Changes in Levels of Intermediates of the C₄ Cycle and Reductive Pentose Phosphate Pathway during Induction of Photosynthesis in Maize Leaves

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ABSTRACT
Changes in the level of metabolites of the C₄ cycle and reductive pentose phosphate (RPP) pathway were measured simultaneously with induction of photosynthesis in maize (Zea mays L.) to evaluate what may limit carbon assimilation during induction in a C₄ plant.

After 20 minutes in the dark, there was an immediate rise in photosynthesis during the first 30 seconds of illumination, followed by a gradual rise approaching steady-state rate after 20 minutes of illumination. Among metabolites of the C₄ cycle, there was a net increase in the level of C₄ compounds (the sum of pyruvate, alanine, and phosphoenolpyruvate) during the first 30 seconds of illumination, while there was a net decrease in the level of C₄ acids (malate plus aspartate). The total level of metabolites of the C₄ cycle underwent a sharp increase during this period. At the same time, there was a sharp rise in the level of intermediates of the RPP pathway (ribulose-1,5-bis-phosphate, 3-phosphoglycerate, dihydroxyacetonephosphate, and fructose-1,6-bisphosphate) during the first minute of illumination. The net increase of carbon among intermediates of the C₄ cycle and RPP pathway was far above that of carbon input from CO₂ fixation, and the increase in intermediates of the RPP pathway could not be accounted for by decarboxylation of C₄ acids, suggesting that an endogenous source of carbon supplies the cycle. After 3 minutes of illumination, there was a gradual rise in the levels of intermediates of the C₄ cycle and in the total level of metabolites measured in the RPP pathway. This rise in metabolite levels occurs as photosynthesis gradually increases and may be required for carbon assimilation to reach maximum rates in C₄ plants. This latter stage of inductive autocatalysis through the RPP pathway may contribute to the final build-up of these intermediates.

In C₄ plants, NADP-malate dehydrogenase and pyruvate, Pi dikinase of the C₄ cycle and fructose-1,6-bisphosphatase of the RPP pathway are known to be light-activated in vivo (7, 21). Recently, the degree of light activation of these photosynthetic enzymes relative to the rate of leaf photosynthesis in a C₄ species, maize, was evaluated during the induction period, and under steady-state photosynthesis at varying light intensities (21). When maize leaves were illuminated following a 20-min dark treatment, these enzymes reached their maximum levels of activation within 2.5 min, although photosynthesis had reached only half its maximum rate.

While the stomata of C₃ plants are partially closed in darkness, the stomata of C₄ species are fully closed (1). This suggests that stomatal conductance could limit photosynthetic CO₂ assimilation during the induction period, especially in C₄ plants. In evaluating this possibility in a previous study (20), when maize plants were illuminated after a 20-min dark treatment, the rate of CO₂ assimilation increased at approximately the same rate as stomatal conductance, suggesting that stomatal conductance might limit the rate of photosynthesis during the induction period. However, during the first 3 to 5 min of illumination, the intercellular concentration of CO₂ gradually decreased from 420 μL·L⁻¹ in the dark to below atmospheric level and then reached a steady-state concentration of approximately 150 μL·L⁻¹. If stomata exerted a limitation during the induction of photosynthesis, a reverse pattern would be expected, with intercellular CO₂ concentration initially being very low upon illumination and then increasing with time as stomatal conductance increased. An internal CO₂ concentration of 150 μL·L⁻¹ was close to saturating levels for maximum rate of photosynthesis by maize under optimal conditions (20). Thus, we concluded that stomatal conductance does not limit photosynthesis during the induction period.

These results indicated that, during the induction process in addition to the light activation of enzymes, some other factor(s) is limiting before photosynthesis in this C₄ plant reaches its maximum rate. This limitation may be the build-up of sufficient concentrations of certain metabolites of the C₄ cycle and/or the RPP pathway. Thus, the purpose of this study was to evaluate the change in levels of various photosynthetic metabolites during induction of photosynthesis in maize.

MATERIALS AND METHODS
Plant Material. Seeds of Zea mays L. (variety Chuseishu-B) were obtained from Nihonsogyo, Tokyo, Japan. Plants were grown outside (June 15 to August 5, 1984). Each plant was cultured in a 3-L pot containing a mixture of vermiculite and soil with sufficient nutrients as described previously (19). The largest fully expanded leaves (about 1 m long × 10 cm wide) of plants 6 to 7 weeks old were used for the experiments.

Treatment of Leaves and Gas Exchange Measurements. Plants were transferred from outdoor to laboratory around 10:30 AM to 1:30 PM on a sunny day. About 5 min elapsed between the transfer of the plants to laboratory and the start of the experiments. Plants were illuminated at 1,100 μE·m⁻²·s⁻¹ PAR for 30 min and then placed in darkness for 20 min prior to measure-
ments during induction.

CO₂ exchange of single attached leaves was measured in an acrylic plastic leaf chamber with an open IR gas analysis system as previously described (20). Photosynthesis was measured under a light intensity of 1,100 μE·m⁻²·s⁻¹ PAR. The CO₂ concentration in the air was 330 μL·L⁻¹. The leaf temperature was maintained at 30°C ± 0.5°C.

For metabolite measurements, leaves similar in age to those in the experiments of CO₂ assimilation were used. Single attached leaves were enclosed in a one side open (two sides were closed by plastic film) acrylic plastic leaf chamber (11 cm wide × 40 cm long × 9 cm high). The air stream containing 330 μL·L⁻¹ CO₂ was passed into the leaf chamber at a constant rate of 3.3 L·min⁻¹, since the leaf chamber was open at one side the exact CO₂ concentration in the chamber was not known. The photosynthetic photon flux density was 1,100 μE·m⁻²·s⁻¹ at the surface of the leaves. The leaf temperature was maintained at 30 ± 0.5°C. At each sampling time, leaf segments (approximately 3 × 10 cm) were cut from the midsection of the leaf and were used for the measurements of metabolites.

**Measurements of Metabolites.** Particular care was taken to ensure uniform sampling for determinations of metabolite levels during induction; midsections of large leaves were used (Table I). Eight samples (enough for one experiment) were obtained from the midsection of a single leaf.

Samples of leaf segments were frozen in liquid N₂ in the light or in darkness (under a low light intensity of green safe light) and stored at −80°C until the assay of metabolites. The liquid N₂ was poured into a centrifuge tube in which a frozen sample was stored. Midrubs were removed and discarded. Leaf samples were pulverized in a liquid N₂-cooled mortar and pestle. A pellet of 1.4 ml of frozen 3% HClO₄ was added to the powder and gently pulverized with it according to the method of Leegood and Furbank (13). The mortar and pestle were rinsed three times with 3 ml of ice-cold 3% HClO₄. The combined mixture was left for 1 h on ice and centrifuged at 3,000g for 10 min at 4°C. The supernatant was retained and the pellet was washed with 1 ml of ice-cold 3% HClO₄ and centrifuged again. The pellet was reserved for the determination of pheophytin.

UV absorbance of the combined supernatant solutions were reduced to a very low level by mixing the extracts with activated charcoal (Sigma C-4386) for 5 min on ice. Preliminary experiments revealed that 14 mg of charcoal are needed for 1 ml of solution with a 4:6 of 20 in order to reduce the UV absorbance. The extracts were centrifuged at 10,000g for 5 min at 4°C. The supernatant fractions were neutralized to about pH 6.0 by addition of 2.5 mM K₂CO₃ and centrifuged at 10,000g for 5 min at 4°C. The supernatant was immediately used for the determination of metabolites by coupled-enzyme assay techniques (14) in a Hitachi 220-A spectrophotometer. The values obtained for FBP is a maximum estimate since the assay used also measures half the sedoheptulose-bisphosphate pool, due to the action of aldolase.

RuBP and PGA were determined by a two-step method, in a mixture (0.5 ml) of 10 mM NaHCO₃, 60 mM Hepes-KOH (pH 8.0), 10 mM KCl, 30 mM MgCl₂, 1 mM DTE, and leaf extract, with or without 1 unit of purified and activated spinach RuBP carboxylase, respectively. RuBP carboxylase was purified from spinach leaves using Sephadex G-200 and DEAE-cellulose. After 20 min, the reactions were stopped by adding 50 μl of 20% HClO₄ and neutralized with 2.5 mM K₂CO₃. The reaction mixtures were centrifuged at 10,000g for 5 min at 4°C, and supernatants were used for the second-step analysis. The PGA generated from the RuBP (samples containing RuBP carboxylase) and the PGA which originally existed in the extracts (samples without RuBP carboxylase) were determined in a mixture (1.0 ml) of 5 mM ATP, 0.2 mM NADH, 5 mM phosphocreatine, 40 mM Hepes-KOH (pH 7.8), 10 units of creatine phosphokinase, 5 units of glyceraldehyde-3-phosphate dehydrogenase, and 5 units of PGA kinase. Alanine was determined in a mixture (1.0 ml) of 16 mM Tris-400 mM hydrazine-HCl (pH 10.0), 1 mM NAD, and 1.5 units of alanine dehydrogenase (Boehringer-Mannheim).

**Chl Determination.** Chl was measured by the method of Arnon (2). Pheophytin was extracted from HClO₄ residues into 80% acetone using a Teflon/glass homogenizer. Pheophytin was measured by the method of Vernon (23) and converted to Chl units.

**RESULTS AND DISCUSSION**

There was a reasonable recovery of metabolites from leaf tissue based on the percentage recovery of authentic compounds from extraction and analysis procedure (72% for PEP, 86 to 97% for all other metabolites; Table I). In determining the metabolite content of leaf tissue, corrections were made based on the average recoveries shown in Table I.

The concentration of malate in maize leaves differed significantly from leaf to leaf (data not shown; also see Fig. 1). This may be due to a large fraction of the total malate pool being photosynthetically inactive (8). Therefore, particular care was taken to ensure uniform sampling for determinations of metabolite levels; the middle sections of a large leaf (about 1 m long × 10 cm wide) were used. The SD of metabolite levels in different sections of the middle portion of the same maize leaf was low (Table I).

A steady-state rate of CO₂ assimilation was reached after 20 to 25 min of illumination (Fig. 1) which is consistent with our previous results (20, 21). Changes in levels of intermediates of the C₃ cycle and RPP pathway during the induction period are shown in Figure 1. There was an initial rapid decrease in pyruvate during the first min of illumination which was accompanied by an increase in PEP. Thereafter, pyruvate levels rose substantially during the first min of illumination, which is consistent with our previous results (20, 21).

Table I. Recoveries of Metabolites from Maize Leaves and Metabolite Levels in the Middle Section of a Maize Leaf

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Recovery (n = 6)</th>
<th>Concentration (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% ± SD (nmol·mg⁻¹ Chl average ± SD)</td>
<td></td>
</tr>
<tr>
<td>PGA</td>
<td>87 ± 3</td>
<td>429 ± 38</td>
</tr>
<tr>
<td>RuBP</td>
<td>86 ± 2</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>PEP</td>
<td>72 ± 3</td>
<td>59 ± 7</td>
</tr>
<tr>
<td>DHAP</td>
<td>91 ± 4</td>
<td>234 ± 22</td>
</tr>
<tr>
<td>FBP</td>
<td>95 ± 4</td>
<td>ND*</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>97 ± 2</td>
<td>56 ± 4</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>95 ± 3</td>
<td>116 ± 10</td>
</tr>
<tr>
<td>Malate</td>
<td>95 ± 3</td>
<td>1,284 ± 127</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>92 ± 4</td>
<td>128 ± 18</td>
</tr>
<tr>
<td>Alanine</td>
<td>90 ± 3</td>
<td>545 ± 70</td>
</tr>
<tr>
<td>Aspartate</td>
<td>92 ± 2</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not determined.
over the 20-min illumination period. PEP reached a peak after 1 min, and following a sharp decline.

There was little change in the concentration of malate during the first 30 s of illumination; thereafter, malate levels decreased substantially and then after 1.5 to 2 min of illumination increased gradually. There was an initial rapid decrease in the level of aspartate during the first min of illumination; then aspartate levels increased and became constant after 5 to 10 min of illumination. At the onset of illumination, aspartate may be converted to malate in the mesophyll cells due to a higher amount of NADPH in the light compared to that in the dark (6). Alternatively, aspartate may be converted to malate prior to malate decarboxylation in bundle sheath cells (18). It has been suggested that aspartate might act as a dark reservoir of CO₂ in maize (4). Thus, the relative slow change in the level of malate during the first 30 s may be due to some conversion of aspartate to malate during that period, masking utilization of malate in decarboxylation. After 30 s of illumination, the rate of aspartate conversion to malate may be slower than malate decarboxylation resulting in a net decrease in malate level. In general, the concentration of C₄ acids (malate + aspartate) decreased during the initial phase of the induction period (see also Figs. 2 and 3). There was a big peak in the concentration of alanine around 30 s to 1 min of illumination, after which its level decreased to 3 min. There was a rapid increase in the concentration of RuBP up to 2 min and thereafter its level gradually decreased and reached a steady level after about 10 min. The concentration of FBP showed similar changes to those of RuBP. The level of PGA rapidly increased during the first 30 s of illumination and thereafter stayed constant for about 3 min; then its level increased to 10 min of illumination and remained constant thereafter. The DHAP level, which was almost nil at time zero, reached a big peak after 1 min of illumination, then decreased rapidly up to 3 min, and gradually increased to a steady level thereafter. The level of fructose-6-phosphate and glucose-6-phosphate showed a small peak around 1 min and then gradually increased.

To assess the potential role of a build-up of the concentrations of intermediates of the C₄ cycle and RPP pathway during induction, the following evaluations were made on the data of Figure
The changes in the total µmols of C• mg⁻¹ Chl of the measured C₄ acids (malate + aspartate), 3-carbon compounds of the C₄ cycle (PEP + pyruvate + alanine), and the measured RPP pathway intermediates (PGA + DHAP + FBP + fructose-6-phosphate + RuBP) during the induction period are shown in Figure 2. The amount of oxaloacetate in maize leaves under steady-state photosynthesis is very low (8) and its amount was not determined in this study. There was an initial decrease followed by a gradual increase in the total C atoms of the measured C₄ acids. Three-carbon metabolites of the C₄ cycle (PEP + pyruvate + alanine) initially attained a peak level around the first 30 s of illumination and after declining rapidly, showed a small increase in the following period. The C atoms in the intermediates of the RPP pathway showed a rapid, large increase during the first min of illumination, a small peak at 1 min, and then a gradual increase after 3 min.

In a previous study, after about 2.5 min of illumination, the enzymes NADP-malate dehydrogenase, fructose-1,6-bisphosphatase, and pyruvate, Pi dikinase were fully activated, although photosynthesis had attained only half of its maximum activity (21), or about 40% of its maximum activity in the experiments shown in Figure 1. During this initial phase of induction, light activation of the enzymes plays an important role in increasing the rate of CO₂ assimilation. Secondary increases in the level of intermediates of the RPP pathway and increases in the level of the C₄ cycle intermediates (Fig. 2; see also Fig. 3), particularly during the latter phase of induction, indicate that the build-up of certain metabolites is required to reach a steady-state of photosynthesis.

The concentration of RuBP was about 40 nmol·mg⁻¹ Chl after 20 min of illumination (Fig. 1) and was 56 nmol·mg⁻¹ Chl under a steady-state photosynthesis (Table I). To examine the concentration of RuBP relative to the concentration of RuBP binding site on RuBP carboxylase, the following calculations were made. The activity of RuBP carboxylase in the leaves used in this study was estimated to be 240 to 280 µmol CO₂ fixed·mg⁻¹ Chl·h⁻¹, based on previous data which showed an approximate 1:1 relationship between photosynthesis and RuBP carboxylase activity in maize under light saturated conditions (19). If we take the specific activity of RuBP carboxylase of 2.4 to 5.6 units·mg⁻¹ protein (15, 17), and a carboxylase activity of 260 µmol·mg⁻¹ Chl·h⁻¹, then the calculated content of RuBP carboxylase would be 0.8 to 1.8 mg·mg⁻¹ Chl. The quantity of RuBP carboxylase in maize leaves of 1 to 1.13 mg·mg⁻¹ Chl has been reported (11, 16). Recently, we found that maize RuBP carboxylase was fully activated under high light intensity after photosynthesis reached a steady-state rate (22). RuBP carboxylase content of 0.8 to 1.8 mg·mg⁻¹ Chl corresponds to 11.6 to 26.2 nmol RuBP binding sites on RuBP carboxylase·mg⁻¹ Chl based on a mol wt of 550,000 and eight binding sites per molecule. The following calculations of concentrations of RuBP and RuBP binding site were made assuming that RuBP and RuBP carboxylase are retained in the bundle sheath chloroplasts and 40% of the Chl in maize leaves is in bundle sheath chloroplasts (10) with a stromal volume of 25 µl·mg⁻¹ Chl. From this analysis, the concentration of RuBP (4 to 5.6 mm) is 1.5 to 4.7 times higher than that of RuBP binding site on RuBP carboxylase (1.2 to 2.6 mm) after reaching a steady state of photosynthesis. Yeoh et al. (24) reported the Kₘ (RuBP) of the RuBP carboxylase from maize leaves is 18 µM. Under steady-state photosynthesis, the CO₂ concentration in maize bundle sheath chloroplasts is believed to be several times higher than that in solution equilibrated with atmospheric CO₂ (9). These facts indicate that the concentration of RuBP exceeded that of RuBP binding sites on the enzyme and also was extremely high relative to the Kₘ (RuBP) concentration of maize RuBP carboxylase at high CO₂ under steady state of photosynthesis. According to one model of photosynthesis described by Farquhar and Sharkey (5), photo- synthesis can be limited by a restricted regeneration of RuBP at high CO₂ in C₄ photosynthesis. Recently, Badger et al. (3) found that RuBP levels exceeded the concentration of RuBP binding site at 2% O₂ and high CO₂ but not at 21% O₂ and high CO₂ with bean leaves. Findings in the present study provide another case for deviation from the above photosynthesis model indicating that further studies are needed to elucidate the limiting factor for photosynthesis at high CO₂ in C₃ and C₄ plants.

After 2 min of illumination, the concentration of RuBP was about 90 nmol·mg⁻¹ Chl (Fig. 1). The concentration of RuBP (9 mm) after 2 min of illumination is 3.5 to 7.5 times higher than that of the RuBP binding sites on RuBP carboxylase based on the same assumptions as already mentioned. This transient peak of RuBP level (which has also been observed in wheat; J. Kobza and G. Edwards, personal communication) is a further case of excess RuBP concentration over RuBP binding sites. However, this peak occurs during the transient initial phase of induction when photosynthesis rate is low. It is unknown whether there is already a CO₂ enrichment in bundle sheath chloroplasts during the initial phase of induction. One interpretation for this large transient increase in RuBP is that the CO₂ concentration in bundle sheath chloroplasts is initially not saturating for the
carboxylation of RuBP. A second possibility is that RuBP carboxylase is not fully activated during the initial phase of induction. Previously, we found that RuBP carboxylase of C₄ plants was fully activated under high light intensity after photosynthesis reached a steady-state rate (22). However, the degree of the activation of RuBP carboxylase during the induction period has not been evaluated. The mechanisms for the transient peak of RuBP during induction should be studied.

Although the mechanism of the accumulation of RuBP during the initial phase of induction is unknown, the source of carbon for the increase in RuBP and other intermediates of the RPP pathway needs to be evaluated. Because carboxylation of RuBP seems to be limited, an autocatalytic build-up of intermediates of the RPP pathway seems unlikely. Whether the amount of carbon fixed during the initial phase of induction could account for the build-up of the intermediates of the RPP pathway was assessed. The total carbon fixed during the initial 30 s of illumination was 185 nmol CO₂·mg⁻¹ Chl (Fig. 2). On the other hand, the amount of carbon accumulated in the measured RPP pathway intermediates was 836 natom C·mg⁻¹ Chl during this period (Fig. 2). These results indicate that the amount of CO₂ assimilated was far below the level needed to account for the observed build-up of the intermediates of the RPP pathway. Another possible source of carbon to account for that accumulated in the RPP pathway intermediates is from intermediates of the C₄ cycle. The amount of carbon lost from the C₄ acids (malate + aspartate) was 280 natom C·mg⁻¹ Chl during the first 30 s of illumination and only one-fourth of this (70 natom C·mg⁻¹ Chl) could serve as a donor of CO₂ from C₄ acid carboxylation (Fig. 2). Thus, there is still a big discrepancy between the amount of total carbon input from CO₂ fixation, the apparent carboxylation of C₄ acids and the amount of carbon accumulated in the measured RPP pathway intermediates. Furthermore, there was a big increase of 598 nmol·mg⁻¹ Chl or 1794 natom C·mg⁻¹ Chl in the level of three-carbon compounds of the C₄ cycle (PEP + pyruvate + alanine) during the first 30 s of illumination (Figs. 2 and 3). Thus, there must be carbon input into the C₄ cycle from an unknown source to account for the net rise in level of these three-carbon compounds (Figs. 2 and 3).

The possible contribution of internal CO₂ to the build-up of the intermediates of the RPP pathway during the initial phase of induction was assessed. The intercellular CO₂ concentration decreased by about 80 µL·L⁻¹ during the first 30 s of illumination (20). The Chl content and thickness of the leaves used in this study were 4.9 mg Chl·dm⁻² and roughly 150 µm, respectively. Then the calculated quantity of CO₂ used from the internal CO₂ pool is roughly only 1 nmol·mg⁻¹ Chl during the initial 30 s of illumination. Therefore, the contribution of internal CO₂ to the build-up of the intermediates of the RPP pathway appears negligible. Even considering that some internal CO₂ will be available in the aqueous phase in the cells in the form of bicarbonate, this source would also appear insignificant since the cytoplasm of the leaf occupies such a small part of the total internal volume of the leaf.

Hatch (8) reported that there was a substantial increase in pools of PEP, oxaloacetate, and PGA during induction in leaves of Chlora gayana, a C₄ carboxykinase type C₄ plant. However, little was known about the changes in levels of photosynthetic metabolites during induction in maize. Recently, Leegood and Furbank (13) observed a similar phenomenon as that described above in that the amount of carbon fixed during an initial phase of induction in maize leaves was less than the amount of carbon accumulated in the intermediates of the RPP pathway (RuBP not measured). They concluded that aspartate and alanine could partly contribute to the build-up of phosphorylated intermediates of the RPP pathway (13). However, initially there was a small increase in alanine concentration (13). Currently no measured metabolites can totally explain the build-up of the intermediates of the RPP pathway and the increase in the alanine levels during initial phase of induction. The peaks of alanine, DHAP, and RuBP and the increase in the levels of PGA during the initial phase of induction suggest that there could be carbon supply from some compound(s) which was not measured but highly related to these 3-carbon compounds, or from compound(s) which existed in other than the measured material (e.g. midrib). Recently, Leegood (12) found that malate, aspartate, and alanine were present in the nonphotosynthetic tissue of the midrib of maize leaves in large quantities. The sources of carbon for the nonautocatalytic build-up of the intermediates of the RPP pathway and also for the increase in the level of alanine will be examined in subsequent work.

In contrast to the present results, Leegood and Furbank (13) suggested that during induction, intermediates of the RPP pathway may be initially built up partly at the expense of the C₄ cycle intermediates or their precursors. This discrepancy might be due to a difference in the length of dark-pretreatment (i.e. 20 min in the present study and 15 h in theirs) and growth conditions (plants grown outdoors under full sunlight in the present study and under greenhouse conditions in their study), but the basis for this discrepancy remains to be elucidated.

If the C₄ cycle were closed, the total µmol of intermediates of the C₄ cycle should stay constant, even though µatom of C in intermediates may increase as a result of carboxylation. However, there were substantial changes in the level of intermediates in the C₄ cycle (Fig. 3). These results indicate that in the initial phase of induction (0 to 30 s) there is input of carbon into the C₄ cycle from an unknown source. During the latter stage of induction (5 to 20 min), there is a gradual increase in level of C₄ cycle intermediates (Fig. 3) which may be due to C input from CO₂ fixation in the RPP pathway. One possible way in which this may occur is by conversion of PGA of the RPP pathway to PEP of the C₄ cycle. Thus, during induction, net interconversions of carbon between the C₄ cycle and the RPP pathway in addition to carbon supply to the C₄ cycle and RPP pathway from unknown sources likely occurs prior to reaching a steady-state of CO₂ assimilation.

In summary, an ultimate build-up of both the intermediates of the C₄ cycle and the RPP pathway appears essential to attain a maximum rate of photosynthesis during the induction period. The source of carbon for the RPP pathway and the mechanism(s)
of interaction of the C4 cycle and the RPP pathway during induction remain to be investigated.

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