Postillumination Burst of Carbon Dioxide in Crassulacean Acid Metabolism Plants

CLIFTON E. CREWS, H. MAX VINES, AND CLANTON C. BLACK, JR.
Departments of Horticulture and of Botany, University of Georgia, Athens, Georgia 30602

ABSTRACT

Immediately following exposure to light, a postillumination burst of CO_{2} has been detected in Crassulaceae acid metabolism plants. A detailed study with pineapple (Ananas comosus) leaves indicates that the postillumination burst changes its amplitude and kinetics during the course of a day. In air, the postillumination burst in pineapple leaves generally is exhibited as two peaks. The postillumination burst is sensitive to atmospheric CO_{2} and O_{2} concentrations as well as to the light intensity under which plants are grown. We propose that the CO_{2} released in the first postillumination burst peak is indicative of photorespiration since it is sensitive to either O_{2} or CO_{2} concentration while the second CO_{2} evolution peak is likely due to decarboxylation of organic acids involved in Crassulacean acid metabolism.

In marked contrast to other higher plants, the postillumination burst in Crassulaceae acid metabolism plants can be equal to or greater than the rate of photosynthesis. Photosynthesis in pineapple leaves also varies throughout a day. Both photosynthesis and the postillumination burst have a daily variation which apparently is a complex function of degree of leaf acidity, growth light intensity, ambient gas phase, and the time a plant has been exposed to a given gas.

In leaves of some higher plants, dark respiration immediately following illumination is demonstrated as an excessive eff lux of CO_{2} which may last for several min before steady state dark respiration is attained. This initial burst of CO_{2} has been termed the PIB. The existence of an excessive efflux, or burst of CO_{2}, immediately following illumination was initially reported by Decker in 1955 (9). He reasoned that this phenomenon, which he called a “CO_{2} outburst,” was a product of light respiration. Hence, he interpreted his data as showing the existence of photorespiration which is not the same as dark respiration. Later it was reported that the PIB was photostimulated by increasing the light intensity of the prior illumination period (10, 29). Tregunna et al. (30) found that in tobacco leaves the initial PIB substrate was a recent product of photosynthesis. They also observed that increasing increments of light intensity from 100 to 1500 ft-c increased the initial dark CO_{2} production in green soybean and pepperomia leaves but inhibited it in green corn leaves. From these data, they projected a relationship among light, the PIB, and the photosynthetic mechanisms of leaves.

The mechanisms involved in the PIB have been studied by Björkman (3) who proposed that a light inhibition of glycolysis is released immediately upon darkening, resulting in a surge of oxidation that yields an excess quantity of CO_{2}. The discovery of the C_{4} pathway (14, 19) in species such as corn and sugarcane and the apparent absence of photorespiration in these species initially suggested that the PIB and photorespiration would be absent in C_{4} plants. Indeed corn did not demonstrate a PIB (30). However, these postulations were altered by reports of PIBs in Amaranthus edulis and Atriplex rosea (3, 4) as well as reports on the enzymes of photorespiration in other C_{4} plants (5, 28). With C_{4} species the PIB was not detected with low O_{2}; in contrast to reports with C_{3} species. Downton (12) correlated the presence or absence of the PIB among C_{4} plants with the presence of a major photosynthesis product, noting that plants which exhibit the PIB initially produce aspartate as their major C_{4} acid while plants exhibiting no PIB produce malate as their major C_{4} acid. NADPH is the redundant for continued fixation of CO_{2} into the C_{4} cycle; so Downton reasoned that “malate formers” produce both CO_{2} and NADPH in the dark such that CO_{2} can continue to be fixed via the C_{4} cycle, assuming ATP is available. Conversely, the dark decarboxylation of aspartate, via then unknown enzymes, in other C_{4} plants could release CO_{2} without producing NADPH. So in darkness the CO_{2} presumably could not be fixed by the C_{4} cycle, therefore, the PIB was exhibited by “aspartate formers.” However, that theory seems no longer tenable with the demonstration of an NAD, malic enzyme in Amaranthus and Atriplex which could form NADH and CO_{2} in the dark (13).

We have been concerned with the net assimilation of CO_{2} by higher plants. CAM plants may be broadly characterized as assimilating major quantities of CO_{2} at night in contrast to C_{3} and C_{4} plants which primarily assimilate CO_{2} during the day. Because we knew that CAM plants were quite sensitive to their environments (26) and that environmentally one could change the pathway of CO_{2} assimilation (2, 5), we began a systematic study of CO_{2} metabolism with CAM plants grown in controlled environments. We found a pronounced PIB in CAM plant leaves. Previous data on a PIB in CAM plants could not be located, so a systematic, comparative physiological study was initiated to determine the effects of O_{2}, CO_{2}, and growth light intensity on the PIB in CAM plants. We present here, some of these physiological characteristics of the PIB in CAM plants.

1 This research supported in part by a Dawson Postdoctoral Fellowship and by National Science Foundation Grant GB-20661.

2 Abbreviations: PIB: postillumination CO_{2} burst, reported in mg of CO_{2} dm^{-2} of leaf surface hr^{-1}; CAM: Crassulacean acid metabolism; PEP: phosphoenolpyruvate; C_{4}: C_{4}-dicarboxylic acid; C_{3}: reductive pentose phosphate; RuDP: ribulose 1.5-diphosphate; OAA: oxalacetate.
MATERIALS AND METHODS

Growth of Plant Material. Pineapples (Ananas comosus), obtained from a local grocery, were rooted and grown following a procedure obtained from Dr. Duane Bartholomew at the University of Hawaii. Plants were grown in a greenhouse with day and night temperatures of 27°C and 21°C, respectively. Due to the sensitive nature of CAM plants to environmental changes (2), they were maintained in growth chambers at least four weeks prior to an investigation. Air was rapidly circulated in the growth chamber while the temperature, photoperiod, and light intensity were regulated. The growth chamber temperature was maintained at 30°C day and 20°C night while light intensities of 2000, 5000, and 7500 ft·c were obtained by placing plants at different heights in the same growth chamber. Plants were exposed to a 12-hour light-dark cycle.

Leaf CO₂ Exchange Chamber. The leaf CO₂ exchange chamber was designed to utilize the air seal technique proposed by Wolf et al. (33). Minimum chamber volume is essential in measuring the PIB; therefore, the Plexiglas chamber was contoured to the shape of the typical pineapple “D” leaf (Fig. 1). Treatment gases were bubbled through water before entering the chamber at a flow rate of 0.5 l/min. The chamber air turnover rate was 18 times per min. CO₂ measurements were obtained by differential analysis of intake and exhaust gases with a Beckman model 215B infrared gas analyzer and recorded on a Sargent model SRG recorder. The instrument response time of our experimental apparatus was 5 to 7 sec.

Leaf temperature was maintained between 27°C to 29°C and was monitored with a Yellow Springs Instrument thermistor. A schematic of the CO₂ analysis system is shown in Figure 1. Leaf surface area was measured using a Hayashi Denko automatic leaf area meter.

Measurement Procedures. Measurements of photosynthesis and the PIB were made at 2, 21, and 99% O₂, using compressed air and commercial gas mixtures of O₂, N₂, and CO₂. The 2, 21, and 99% O₂ mixtures contained 324 μl/l, 338 μl/l, and 320 μl/l of CO₂, respectively. To examine a higher CO₂ concentration, a mixture of 21% O₂ and 934 μl/l CO₂ was purchased. Light intensity of 5000 ft·c was maintained in the leaf CO₂ exchange chamber for all of the PIB measurements.

When exchange was measured, plants were removed from growth chambers at 0800 AM and relocated in the darkened experimental setup. To allow a plant sufficient time to equilibrate to its new environment, leaves were immediately placed in the leaf CO₂ exchange chambers at the prescribed O₂ and CO₂ levels. Illuminations normally began at 0900 AM and, in studies such as those reported in Figure 4 or 6, measurements were made approximately every 30 min throughout the day and continued until the PIB was not detectable. Each treatment was replicated a minimum of four times.

RESULTS

In preliminary studies we measured the daily CO₂ exchange pattern and titratable acidity of intact CAM leaves. The results of such a study with pineapple leaves are shown in Figure 2. Growth light intensity did not affect the diurnal fluctuations of titratable acidity. However, some differences were observed in the CO₂ uptake patterns. Growth light intensities of 5000 and 7500 ft·c increased CO₂ assimilation much earlier than 2000 ft·c during initial day and night periods. It should be noted that we consistently observed CO₂ uptake in the light with CAM leaves particularly in the latter half of the photoperiod (Fig. 2) so that broadly characterizing CAM CO₂ uptake as occurring during the night is correct; but a substantial CO₂ uptake also may occur during the day.

In the course of these daily CO₂ uptake studies, we consistently noted a PIB (Fig. 3) of unusual shape and kinetics when the normal night period began. For discussion we divided this PIB into the primary CO₂ release peak and the secondary peak (Fig. 3).

Other CAM plants, including Kalanchoe daigremontiana, K. pinata, K. tubiflora, Sedum telephodes, and Crassula argentea, showed titratable acidity and CO₂ gas exchange patterns similar to those of pineapple in Figure 2. A definite PIB also
found inconsistencies when comparing PIB amplitudes and kinetics in CAM plants. In order to sort out these inconsistencies, our work focused on the PIB of pineapple.

The general character of the PIB in pineapple leaves throughout a day in a variety of atmospheres is depicted by the recorder traces in Figure 4. After darkening, lag periods of up to 45 sec were observed prior to the first efflux of CO₂ (Fig. 3, 4). Therefore, the kinetic responses of CAM PIBs are much slower than those reported for other higher plants (3, 7-10). In air, the primary PIB occurred about 2 min after darkening, followed by the greatly reduced secondary burst 2 to 4 min later. As we began to realize that the PIB also changed throughout a day (Fig. 4), we made random checks in other experiments on 24 hr CO₂ exchange, and results such as those in Figure 4 were reproducible at any given time in a light cycle. These preliminary investigations indicated a PIB in CAM plants that differed from the PIB in other higher plants. Therefore, special emphasis was placed on the effects of O₂, CO₂, and growth-light intensity on the PIB in pineapple leaves.

O₂ Concentration and PIB. The recorder traces in Figure 4 illustrate the effects of ambient O₂ on both PIB amplitudes and kinetics with pineapples grown under the captioned light intensities. Clearly the PIB is a complex function of time in the photoperiod, the light intensity under which plants were cultured, the ambient O₂ concentration, and the length of time in a day the leaves were exposed to a given O₂ concentration (Fig. 4).

At all growth light intensities the PIBs observed under 2% O₂ peaked and disappeared earlier in the illumination period than the PIBs produced with 21% O₂. Additionally, PIB kinetics and amplitudes were greatly altered and/or reduced by 2% O₂ when compared to 21% O₂. However, such alterations by 2% O₂ usually did not occur until 3 to 5 hr into the illumination period, with PIBs completely diminishing after 7 to 8 hr of light. The response time and amplitude of individual PIBs produced under 2% O₂ was dependent upon the growth light intensity. PIBs under 2% O₂ observed with plants grown under 2000 ft-c of light required 5 to 6 min after darkening before obtaining maximum amplitudes. In contrast, maximum PIB amplitudes at 2% O₂ from plants grown at 5000 and 7500 ft-c of light occurred within the initial 4 min of darkness (Fig. 4).

PIBs produced under 21% O₂ did not begin to dissipate until late in the illumination period, with nearly complete disappearance occurring only after 10 to 11 hr of illumination. The recorder traces also indicate the PIBs under air changed their shape and amplitude throughout a day. The response time of PIBs under 21% O₂ at all growth light intensities, was much faster than under 2%, requiring only 1 to 2 min after darkening to obtain maximum amplitudes (Fig. 4).

An increase in O₂ concentration to 99% initially reduced PIBs in plants grown under 5000 ft-c of light. Five to 7 hr of light was required for PIB amplitudes under 99% O₂ to exceed those produced in 2% or 21% O₂. The PIB under 99% O₂ diminished only late in the light period. Maximum PIB amplitudes under 99% O₂ were larger than those under 2% or 21% O₂-reaching a maximum rate of 8.5 mg of CO₂ evolved dm⁻² hr⁻¹ (Fig. 4).

Growth Light Intensity and PIB. Increases in the light intensity used to grow plants produced an increase in the maximum amplitude of the PIB (Fig. 5). With plants grown at 200 ft-c, the maximum PIB was only 0.2 mg of CO₂ dm⁻² hr⁻¹. Growth regimes of 2000 ft-c or greater produced PIBs with maximum amplitudes in excess of 5 mg of CO₂ dm⁻² hr⁻¹. The maximum PIB in air, as depicted in Figure 5, was dependent upon growth light intensity between 200 and 5000 ft-c, but independent of intensity above about 2000 ft-c.

CO₂ Concentration and PIB. An increase in CO₂ concentration to 934 µl/l initially reduced the PIB of plants grown at both 2000 and 5000 ft-c (only Fig. 6 gives the 5000 ft-c data). The maximum PIBs under 934 µl/l CO₂ were observed approximately 6 hr into the light period. The shapes and amplitudes of PIBs under 934 µl/l CO₂ and 2% O₂ were similar to those obtained with 320 µl/l CO₂ and 2% O₂ (shown in Fig. 7).

Influence of O₂ and CO₂ Concentration on CO₂ Assimilation. In pineapple, light and dark CO₂ assimilation under air was approximately equal with plants from all light growth regimes (Fig. 2). During the initial 4 hr of illumination CO₂ uptake in pineapple leaves grown at 2000 ft-c was greatly reduced. Higher light growth regimes of 5000 and 7500 ft-c resulted in slightly more CO₂ uptake during the initial light period. Maximum CO₂ uptake occurred about 8 hr into the light period at all light growth intensities. Immediately after illumination, CO₂ uptake at all growth light intensities decreased for 1 to 2 hr followed by an increased dark CO₂ uptake. The maximum dark CO₂ uptake occurred 8 to 10 hr after illumination and then declined (Fig. 2). At growth light intensities of 2000 and 7500 ft-c, CO₂ uptake under 2% and 21% O₂ was approximately equal throughout the day (Fig. 4). However, in pineapple leaves grown at 5000 ft-c of light, 2% O₂ did increase CO₂ uptake after 3 to 5 hr of light. In contrast, 99% O₂ greatly reduced CO₂ uptake for 8 hr into the light period followed by a rapid increase in CO₂ uptake.

At a light growth intensity of 5000 ft-c, CO₂ uptake under both 338 µl/l or 934 µl/l CO₂ in 21% O₂ was approximately equal for 6 to 7 hr into the light period. Shortly thereafter, a rapid increase in CO₂ uptake was observed under 934 µl/l CO₂ (Fig. 6).

DISCUSSION

If it is assumed that CO₂ metabolism in CAM plants proceeds via photosynthetic and respiratory pathways outlined earlier (5), then the PIB in CAM plants has some unique features when compared to the PIB in C₃ or C₄ plants (3, 7-9, 12, 29-31). First, to our knowledge, the observation of a changing PIB throughout a day (Fig. 4) is observed only with CAM plants. Not only does the amplitude of the PIB in air change during a day but the kinetics changes markedly (Fig. 4). Second, the absolute rate of the PIB can be equal to or much greater than the rate of leaf photosynthesis (Figs. 2, 4, 6, and 7). This is in marked contrast to C₃ and C₄ plants where the
FIG. 4. Recorder tracings showing daily changes in PIB kinetics and amplitudes at various O₂ concentrations with pineapples grown at the captioned light intensities. Numerals above each PIB trace represent the time of day. Horizontal lines of dots are time in minutes while each vertical dash is a mg of CO₂ cm⁻² leaf area hr⁻¹ release (below zero) or uptake (above zero). Similar units also are used in Fig. 7.
maximum rate of the PIB is only 10 to 20% of the rate of photosynthesis (7, 9, 12). Third, in experiments with C₃ and C₄ plant leaves, as gases in the atmosphere of a leaf chamber are switched (3, 7), the rates of leaf CO₂ exchange will change very rapidly (under 1 min) to a new steady state and changes occur in the subsequent PIB. With CAM leaves rapid switching (with seconds or minutes intervals) of O₂ or CO₂, or both, in a leaf chamber have almost no immediate effect on photosynthesis or the PIB. However, if CAM leaves are maintained for hours in a given gas, then both the PIB and the rate of photosynthesis will respond to O₂ and CO₂ (Figs. 4, 6, and 7). In general, gas exchange studies with CAM leaves require much longer to reach “steady state,” if such a condition ever occurs. In addition the maximum amplitude of the PIB in CAM leaves in air requires 2 to 3 min to peak (Fig. 4) in contrast to the PIB in C₃ and C₄ plants which usually peaks in less than 1 min (3, 7, 9, 12).

In C₃ and C₄ plants there is no daily change in titratable acidity (5). With CAM plants in air the rapid loss of titratable acidity early in the day, presumably via decarboxylation of organic acids, occurs concurrently with a rapid amplification of the PIB amplitude (Fig. 2). The increased amplitude of the PIB probably results from PEP carboxykinase activity in pineapple (11). The PIB continued to be observed during periods of stable titratable acidity in air, but the amplitude decreased markedly (Fig. 2). The PIB also was associated with an increased growth light intensity (Fig. 2, 5) which can be explained as a result of increased photosynthesis at higher growth light intensities via the glycolate pathway. As previously reported (10, 29), an increase in light intensity increased photosynthesis through an increased production of glycolate which should occur in plants grown at higher light intensities. Bowes et al. (6) have reported that glycolate can be synthesized through an O₂-dependent cleavage of RuDP to phosphoglycolate followed by hydrolysis with phosphoglycolate phosphatase (1, 23) to form glycolate. In addition, high light intensities tend to make the chloroplast stroma and cells more alkaline, which may favor oxygenation rather than carboxylation of RuDP (1, 22).

We postulate that the major loss of CO₂ through the PIB in CAM plants involves two decarboxylations. Two peaks are clearly evident in ambient air (Figs. 3, 4, and 7), but either decreasing O₂ or increasing CO₂ (Figs. 4 and 7) can eliminate the primary peak without greatly affecting the secondary peak. We postulate that during the primary PIB and CAM plants, photorespiration via the glycolate pathway (27, 28) contribute most of the CO₂. Under low O₂, the degree of CO₂ competing with CO₂ in the carboxylation of RuDP would be limited; therefore, less RuDP would be used to form phosphoglycolate (21) which would furnish the CO₂ released in the primary PIB peak. A suppression of the primary PIB peak with a CO₂ concentration of 934 µl/l also supports the same postulation (Fig. 7).

We postulate that most of the CO₂ released in the secondary peak is not due to photorespiration or dark respiration; rather it is a result of PEP carboxykinase activity. The persistent secondary peak under 21% O₂ (Figs. 4 and 7), low ambient O₂ (Figs. 4 and 7), and high CO₂ (Fig. 7) connotes another mechanism, because neither O₂ nor CO₂ concentration greatly influenced the secondary peak when compared to air (Fig. 7). Dittrich et al. (11) have reported that PEP carboxykinase is the major decarboxylating enzyme in pineapple leaves. Malate, the major organic acid in CAM, must be converted to OAA by malic dehydrogenase prior to decarboxylation by PEP carboxykinase to form PEP + CO₂ + ADP. The released CO₂ presumably is reassimilated during periods of illumination (5); however, upon darkening, photosynthetic NADPH may become a limiting factor, reducing the amount of CO₂ fixed, thereby allowing an efflux of CO₂. The dark decarboxylation of OAA may account for most of the CO₂ loss during the transient secondary PIB in pineapple leaves. Presumably this
CO₂ loss ceases in the dark, because ATP synthesis also is reduced. The lack of great influence of O₂ or CO₂ concentration on the secondary peak (Fig. 7) also is consistent with this theory for PEP carboxykinase would not likely be influenced by such changes in O₂ or CO₂ concentration. Secondary CO₂ bursts and even other dark CO₂ peaks have been reported in *Panicum burchii* (7) and tobacco (15) and in other plants, but these transient CO₂ peaks have not been the subject of intensive research.

A study of the data on photosynthetic CO₂ assimilation (Figs. 2–4, 6, 7) makes it clear that photosynthesis varies throughout the photoperiod and, like the PIB, photosynthesis is apparently a function of degree of acidity (Fig. 2), growth light intensity (Fig. 2), the ambient gas phase (Figs. 4, 6, and 7), and the time of exposure to O₂ and CO₂ (Figs. 4 and 6).

Photosynthesis during the initial illumination periods (Fig. 2) exhibited reduced rates of exogenous CO₂ fixation which probably are associated with large malate pools (24). According to Kluge (16–18), increased malate in the cytoplasm may cause a feedback inhibition of PEP carboxylase; therefore, RuDP would have a greater probability of fixing CO₂. The decarboxylation of organic acids (11) would release CO₂ to be fixed by RuDP carboxylase to form carbohydrates (18, 20, 32). This supply of endogenous CO₂ may simply reduce the requirement for exogenous CO₂, thereby reducing net photosynthesis early in the day. Later in the day, the organic acid content and CO₂ pools change so that both PEP and RuDP carboxylase could be active during photosynthetic CO₂ fixation.

**Acknowledgments**—We are grateful to Dr. D. Bartholomew for detailed information on culturing pineapples. Thanks also are extended to Dr. R. H. Brown for his stimulating discussions during the course of this study.

**LITERATURE CITED**


