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Regulation of photosynthesis in leaves of C_4 plants following a transition from high to low light

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The aim of this work was to investigate the factors which regulate photosynthetic carbon assimilation in leaves of maize (*Zea mays*) and *Amaranthus edulis* following a decrease in photon flux density. The principal observations in maize were that, after a decrease in photon flux density; (i) there was a transient inhibition of the rate of carbon assimilation; (ii) the pool of phospho*enol*pyruvate rose while the flux decreased, indicating rapid regulation of the activity of phospho*enol*pyruvate carboxylase; (iii) the triose-*P*/glycerate-3-*P* ratio declined drastically; at the same time the pool of pyruvate increased several-fold; (iv) the activation state of $NADP^+$-malate dehydrogenase declined rapidly, while the activation states of pyruvate, P_i dikinase, fructose-1,6-bisphosphatase and sucrose phosphate synthase changed relatively slowly; (v) there was a rapid decrease in non-photochemical quenching of chlorophyll fluorescence and a rapid oxidation and re-reduction of Q_A followed by a slow oxidation of Q_A; (vi) in *A. edulis* the content of aspartate and of the total pool of C_4-cycle intermediates increased. The observations are discussed in terms of the regulation of C_4 photosynthesis and in terms of the supply of, and demand for, the products of electron transport by carbon metabolism. It is suggested that the transient inhibition of photosynthetic carbon assimilation in maize leaves is accompanied by a high demand for ATP which is met by cyclic electron transport.

Introduction

When illuminated leaves are subjected to a sudden decrease in PFD, a transient inhibition occurs in the rate of photosynthetic carbon assimilation before it proceeds at a new steady-state rate. This transient comprises two phenomena. The first is a post-lower-PFD CO_2 burst which probably results from the continued turnover of photorespiratory intermediates which derive from the period spent at high PFD [1,2]. The second is a true inhibition of the rate of photosynthetic carbon assimilation [3,4] which reflects regulatory imbalances within the system. During a light transition, regulation must act to minimise such imbalances between the reactions of electron transport (supply of ATP and NADPH) and those of photosynthetic carbon assimilation (demand for ATP and NADPH). Prinsley et al. [4] observed that in spinach leaves the amount of ATP and NADPH (also indicated by the triose-*P*/PGA ratio [5]) was reduced following the transition to a level below that subsequently reached in the steady-state in low light. Various explanations have been advanced to explain the undershoot in the amounts of ATP and NADPH. For example, on the basis of a relatively slow decrease in non-photochemical quenching and light-scattering following a decrease in PFD in leaves of *Hedera helix*, Sivak and Walker [6] proposed that a high transthylakoid pH present under high PFD relaxed slowly following the light transition, resulting in a temporary inhibition of electron transport by back pressure from an excessive proton gradient. On the other hand, Heber et al. [5] have shown that triose-*P* builds up to high concentrations in the medium surrounding isolated spinach chloroplasts in high light and that triose-*P* oxidation in low light may then account for the transient inhibition of carbon assimilation.

In order to understand the interactions between electron transport (supply) and CO_2 assimilation (demand) in leaves, we need to be able to measure the behaviour of both of these processes in parallel. The aim of this

Abbreviations: PGA, glycerate-3-*P*; RuBP, ribulose-1,5-bisphosphate; FBP, fructose-1,6-bisphosphate; PEP, phospho*enol*pyruvate; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; F_o, fluorescence level when all Photosystem II centres are open; F_m, fluorescence yield when all centres are closed; q_Q, photochemical fluorescence quenching; q_{NP}, non-photochemical fluorescence quenching; PFD, photon flux density.

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work was to investigate the factors which regulate photosynthetic carbon assimilation in leaves of maize (*Zea mays*) and *Amaranthus edulis* following a decrease in photon flux density by measurements of gas-exchange, metabolites, enzymes and the photochemical and non-photochemical components of chlorophyll fluorescence quenching.

Materials and Methods

Plant material. Maize (*Z. mays*) and *A. edulis* were grown for 4 weeks in a glasshouse in a mixture of compost and vermiculite in 5–1 pots (one plant per pot) with a minimum temperature of 15 °C, and a maximum of 30 °C, and supplementary illumination for 12 h per day. Barley (*Hordeum vulgare*) was grown in vermiculite under similar conditions for 10 days. Twice a week maize and *A. edulis* were fed a solution of Solufeed F (ICI Plant Protection Division, Haslemere, U.K.) with supplementary 10 mM $Ca(NO_3)_2$.

Gas exchange measurements. CO_2 uptake by attached leaves was measured in 20% O_2 and 370 ppm CO_2 in an open system [7]. Water at 25 °C was circulated through an aluminium chamber modified from the design of Harris et al. [7], and the leaf temperature was monitored by a thermocouple attached to the underside of the leaf. An attached leaf was sealed between the lower and upper halves of the chamber by foam rings. The windows of the chamber were parafilm discs held in place by O-rings and flanged aluminium cylinders. Attachments on the top cylinder held two branches of a fibre optic (from a Schott KL 1500 projector) at an angle of 45° to illuminate the leaf from above.

Chlorophyll fluorescence measurement. Chlorophyll fluorescence was analysed by a pulse amplitude modulation fluorometer (PAM-101, H. Walz, Effeltrich, F.R.G.). The fibre-optic probe was located under the lower window of the chamber. Saturating pulses of light (2500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, duration 1 s) were provided to obtain transient reduction of Q_A. F_m was measured after prolonged dark pretreatment (greater than 1 h) of the leaf. Photochemical (q_Q) and non-photochemical quenching (q_{NP}) were calculated according to Bilger and Schreiber [8]. For Fig. 1 (inset) and Fig. 5, illumination was provided by a Leitz 250W projector and was passed through a heat reflecting mirror (Oriel Corp., Stamford, CA, U.S.A.). The PFD was decreased by the insertion of neutral density filters so as to avoid the very brief dark period which resulted from switching the Schott projector, although in practice this had little effect on the changes in chlorophyll fluorescence and made no difference to metabolic changes.

Freeze-clamping and extraction. Leaf metabolism was stopped by freeze-clamping the leaf between two removable copper discs, cooled to the temperature of liquid N_2 and attached to two solenoids which were fired remotely. The frozen leaf disc (11.2 cm^2) was put into an aluminium foil envelope under N_2 and stored in liquid N_2. For analysis, the leaf disc was divided into two approximately equal parts. One half was extracted in $HClO_4$ as in Ref. 9; pieces of the other half were used for measurement of enzyme activities.

Measurement of metabolites. All metabolites were measured in a dual-wavelength spectrophotometer (340–400 nm) as described in [10]. PGA and RuBP were measured in a single assay step. RuBP carboxylase purified from wheat leaves [11] (a gift from the Department of Biochemistry, Rothamsted Experimental Station, U.K.) was pre-activated by incubating 1 mg protein in 20 µl of 20 mM $NaHCO_3$ and 20 mM $MgCl_2$ at 40 °C for 40 min. To minimise drift, the extract was preincubated [12] in 50 mM Tricine (pH 8.0), 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM ATP for 20 min before assay. 80 µM NADH was then added, followed by 0.36 U phosphoglycerate kinase and 0.18 U glyceraldehyde-3-*P* dehydrogenase to determine PGA, and 50 µg RuBP carboxylase to determine RuBP.

Measurement of enzyme activities. Frozen leaf samples stored in liquid N_2 were used for enzyme assays. Leaf pieces (20–30 µg chlorophyll) were rapidly homogenised (less than 20 s) in a glass-in-glass homogeniser. For all assays except that for sucrose phosphate synthase, the extract was centrifuged (Eppendorf 5414) for 10 s, and 50 µl of the supernatant was added immediately to the assay cuvette (1 ml). All assays were linear. Enzymes were assayed as follows:

NADP-malate dehydrogenase [13]: extraction in 100 mM Tris-HCl (pH 7.8), 0.05% (v/v) Triton X-100, 1 mM EDTA, 15 mM 2-mercaptoethanol and 50 $mg \cdot ml^{-1}$ PVP-40 and assay in 100 mM Tris-HCl (pH 7.8), 0.05% (v/v) Triton X-100, 1 mM EDTA, 15 mM 2-mercaptoethanol, 0.5 mM OAA and 0.4 mM NADH.

Fructose-1,6-bisphosphatase [14]: extraction in 100 mM Tris-HCl (pH 7.8), 0.05% (v/v) Triton X-100, 1 mM EDTA, 10 mM $MgCl_2$, 0.25 mM FBP and 50 $mg \cdot ml^{-1}$ PVP-40 and assay in 100 mM Tris-HCl (pH 7.8), 0.05% (v/v) Triton X-100, 1 mM EDTA, 10 mM $MgCl_2$, 0.25 mM FBP, 0.5 mM NADP, 2U glucose-6-*P* dehydrogenase and 4 U phosphoglucose isomerase.

Pyruvate, P_i dikinase [15]: extraction in 50 mM Hepes (pH 7.8), 8 mM $MgSO_4$, 5 mM DTT and 50 $mg \cdot ml^{-1}$ PVP-40 and assay in 50 mM Hepes (pH 7.8), 8 mM $MgSO_4$, 5 mM DTT, 10 mM $KHCO_3$, 1 mM ATP, 1 mM glucose-6-*P*, 5 mM $(NH_4)_2SO_4$, 5 mM pyruvate, 2.5 mM KH_2PO_4, 0.2 mM NADH, 0.05% (v/v) Triton X-100, 1 U malate dehydrogenase and 2 U PEP carboxylase.

Sucrose phosphate synthase [16]: extraction in 50 mM Hepes (pH 7.4), 5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 10% (v/v) glycerol and 0.1% (v/v) Triton X-100. 50 µl extract was added to 150 µl 2 mM F6P, 10 mM G6P, 3 mM UDPG, 2 mM KH_2PO_4, 50 mM

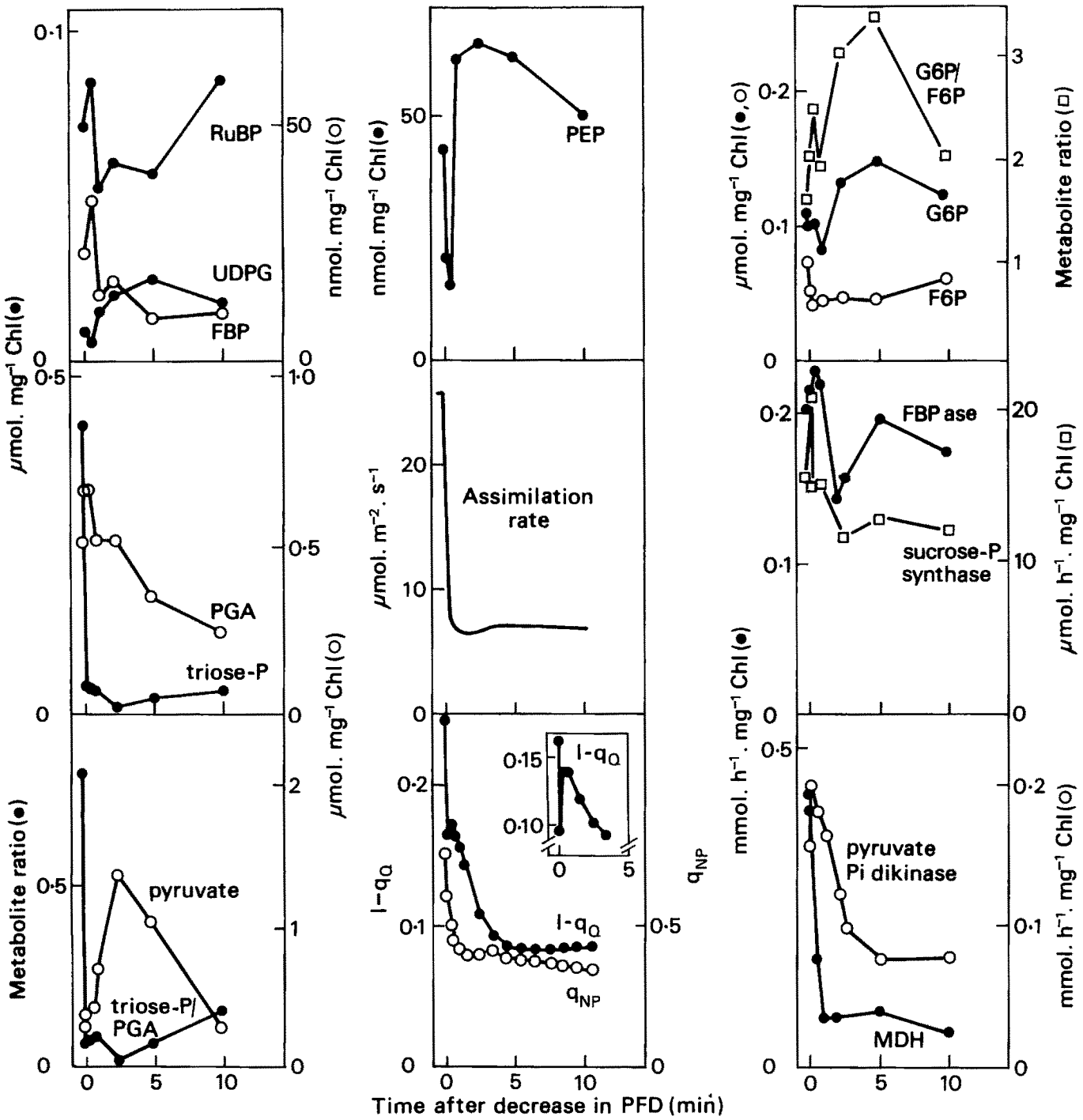

Fig. 1. Influence of a decrease in PFD from 1700 to 140 μmol quanta·m^{-2}·s^{-1} upon the CO_2 assimilation rate, the amounts of metabolites and the ratios of triose-*P* to PGA and glucose-6-*P* to fructose-6-*P*, $1-q_Q$, non-photochemical quenching and the activities of NADP$^+$-dependent malate dehydrogenase (MDH), fructose-1,6-bisphosphatase (FBPase), sucrose phosphate synthase and pyruvate, P_i dikinase in leaves of *Z. mays*. Standard errors (not shown) were all less than 10% of the means ($n = 3$). The chlorophyll content of these leaves was 350 mg·m^{-2}.

Hepes (pH 7.3), 2.5 mM $MgCl_2$ and 0.5 mM EDTA. The control lacked hexose phosphate. After incubation at 25 °C for 20 min, the reaction was stopped by heating in a boiling water bath for 4 min. After centrifugation 50 μl of the incubation mixture was assayed for UDP formation in 50 mM Hepes (pH 7.0), 5 mM $MgCl_2$, 5 mM KCl, 2.5 mM PEP, 60 μM NADH, 0.4 U lactate dehydrogenase and 0.6 U pyruvate kinase.

Chlorophyll and phaeophytin. The chlorophyll content of the crude extract was determined according to Bruinsma [17]. Phaeophytin was determined by the method of Vernon [18].

Results

Leaves of maize and *A. edulis* were subjected to a variety of transitions between high and low PFD, vary-

ing between 3-fold and 100-fold. The behaviour of all metabolite pools was qualitatively similar, whatever the magnitude of the light transition. Fig. 1 shows some of the changes in maize leaves which accompanied a 12-fold decrease in PFD. There was a typical transient inhibition of the assimilation rate which lasted several minutes. Large changes in metabolite pools followed the transient. The amount of PEP fell immediately, but by 1 min after the transient it had increased to an amount above the content at high PFD. After 10 min the PEP pool reached its new steady-state level, which was maintained for the next 30 min (data not shown). The amount of pyruvate rose dramatically, as much as 5-fold, and declined after several minutes. The PGA pool rose steeply following a decrease in PFD and then declined slowly. In contrast the amount of triose-*P* fell rapidly, falling to minimum after 2 min and then rose slowly. The triose-*P*/PGA ratio therefore declined rapidly and for a considerable period it remained well below the value subsequently reached in the steady-state in low light (0.45). The amounts of both F6P and G6P fell initially. The content of F6P then increased slightly, but the amount of G6P increased substantially. The G6P/F6P ratio rose overall during the time-course of the experiment, reflecting the fact that the ratio is higher in low light than in high light in the steady-state [19]. FBP showed a peak during the first minute before declining to a new lower steady-state content [19]. RuBP also showed a transient peak, followed by a small decline and rise to a content of RuBP similar to that in high light. The amount of UDP-glucose rose, following a small decline.

Of the four enzymes measured, only the activity of $NADP^+$-malate dehydrogenase declined rapidly in response to lowered PFD (Fig. 1). By comparison, the activity of pyruvate, P_i dikinase declined slowly to its new steady-state activity in low light. Sucrose phosphate synthase and FBPase showed overall changes which were rather small following a 12-fold change in PFD. Fig. 2 illustrates changes in the activities of these enzymes in maize leaves which were observed following a larger (70-fold) change in PFD. Again, malate dehydrogenase showed a rapid decline in activity. Sucrose phosphate synthase and FBPase showed large decreases in activity and in both the activity increased again between 7 and 15 min. The activity of pyruvate, P_i dikinase declined relatively slowly, as in the smaller light transition (Fig. 1).

In $NADP^+$-malic enzyme plants such as maize, measurement of the total leaf pool of malate provides little

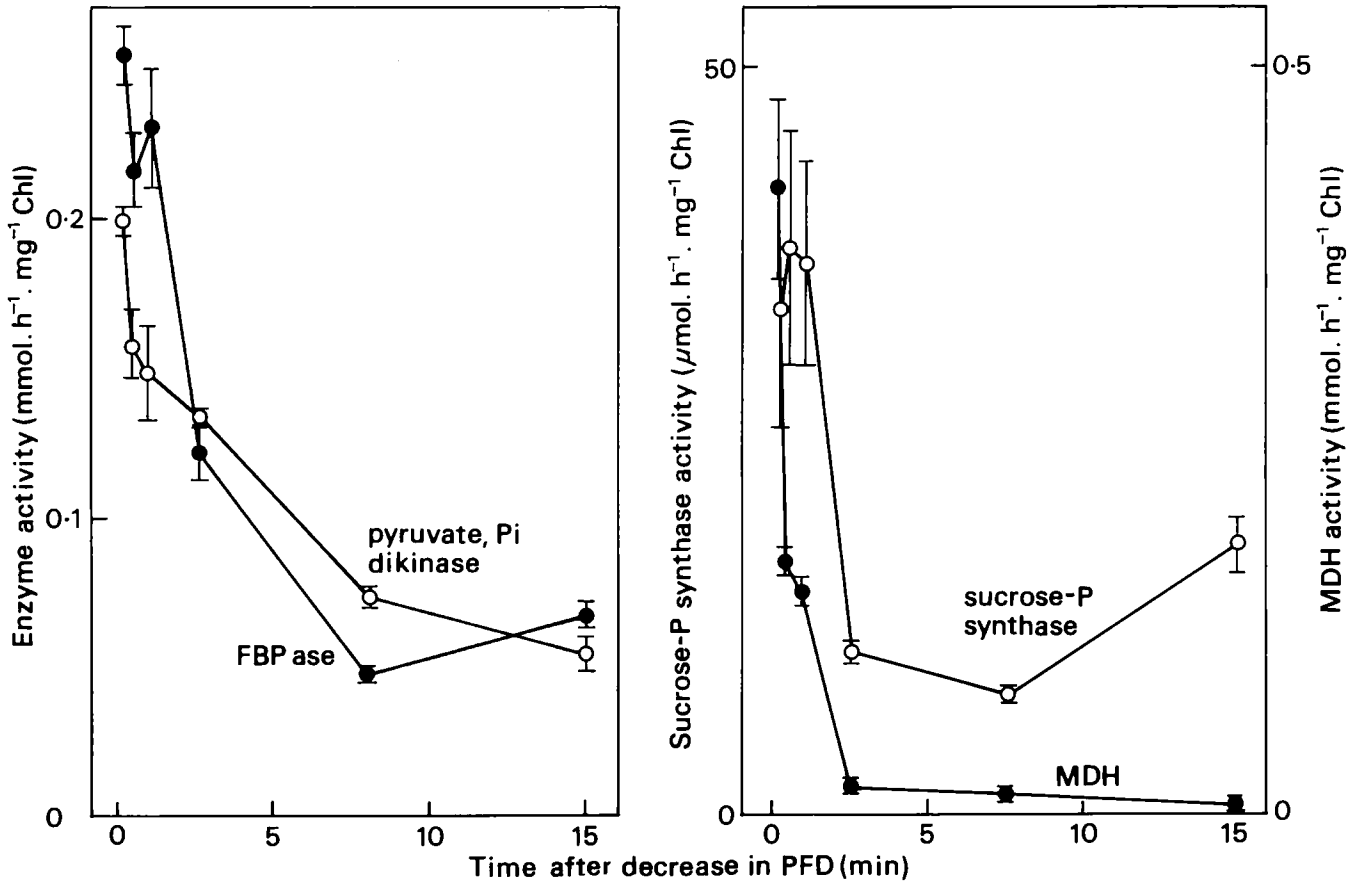

Fig. 2. Influence of a decrease in PFD from 1240 to 17 μmol quanta$\cdot m^{-2}\cdot s^{-1}$ upon the extractable activities of $NADP^+$-dependent malate dehydrogenase (MDH), fructose-1,6-bisphosphatase (FBPase), sucrose phosphate synthase and pyruvate, P_i dikinase in leaves of *Z. mays*. Results are means ± S.E. of three separate determinations.

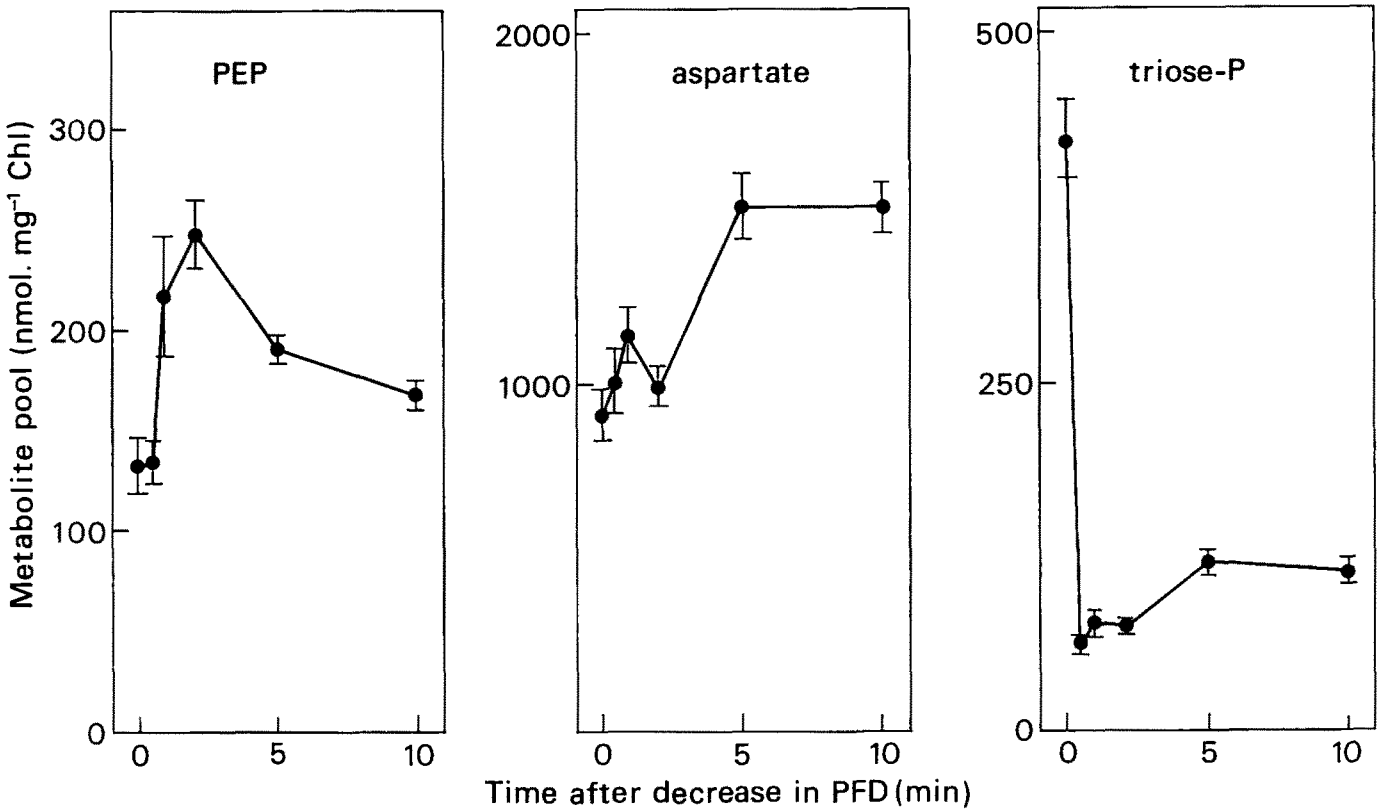

Fig. 3. Influence of a decrease in PFD from 1700 to 140 μmol quanta·m^{-2}·s^{-1} upon the amounts of PEP, aspartate and triose-*P* in leaves of *A. edulis*. Results are means ± S.E. of five separate determinations.

useful information about the behaviour of the C_4 acid pool because the photosynthetically-active pool of malate is masked by a large storage pool [9]. The behaviour of the pool of C_4 acids was therefore determined in leaves of the NAD$^+$-malic enzyme type C_4 plant *A. edulis*, in which all the metabolite pools constituting the C_4-cycle are largely photosynthetically active [19]. Fig. 3 illustrates changes which occurred in the amounts of PEP, aspartate and triose-P following a 10-fold decrease in PFD. The pools of both PEP and aspartate increased and the pool of triose-*P* declined drastically. Fig. 4 shows how the total leaf pool of metabolites of the C_4 cycle and the sum of the amounts of PGA and triose-P changed following a decrease in PFD in leaves of *A. edulis*. Metabolites of the C_4-cycle increased transiently, but overall they remained constant, while the pool of PGA + triose-*P* declined quite slowly by about half.

Fig. 5 shows how the components of chlorophyll fluorescence quenching changed following a decrease in PFD in leaves of maize, and in leaves of the C_3 plant, barley, for comparison. After establishment of F_o and F_m in dark-adapted leaves, they were illuminated at a PFD of 1700 μmol quanta · m^{-2} · s^{-1} until a steady-state rate of photosynthesis was achieved, F_o was then redetermined. In barley, F_o was quenched (F_o') after a period in high light [8] and there was a slow recovery to the original F_o level of fluorescence. In maize, there was no quenching of F_o at the PFD used here (and non-photochemical quenching was lower than in barley). Instead, fluorescence increased following darkening and decreased slowly to the original F_o. The leaves were

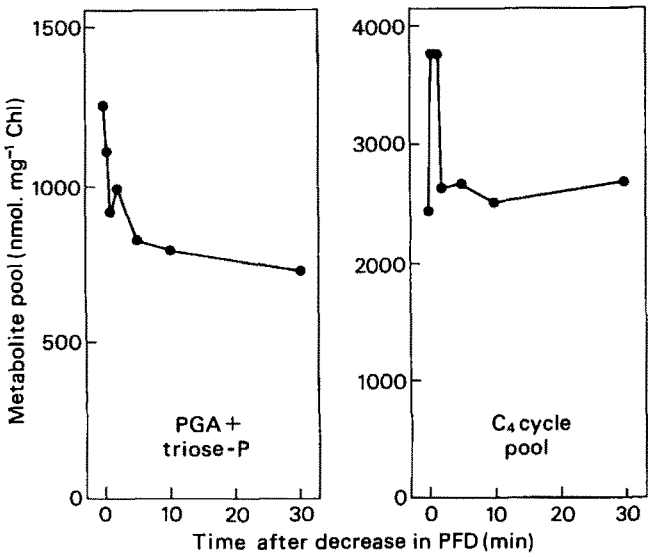

Fig. 4. Influence of a decrease in PFD from 1500 to 150 μmol quanta·m^{-2}·s^{-1} upon the sum of the amounts of PGA and triose-*P* (A) and the sum of the metabolites of the C_4-cycle pool (aspartate + alanine + OAA + pyruvate + PEP) (B) in leaves of *A. edulis*. Results are means of two separate determinations. All metabolites are considered equivalent on a molar basis.

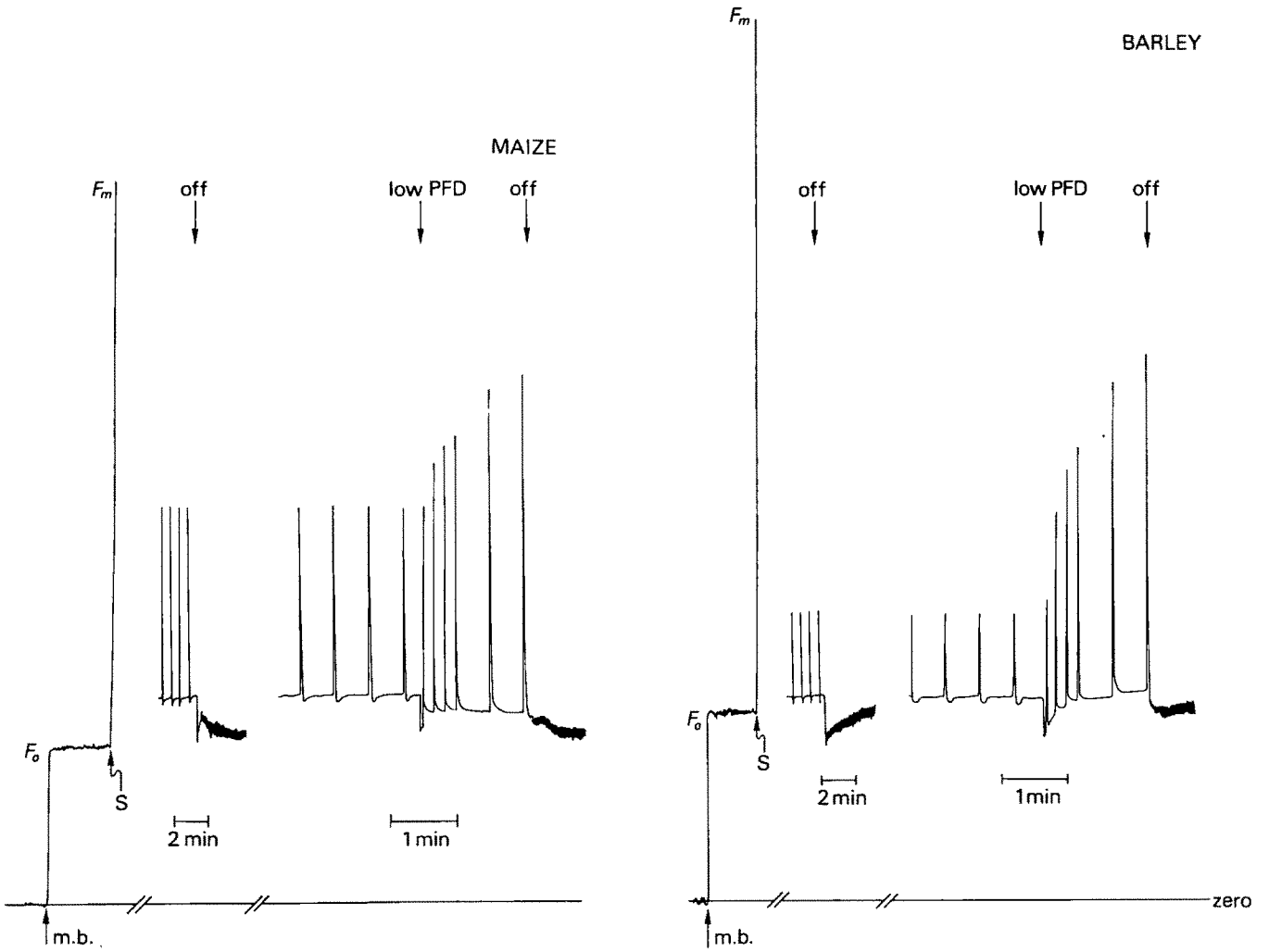

Fig. 5. Influence of a decrease in PFD from 1700 to 140 μmol quanta·m^{-2}·s^{-1} upon chlorophyll fluorescence yield in leaves of *Z. mays* and barley. F_o and F_m were determined in a fully dark-adapted leaf (left-hand of each trace). The leaf was then illuminated (1700 μmol quanta·m^{-2}·s^{-1}) until steady-state photosynthesis was achieved. The change in F_o was then measured during a brief dark interval (centre of each trace). The leaf was re-illuminated (1700 μmol quanta·m^{-2}·s^{-1}) until steady-state photosynthesis was achieved. The PFD was then decreased to 140 μmol quanta·m^{-2}·s^{-1}. Finally the leaf was darkened and the change in F_o was monitored (right-hand of each trace). m.b., measuring beam on; S, application of saturating light pulse.

then reilluminated at a PFD of 1700 μmol quanta · m^{-2} · s^{-1} until a steady-state rate of photosynthesis was achieved, before transition to a low PFD. Immediately following a decrease in PFD, fluorescence was quenched close to the F_o level in maize, or to the quenched F_o' level in barley. This shows that Q_A became strongly oxidised following the light transition. Q_A was then re-reduced in both barley and maize. Upon darkening from low light, there was no quenching of F_o in barley [8], while in maize there was a small transient rise in fluorescence.

In the experiment in maize leaves shown in Fig. 1, the redox state of Q_A ($1-q_Q$) estimated 10 s after the decrease in PFD is shown, but in the inset, a point 3 s after the PFD was decreased is shown and again demonstrates the oxidation and re-reduction of Q_A seen in Fig. 5. Re-reduction of Q_A was followed by a slow oxidation over a period of up to 5 min, with Q_A achieving a new steady-state redox state coincidentally with the recovery of the assimilation rate. Fig. 1 shows that non-photochemical quenching declined rapidly following the lowering of the PFD in both barley and maize (Fig. 5). In maize, two principal phases were evident (Fig. 1). The first phase declined rapidly within about 30 s, thereafter non-photochemical quenching declined very slowly.

Discussion

Enzymic regulation of carbon assimilation following a transition in PFD

The data provide evidence for regulation of a number of enzymes of the C_4 and Calvin cycles. The transient decrease in the PEP pool in maize leaves which

occurred after transfer to low PFD reflects continued consumption of PEP by PEP carboxylase, but it was followed by an increase in the PEP pool while the flux decreased (Fig. 1). The latter change also occurred in leaves of *A. edulis* (Fig. 3). This behaviour shows that PEP carboxylase activity was rapidly modulated after a light transition. There is additional evidence for light modulation of the activity of PEP carboxylase in vivo. During a dark-to-light transition in maize the amount of PEP declined while the flux increased [26]. The pool of PEP also remains stable during steady-state photosynthesis in varying PFD in leaves both of maize and *A. edulis* [19,27]. This stability can also be observed in Fig. 1. The activity of PEP carboxylase is regulated by light-dependent protein phosphorylation [20,21] and by a number of metabolites of physiological relevance. Of particular importance are the C_4 acid inhibitors, malate and aspartate [22], and the activators, hexose-*P* and triose-*P* [23–25]. The metabolite data indicate how regulation of PEP carboxylase might be achieved following a decrease in PFD. Figs. 1 and 3 show that the activators triose-*P* and hexose-*P* both declined whereas the inhibitor, aspartate, increased following a decrease in PFD. These changes alone would be expected to play an important role in decreasing the activity of PEP carboxylase, especially as changes in the phosphorylation state of the enzyme apparently occur too slowly to explain rapid modulation of enzyme activity [21].

The increase which occurred in the level of aspartate in leaves of *A. edulis* following a decrease in irradiance demonstrates modulation of its decarboxylation in the bundle-sheath. This is consistent with measurements of the aspartate pool during steady-state photosynthesis with varying PFD in leaves of *A. edulis* which show that decarboxylation of C_4 acids is light-regulated in vivo [19]. Similarly, malate decarboxylation by isolated bundle-sheath strands of maize is regulated by light [28].

The rise in pyruvate which occurred following a decrease in PFD was similar to that which occurs when maize leaves are darkened [9,29]. The increase in pyruvate could result either from a lack of the ability of pyruvate, P_i dikinase to metabolise pyruvate or from a lack of ATP. The first is unlikely as the activation state of pyruvate, P_i dikinase remained high following the transition and during the transient inhibition of photosynthesis, indeed its activation state 2 min after the transition was the same as under high PFD. Even after larger changes in PFD, its activation state changed relatively slowly (Fig. 2). Any accumulation of pyruvate, resulting in an increase in the ratio of pyruvate to PEP, as occurs following the light transition (Fig. 1), tends to maintain the enzyme in an active state [29] and may account for the observed behaviour. It can be inferred from these observations that the build-up of pyruvate results from a lack of ATP.

In C_3 plants, amounts of RuBP rapidly fall below the concentration of binding sites on RuBP carboxylase when the PFD is lowered [4,30,31], and then rise. It has been proposed that this is due to slow modulation of the activity of RuBP carboxylase [30,31], although the behaviour is probably due to a low capacity for RuBP regeneration rather than to poor regulation of RuBP utilisation [4]. In maize, instead of the precipitous fall in RuBP that is observed in C_3 plants, there was a transient peak indicating rapid modulation of RuBP consumption. The steady-state content of RuBP was also similar in high and low light [19,27]. The amount of RuBP was maintained at high levels relative to RuBP binding sites following the transition (binding sites are equivalent to about 30 nmol/mg chlorophyll [19]). In *Chlorella* (another CO_2-saturated system), the RuBP pool behaved in a manner similar to that in maize [32]. The transient increase in the amount of FBP indicates rapid regulation of the activity of FBPase, a feature observed by Taylor et al. [32] who also noted transient increases in the pools of FBP and sedoheptulose 1,7-bisphosphate in *Chlorella.* However, the changes in the activation state of FBPase (Fig. 1) do not suggest how this occurs. Similarly, the rise in UDP-glucose (which also increases in *Chlorella* [32] and in which the rate of sucrose synthesis shows a momentary lag) cannot be explained by the small changes in the activation state of sucrose-*P* synthase. The rapid decline in F6P (Fig. 1) may be a factor which restricts metabolism of UDP-glucose by sucrose-*P* synthase.

Maintenance of metabolite pools

An important aspect of C_4 photosynthesis is the development of large pools of metabolites during induction, and their maintenance during steady-state photosynthesis [9,33]. These large pools reflect the requirement for metabolite gradients of the C_3 and C_4 acids, PGA and triose-*P* which exist between the mesophyll and bundle-sheath. Although smaller metabolite gradients would be required in low light, discharge of the pools that sustain these gradients, e.g. by product synthesis, would result in a prolonged period of induction if the PFD increased again. The data for *A. edulis* (Fig. 4) demonstrate that, over this period of time, pools of metabolites will be conserved. It is important to note that the C_4 cycle is not a closed system, because it is connected to the Calvin cycle via interconversion of PEP and PGA in the reactions catalysed by phosphoglycerate mutase and enolase [26]. Any fall in PGA + triose-*P* or Calvin cycle intermediates can therefore be mitigated by the stability of the total content of C_4-cycle intermediates. The decrease in the pool of PGA + triose-*P* was 500 nmol/mg chlorophyll, compared with a total C_4-cycle pool of 2500 nmol/mg chlorophyll. Short-term fluctuations in light would therefore result in no large overall change in the level of metabolites,

but merely in the redistribution of carbon between them, and autocatalytic build-up of intermediates would be unnecessary [9].

Relationships between electron transport and carbon assimulation

In maize, despite the presence of two cell types, interpretation of chlorophyll fluorescence is simplified because the vast majority of variable chlorophyll fluorescence emanates from the mesophyll [34,35] and therefore reflects events occurring in the mesophyll. Q_A was immediately oxidised upon lowering the PFD, which might be predicted, but the re-reduction which followed requires explanation and could reflect at least two possibilities. The first could be a rapid re-reduction of intersystem electron acceptors such as plastoquinone, which would in turn block the oxidation of Q_A. The second is a reversal of the energy-dependent quenching mechanism which maintains Q_A in an oxidised state, as proposed by Weis and Berry [36], and which would accompany a decline in q_{NP}.

Non-photochemical quenching of chlorophyll fluorescence is dependent upon a number of different processes, including thylakoid energisation, state transitions and photoinhibition [37]. In maize plastoquinone-dependent quenching of chlorophyll fluorescence [13,35,38] may also be important. Several components in the dark relaxation of non-photochemical quenching have been identified, of which only the fastest component (q_E) is likely to reflect ΔpH [12,37]. If the rapid decline in fluorescence in maize (Fig. 1) is due to the decay of ΔpH, then it would provide a maximum estimate for the time taken, which in this instance was about 30 s.

There are additional metabolic probes of the relationship between electron transport and carbon assimilation. The first is the measurement of $NADP^+$-dependent malate dehydrogenase activity (which is confined to the mesophyll) as a probe of the redox state of the terminal acceptors of Photosystem I [13]. In many instances there is also a good correlation during steady-state photosynthesis between the redox state of Q_A and the activation state of malate dehydrogenase [13,39]. The second is the use of the triose-*P*/PGA ratio as a probe of the supply of ATP and NADPH (the assimilatory force [5]). Fig. 1 shows that the triose-*P*/PGA ratio declined rapidly and for a considerable period it remained well below the value subsequently reached in the steady state in low light, as occurs in spinach leaves [4]. However, the information which the triose-*P*/PGA ratio provides about the regulation of the system is ambiguous. It does not reveal whether the problem is one of *supply* (i.e., electron transport is unable to meet the requirement for ATP and NADPH, a situation which might obtain if, for example, ΔpH persisted following the transition) or whether the problem is one of excessive *demand* for ATP and NADPH. In maize the pyruvate pool can be considered as an additional metabolic probe of the relationship between supply and demand.

In isolated maize mesophyll chloroplasts, which are capable of converting pyruvate to PEP at the sole expense of ATP, the addition of pyruvate results in an enhanced slow phase of the electrochromic shift (P518) [35] indicating electrogenic electron transfer through the cytochrome *bf* complex [40]. In maize this is probably associated with the cyclic pathway of electron flow [35,41]. During pyruvate metabolism in isolated chloroplasts both plastoquinone and Q_A are maintained in a highly reduced state, as indicated by chlorophyll fluorescence induction studies and by the effect of DCMU addition upon steady-state chlorophyll fluorescence [13,35] and ΔpH (as indicated by the extent of non-photochemical quenching and by 9-aminoacridine fluorescence) and the ATP/ADP ratio are low [13,35,41].

The current results indicate that a situation obtains following a decrease in PFD in which (a) ΔpH decreases rapidly (indicated by the rapid decay of non-photochemical quenching), (b) the acceptor side of Photosystem I becomes relatively oxidised (indicated by the change in the activity of $NADP^+$-malate dehydrogenase), (c) Q_A is transiently re-reduced (but nevertheless remains oxidised relative to its redox state in high light), (d) the amounts of ATP and NADPH are low (indicated by the low triose-*P*/PGA ratio). A hypothesis which can be advanced by analogy with the behaviour of isolated maize mesophyll chloroplasts is that the accumulation of pyruvate would lower ΔpH and stimulate cyclic electron flow to generate additional ATP with resultant reduction of plastoquinone and hence of Q_A.

On the origins of the transient inhibition of carbon assimilation following a decrease in PFD

As in any metabolic system, the control of carbon assimilation during a light transition will not be invested in a single step, but will be shared [42]. It is clear from these results that regulation of the response is shared between electron transport and carbon metabolism. A number of factors have been proposed to contribute to the transient inhibition of carbon assimilation, for example, an overshoot in sucrose synthesis [3], slow modulation of the activation state of RuBP carboxylase [30,31], a high ΔpH which persists in low light [6], triose-*P* oxidation [5] and transient hydrolysis of ATP [43]. None of these can 'explain' the transient inhibition any more than their quantitative contribution can be assessed. From the results presented here we suggest that ΔpH may fall rapidly. Triose-*P* oxidation in low light is also unlikely to be a major factor, as triose-*P* declined prior to the transient, there was a decline in PGA during the transient (Fig. 1) and there was no

evidence for slow oxidation of the acceptor side of Photosystem I. RuBP levels did not decline markedly as they did in C_3 plants [3,30,31], suggesting that that slow modulation of RuBP carboxylase activity is not an explanation for the transient inhibition. The lack of any evidence of a massive drainage of intermediates of the Calvin cycle (RuBP, FBP, PGA) or of intermediates involved in product synthesis (hexose-*P* and UDP-glucose) suggests that a continuation of sucrose synthesis at a high rate does not impose a stress on the system. The rapid decrease in triose-*P* would also ensure that sucrose synthesis rapidly became substrate limited [44]. Indeed, the induction loss (the difference between the amount of CO_2 which was actually fixed and that which would have been fixed had there been a step change in photosynthetic rate [45,46]) was about 96 μmolC $\cdot$ m^{-2} (274 nmolC/mg chlorophyll) in the example shown in Fig. 1, which represents only 4 s steady-state photosynthesis at the high PFD and only 15 s photosynthesis when the rate was at its minimum. It therefore seems more likely that the rate is held below its maximum potential during the transient inhibition by factors other than the unregulated drainage of carbon skeletons. A similar conclusion concerning the relative importance of product synthesis and of the ability to reduce PGA following a light transition in barley leaves in saturating CO_2 has been reached by Stitt et al. [47].

All the evidence from metabolites points to rapid modulation of fluxes. What regulation cannot readily overcome is the fall in the triose-*P*/PGA ratio which occurs as a result of a decrease in the rate of electron transport and the provision of ATP and NADPH, coupled with the large increase in the amount of pyruvate. The accumulation of pyruvate exacerbates a situation in which the ATP and NADPH are already in short supply. A switch from linear to cyclic electron flow, as occurs in isolated chloroplasts under these conditions, may underlie the transient inhibition of the rate of carbon assimilation.

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References

1 Vines, H.M., Armitage, A.M., Chen, S., Tu, Z. and Black, C.C. (1982) Plant Physiol. 70, 629–631.
2 Vines, H.M., Tu, Z., Armitage, A.M., Chen, S. and Black, C.C. (1983) Plant Physiol. 73, 25–30.
3 Prinsley, R.T., Hunt, S., Smith, A.M. and Leegood, R.C. (1986) Planta 167, 414–420.
4 Prinsley, R.T., Dietz, K.-J. and Leegood, R.C. (1986) Biochim. Biophys. Acta 849, 254–263.
5 Heber, U., Neimanis, S., Dietz, K.-J. and Viil, J. (1986) Biochim. Biophys. Acta 852, 144–155.
6 Sivak, M.N. and Walker, D.A. (1987) in Progress in Photosynthesis Research, Vol. III (Biggins, J., ed.), pp. 313–316, Martinus Nijhoff, Dordrecht.
7 Harris, G.C., Cheesbrough, J.K. and Walker, D.A. (1983) Plant Physiol. 71, 102–107.
8 Bilger, W. and Schreiber, U. (1986) Photos. Res. 10, 303–308.
9 Leegood, R.C. and Furbank, R.T. (1984) Planta 162, 450–456.
10 Lowry, O.H. and Passonneau, J.V. (1972) A Flexible System of Enzymatic Analysis, Academic Press, New York.
11 Schmidt, C.N.G., Cornelius, M.J., Burton, S., Parry, M.A.J., Millard, B.N., Keys, A.J. and Gutteridge, S. (1984) Photosyn. Res. 5, 47–62.
12 Leegood, R.C., Labate, C.A., Huber, S.C., Neuhaus, H.E. and Stitt, M. (1988) Planta 176, 117–126.
13 Leegood, R.C. and Walker, D.A. (1983) Plant Physiol. 71, 513–518.
14 Leegood, R.C. and Walker, D.A. (1982) Planta 156, 449–456.
15 Chapman, K.S.R. and Hatch, M.D. (1981) Arch. Biochem. Biophys. 210, 82–89.
16 Stitt, M., Wilke, I., Feil, R. and Heldt, H.W. (1988) Planta 174, 217–230.
17 Bruinsma, J. (1961) Biochim. Biophys. Acta 52, 576–578.
18 Vernon, L.P. (1960) Anal. Chem. 32, 1144–1150.
19 Leegood, R.C. and Von Caemmerer, S. (1988) Planta 174, 253–262.
20 Nimmo, G.A., McNaughton, G.A.L., Fewson, C.A., Wilkins, M.B. and Nimmo, H.G. (1987) FEBS Lett. 213, 18–22.
21 Jiao, J.-A. and Chollet, R. (1988) Arch. Biochem. Biophys. 261, 409–417.
22 Huber, S.C. and Edwards, G.E. (1975) Can. J. Bot. 53, 1925–1933.
23 Ting, I.P. and Osmond, C.B. (1973) Plant Sci. Lett. 1, 123–128.
24 Ting, I.P. and Osmond, C.B. (1973) Plant Physiol. 51, 439–447.
25 Doncaster, H.D. and Leegood, R.C. (1987) Plant Physiol. 84, 82–87.
26 Furbank, R.T. and Leegood, R.C. (1984) Planta 162, 457–462.
27 Usuda, H. (1987) Plant Physiol. 84, 549–554.
28 Hatch, M.D. and Kagawa, T. (1976) Arch. Biochem. Biophys. 175, 39–53.
29 Burnell, J.M., Jenkins, C.L.D. and Hatch, M.D. (1986) Aust. J. Plant Physiol. 13, 203–210.
30 Perchorowicz, J.T., Raynes, D.A. and Jensen, R.G. (1981) Proc. Natl. Acad. Sci. USA 78, 2985–2989.
31 Mott, K.A., Jensen, R.G., O'Leary, J.W. and Berry, J.A. (1984) Plant Physiol. 76, 968–971.
32 Taylor, S.E., Terry, N. and Bassham, J.A. (1986) Plant Cell Physiol. 27, 1169–1175.
33 Leegood, R.C. (1985) Planta 164, 163–171.
34 Leegood, R.C., Crowther, D., Walker, D.A. and Hind, G. (1983) Biochim. Biophys. Acta 722, 116–126.
35 Crowther, D., Leegood, R.C., Walker, D.A. and Hind, G. (1983) Biochim. Biophys. Acta 722, 127–136.
36 Weis, E. and Berry, J.A. (1987) Biochim. Biophys. Acta 894, 198–208.
37 Horton, P. and Hague, A. (1988) Biochim. Biophys. Acta 932, 107–115.
38 Vernotte, C., Etienne, A.L. and Briantais, J.-M. (1979) Biochim. Biophys. Acta 545, 519–527.
39 Scheibe, R. and Stitt, M. (1988) Plant Physiol. Biochem. 26, 473–481.
40 Rich, P. (1988) Biochim. Biophys. Acta 932, 33–42.
41 Fernyhough, P., Foyer, C. and Horton, P. (1983) Biochim. Biophys. Acta 725, 155–161.
42 Kacser, H. and Burns, J.A. (1973) Symp. Soc. Exp. Biol. 27, 65–104.
43 Strotmann, H., Kiefer, K. and Altvater-Mackensen, R. (1986) Biochim. Biophys. Acta 850, 90–96.
44 Stitt, M. and Heldt, H.W. (1985) Planta 164, 179–188.
45 Prinsley, R.T. and Leegood, R.C. (1986) Biochim. Biophys. Acta 849, 244–253.
46 McAlister, E.D. (1937) Smithsonian Misc. Coll. 95, 1–17.
47 Stitt, M., Scheibe, R. and Feil, R. (1989) Biochim. Biophys. Acta 973, 241–249.