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Concurrent Measurements of Oxygen and Carbon Dioxide Exchange during Lightflecks in Maize (*Zea mays* L.)[1]

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Leaves of maize (*Zea mays* L.) were enclosed in a temperature-controlled cuvette under 35 Pa (350 μbars) CO_2 and 0.2 kPa (0.2%) O_2 and exposed to short periods (1–30 s) of illumination (lightflecks). The rate and total amount of CO_2 assimilated and O_2 evolved were measured. The O_2 evolution rate was taken as an indicator of the rate of photosynthetic noncyclic electron transport (NCET). In this C_4 species, the response of electron transport during the lightflecks qualitatively mimicked that of C_3 species previously tested, whereas the response of CO_2 assimilation differed. Under short-duration lightflecks at high photon flux density (PFD), the mean rate of O_2 evolution was greater than the steady-state rate of O_2 evolution under the same PFD due to a burst of O_2 evolution at the beginning of the lightfleck. This O_2 burst was taken as indicating a high level of NCET involved in the buildup of assimilatory charge via ATP, NADPH, and reduced or phosphorylated metabolites. However, as lightfleck duration decreased, the amount of CO_2 assimilated per unit time of the lightfleck (the mean rate of CO_2 assimilation) decreased. There was also a burst of CO_2 from the leaf at the beginning of low-PFD lightflecks that further reduced the assimilation during these lightflecks. The results are discussed in terms of the buildup of assimilatory charge through the synthesis of high-energy metabolites specific to C_4 metabolism. It is speculated that the inefficiency of carbon uptake during brief light transients in the C_4 species, relative to C_3 species, is due to the futile synthesis of C_4 cycle intermediates.

The relationship between the rate of O_2 evolution and the rate of CO_2 assimilation during light transients (lightflecks) has previously been described for the C_3 shade species *Alocasia macrorrhiza* (Kirschbaum and Pearcy, 1988) and for the high-light C_3 species sunflower (Laisk et al., 1992). In both species it was found that at saturating incident PFD the rate of NCET (as determined from O_2 evolution) was higher at the beginning of the lightfleck than during steady-state photosynthesis and was higher than required to support the contemporaneous rate of CO_2 assimilation during the lightfleck. The electron flow transiently in excess of that required for CO_2 assimilation was discussed in terms of energy storage in the form of reduced metabolites. The buildup of high-energy metabolites has been termed the assimilatory charge (Laisk et al., 1987; Pearcy, 1990) and described as analogous to the capacitance of an electrical system (Pearcy, 1990). The magnitude of the assimilatory charge, which is the capacity of the chloroplast to store light-generated chemical energy for later use, is dependent on the size of the substrate pools, which are reduced and/or energized by the photochemical energy generated by illumination.

In C_3 plants, the assimilatory charge is principally embodied in the buildup of ribulose 1,5-bisphosphate and triose-P pools during the light transient (Sharkey et al., 1986). These pools of high-energy RPP pathway metabolites can maintain CO_2 assimilation for a period after termination of illumination. In fact, the carbon gain due to short (1–10 s) lightflecks in the understory is often greater than would be predicted by multiplying the steady-state CO_2 assimilation rate by the summed illumination time (Pearcy, 1990).

Although species with the C_4 mechanism of photosynthetic CO_2 assimilation are only occasionally found in the understory (Pearcy and Calkin, 1983), lower leaves in canopies of C_4 crop species often receive intermittent illumination due to shading by the upper leaves. From consideration of the mechanism of C_4 photosynthesis, it seems likely that the photosynthetic behavior of C_4 species during and after transient illumination would be different from that of C_3 species.

In addition to the metabolite pools that may contribute to assimilatory charge in C_3 plants, C_4 species have others that are intrinsic to the CO_2-concentrating mechanism. The linkage between assimilatory charge and CO_2 assimilation is further complicated in C_4 species of the NADP-ME type such as maize because NCET, and the consequent production of reductant and ATP, is located primarily in the mesophyll cells (Leegood et al., 1983). The assimilatory charge must be transported to the site of the RPP pathway, which is confined to the bundle sheath tissue.

In NADP-ME type C_4 species, the principal metabolite for transfer of CO_2 and reducing potential from the mesophyll to the bundle sheath is malate. During steady-state C_4 photosynthesis, malate is transported from the mesophyll to the bundle sheath cells for oxidative decarboxylation by NADP-ME. The NADPH and released CO_2 are then available to the RPP pathway. During steady-state photosynthesis, the rate of malate production is linked to the rate of production of PGA, and its synthesis is controlled by the mesophyll enzymes PPDK, PEPC, and MDH.

Because the synthesis of malate from pyruvate requires

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Abbreviations: C_i, CO_2 concentration in the substomatal cavity of the leaf; MDH, malate dehydrogenase; NADP-ME, NADP-malic enzyme; NCET, noncyclic electron transport; PEP, phospho*enol*pyruvate; PEPC, phospho*enol*pyruvate carboxylase; PFD, photon flux density; PGA, 3-phosphoglycerate; PPDK, pyruvate-Pi dikinase; RPP, reductive pentose phosphate.

both ATP and NADPH, generation of malate from a pyruvate pool during a light transient would create a store of assimilatory charge in the mesophyll tissue. The reducing equivalents stored in malate would later be made available to the RPP pathway in the bundle sheath by the synthesis of NADPH in the conversion of malate to pyruvate by NADP-ME. The energy from the ATP used in the synthesis of malate from pyruvate in the mesophyll tissue would not be available to the RPP pathway but, rather, is the energy cost of the CO_2-concentrating mechanism.

Because the storage of assimilatory charge in C_4 species is spatially separate from its site of utilization and because of the energy cost of maintaining a high concentration of CO_2 in the bundle sheath, it seemed interesting to investigate whether short-duration light energy is managed as efficiently as in C_3 species.

MATERIALS AND METHODS

Plant Material

Zea mays L. (hybrid field corn supplied by Northrup King, Golden Valley, MN) was grown from seed in a controlled-environment growth chamber at a PFD of 500 μmol photons m^{-2} s^{-1} during a 16-h photoperiod. Day/night temperatures were 28/22°C. Plants were grown in potting soil (UC mix) amended with 20% pumice rock and were watered daily with half-strength modified Hoagland solution. All experiments were performed on the third emergent leaf when it had just ceased expansion.

Gas Exchange

Gas exchange measurements were performed as described by Kirschbaum and Pearcy (1988). Briefly, a section of the attached leaf approximately 15 cm from the tip was placed in a cuvette with the upper surface of the leaf sealed with vacuum grease to the upper water-jacketed glass lid of the cuvette. This improves the thermal stability of the leaf during light transients. Maize is amphistomatous, and gas exchange occurred through the lower surface of the leaf, which was in contact with the air stream. Leaf temperature was maintained at 25 ± 0.1°C under all conditions. The ambient CO_2 concentration was maintained at 35 Pa (except for C_i response measurements).

Leaf CO_2 uptake was measured with a differential IRGA (model 6250; Li-Cor, Lincoln, NE). O_2 evolution was measured with a zirconium oxide ceramic cell O_2 analyzer (model S-3A; Applied Electrochemistry, Inc., Sunnyvale, CA), as described by Björkman and Gauhl (1970). To ensure a satisfactory sensitivity and signal to noise ratio in the O_2 measurements, all experiments were conducted at 0.2 kPa O_2. It was separately determined that the response of CO_2 assimilation to lightflecks in maize is the same under either 21 or 0.2 kPa O_2. Calculation of gas exchange parameters was as described by von Caemmerer and Farquhar (1981).

Before lightfleck measurements were begun, the leaf was allowed to achieve steady-state photosynthesis under 21 kPa O_2 at the PFD of the experiment or, for PFD response experiments, at 300 μmol of photons m^{-2} s^{-1}. The O_2 concentration was then lowered to 0.2 kPa. Before a lightfleck was administered, the leaf was allowed to achieve steady-state photosynthesis at the PFD of the lightfleck. Thirty seconds before and for 60 s after the lightfleck the PFD was lowered to 70 μmol of photons m^{-2} s^{-1}, which approximates the illumination on a shaded leaf.

All data were collected on a data acquisition system (model HP3497A; Hewlett-Packard, Palo Alto, CA) every few minutes during steady state and five times per s during lightfleck measurements. The total amount of O_2 evolved or CO_2 assimilated due to a lightfleck was determined from the area under the curve of the gas exchange rate versus time above a baseline given by the steady-state rate in low PFD. From this, the mean rates of CO_2 or O_2 exchange were calculated by dividing the total amount of gas exchanged by the duration of the lightfleck.

RESULTS

For comparison with the lightfleck data presented below, Figure 1 shows the steady-state responses of a typical maize leaf to PFD and to C_i. There was no significant difference between the O_2 and CO_2 exchange rates at any PFD or C_i. By contrast, O_2 and CO_2 exchange rates during lightflecks differed both transiently and in the integrated totals occurring due to the lightflecks (Fig. 2). At the lowest lightfleck PFD, the rate of O_2 evolution (Fig. 2A) increased almost instantaneously to a value that then remained constant during the remainder of the lightfleck. When the leaf was exposed to higher PFD lightflecks, the rate of O_2 evolution exhibited an initial overshoot, lasting for 6 to 8 s, before arriving at the subsequent steady-state rate. At subsaturating PFDs the magnitude of both the overshoot and the steady-state rate were proportional to the lightfleck PFD. At those PFDs saturating

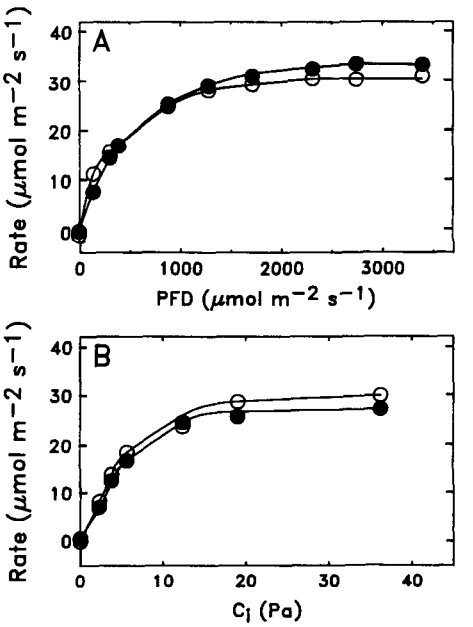

Figure 1. Responses of O_2 evolution (●) and CO_2 assimilation (○) in a maize leaf to PFD at an ambient CO_2 concentration of 35 Pa (A) and to C_i at a PFD of 1600 μmol of photons m^{-2} s^{-1} (B).

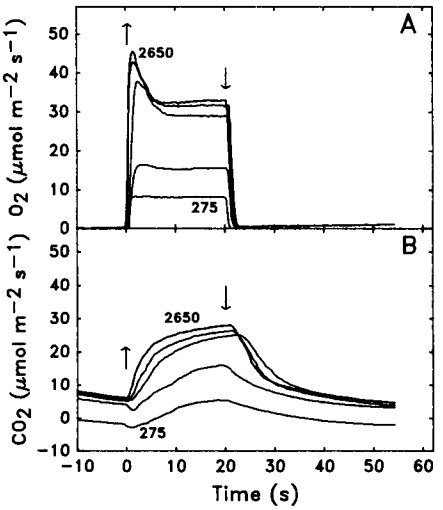

Figure 2. Simultaneous time course of O_2 evolution (A) and CO_2 assimilation (B) occurring in response to 20-s lightflecks at different PFDs (275, 430, 1300, 1800, and 2650 μmol of photons m^{-2} s^{-1}). Results for both O_2 and CO_2 are from one leaf that was typical of a series of replicate determinations with other leaves.

for CO_2 assimilation the magnitudes of both the initial overshoot and the steady-state rate of O_2 evolution present later in the lightfleck were independent of the PFD during the lightfleck.

At all times during the lightfleck and at any PFD, the rate of O_2 evolution was greater than the contemporaneous rate of CO_2 assimilation (Fig. 2B). This was especially true early in the lightflecks because the CO_2 assimilation rate increased more slowly than the O_2 evolution rate. In fact, at the two lowest PFDs there was a CO_2 burst at the beginning of the lightfleck, evidenced by a decrease in the measured assimilation rate, which is discussed below. Toward the end of the 20-s lightfleck, when CO_2 assimilation approached equilibrium and O_2 evolution was at equilibrium, the CO_2 assimilation rate was usually about 85% of that of the rate of O_2 evolution. At the lowest PFD, however, the rate of CO_2 assimilation never exceeded 65% of the rate of O_2 evolution.

CO_2 assimilation persisted at a rate elevated above the steady-state rate for the background PFD for approximately 30 s after low PFD lightflecks and for more than 60 s after high-PFD lightflecks. Following low-PFD lightflecks, the CO_2 assimilation rate began to decrease 2 s after termination of the light transient. However, after high-PFD lightflecks, the measured rate of CO_2 assimilation continued to increase for 1 to 2 s after the lightfleck was terminated. This was not an artifact of a time lag caused by the movement of gas from the leaf chamber to the CO_2 analyzer because such lags were compensated for in the calculations.

The relationship between the mean rate of O_2 evolution during a lightfleck and the lightfleck duration varied with the lightfleck PFD (Fig. 3A). At low PFD the mean O_2 evolution rate was independent of lightfleck duration, reflecting the constant O_2 evolution rates throughout the lightfleck (Fig. 2A). These rates were equal to the steady-state rate of O_2 evolution for a given leaf at that PFD. Because of the overshoot in O_2 evolution during high-PFD lightflecks, mean O_2 evolution rates increased as the lightfleck duration decreased. For 1-s lightflecks at the highest PFD, the mean O_2 evolution rate was 30% higher than the steady-state rate because all of the O_2 evolution occurred during the initial overshoot.

However, the mean rate of CO_2 assimilation became lower as lightfleck duration decreased (Fig. 3B), the opposite of the response seen for O_2 evolution. Proportionally, the decrease in the mean CO_2 assimilation rate was greatest with low-PFD lightflecks. At the lowest PFDs, the amount of CO_2 fixed during these short-duration lightflecks was depressed because of the CO_2 burst at the beginning of the lightfleck (Fig. 2B). This abrupt decrease in the measured assimilation rate at the onset of the lightfleck was not due to a depletion of the intercellular CO_2. In fact, C_i increased during this period and was always in excess of saturating values (not shown). The CO_2 burst was most apparent during lightflecks of 5 s or less and was seen only during low-PFD lightflecks. When the PFD during the lightfleck was greater than about 500 μmol of photons m^{-2} s^{-1}, the CO_2 assimilation rate increased immediately upon illumination.

The ratio of CO_2 assimilated to O_2 evolved gives the proportion of the NCET (seen as O_2 evolution) that resulted in net CO_2 uptake by the leaf (Fig. 4). Because the oxidation of 2 H_2O (producing 1 O_2) is required for reduction of 1 CO_2 in either C_3 or C_4 photosynthetic types, a ratio of 1 would mean that all NCET was dedicated to CO_2 assimilation.

In contrast to steady-state conditions, in which apparently all of NCET is devoted to CO_2 fixation (Fig. 1), the ratio of CO_2 to O_2 exchange during lightflecks was considerably less than 1. The ratio was highest at high-PFD lightflecks and for long-duration lightflecks but was still only 0.7. Thus, 30% of

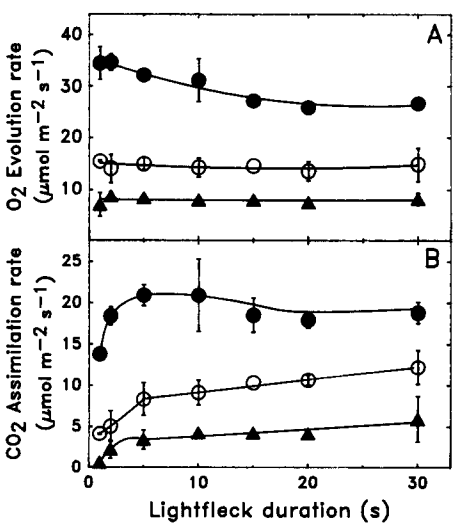

Figure 3. Mean rates of O_2 evolution (A) and CO_2 assimilation (B) due to lightflecks at 275 (▲), 430 (○), and 1800 (●) μmol of photons m^{-2} s^{-1} as a function of lightfleck duration. The mean rates were calculated by dividing the total O_2 evolved or CO_2 assimilated due to a lightfleck by the duration of the lightfleck. Each lightfleck duration series is the mean of three replicate experiments. The error bars indicate ± 1 SD.

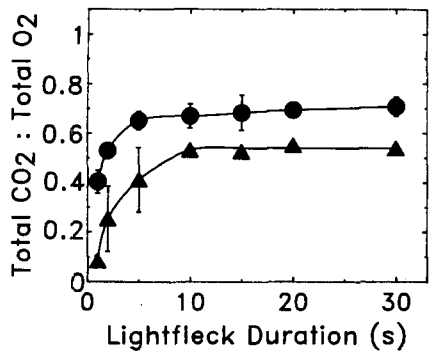

Figure 4. The ratio of the total amount of CO_2 assimilated to the total amount of O_2 evolved due to lightflecks of various durations at 275 (▲) and 1800 (●) μmol of photons m^{-2} s^{-1}. The data are the means of three replicate runs at each PFD and are derived from the same data used to derive the data depicted in Figure 3. The error bars represent ± 1 SD.

NCET went to reactions other than net CO_2 fixation under these conditions. As lightfleck duration decreased to 1 s, the ratio decreased to 0.4 for high-PFD lightflecks; for low-PFD lightflecks the ratio decreased from 0.5 to 0.1 as lightfleck duration decreased. Thus, for a 1-s low-PFD lightfleck, only 10% of the NCET went to CO_2 fixation.

In Figure 5 the mean rates of O_2 evolution and CO_2 assimilation during 2-s lightflecks are compared to the steady-state assimilation rate as a function of PFD. The depression in mean CO_2 assimilation rates during lightflecks as compared to steady-state rates was apparent over the entire range of PFDs. At low PFDs the mean O_2 evolution rates during the 2-s lightflecks were essentially equal to the steady-state rates of CO_2 assimilation. These rates were, in turn, equal to the steady-state rates of O_2 evolution (not shown). As PFD increased, the mean O_2 evolution rate during the lightfleck became increasingly greater than the steady-state CO_2 assimilation rate. For the leaf whose response is shown in Figure 5, the mean rate of O_2 evolution was 40% greater than the steady-state rate of CO_2 assimilation and more than 65% greater than the mean rate of CO_2 assimilation during the lightfleck, at the maximum PFD tested.

DISCUSSION

NCET

The response of photosynthetic electron transport, as measured by O_2 evolution (Fig. 2A), was qualitatively identical with that seen in the C_3 forest understory species *A. macrorrhiza* (Kirschbaum and Pearcy, 1988) and in sunflower (Laisk et al., 1992). In both the C_3 species and in maize there was an initial overshoot of electron flow above the eventual rate achieved in the lightfleck when the lightfleck level approached or was saturating. Because of this initial burst, the mean O_2 evolution rate increased significantly as lightfleck duration decreased. In C_3 species the overshoot has been hypothesized to be due primarily to the rapid reduction of RPP pathway metabolite pools, especially conversion of PGA to triose-P (Kirschbaum and Pearcy, 1988). Additionally, reduction of plastoquinone may contribute to the size of the burst (Laisk et al., 1992). The triose-P is available for subsequent use in CO_2 assimilation, by the RPP pathway, in the light or in a subsequent dark period. Also, the phosphorylation of ribulose-5-P provides a sink for the ATP generated by the transport of electrons through the plastoquinone pool.

In NADP-ME-type C_4 plants such as maize, virtually all of the NCET occurs in the mesophyll chloroplasts, but PGA, the primary electron acceptor, is generated in the bundle sheath cell chloroplasts. The transfer of light-generated reducing potential from the mesophyll to the bundle sheath PGA pool is mediated in two ways. Malate is shuttled from the mesophyll to the bundle sheath cells, where it is oxidatively decarboxylated to pyruvate, producing NADPH. PGA also diffuses out to the mesophyll for reduction to triose-P, which diffuses back into the bundle sheath tissue and reenters the RPP pathway. The overshoot in electron transport in maize mesophyll chloroplasts could be due either to the rapid synthesis of malate from pyruvate (which would require both NADPH and ATP) or to the reduction of the imported PGA (requiring NADPH only). Also, under physiological conditions in which there is an adequate concentration of NADPH, the conversion of aspartate to malate in the mesophyll cells (Shieh et al., 1982), via oxaloacetate generated by aspartate aminotransferase, can be a sink for NCET. In a study by Usuda (1985) of maize, the aspartate pool size decreased rapidly at the onset of illumination; during the same period PEP concentrations increased because of the phosphorylation of pyruvate. The conversion of PEP from pyruvate requires two ATP, which are the product of NCET.

In our experiments, the leaf was shaded for only 30 s before reillumination by the lightfleck. The CO_2 assimilation, occurring in response to reillumination, therefore, corresponds to the initial "fast" phase of the biphasic induction in C_4 plants (Leegood and Furbank, 1984; Furbank and Walker, 1985). During this fast phase the rate of CO_2 assimilation is dependent on the availability of extant C_4 cycle intermediates.

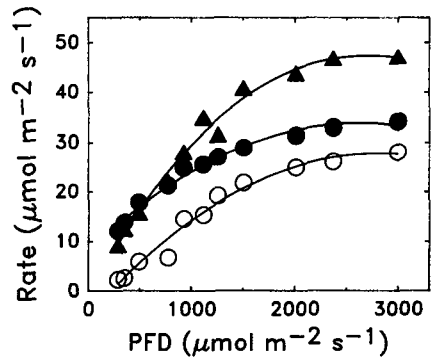

Figure 5. PFD response of net steady-state CO_2 assimilation (●) and of the mean rates of CO_2 assimilation (○) and O_2 evolution (▲) due to 2-s lightflecks at the various PFDs. The mean rate of CO_2 assimilation or O_2 evolution was calculated from the total amount of CO_2 assimilated or O_2 evolved due to a 2-s lightfleck divided by the duration of the lightfleck. The results are from the same leaf and are typical of several replicate measurements on different leaves.

Furbank and Walker (1985) interpreted their results as indicating that after a 4-min dark period there is still a high level of C_4 intermediates compared to totally dark-adapted leaves. PPDK (Yamamoto et al., 1974; Nakamoto and Edwards, 1983), PEPC (Karabourniotis et al., 1983), and MDH (Johnson and Hatch, 1970) would still be appreciably activated after the 30-s shade period, and a significant portion of the *trans*-thylakoid pH gradient would persist (Demmig and Winter, 1988). Because pyruvate levels in maize increase dramatically when an illuminated maize leaf is darkened (Leegood and Furbank, 1984), it is reasonable that after a 30-s shade period there would be sufficient pyruvate and sufficient PPDK, PEPC, and MDH activity to allow a pyruvate pool to be rapidly metabolized to PEP and then to malate utilizing the products (ATP and NADPH) of NCET.

CO_2 Assimilation

The most striking difference between the response of maize to lightflecks and that of C_3 species reported earlier was in the response of CO_2 assimilation. In *Alocasia* (Kirschbaum and Pearcy, 1988), soybean (Pons and Pearcy, 1992), and various tropical species (Chazdon and Pearcy, 1986), the mean rate of CO_2 assimilation increased as lightfleck duration decreased; in maize it decreased (Fig. 3B).

As with C_3 species, sufficient assimilatory charge was stored during lightflecks in maize to allow net CO_2 assimilation to continue at a significant rate for about 30 s after the lightfleck (Fig. 2B). At all PFDs, the relationship between lightfleck duration and the amount of CO_2 assimilated increased in an approximately linear fashion with increased lightfleck duration (not shown). However, with lightflecks shorter than 10 s, the efficiency of incorporation of light-induced electron flow into fixed carbon decreased as lightfleck duration decreased. Therefore, for these short lightflecks the mean rate of CO_2 assimilation decreased as the lightfleck duration decreased at both high and low PFD. The proportional decrease was greater at lower PFD (Fig. 3B).

The low mean rate of CO_2 assimilation during low-PFD lightflecks can be accounted for by the CO_2 burst observed at the beginning of these lightflecks (Fig. 2B). The CO_2 burst results in a net loss of carbon from the leaf and is most plausibly explained as a more rapid release of CO_2 in the bundle sheath by NADP-ME than can be immediately accommodated by the RPP pathway. An incapacity of the RPP pathway could result from limited reducing potential in the bundle sheath, which is likely the case after a period of low PFD. The assimilation of one CO_2 via ribulose 1,5-bisphosphate carboxylase generates two PGA, which require four reducing equivalents (two NADPH) for reduction to triose-P; this reduction is essential for the continued cycling of the RPP pathway.

Decarboxylation of malate generates only one NADPH or one-half of the equivalents required. The other two must be imported from the mesophyll via a PGA-triose-P exchange, which may not be operating maximally at the very beginning of the lightfleck. If, momentarily at the beginning of a lightfleck, the supply of reductant to the RPP pathway is restricted to that produced by the NADP-ME reaction, only one-half of the CO_2 produced by decarboxylation can be assimilated. At high PFD, the decline in the mean CO_2 assimilation rate for short-duration lightflecks was not accompanied by a noticeable CO_2 burst. It may be that the burst was masked by the greater volumes of assimilated CO_2. This decline in the mean assimilation rate may, nevertheless, indicate inadequate assimilatory capacity of the bundle sheath tissue when the light transient is of short duration.

The inefficiency of CO_2 assimilation during brief light transients was independent of PFD (Fig. 5). For 2-s lightflecks the mean rate of CO_2 assimilation was lower than the steady-state rate over the range of PFD. This is in contrast to the response of the C_3 species *Alocasia* (Kirschbaum and Pearcy, 1988), soybean (Pons and Pearcy, 1992), and others (Chazdon and Pearcy, 1986). In these species the efficiency of light utilization for CO_2 assimilation during short lightflecks (the lightfleck utilization efficiency [Pearcy, 1990]) became progressively greater than the steady-state rate as PFD increased.

Relationship between NCET and CO_2 Assimilation

The ratio of CO_2 assimilated to O_2 evolved during steady-state photosynthesis was approximately 1.0 at all PFDs (Fig. 1), whereas it never exceeded 0.7 during lightflecks and was much less for short lightflecks (Fig. 4). This seems to indicate that, despite the high rate of production of assimilatory charge at the beginning of the lightflecks, if the lightfleck is of short duration, much of the stored energy cannot be used for CO_2 fixation. Apparently, in maize the coordinate metabolic processes necessary for utilization of electron transport products for CO_2 assimilation operate efficiently only under protracted illumination. The much higher mean rate of O_2 evolution relative to CO_2 assimilation, seen especially during short-duration lightflecks at low PFD, implies a large dissipation of reducing potential, perhaps through the futile synthesis and decarboxylation of malate.

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