Journal of Experimental Botany, Vol. 51, GMP Special Issue,
pp. 391–397, February 2000

Journal of
Experimental
Botany

Is there scope for improving balance between RuBP-regeneration and carboxylation capacities in wheat at elevated CO_2?

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Received 19 April 1999; Accepted 29 September 1999

Abstract

Carboxylation and RuBP-regeneration capacities, which determine light-saturated photosynthetic rate, were analysed in leaves of spring wheat (*Triticum aestivum* L. cv. Minaret) grown under different atmospheric CO_2 partial pressure (pC_a) and N supply regimes. Capacities were estimated from a large number of gas exchange, Rubisco and ATP-synthase content measurements, and from these, the pC_a at which the two capacities are equal was derived, to allow direct comparison with growth pC_a. Acclimation of the balance between the two capacities to growth at elevated pC_a in wheat was only partial and appears to occur mostly in older flag leaves and at low N. However, in contrast to conclusions drawn from previous analyses of these data, there was evidence of a specific effect of growth at 70 Pa pC_a, where carboxylation capacity is reduced more than RuBP-regeneration capacity for a given leaf N content. A model was used to estimate the effects of fluctuations in PPFD and temperature in the growth environment on the optimal balance between these capacities. This showed that the observed balance between carboxylation and RuBP-regeneration capacities in young wheat leaves could be consistent with adaptation to the current, or even the preindustrial pC_a.

Key words: *Triticum aestivum*, carboxylation, RuBP regeneration, carbon dioxide, nitrogen, photosynthesis.

Introduction

The current atmospheric CO_2 partial pressure (pC_a) is already the highest experienced by plants at any time over at least the last 160 000 years (Barnola *et al.*, 1987), possibly for several million years, and is rapidly increasing. There may therefore be opportunities to improve the adaptation of photosynthesis of C_3 crops, since their recent ancestors evolved under lower pC_a. Furthermore, there is evidence that breeding has increased stomatal conductance in wheat (Fischer *et al.*, 1998), thereby further increasing the CO_2 at the site of carboxylation (pC_c) compared to that experienced by wheat ancestors.

One consequence of increasing pC_a is that carboxylation by Rubisco becomes more efficient compared with RuBP-regeneration and other processes. For maximum photosynthesis (A) per unit leaf N (N-use efficiency), a decrease in Rubisco relative to RuBP-regeneration components is therefore theoretically required at high pC_a (Medlyn, 1996; Sage, 1994). This theory is supported by observations in plants where the amount of Rubisco has been decreased by genetic manipulation. In tobacco, the amount of Rubisco in leaves of plants grown at ambient

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Abbreviations: α, fraction of incident light usefully absorbed by photosystems; β, pC_i/pC_a ratio; Γ_*, photorespiratory compensation point; θ, curvature of photosynthetic light response; τ, Rubisco specificity factor; A, light-saturated net CO_2 assimilation rate; A_g, light-saturated CO_2 assimilation rate gross of non-photorespiratory respiration; g_w, conductance for diffusion of CO_2 from intercellular space to site of carboxylation; J_{max}, maximum rate of non-cyclic photosynthetic electron transport; K_c, Rubisco Michaelis constant for CO_2; K_o, Rubisco Michaelis constant for O_2; N_{RC}, relative N cost of investing in RuBP-regeneration capacity compared to carboxylation capacity; pC_a, atmospheric CO_2 partial pressure; $pC_{a,bal}$, pC_a at which carboxylation and RuBP-regeneration capacities are equal; pC_c, CO_2 partial pressure at site of carboxylation; $pC_{c,bal}$, pC_c at which carboxylation and RuBP-regeneration capacities are equal; pC_i, CO_2 partial pressure in intercellular space; PPFD, photosynthetically-active photon flux density; RuBP, ribulose-1,5-bisphosphate; Rubisco, RuBP carboxylase/oxygenase; V_{cmax}, maximum rate of carboxylation.

pC_a largely determines light-saturated photosynthetic rate, but does not limit photosynthesis at elevated pC_a (Quick *et al.*, 1992) and the pC_a at which RuBP-regeneration begins to limit photosynthesis is much higher in plants with decreased Rubisco content (Masle *et al.*, 1993; von Caemmerer *et al.*, 1994). In a comparison of rice lines grown at ambient pC_a, those with decreased Rubisco content had a greater N-use efficiency measured at high pC_a (Makino *et al.*, 1997). However, there remains the question of whether there are acclimatory responses to long-term growth at elevated CO_2 such that any excess investment in Rubisco is decreased or removed. Reviews of gas exchange studies (Medlyn, 1996; Sage, 1994) have concluded that there is evidence of some rebalancing occurring under certain conditions (particularly nutrient deficiency), but usually it is less than the predicted optimum and often there is none. A large (60%) decrease in Rubisco content relative to wild type in transgenic tobacco plants had a smaller relative effect on growth at elevated compared to ambient pC_a (Masle *et al.*, 1993). Had perfect acclimation occurred, Rubisco would be equally limiting at both pC_a and the relative effect of the reduction in content would be about the same. Furthermore, sample A–pC_i responses again showed only a small shift in favour of RuBP-regeneration over carboxylation capacity in wild-type leaves (Masle *et al.*, 1993).

In recent work (Theobald *et al.*, 1998), gas-exchange and leaf composition measurements were used to estimate the amount of excess Rubisco at elevated pC_a in flag-2, flag-1 and flag leaves of wheat grown at different pC_a and N supply regimes. Most of the excess Rubisco at high pC_a persisted even in plants grown at high pC_a. It was also concluded that what decrease in excess there was in these plants occurred probably as a consequence of elevated growth pC_a inducing a reduction in leaf N content, which was strongly associated with a decrease in the fraction of Rubisco that was in excess. Here, the gas-exchange measurements are re-examined specifically to estimate the value of RuBP regeneration capacity and the effects of growth pC_a and N on the balance between RuBP-regeneration and carboxylation capacities in wheat. This new analysis has somewhat modified one of the original conclusions.

In order to interpret how these results compare with theoretical optima, it is necessary to remember that photosynthetic components are often not optimized such as to maximize photosynthesis for (unnatural) constant conditions, but rather appear to invest such that they can exploit changes that would be expected in a natural environment (Stitt and Schulze, 1994). In the case of the balance between carboxylation and RuBP-regeneration capacities, the optimum is affected by temperature and PPFD, which fluctuate too rapidly to be acclimated to in a natural environment. A model was therefore developed which describes the balance of carboxylation and RuBP-regeneration capacities as a function of pC_a, temperature and PPFD. This model allows the investigation of how the optimal balance of capacities under the measuring conditions of light-saturation and 20 °C will differ from the optimal balance for a natural environment.

Materials and methods

Experimental

The experimental methods have been described in detail (Theobald *et al.*, 1998), but a brief summary is given here. Spring wheat stands were grown from emergence to maturity at 600 μmol m^{-2} s^{-1} PPFD, day/night temperature 20/12 °C, and vapour-pressure deficit 0.8/0.4 kPa VPD. They were grown either under ambient (36 Pa) or elevated (70 Pa) CO_2 partial pressures and with low or high N supply. Response of light-saturated photosynthesis to internal CO_2 partial pressure (pC_i) was measured at intervals in flag-2, flag-1 and flag leaves. The same leaves were sampled to determine leaf N, Rubisco and ATP-synthase contents, and Rubisco activation state.

Carboxylation capacity (V_{cmax}) was estimated from fitting points below a critical pC_i (as detailed in Theobald *et al.*, 1998). RuBP-regeneration capacity (J_{max}) was estimated from fitting points above critical pC_i to the equation (Farquhar and Sharkey, 1994):

$$A_g = J_{max}(pC_c - \Gamma_*)/(4pC_c + 8\Gamma_*) \qquad (1)$$

where A_g is photosynthetic rate gross of dark respiration, Γ_* is photorespiratory compensation point and

$$pC_c = pC_i - A/g_w \qquad (2)$$

where g_w is the transfer conductance from the intercellular space to the site of carboxylation. The critical pC_i was chosen by trying each value in the range 25–35 Pa with 1 Pa steps and finding the value that maximized the overall percentage variance accounted for when the two curves were fitted below and above this point. Points at high pC_i (>60 Pa) which showed signs of limitation by capacity to utilise triose-phosphate (i.e. decreased A with increasing pC_i) were excluded from the fit to Equation 1.

A further parameter was derived from estimates of J_{max} and V_{cmax}. The pC_c at which the two capacities are equal ($pC_{c,bal}$) is given by:

$$pC_{c,bal} = (J_{max}K - 8V_{cmax}\Gamma_*)/(4V_{cmax} - J_{max}) \qquad (3)$$

where K is the effective Michaelis-Menten constant of Rubisco for pC_c. $pC_{c,bal}$ is converted to the corresponding pC_a ($pC_{a,bal}$) by adding A/g_w and multiplying by observed pC_a/pC_i ratio (which was not affected by growth pC_a).

Modelling

The same parameter values used in the fitting procedure were used in the model (Table 1). Additional parameters assumed were necessary to treat the temperature dependence of these parameters, and to investigate the effect of variable PPFD. This latter effect was treated by using the solution to the equation (Farquhar and Sharkey, 1994):

$$\theta J^2 - J(\alpha I/2 + J_{max}) + \alpha I/2 J_{max} = 0 \qquad (4)$$

where θ is the curvature of the response, α is the fraction of incident light usefully absorbed by PSII and I is the PPFD of incident light. For model runs shown here, a light distribution was chosen with a range of 100–1500 mol m^{-2} s^{-1} with the duration of each being the same for 100–600 μmol m^{-2} s^{-1},

Table 1. *Parameters used in fitting procedure and in model*

Estimated values are taken from literature for wheat, where possible.

Parameter	Units	Value at 20 °C	Temperature dependency
K_c Rubisco Michaelis constant for CO_2	µM	10.1[b]	2% of 25 °C value increase per °C[c]
K_o Rubisco Michaelis constant for O_2	µM	383[b]	None[d]
τ Rubisco specificity factor		116[e]	2.9% of 25 °C value decrease per °C[e]
Γ_*	Pa	3.2[a,f]	From that for τ and solubilities[f]
K	Pa	46[a,g]	From that for K_c, K_o and solubilities[g]
g_w	$\mu mol\ m^{-2}\ s^{-1}\ Pa^{-1}$	5.0–2.0[a,h]	None
V_{cmax}	$\mu mol\ m^{-2}\ s^{-1}$	100[i]	$E_a = 58\,600\ J\ mol^{-1}$[b]
J_{max}	$\mu mol\ m^{-2}\ s^{-1}$	178[i]	$E_a = 37\,000\ J\ mol^{-1}$[j]
α		0.65[k]	None
β pC_i/pC_a ratio		0.7[k]	None
θ		0.7–0.98[k]	None
N_{RC}		2.0[l]	None

[a] Values used in fitting procedure.
[b] Makino *et al.* (1988).
[c] Machler *et al.* (1980).
[d] Jordan and Ogren (1984).
[e] Keys (1999).
[f] Derived from τ and temperature dependent solubilities of O_2 and CO_2 in water (Ku and Edwards, 1977).
[g] Derived from K_c and K_o and temperature dependent solubilities of O_2 and CO_2 in water (Ku and Edwards, 1977).
[h] Loreto *et al.*, (1994). Value set to 5 $\mu mol\ m^{-2}\ s^{-1}\ Pa^{-1}$ in model.
[i] Typical values observed for young leaves.
[j] Farquhar *et al.* (1980).
[k] Medlyn (1996).
[l] See text.

and then decreasing linearly to 10% of this at 1500 $\mu mol\ m^{-2}\ s^{-1}$.

For modelling of optimal balance it is also necessary to assume a relative N cost for J_{max} compared to that for V_{cmax} (N_{RC}). If J_{max} can only be increased by increasing the number of photosynthetic cells and chloroplasts per unit leaf area then $N_{RC} \approx 2$ (Table 1).

Results

Figure 1 shows the relationship between J_{max} estimated from gas exchange and the ATP-synthase content of the same leaf section, estimated by Western blots (Theobald *et al.*, 1998). The data are reasonably well correlated ($R^2 = 0.65$), although not as tightly as Rubisco content and V_{cmax} (Theobald *et al.*, 1998).

Changes of J_{max} in flag leaves through time (Fig. 2) showed that low N supply generally decreased J_{max}, and that elevated growth pC_a never significantly increased J_{max} and sometimes induced a decrease. Also shown in Fig. 2 is the balance of carboxylation and RuBP-regeneration capacities, expressed as the pC_a at which they would be equal ($pC_{a,bal}$ calculated from V_{cmax} and J_{max} values using equation 3). Optimal use of resources for the measurement conditions is therefore given by $pC_{a,bal}$ = growth pC_a (i.e. 36 or 70 Pa). In plants grown with pC_a = 36 Pa, the value of $pC_{a,bal}$ is initially above 36 Pa and tends to decrease with time (especially with high N supply), indicating increased limitation by RuBP-regeneration. Growth at 70 Pa pC_a had no significant effect on $pC_{a,bal}$ values in flag leaves at full emergence (first point), and there was no consistent effect for any point for high N plants. However, growth at 70 Pa pC_a did significantly increase $pC_{a,bal}$ values to 55–60 Pa for low N plants in the period 9–16 d after anthesis.

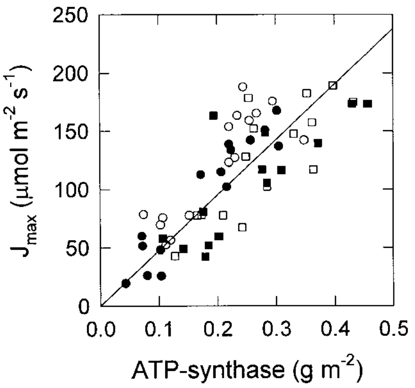

Fig. 1. J_{max} against ATP-synthase content in wheat flag-1 and flag leaves for different growth conditions. Line shows regression $y = 476 \times$ ($R^2 = 0.65$). (Data from Theobald *et al.*, 1998, with a recalculated y-axis.) (○) Low N, 36 Pa CO_2; (●) low N, 70 Pa CO_2; (□) high N, 36Pa CO_2; ■ high N, 70 Pa CO_2.

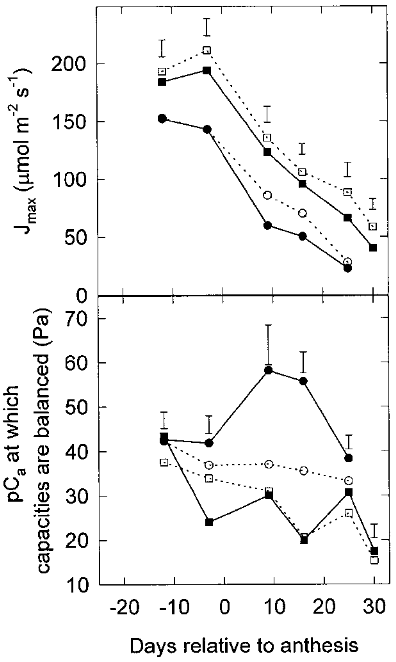

Fig. 2. Changes in J_{max} and $pC_{a,bal}$ through time for flag leaves grown under different conditions as indicated in the key. Each point is mean of parameter estimates from six separate A–pC_i responses. Error bars are SED. (○) Low N, 36 Pa CO_2; (●) low N, 70 Pa CO_2; (□) high N, 36Pa CO_2; ■ high N, 70 Pa CO_2.

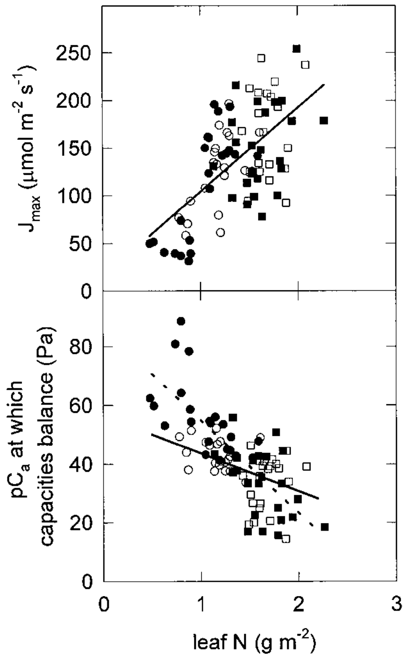

Fig. 3. Leaf N content versus J_{max} and $pC_{a,bal}$ for flag leaves grown under different conditions for the first four measuring times in Fig. 2 (8 samples lost, so $n = 88$). Relationship as tested by linear regression is not significantly different between growth pC_a for J_{max} ($R^2 = 0.39$), but slopes differ significantly for $pC_{a,bal}$ (-13 for 36 Pa, -32 for 70 Pa; $R^2 = 0.54$).

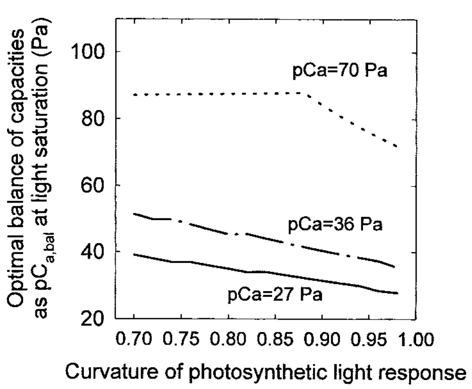

Fig. 4. Model output predicting the optimal balance between RuBP-regeneration and carboxylation capacities ($pC_{a,bal}$ measured at saturating light) for different curvature of light response and a distribution of growth PPFD values.

This rebalancing could be explained as a reduction in leaf N content, which is generally associated with a decrease in carboxylation capacity relative to RuBP-regeneration capacity in our data (Theobald *et al.*, 1998). Figure 3 shows the data from the first four occasions in Fig. 2 replotted against leaf N content for individual leaf segments. J_{max} is significantly, but not very tightly, correlated with leaf N content ($R^2 = 0.38$), and there are no significant differences between growth environments in the relationship. However, the relationship between $pC_{a,bal}$ and leaf N was significantly improved by assuming separate intercepts and slopes for plants grown in the two pC_a environments ($R^2 = 0.53$). These lines suggest that growth at elevated pC_a induces a rebalancing in favour of RuBP-regeneration capacity for leaves with low N content (< 1.5 g m^{-2}) in addition to any effect on leaf N content.

The results of running the model to find the optimal value of relative investment in components responsible for RuBP-regeneration and carboxylation for variable environments are shown in Figs 4 and 5, and are expressed as the $pC_{a,bal}$ that would be observed at 20 °C and saturating light. The more curved the light-response of photosynthesis (i.e. the lower the value of θ), the greater the value of $pC_{a,bal}$ (Fig. 4). As θ approaches 1, where there is no co-limitation, the optimal value approaches the growth pC_a. It is interesting to note that the observed values of $pC_{a,bal}$ from this study are consistent with optimization to the pre-industrial pC_a for reasonable values of θ (~ 0.85). Figure 5 shows the effect of a difference in leaf temperature between growth and measurement conditions. The measured $pC_{a,bal}$ will tend to exceed the growth pC_a if it is optimized to a lower temperature than the measurement temperature, and vice versa.

Discussion

Assuming ATP-synthase is substrate-saturated and fully activated under conditions where RuBP-regeneration

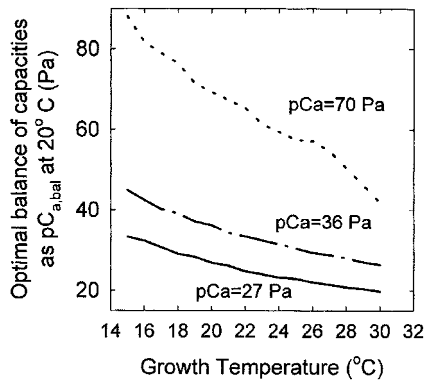

Fig. 5. Model output predicting the optimal balance between RuBP-regeneration and carboxylation capacities ($p\mathrm{C}_{\mathrm{a,bal}}$ measured at 20 °C) for different growth temperatures assuming saturating PPFD.

limits photosynthesis, it is possible to estimate a k_{cat} from the regression line through the origin in Fig. 1. A value of 260 mol ATP mol^{-1} ATP-synthase s^{-1}, is given by assuming 1 ATP : 3 protons : 1 electron. However, these assumptions give a higher value than the ATP-requirement for the observed photosynthetic rates in the range where RuBP-regeneration is assumed limiting, i.e. the points used to estimate J_{max} (data not shown). This is because equation 1, used to estimate J_{max}, assumes limitation of photosynthesis by production of reductant rather than ATP. The two estimates match closely if 1 ATP : 4 protons and 3 protons : 1 electron are assumed (Noctor and Foyer, 2000), giving a k_{cat} of 200 mol ATP mol^{-1} ATP-synthase s^{-1}.

The reanalysis of the gas-exchange data (396 A–$p\mathrm{C}_{\mathrm{i}}$ responses) from previously reported experiments (Theobald *et al.*, 1998) is consistent with the main conclusions arrived at there: that there is a tendency to over-invest in Rubisco for optimization of photosynthesis to growth at 70 Pa $p\mathrm{C}_{\mathrm{a}}$ (shown by $p\mathrm{C}_{\mathrm{a,bal}} < 70$ Pa in Fig. 2), and that this tendency is much greater in leaves with high N contents (Fig. 3). When elevated $p\mathrm{C}_{\mathrm{a}}$ does induce an apparent rebalancing, it is by a reduction in Rubisco content, rather than a stimulation of RuBP-regeneration components, since J_{max} was never increased by growth at elevated $p\mathrm{C}_{\mathrm{a}}$ (Fig. 2), and it appears to occur only in older leaves with low N supply. However, while it was concluded (Theobald *et al.*, 1998) that this rebalancing was entirely explicable as a consequence of elevated $p\mathrm{C}_{\mathrm{a}}$ reducing leaf N content, specific analysis of the relationship between RuBP-regeneration and carboxylation capacities suggests that RuBP-regeneration: carboxylation is increased by growth at elevated $p\mathrm{C}_{\mathrm{a}}$ for a given leaf N content (Fig. 3).

Recent studies on response of photosynthetic capacities to elevated $p\mathrm{C}_{\mathrm{a}}$ have confirmed the conclusions from a review of earlier studies (Sage, 1994), that the response is variable and more likely to occur when N is deficient. In field experiments on wheat, it was found that Rubisco decreased more in response to elevated $p\mathrm{C}_{\mathrm{a}}$ in plants grown with limiting N supply than with free access to N (Rogers *et al.*, 1996) and no evidence was found of a decrease in photosynthetic capacity of young, fully-expanded leaves until grain-fill in a well-fertilized crop (Garcia *et al.*, 1998), although there was some evidence for a decrease in Rubisco in leaves lower in the canopy (Osborne *et al.*, 1998). This latter study also found increased amounts of light-harvesting complex in these leaves, but this is consistent with acclimation to low incident radiation and would not be expected to increase J_{max}. In a controlled-environment experiment on wheat, it has been shown that changes in photosynthetic capacity at elevated $p\mathrm{C}_{\mathrm{a}}$ were completely eliminated by supplying N in proportion to growth, thus removing the indirect effect of $p\mathrm{C}_{\mathrm{a}}$ in increasing the limitation of N on growth (Farage *et al.*, 1998). In a study which looked specifically at the ratio of RuBP-regeneration capacity : carboxylation capacity in soybean (Sims *et al.*, 1998*a*), growth at elevated $p\mathrm{C}_{\mathrm{a}}$ increased the ratio more in plants grown at low N than at high N. However, they found that low N supply alone did not affect, or even increased this ratio. This contrasts with the results of the present study for wheat, where low N supply increased $p\mathrm{C}_{\mathrm{a,bal}}$ (Fig. 2), equivalent to a decrease in the ratio, a finding which is consistent with the observation that the ratio of Rubisco : ATP-synthase contents increases in wheat leaves with high N contents (Theobald *et al.*, 1998).

It is interesting to speculate on the mechanism behind the shift in balance between RuBP-regeneration and carboxylation capacities that sometimes occurs in response to elevated $p\mathrm{C}_{\mathrm{a}}$. It now seems clear that it is not a direct response to $p\mathrm{C}_{\mathrm{a}}$, but rather a consequence of the change in the balance of limitations for plant growth which occurs at elevated $p\mathrm{C}_{\mathrm{a}}$ (Farage *et al.*, 1998; Sage, 1994; Sims *et al.*, 1998*b*). It is often suggested that sugar accumulation in the leaf, which suppresses expression of photosynthetic genes, may be the cause (van Oosten and Besford, 1996), but this also decreases expression of genes encoding components of RuBP-regeneration (Krapp and Stitt, 1995). It may be that the amount of active Rubisco responds more quickly than other components, but this has not been established. In the later leaves of wheat, this mechanism seems less likely as fructan synthesis in the internodes represent a very large sink for sugar export. Leaf carbohydrate content was not increased at elevated $p\mathrm{C}_{\mathrm{a}}$ in the experiments (JC Theobald, unpublished data) and was not correlated with changes in message levels for photosynthetic components in a field experiment on wheat (Nie *et al.*, 1995). The timing of the effect seems more consistent with an increased demand for N from an increased number of grain at elevated $p\mathrm{C}_{\mathrm{a}}$, an interpretation that also applied to a number of open-topped chamber experiments carried out in Europe (Mitchell *et al.*, 1999).

In this paper, the balance of RuBP-regeneration : carboxylation capacities has been presented as $pC_{a,bal}$, which allows comparison with the growth environment pC_a. However, $pC_a = pC_{a,bal}$ does not correspond to optimal use of resources for the growth environment where incident radiation fluctuates, because J_{max} is believed to have an influence on photosynthesis at sub-saturating light intensities (Farquhar *et al.*, 1980). It has been shown that this increases the optimal ratio of $J_{max} : V_{cmax}$, depending on the curvature in equation 4 and the light distribution that is assumed (Medlyn, 1996). An arbitrary light distribution was chosen in this work, which has the necessary property that the duration of intensities approaching full sunlight are shorter than those for lower intensities; other distributions with this property gave similar results. In this case, the curvature (θ) reflects genuine co-limitation between J_{max} and PPFD, rather than any heterogeneity within the leaf. The model shows (Fig. 4) that the observation that $pC_{a,bal}$ usually exceeds the current ambient pC_a (in young leaves $pC_{a,bal} \approx 40$ Pa in these data, Fig. 2), in agreement with results elsewhere (Makino *et al.*, 1988; Masle *et al.*, 1993; von Caemmerer *et al.*, 1994), could be consistent with optimization. Indeed, it could even be consistent with adaptation to the pre-industrial pC_a (Fig. 4).

The model was also used to examine the effect of temperature on the optimal balance of capacities. The specific temperature dependencies in Table 1 are open to some doubt, since published data are sparse, and for K_c and K_o a temperature dependency for spinach was used to correct the published wheat value determined at 25 °C. Nevertheless, it seems certain that J_{max} is less increased by temperature than V_{cmax}, for example, in cotton (Harley *et al.*, 1992). This has the consequence that if leaves optimize their resources to a lower average temperature than they are measured at, the observed $pC_{a,bal}$ will be greater than the growth pC_a (Fig. 5).

For an optimal resource allocation between RuBP-regeneration and carboxylation capacities at a growth pC_a of 70 Pa, the $pC_{a,bal}$ should exceed this value, as shown in Fig. 4. This was not observed in these data (Fig. 3). The only occasions on which partial rebalancing did occur in response to elevated growth pC_a, were between 6 d and 15 d after anthesis in the low N treatment (Fig. 3), possibly corresponding to large N demand from the developing grain. It therefore appears that there is a tendency for wheat to over-invest in Rubisco at elevated pC_a, so genetic manipulation to reduce Rubisco relative to RuBP-regeneration capacity could benefit N-use efficiency of photosynthesis under these conditions.

Acknowledgements

Gift of antibodies against CF1 sub-units from Professor JC Gray, University of Cambridge, UK is gratefully acknowledged. We thank Dr PJ Andralojc for assistance and advice with the ATP-synthase work, and SP Driscoll and VJ Mitchell for technical assistance. We thank Professors CB Osmond and SP Long for useful comments on the manuscript. JCT was supported by a grant from the European Union as part of the ESPACE-Wheat project, contract number EV5V-C793–0301. RACM receives support from UK Ministry of Agriculture, Fisheries and Food contract CSA4532. IACR-Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

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