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Effects of temperature on the regulation of photosynthetic carbon assimilation in leaves of maize and barley

Carlos A. Labate*, Michael D. Adcock, and Richard C. Leegood**

Robert Hill Institute and Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK

Abstract. The aim of this work was to examine the effect of temperature in the range 5 to 30° C upon the regulation of photosynthetic carbon assimilation in leaves of the C_4 plant maize (*Zea mays* L.) and the C_3 plant barley (*Hordeum vulgare* L.). Measurements of the CO_2-assimilation rate in relation to the temperature were made at high (735 μbar) and low (143 μbar) intercellular CO_2 pressure in barley and in air in maize. The results show that, as the temperature was decreased, (i) in barley, pools of phosphorylated metabolites, particularly hexose-phosphate, ribulose 1,5-bisphosphate and fructose 1,6-bisphosphate, increased in high and low CO_2; (ii) in maize, pools of glycerate 3-phosphate, triose-phosphate, pyruvate and phosphoenolpyruvate decreased, reflecting their role in, and dependence on, intercellular transport processes, while pools of hexose-phosphate, ribulose 1,5-bis phosphate and fructose 1,6-bisphosphate remained approximately constant; (iii) the redox state of the primary electron acceptor of photosystem II (Q_A) increased slightly in barley, but rose abruptly below 12° C in maize. Non-photochemical quenching of chlorophyll fluorescence increased slightly in barley and increased to high values below 20° C in maize. The data from barley are consistent with the development of a limitation by phosphate status at low temperatures in high CO_2, and indicate an increasing regulatory importance for regeneration of ribulose 1,5-bisphosphate within the Calvin cycle at low temperatures in low CO_2. The data from maize do not show that any steps of the C_4 cycle are particularly cold-sensitive, but do indicate that a restriction in electron transport occurs at low temperature. In both plants the data indicate that regulation of product synthesis results in the maintenance of pools of Calvin-cycle intermediates at low temperatures.

Key words: Carbon assimilation, photosynthetic – *Hordeum* (photosynthesis) – Photorespiration – Temperature (low) and carbon assimilation –*Zea* (photosynthesis)

Introduction

Photosynthetic systems have to face large and frequent variations in temperature. Changes in temperature will be accompanied by alterations in the regulation of metabolism which will require modifications in the substrates and regulators of enzymic reactions to be coordinated with temperature-dependent changes in the properties of enzymes. Any differential effects of temperature on such properties are likely to result in alterations in the direction of metabolism (ap Rees et al. 1988). In photosynthetic systems a potential disparity between the temperature dependence of the Calvin cycle, with its high Q_{10} at low temperatures (Baldry et al. 1966), and that of other metabolic processes could arise (Leegood and Walker 1983). These other metabolic processes, such as respiration and product synthesis, which compete with the Calvin cycle for fixed carbon, must then either be regulated at low temperature by lack of substrate availability or by suitable temperature-dependent changes in the properties of enzymes. Stitt and Grosse (1988) have suggested that, as the temperature is decreased, an increase in the physiological K_m (fructose-1,6-bisphosphate) of the cytosolic fructose-1,6-bisphosphatase and a high Q_{10} for sucrose-phosphate synthase may contribute to effective regulation of sucrose synthesis.

In leaves of C_3 plants, a number of additional factors have been identified as playing an important regulatory role in respect of temperature. As well as changes in the rate of product synthesis, it is well established that

* *Permanent address:* Escola Superior de Agricultura "Luiz de Queiroz", Departamento de Genética, Universidade de São Paulo, Piracicaba, C.P. 83, CEP 13400, Brazil

** To whom correspondence should be addressed

Abbreviations: Glc6P = glucose-6-phosphate; Fru6P = fructase-6-phosphate; Fru1,6bisP = fructose-1,6-bisphosphate; PGA = glycerate-3-phosphate; p_i = intercellular partial pressure of CO_2; RuBP = ribulose-1,5-bisphosphate; triose-P = sum of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate

the proportion of fixed carbon entering the photorespiratory pathway increases greatly as the temperature is increased. At high, supra-optimal temperatures, regulation by, and inactivation of, ribulose-1,5-bisphosphate (RuBP) carboxylase-oxygenase and thylakoid reactions become important (Berry and Björkman 1980; Leegood 1985; Kobza and Edwards 1987; Weis and Berry 1988). On the other hand, it is clear from such phenomena as the occurrence of oscillatory behaviour, lack of CO_2 sensitivity, lack of O_2 sensitivity and the direct stimulation of photosynthesis by orthophosphate (Pi; Leegood and Furbank 1986; Sharkey et al. 1986) that a restriction of sucrose synthesis, and hence of phosphate recycling and photophosphorylation, occurs at low temperatures.

We are still largely ignorant of the metabolic changes which occur when the leaves of C_4 plants are subjected to abrupt changes to low temperatures. Temperature optima in C_4 plants are usually higher than in C_3 plants and C_4 plants are largely absent from cold environments. Although many C_4 plants, including maize, are prone to chilling injury at low temperatures (e.g. Long et al. 1983), and enzymes such as pyruvate, Pi dikinase are cold-inactivated (see Berry and Björkman 1980 for a review), $^{14}CO_2$-labelling studies suggest that there are no particularly cold-sensitive steps in the C_4 pathway in vivo in plants grown at low temperatures (Caldwell et al. 1977). However, while photorespiration is largely suppressed in C_4 plants, glycerate-3-phosphate (PGA) reduction in the mesophyll and product synthesis are processes which effectively withdraw carbon from the Calvin cycle and which must be regulated when changes in environmental conditions occur.

The aim of this study was to investigate and compare the influence of temperature on the regulation of photosynthetic carbon assimilation in leaves of the C_4 plant maize (*Zea mays* L.) and the C_3 plant barley (*Hordeum vulgare* L.), grown at high temperature (30° C), but photosynthesising at a range of temperatures between 5° and 30° C.

Material and methods

Material. Maize (*Zea mays* L. cv. Kelvedon Glory; Nickersons R.P.B., High Wycombe, Bucks., UK) was grown in a mixture of soil and vermiculite in 21 pots. Barley (*Hordeum vulgare* L. cv Sonja, Nickersons R.P.B) was grown in compost in 3.5-inch square pots for 10 d in a greenhouse, as described previously (Labate and Leegood 1989). Plants were grown in a greenhouse under a maximum photosynthetic photon quantum flux density of 500–600 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ at mid-day. Air temperature was maintained at an average of 30° C during the day and 18° C at night.

Measurement of gas-exchange and chlorophyll fluorescence. For each experiment involving comparison of gas-exchange analysis characteristics, all the leaves were taken from one pot of 10-d-old seedlings. Measurements of the rate of CO_2 assimilation and transpiration were made in an open gas-exchange system as described by Harris et al. (1983). Once the leaves had reached a steady-state rate of photosynthesis, they were freeze-clamped as described by Doncaster et al. (1989). Maize leaves were illuminated for about 90 min at the low temperatures before the achievement of steady-state rates of CO_2 assimilation. Chlorophyll fluorescence was analysed by a pulse amplitude modulation fluorometer (PAM-101; H. Walz, Effeltrich, FRG) as described by Doncaster et al. (1989). The quantum efficiency for electron transport by photosystem II (Φ_{PSII}) was estimated from chlorophyll-fluorescence data during steady-state photosynthesis as described by Genty et al. (1989); $\Phi_{PSII} = (F_m - F_s)/F_m$, where F_m is the level of chlorophyll fluorescence when all photosystem II traps are closed and F_s is the steady-state level of chlorophyll fluorescence.

Metabolite measurement. Leaf samples were freeze-clamped and stored in envelopes of aluminium foil in liquid N_2 prior to extraction in $HClO_4$ (maize) or $CHCl_3/CH_3OH$ (barley) as described previously (Labate and Leegood 1989). Metabolites were determined by the methods of Lowry and Passonneau (1972) in a Hitachi (Tokyo, Japan) 557 dual-wavelength spectrophotometer (340–400 nm). Both PGA and RuBP were measured as described by Doncaster et al. (1989). For the measurement of fructose-1,6-bisphosphate (Fru2,6bisP) in barley, extracts were assayed as described by Labate and Leegood (1989).

Results and discussion

The response of photosynthesis in barley leaves to temperature at different intercellular partial pressures of CO_2 (p_i). Barley leaves were illuminated at temperatures between 5 and 30° C and the ambient CO_2 concentration was adjusted so as to keep p_i constant at values of around 143 or 735 μbar. Experiments were done at the higher p_i to minimise photorespiration and to impose a limitation due to phosphate recycling at low temperatures (Leegood and Furbank 1986; Labate and Leegood 1988; Stitt and Grosse 1988), and at the lower p_i to provide conditions in which the rate of photorespiration would be increased greatly at all, but particularly at high, temperatures. In order to avoid changes in photosynthesis due to the onset of light limitation at higher temperatures, we ensured that photosynthesis was light-saturated (1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at all temperatures at high and low p_i.

Figure 1 illustrates the behaviour of the assimilation rate (A), metabolite pools and the photochemical ($1 - q_Q$) and non-photochemical (q_{NP}) components of chlorophyll fluorescence quenching in barley leaves with temperature at the two intercellular partial pressures of CO_2. The assimilation rate showed a steady increase with temperature at high p_i but reached a maximum at 15° C at low p_i, as shown previously (Labate and Leegood 1988). The rate of CO_2 assimilation was virtually insensitive to an increase in CO_2 concentration at 5° and 10° C.

The amount of phosphate in the major phosphorylated intermediates increased with a decrease in the temperature at both high and low CO_2. The amounts of both RuBP and Fru1,6bisP increased as the temperature was decreased at both CO_2 concentrations, but the increase in Fru1,6bisP at low p_i was much more marked than at high p_i. Although the RuBP pool was higher at low than at high p_i, in accord with previous observations (e.g. Badger et al. 1984), and it remained higher at all temperatures, the pattern of change was not greatly different, despite the very different contribution that photorespiration would have made at different CO_2 concen-

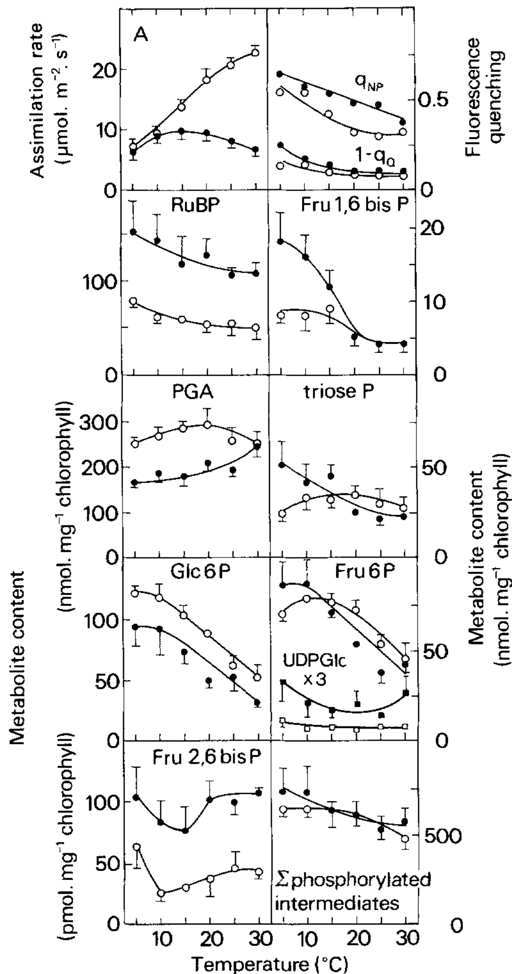

Fig. 1. Changes in the CO_2-assimilation rate (A), metabolite pools and the photochemical (1-q_Q) and non-photochemical (q_{NP}) components of chlorophyll fluorescence quenching in barley leaves at different temperatures at two intercellular partial pressures of CO_2: ●, 143±2 μbar; ○, 735±33 μbar. The photon flux density was 1000 μmol quanta·m^{-2}·s^{-1}. Data are means of three replicates, with SE bars shown

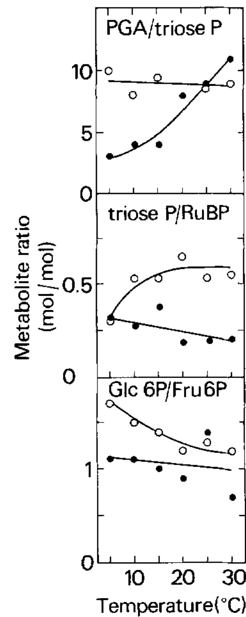

Fig. 2. Changes in metabolite ratios in barley leaves at different temperatures at two intercellular partial pressures of CO_2: ●, 143±2 μbar; ○, 735±33 μbar. Data are means of three replicates from Fig. 1

trations, especially at the lower temperatures. A rise in the RuBP pool as the temperature is decreased has also been observed by Dietz and Heber (1986), and at temperatures below 5° C by Leegood (1985). The content of PGA fell as the temperature was decreased at low p_i but changed relatively little with temperature at high p_i.

The amounts of both glucose-6-phosphate (Glc6P) and fructose-6-phosphate (Fru6P) increased as the temperature was decreased at both concentrations of CO_2, although below 10° C there was a flattening at low p_i and a fall in Fru6P at high p_i. The ratio Glc6P/Fru6P (Fig. 2) appeared to rise slightly at low p_i as the temperature was decreased (with the exception of the ratio at 25° C), but rose more steeply as the temperature was decreased at high p_i. The content of uridine 5′-diphosphate-glucose (UDPGlc) increased at low temperature, particularly at low p_i. A rise in the content of hexose-phosphate (hexose-P) with a decrease in temperature has also previously been observed in wheat leaves (Arrabaca et al. 1981; Kobza and Edwards 1987)) and in spinach leaves in high CO_2 (Dietz and Heber 1986; Stitt and Grosse 1988) and in ambient CO_2 (Leegood 1985).

The pool of fructose-2,6-bisphosphate (Fru2,6bisP) showed a similar pattern in relation to temperature at both high and low p_i, in showing a minimum between 10 and 15° C (see also Stitt and Grosse 1988). At high p_i there was a rise in Fru2,6bisP and a fall in triose-phosphate (triose-P) as the temperature was decreased from 10° to 5° C, but at low p_i the rise in Fru2,6bisP at the lower temperatures was accompanied by a rise in triose-P. At higher temperatures the rise in Fru2,6bisP was more marked at lower p_i. Under these conditions photorespiration would tend to compete with sucrose synthesis for assimilate carbon to a far greater degree than would occur at high p_i. The fact that the contents of triose-P, hexose-P and UDP-Glc at higher temperatures were not much less at low p_i, than at high p_i, possibly reflects effective regulation of the activity of fructose-1,6-bisphosphatase (by Fru2,6bisP) and sucrose-phosphate synthase by CO_2.

The response of photosynthesis in maize leaves to changes in temperature. Maize leaves were illuminated at temperatures between 8° and 30° C at ambient CO_2. Figure 3 illustrates the behaviour of the assimilation rate (A), metabolite pools and the photochemical ($1-q_Q$) and non-photochemical (q_{NP}) components of chlorophyll fluorescence quenching in maize leaves with temperature.

The increase in the assimilation rate with temperature (Fig. 3) was steeper than the response of the assimilation rate to temperature in barley leaves in high CO_2 (Fig. 1). The amount of Glc6P remained constant as the temperature was decreased and there was a fall in Fru6P at low temperature. There was a rise in Glc6P/Fru6P ratio from a value of about 2 at 30° C to above 3 below 15° C, indicating a shift towards the cytosolic compart-

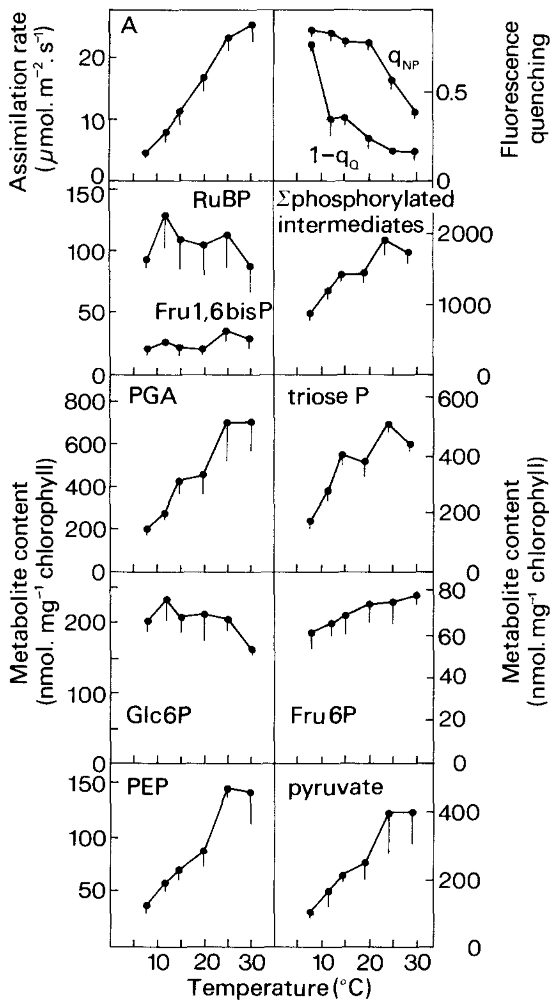

Fig. 3. Changes in the CO_2 assimilation rate (A), metabolite pools and the photochemical (1-q_Q) and non-photochemical (q_{NP}) components of chlorophyll fluorescence quenching in maize leaves at different temperatures. The photon flux density was 1050 μmol quanta·m^{-2}·s^{-1}. Data are means of three replicates, with SE bars shown. *PEP* = phosphoenolpyruvate

mentation of these compounds (Gerhardt et al. 1987). The amounts of both RuBP and Fru1,6bisP remained more or less constant as the temperature was decreased, indicating the development of a restriction on their consumption at lower temperatures. The amounts of phosphoenolpyruvate and pyruvate and the sum of phosphorylated intermediates all decreased with a decrease in temperature below 25° C. The content of PGA fell about threefold as the temperature was decreased from 30 to 8° C while the content of triose-P also decreased markedly with decreasing temperature below 25° C.

Interactions between electron transport and carbon assimilation in barley and maize leaves. At least three factors will influence the balance between electron transport and carbon assimilation at low temperatures. These are (i) a restriction of sucrose synthesis in high CO_2, which will lead to orthophosphate (Pi) limitation of electron transport, (ii) the occurrence of photorespiration in low CO_2, which will dissipate energy, and (iii) if carbon metabolism is restricted at low temperatures, photosynthetic control will lead to a restriction of electron transport, accompanied by an increased transthylakoid pH (and increased non-photochemical quenching), reduction of Q_A and oxidation of the acceptor side of photosystem I, leading to inactivation of light-activated enzymes (Leegood et al. 1989; Neuhaus and Stitt 1989).

The PGA/triose-P ratio can be used as an indicator of the assimilatory force, the combined redox and phosphorylation potential (Dietz and Heber 1986). In barley, the PGA/triose-P ratio (Fig. 2) remained virtually constant at different temperatures in high CO_2, while it declined with decreasing temperature in low CO_2. Previous work in C_3 plants has shown that the PGA/triose-P ratio remains constant or rises as the temperature is decreased in spinach leaves at ambient (Leegood 1985) or high CO_2 (Dietz and Heber 1986; Stitt and Grosse 1988) or rises in wheat leaves at ambient or high CO_2 (Kobza and Edwards 1987). Dietz and Heber (1986) suggested that this rise in the ratio at low temperatures was simply due to the influence of temperature on the ability of electron transport to generate ATP and NADPH, but it could equally well be interpreted as the onset of a sub-optimal phosphate status at low temperatures and high CO_2, leading to a restriction of photophosphorylation (Leegood and Furbank 1986; Sharkey et al. 1986). Conversely, the fall in the PGA/triose-P ratio as the temperature was decreased at low p_i would result from a decrease in the amount of energy dissipated by the lowered rates of photosynthesis and photorespiration at lower temperatures, and hence a decrease in the amount of ATP and NADPH consumed in carbon assimilation.

In barley, both the redox state of the pimary electron acceptor of photosystem II (Q_A; shown as $1-q_Q$) and the non-photochemical (q_{NP}) component of chlorophyll fluorescence quenching increased as the temperature was decreased (Fig. 1), showing that at both concentrations of CO_2 the energy provided by electron transport was not being utilised by metabolism at low temperatures. At high p_i the increase in non-photochemical fluorescence quenching between 20° and 5° C (suggesting an increase in energisation) would presumably result from a restriction on photophosphorylation, caused by a restriction on the rate of sucrose synthesis. At low p_i, the increase in non-photochemical quenching would again result from a decrease in the rate of photosynthetic and photorespiratory energy dissipation at lower temperature and is consistent with the observed fall in the PGA/triose-P ratio.

In maize, the PGA/triose-P ratio (Fig. 4) declined with decreasing temperature to 12° C and then showed a rise at 8° C. At 8° C a restriction on electron transport therefore appears. This is because a decrease in the utilisation of the products of electron transport by metabolism would be expected to lead to a fall in the PGA/triose-P ratio, not the rise which is observed. The photochemical ($1-q_Q$) and non-photochemical (q_{NP}) components of chlorophyll fluorescence quenching increased as the temperature decreased (Fig. 3). Both components changed to a much greater extent than in barley leaves

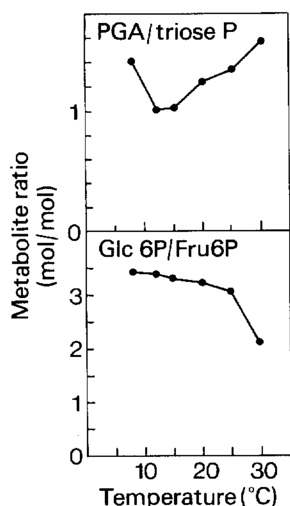

Fig. 4. Changes in metabolite ratios in maize leaves at different temperatures. Data are means of three replicates from Fig. 3

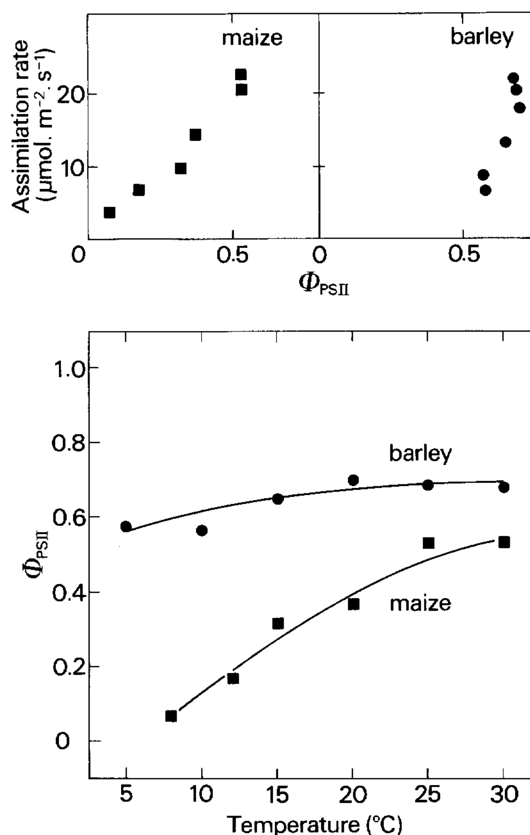

Fig. 5. Changes in the quantum efficiency for electron transport in photosystem II (Φ_{PSII}) in maize (■) and barley (●) leaves at different temperatures (*lower figure*) and the relationship between the assimilation rate and Φ_{PSII} in maize (■) and barley (●) leaves (*upper figure*). The intercellular partial pressure of CO_2 in barley was 735±33 μbar. The photon flux density was 1000 μmol quanta$\cdot m^{-2} \cdot s^{-1}$ for barley and 1050 μmol quanta$\cdot m^{-2} \cdot s^{-1}$ for maize

(Fig. 1) and there were particularly large increases in q_{NP} between 30° and 20° C and in Q_A reduction between 12° and 8° C. Non-photochemical quenching reached a maximum (greater than 0.8) at around 20° C and then Q_A became increasingly reduced. It is at this temperature that light begins to saturate the rate of carbon assimilation (data not shown). These changes in fluorescence quenching strongly resemble the changes which occur in maize leaves in relation to changes in CO_2 concentration (Leegood et al. 1989) and are again consistent with the occurrence of photosynthetic control and the notion that a high ΔpH at low temperature (or at low CO_2) restricts intersystem electron transfer.

At lower temperatures the decline in the efficacy of electron transport in maize which is indicated by these data is also accompanied by a gradual decline in the quantum efficiency for electron transport by photosystem II (Φ_{PSII}). Genty et al. (1989) have shown that this fluorescence parameter is directly proportional to the product of photochemical fluorescence quenching and the efficiency of excitation capture by open photosystem-II reaction centres. A comparison of temperature-dependent changes in Φ_{PSII} in maize and barley leaves and the relationship between Φ_{PSII} and the assimilation rate at different temperatures is shown in Fig. 5. In maize there was a large decline in Φ_{PSII} with decreasing temperature and there was a strong positive correlation with the assimilation rate measured at the same temperatures. Since, under conditions of CO_2 saturation, changes in the assimilation rate must approximate to changes in the rate of electron transport, this shows that changes in the efficiency of photosystem II (predicted by Φ_{PSII}) are largely explained by changes in the redox stat of Q_A and the efficiency of excitation energy capture. However, in barley leaves, Φ_{PSII} decreased only slightly as the temperature was decreased whereas the assimilation rate in high CO_2 decreased strongly. This indicates that additional factor(s) must determine photosystem-II efficiency in barley (e.g. PSII cycling and- or the Mehler reaction, see Rees and Horton (1990a, b)).

A consequence of this difference in the regulation of electron transport with temperature is that maize leaves are particularly prone to photoinhibition at low temperatures. For example, an appreciable reduction in the subsequent rates of photosynthesis occurs even after chilling maize leaves for an hour or two in the light (Long et al. 1983). This contrasts with the ability of barley to resist over-reduction of Q_A at low temperatures, apparently because it has additional energy-dissipating mechanisms.

Interactions between sucrose synthesis, photorespiration and photosynthesis. In high CO_2 a selective restriction to sucrose synthesis at low temperatures would be expected to lead to an accumulation of metabolites such as hexose-P and triose-P in the cytosol. The increase in the ratio Glc6P/Fru6P (particularly at high p_i) (Fig. 2) at low temperatures bears out this view. However, there was actually a decrease in triose-P at high p_i at low temperatures. This is likely to be due to a lack of Pi recycling by sucrose synthesis leading to a restriction of ATP production and, in turn, the conversion of PGA to triose-P. Hence, despite a falling rate of photosynthesis, which should lead to a decreased utilisation of ATP and NADPH, there is a constancy in the PGA/triose-P ratio with changing temperature at high p_i (Fig. 2).

In low CO_2 the regulation of sucrose synthesis will be very different. In leaves of *Phaseolus vulgaris* in low CO_2, there is no selective decrease in the synthesis of sucrose and its synthesis is favoured over that of starch (Sharkey et al. 1985). Amounts of triose-P in low CO_2 were actually higher at lower, than at higher, temperature. The rises in the amounts of triose-P, UDPGlc and, most remarkably, hexose-P, probably reflect the necessity for an increase in substrate pools to support sucrose synthesis at low temperatures in the face of decreasing substrate affinities and enzyme velocities rather than being symptomatic of a restriction on sucrose synthesis. The increase in the amount of phosphate in the major phosphorylated intermediates was about 150 $nmol \cdot mg^{-1}$ chlorophyll at high p_i and was about 250 $nmol \cdot mg^{-1}$ chlorophyll at low p_i, when contents of metabolites were compared between 30° and 5° C. If these changes are confined to the chloroplasts and the cytosol with a combined volume of 45 $\mu l \cdot mg^{-1}$ chlorophyll (Gerhardt et al. 1987), then these temperature-dependent increases in metabolite pools would be accompanied by decreases in Pi of approx. 3 and 6 mM at high and low p_i, respectivily. This change could contribute to the phosphate limitation of photosynthesis, if Pi is not readily available from the vacuole (Bligny et al. 1989). This may also be an important factor in mediating feedback effects on photosynthesis at low temperatures (Foyer 1987).

At low p_i, photorespiration clearly ameliorates the phosphate limitation which occurs at low temperatures in high CO_2, because the rates of CO_2 assimilation are virtually identical at the two CO_2 concentrations. This effect of photorespiration is evident as an increase in energisation (q_{NP}) and improved PGA reduction at low CO_2. This can be viewed as the basis of the observation of 'thresholds' in oscillatory behaviour (i.e. oscillations, which are symptomatic of phosphate limitation, are only observed in high CO_2 or low O_2 when photorespiration has been largely suppressed) (Sivak and Walker 1986; Leegood and Furbank 1986). At low p_i it is also apparent that increased photorespiration does not result in a drainage of intermediates of photosynthetic carbon assimilation. Thus total metabolite pools at low p_i, as well as the pool of RuBP, were equal to, or higher than, those at high p_i at all temperatures (Fig. 1). This emphasis the effectiveness of metabolic control within the Calvin cycle and sucrose metabolism even when photorespiratory fluxes are large.

Temperature-dependent regulation of the Calvin cycle in barley leaves. The ratio triose-P/RuBP can also be used as an indicator of the ability of the system to regenerate RuBP (Stitt and Grosse 1988). At high p_i the triose-P/RuBP ratio remained constant between 30° and 10° C but decreased at 5° C. Similarly, the mass-action ratio employed by Dietz and Heber (1986) to describe the reactions of RuBP regeneration decreased when the temperature was decreased from 30° to 12° C (in chloroplasts isolated by non-aqueous means from spinach leaves photosynthesising in saturating CO_2). At low p_i, the ratio triose-P/RuBP increased as the temperature was decreased (Fig. 2). The decrease in the ratio triose-P/RuBP at 5° C at high p_i occurs when the PGA/triose-P ratio is constant and under conditions in which the supply of ATP and NADPH is likely to be restricted, as discussed above (see also Dietz and Heber, 1986). It is apparent that regeneration of RuBP from triose-P is less sensitive to this restriction in the supply of energy than is the conversion of PGA to triose-P. Similar behaviour has also been observed as a transient inhibition of PGA reduction by ribose-5-phosphate in the reconstituted chloroplast system (Slabas and Walker 1976; Carver et al. 1983). At low p_i the increase in the triose-P/RuBP ratio as the temperature is decreased, despite the large fall in the PGA/triose-P ratio, suggests that regeneration of RuBP becomes restricted as the temperature is lowered. This decrease in the utilisation of the products of electron transport within the Calvin cycle is also evidenced by the rise in Fru1,6bisP at low temperature (Fig. 1) and suggests that enzymic regulation within the Calvin cycle rather than the supply of energy is responsible.

Why should there be a decreased regenerative capacity in the Calvin cycle at low p_i at lower temperatures? Two factors will be important, (i) photosynthetic control will lead to inactivation of light-activated enzymes such as fructose-1,6-bisphosphatase at low temperatures (data not shown) which could then result in the build-up of metabolites such as Fru1,6bisP; (ii) although increased amounts of stromal metabolites (achieved by effective regulation of sucrose synthesis) will partially compensate for declining activity of the Calvin-cycle enzymes at low temperatures and allow RuBP regeneration to be maintained over a wide range of leaf temperatures (Stitt and Grosse 1988), the autocatalytic nature of the Calvin cycle results in a very high Q_{10} at low temperatures (Baldry et al. 1966) which increased pools of substrates may not be able wholly to offset. Stitt and Grosse (1988) have also suggested that, at higher temperatures (30° C), low metabolite pools may be insufficient to maintain RuBP regeneration in the Calvin cycle. However, from these results it is apparent that, even with low metabolite pools and a high photorespiratory drain at low p_i, RuBP regeneration is not being adversely affected by high temperatures.

The basis of temperature-dependent changes in metabolite pools in maize. In maize, the exceptionally large leaf pools of photosynthetic intermediates such as PGA, triose-P and pyruvate reflect the demands of intercellular transport (Leegood and Osmond 1990). In general it would be expected that, as the photosynthetic flux falls, so metabolite gradients of C_4 acids, PGA, triose-P and pyruvate between the bundle-sheath and mesophyll would decline. This is undoubtedly the reason for the decline in metabolite pools with a decrease in temperature seen in Fig. 3. However, it should be emphasised that the total metabolite pools, even at 8° C, are appreciably higher than total metabolite pools in barley leaves at a comparable temperature (Fig. 1). Hence the decrease in triose-P reflects a decrease in PGA transported from the mesophyll and a declining gradient, rather than decreased phosphate recycling, as seen in barley (Fig. 1).

Similarly, the amount of phosphoenolpyruvate in maize leaves decreases because it is in equilibrium with PGA and the amount of PGA exported to the mesophyll decreases as the flux decreases (Leegood and Osmond 1990; Leegood and von Caemmerer 1989). Intermediates of the Calvin cycle or of product synthesis behaved differently from the metabolites involved in intercellular transport. Thus RuBP, Fru1,6bisP and hexose-P remained relatively constant, as in barley. In similar experiments with leaves of another C_4 plant, *Amaranthus edulis*, the pool of Fru1,6bisP increased fivefold and total hexose-P increased by 70% between 30° and 8° C, while intermediates of the C_4 cycle declined, as in maize (data not shown). As in barley, maintenance of the hexose-P pool in maize and in *A. edulis* presumably results from specific temperature-dependent regulation of the enzymes of starch and-or sucrose synthesis.

The decrease in the sum of esterified phosphate as the temperature was decreased in maize leaves was approx. 1000 $nmol \cdot mg^{-1}$ chlorophyll. Such a change would lead to a large accumulation of Pi at low temperatures (a minimum increase of 20 mM if equally distribuded between the chloroplasts and cytosol), unlike the decrease observed in barley (Fig. 1). This is possibly the reason why no symptoms of a restriction of sucrose synthesis are apparent at low temperatures in maize.

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