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Postillumination Burst of Carbon Dioxide in Crassulacean Acid Metabolism Plants[1]

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ABSTRACT

Immediately following exposure to light, a postillumination burst of CO_2 has been detected in Crassulacean 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 Crassulacean 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 efflux 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.[2] The existence of an excessive effflux, 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 peperomia 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 deleted 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 reductant for continued fixation of CO_2 into the C_3 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_3 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_3 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.

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[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: oxaloacetate.

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 sensative 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 CO_2 Exchange Chamber. The leaf CO_2 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. CO_2 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 thermister. A schematic of the CO_2 analysis system is shown in Figure 1. Leaf surface area was measured using a Hayaski Denko automatic leaf area meter.

Measurement Procedures. Measurements of photosynthesis and the PIB were made at 2, 21, and 99% O_2 using compressed air and commercial gas mixtures of O_2, N_2, and CO_2. The 2, 21, and 99% O_2 mixtures contained 324 μl/l, 338 μl/l, and 320 μl/l of CO_2, respectively. To examine a higher CO_2 concentration, a mixture of 21% O_2 and 934 μl/l CO_2 was purchased. Light intensity of 5000 ft-c was maintained in the leaf CO_2 exchange chamber for all of the PIB measurements.

When CO_2 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 CO_2 exchange chambers at the prescribed O_2 and CO_2 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 CO_2 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 CO_2 uptake patterns. Growth light intensities of 5000 and 7500 ft-c increased CO_2 assimilation much earlier than 2000 ft-c during initial day and night periods. It should be noted that we consistently observed CO_2 uptake in the light with CAM leaves particularly in the latter half of the photoperiod (Fig. 2) so that broadly characterizing CAM CO_2 uptake as occurring during the night is correct; but a substantial CO_2 uptake also may occur during the day.

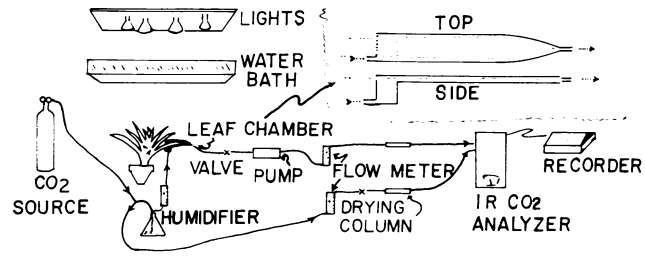

FIG. 1. Schematic of the CO_2 exchange analysis system.

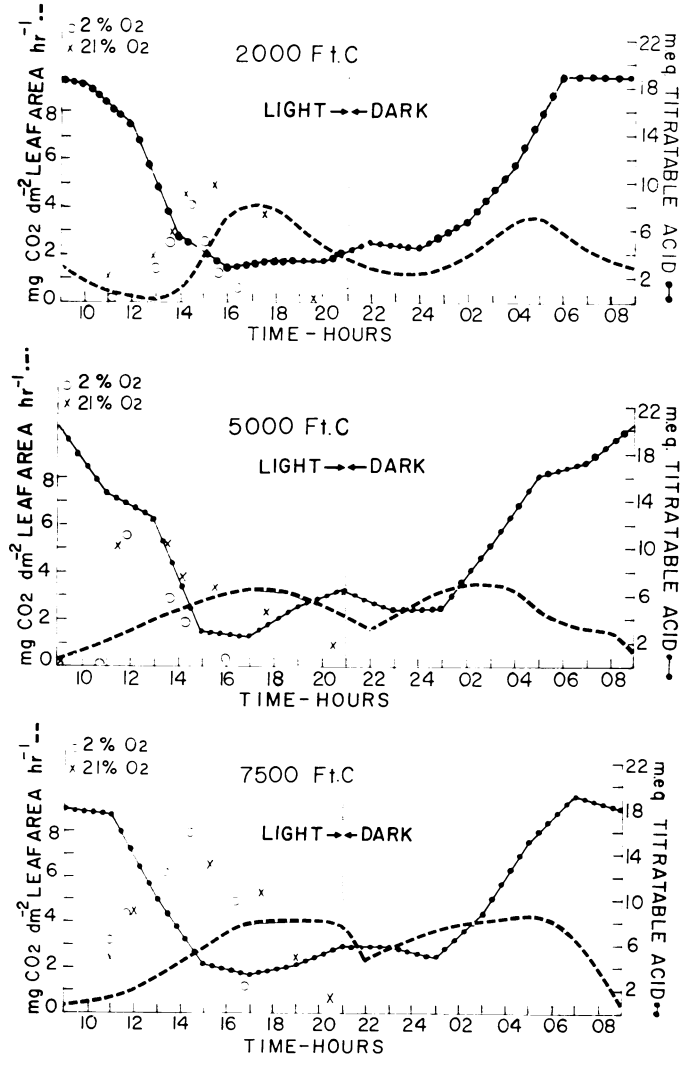

FIG. 2. Daily changes in titratable acidity (●——●), CO_2 uptake (– – –), and the maximum amplitude of the PIB in pineapple leaves grown at three light intensities. Daily changes in PIB amplitudes were measured in 2% O_2 (○) and 21% O_2 (×) with 320 ml/l CO_2. Note that PIB data are for CO_2 evolution.

In the course of these daily CO_2 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 CO_2 release peak and the secondary peak (Fig. 3).

Other CAM plants, including *Kalanchoe daigremontiana, K. pinata, K. tubiflora, Sedum telephodies,* and *Crassula argentea,* showed titratable acidity and CO_2 gas exchange patterns similar to those of pineapple in Figure 2. A definite PIB also

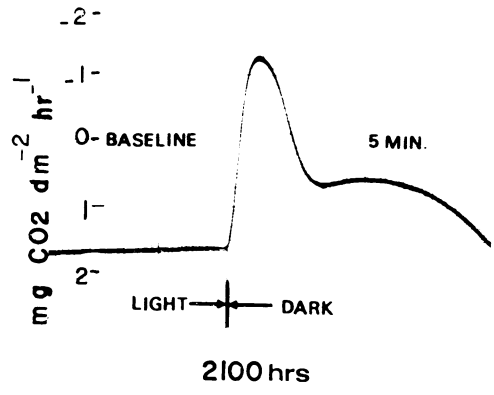

FIG. 3. Recorder tracing at 2100 hr showing the PIB initially observed in air with a pineapple leaf growing under 2000 ft-c of light.

was found in all of these CAM species; but we noted numerous 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 CO_2 (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 CO_2 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 O_2, CO_2, and growth-light intensity on the PIB in pineapple leaves.

O_2 Concentration and PIB. The recorder traces in Figure 4 illustrate the effects of ambient O_2 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 O_2 concentration, and the length of time in a day the leaves were exposed to a given O_2 concentration (Fig. 4).

At all growth light intensities the PIBs observed under 2% O_2 peaked and disappeared earlier in the illumination period than the PIBs produced with 21% O_2. Additionally, PIB kinetics and amplitudes were greatly altered and/or reduced by 2% O_2 when compared to 21% O_2. However, such alterations by 2% O_2 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% O_2 was dependent upon the growth light intensity. PIBs under 2% O_2 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% O_2 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% O_2 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% O_2, 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 O_2 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% O_2 to exceed those produced in 2% or 21% O_2. The PIB under 99% O_2 diminished only late in the light period. Maximum PIB amplitudes under 99% O_2 were larger than those under 2% or 21% O_2—reaching a maximum rate of 8.5 mg of CO_2 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 CO_2 dm^{-1} hr^{-1}. Growth regimes of 2000 ft-c or greater produced PIBs with maximum amplitudes in excess of 5 mg of CO_2 dm^{-2} hr^{-1}. The maximum PIB in air, as depicted in Figure 5, was dependent upon growth light intensity between 200 and about 2000 ft-c, but independent of intensity above about 2000 ft-c.

CO_2 Concentration and PIB. An increase in CO_2 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 CO_2 were observed approximately 6 hr into the light period. The shapes and amplitudes of PIBs under 934 μl/l CO_2 and 21% O_2 were similar to those obtained with 320 μl/l CO_2 and 2% O_2 (shown in Fig. 7).

Influence of O_2 and CO_2 Concentration on CO_2 Assimilation. In pineapple, light and dark CO_2 assimilation under air was approximately equal with plants from all light growth regimes (Fig. 2). During the initial 4 hr of illumination CO_2 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 CO_2 uptake during the initial light periods. Maximum CO_2 uptake occurred about 8 hr into the light period at all light growth intensities. Immediately after illumination, CO_2 uptake at all growth light intensities decreased for 1 to 2 hr followed by an increased dark CO_2 uptake. The maximum dark CO_2 uptake occurred 8 to 10 hr after illumination and then declined (Fig. 2). At growth light intensities of 2000 and 7500 ft-c, CO_2 uptake under 2% and 21% O_2 was approximately equal throughout the day (Fig. 4). However, in pineapple leaves grown at 5000 ft-c of light, 2% O_2 did increase CO_2 uptake after 3 to 5 hr of light. In contrast, 99% O_2 greatly reduced CO_2 uptake for 8 hr into the light period followed by a rapid increase in CO_2 uptake.

At a light growth intensity of 5000 ft-c, CO_2 uptake under both 338 μl/l or 934 μl/l CO_2 in 21% O_2 was approximately equal for 6 to 7 hr into the light period. Shortly thereafter, a rapid increase in CO_2 uptake was observed under 934 μl/l CO_2 (Fig. 6).

DISCUSSION

If it is assumed that CO_2 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 C_3 or C_4 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 C_3 and C_4 plants where the

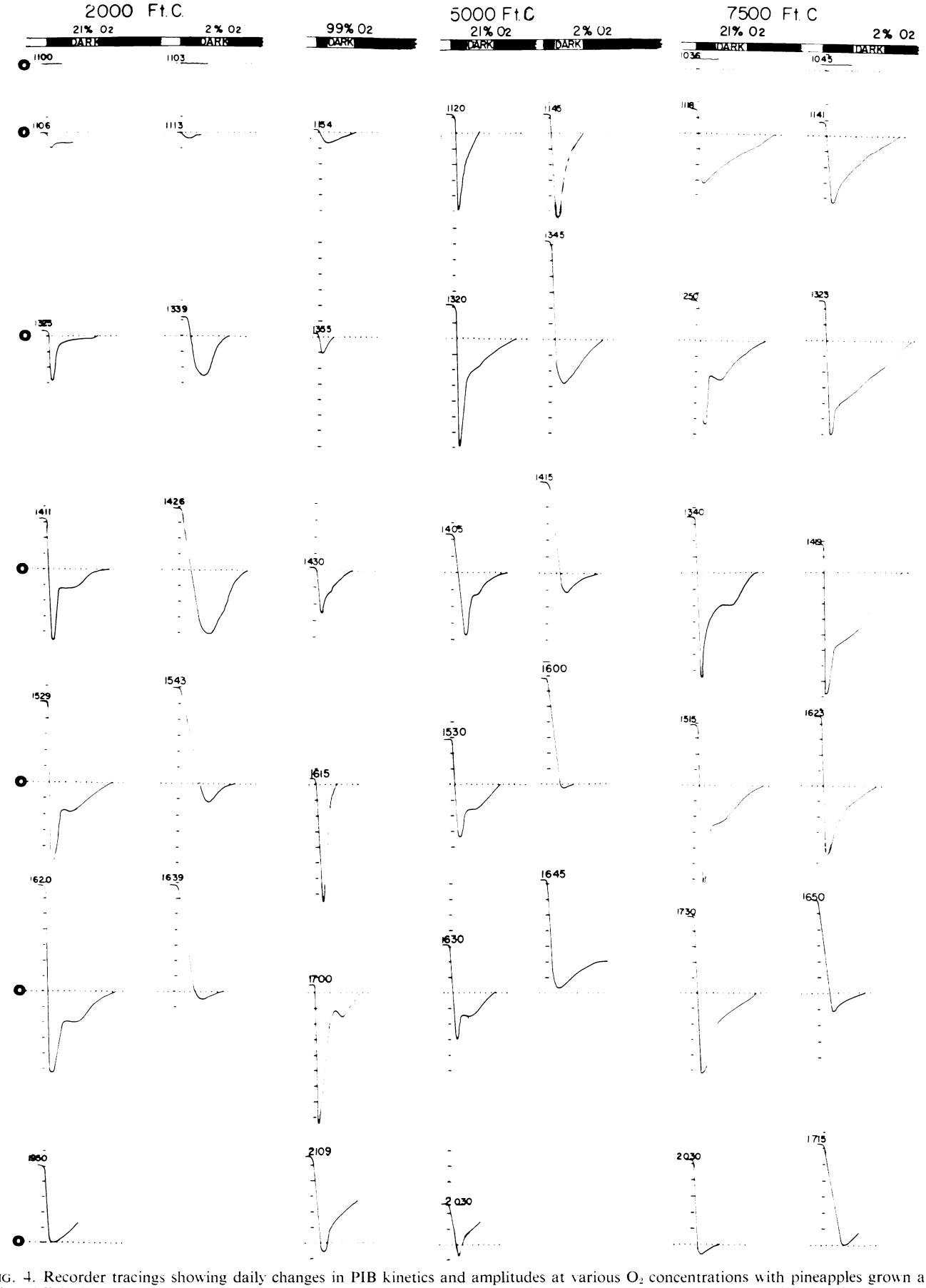

FIG. 4. Recorder tracings showing daily changes in PIB kinetics and amplitudes at various O_2 concentrations with pineapples grown at the captioned light intensities. Numerals above each PIB trace represent the time of day. Horizontal lines of dots are time in minutes while each vertical dash is a mg of CO_2 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 C_3 and C_4 plant leaves, as gases in the atmosphere of a leaf chamber are switched (3, 7), the rates of leaf CO_2 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 O_2 or CO_2, 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 O_2 and CO_2 (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 C_3 and C_4 plants which usually peaks in less than 1 min (3, 7, 9, 12).

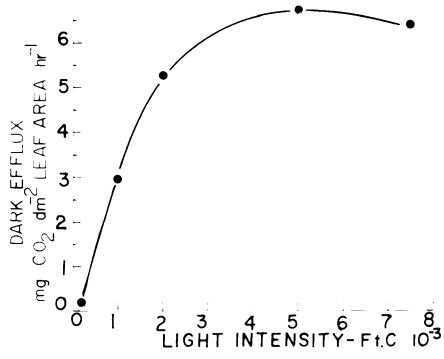

FIG. 5. Maximum daily amplitude of the PIB with pineapple leaves grown at various light intensities.

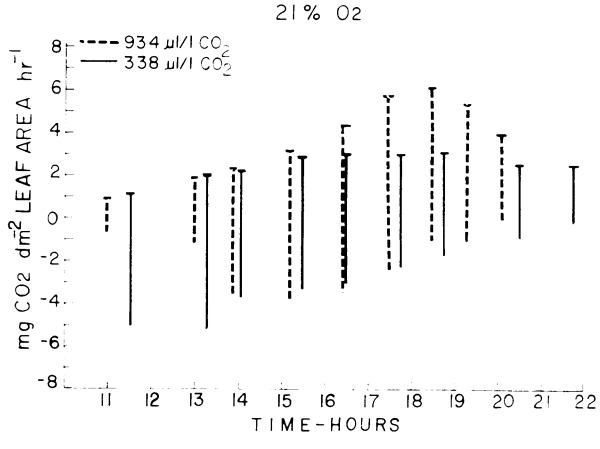

FIG. 6. Effect of CO_2 concentration on photosynthesis and the PIB with pineapple leaves throughout a day. The top of each line represents the photosynthesis rate. The bottom of each line indicates the maximum amplitude of the PIB.

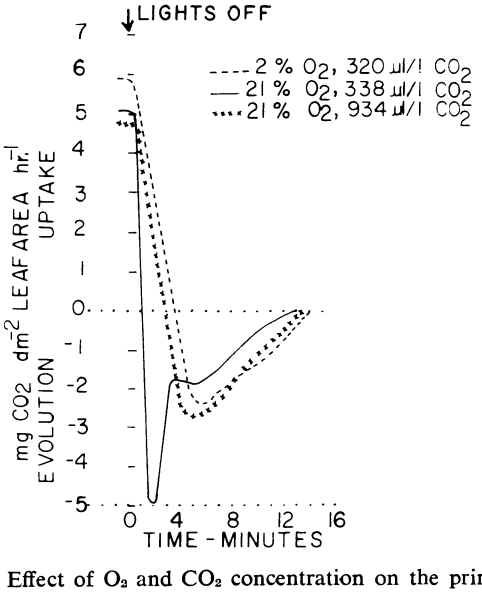

FIG. 7. Effect of O_2 and CO_2 concentration on the primary and secondary peaks of the PIB with pineapple leaves. Measurements were made following approximately 6 hr of light.

In C_3 and C_4 plants there is no daily change in titratable acidity (5, 25). 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 photorespiration at higher growth light intensities via the glycolate pathway. As previously reported (10, 29), an increase in light intensity increased photorespiration 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 O_2-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 CO_2 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 O_2 or increasing CO_2 (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 CO_2. Under low O_2, the degree O_2 competing with CO_2 in the carboxylation of RuDP would be limited; therefore, less RuDP would be used to form phosphoglycolate (21) which would furnish the CO_2 released in the primary PIB peak. A suppression of the primary PIB peak with a CO_2 concentration of 934 μl/l also supports the same postulation (Fig. 7).

We postulate that most of the CO_2 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% O_2 (Figs. 4 and 7), low ambient O_2 (Figs. 4 and 7), and high CO_2 (Fig. 7) connotes another mechanism, because neither O_2 nor CO_2 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 + CO_2 + ADP. The released CO_2 presumably is reassimilated during periods of illumination (5); however, upon darkening, photosynthetic NADPH may become a limiting factor, reducing the amount of CO_2 fixed, thereby allowing an efflux of CO_2. The dark decarboxylation of OAA may account for most of the CO_2 loss during the transient secondary PIB in pineapple leaves. Presumably this

CO_2 loss ceases in the dark, because ATP synthesis also is reduced. The lack of great influence of O_2 or CO_2 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 O_2 or CO_2 concentration. Secondary CO_2 bursts and even other dark CO_2 peaks have been reported in *Panicum bergii* (7) and tobacco (15) and in other plants, but these transient CO_2 peaks have not been the subject of intensive research.

A study of the data on photosynthetic CO_2 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 O_2 and CO_2 (Figs. 4 and 6).

Photosynthesis during the initial illumination periods (Fig. 2) exhibited reduced rates of exogenous CO_2 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 CO_2. The decarboxylation of organic acids (11) would release CO_2 to be fixed by RuDP carboxylase to form carbohydrates (18, 20, 32). This supply of endogenous CO_2 may simply reduce the requirement for exogenous CO_2, thereby reducing net photosynthesis early in the day. Later in the day, the organic acid content and CO_2 pools change so that both PEP and RuDP carboxylase could be active during photosynthetic CO_2 fixation.

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