nutrition are small. Differences in both assimilation and composition become greater between treatments as the leaves age but the amounts of the structural components change in similar proportion; the component or process regulating assimilation cannot be identified. With nitrate deficiency, assimilation per m² is proportional to the amount of RuBPC-o and chlorophyll per m² but assimilation did not increase in proportion to protein or chlorophyll as these increased in amount. It appears that part of the RuBPC-o protein produced at high nitrate fertilization is inactive in the leaf as the specific activity is smaller and also the rate of net assimilation is not proportional to RuBPC-o protein. Evidence for control of assimilation by other enzymes is lacking. The ratio of photorespiration to photosynthesis is not affected by growth conditions; the ratio is greater in warm than cool measurement conditions. Additional nitrate does not, therefore, decrease the efficiency of assimilation by changing photorespiration. During senescence, loss of carboxylation efficiency is greater than loss of photochemical efficiency, but the mechanisms regulating the processes are not understood. Control mechanisms in photosynthetic electron transport and associated processes and those operating on carboxylation, are probably different but not understood. However, development and maintenance of assimilation requires that nitrate supply is sufficient to produce a large content of chlorophyll and proteins, and that the supply of nitrate is adequate to minimize their remobilization with age.

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