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Short Communication

A Transient Burst of CO_2 from Geranium Leaves during Illumination at Various Light Intensities as a Measure of Photorespiration[1]

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ABSTRACT

A transient CO_2 burst is exhibited by irradiated leaves of the C_3 plant geranium (*Pelargonium* X *hortorum*, Bailey) after the irradiance is quickly lowered. The light CO_2 burst appears to be related to photorespiration because of its irradiance dependency and its sensitivity to other environmental components such as CO_2 and O_2 concentration. The term post-lower-irradiance CO_2 burst or PLIB is used to describe the phenomenon. The PLIB appears to be a quantitative measurement of photorespiration with intact geranium leaves. The PLIB has been observed with intact leaves of other C_3 plants but not with C_4 leaves. Therefore, it is proposed that, after maximizing intact leaf photosynthetic rates and leaf chamber gas measuring conditions, photorespiration can be measured with intact C_3 leaves such as geranium as a transient post-lower-irradiance CO_2 burst.

An estimated 30% to 50% of the photosynthetically assimilated carbon is lost through the process of photorespiration, particularly in leaves of C_3 plants (6, 7). Technically, photorespiration is difficult to measure accurately because in light CO_2 is assimilated from the atmosphere while at the same time CO_2 is evolved via photorespiration. Therefore, an accurate and simple method of measuring photorespiration is needed.

This a report of a serendipitous observation made while measuring leaf photosynthetic rates at progressively reduced irradiance levels in the C_3 plant geranium. At each reduced irradiance intensity, a transient CO_2 burst occurred prior to obtaining a steady-state rate of photosynthesis. This CO_2 burst from an illuminated leaf appeared kinetically to be similar to the PIB[4] described initially nearly three decades ago (3, 4). The PIB was originally referred to as a remnant of light-respiration; indeed it was postulated to be a brief remnant of photorespiration inasmuch as it was observed in the dark within 1 to 3 min (3, 4). These early ideas are generally accepted today (2, 5–7). We reasoned that this reduced irradiance intensity CO_2 burst also might be associated with photorespiration. The PLIB is proposed to describe this phenomenon. In this manuscript, we will describe our discovery and measurement of the PLIB in intact geranium leaves and present initial observations regarding the PLIB with other leaves. We will propose that the PLIB may be a direct, easy, and useful measurement of photorespiration in intact leaves.

MATERIALS AND METHODS

One hundred geranium plants (*Pelargonium* X *hortorum*, Bailey cv. Razmatazz) were grown in a standard peat:vermiculite (1:1 v/v) mix in 10-cm pots. Plants were watered to saturation when needed and fertilized weekly with 800 mg/l of 15-0-12.5 N-P-K at each irrigation for 10 weeks and were vigorously growing in a standard greenhouse at 30 ± 5°C day and 21 ± 3°C night.

The fourth leaf from the base was used for all photosynthesis measurements while still attached to an intact plant. All CO_2 measurements were obtained by the standard method differential analysis between CO_2 concentration entering and exiting a photosynthesis chamber using a Beckman model 215B IR CO_2 analyzer. The circular chamber was sealed gas tight with a rubber O ring and play-putty at the leaf petiole entrance. An in-chamber quantum sensor (LI-COR 285), thermister, and low-speed (200 rpm) high-torque circulating fan resulted in accurate and reproducible results with a response time of 3 to 5 s. Irradiance intensity was varied simply by interposing or removing layers of cheesecloth. The volume of the system, including the chamber, was 145 ml, and gas flow was 1.5 L/min. Premixed gases of 2% or 21% O_2 containing ~300 μl/l CO_2 with the balance N_2 were used.

Leaf areas were measured with a Lambda leaf area meter and data calculated according to standard methods (1, 2). The PLIB value in mg of CO_2 dm^{-2} h^{-1} was calculated from the peak recorder deflection as illustrated in Figure 1. The PLIB is a somewhat relative value since a steady state is never reached. A smaller or larger volume gas handling system or a change in flow rate would vary the peak height, but the data in this manuscript were collected using the same physical system so any correction is constant.

RESULTS

In a study to establish the optimum irradiance intensity for geranium growth and photosynthesis, it was observed that a

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[4] Abbreviations: PIB, post-illumination CO_2 burst; PLIB, post-lower-irradiance CO_2 burst.

Table I. *The Influence of 21% and 2% O_2 on Leaf Photosynthesis Compared with the Detection of the PLIB in Various C_3 and C_4 Plants*

	Photosynthesis[a]		Photosynthesis Inhibition at 21% O_2	PLIB in Air[a]	PLIB/Photosynthesis in Air
	21% O_2	2% O_2			
	mg CO_2 dm^{-2} h^{-1}		*%*	*mg CO_2 dm^{-2} h^{-1}*	*%*
C_3 Plants					
Sweet Potato	12.7	18.1	30	1.9	15
Cotton	17.1	23.3	27	2.1	12
Potato	14.5	21.5	33	1.5	10
Broad bean	17.7	25.6	30	3.7	21
Soybean	13.0	18.8	31	0.7	6
Brassica rapa	16.8	25.3	33	2.7	16
Begonia	7.4	10.1	26	0.8	11
Coleus	8.7	12.7	31	0.4	5
Geranium	13.3	19.3	31	4.6	34
C_4 Plants					
Corn	22.0	22.5	2	0	0
Crabgrass	30.4	30.4	0	0	0

[a] Irradiance intensity 600 $\mu E\ m^{-2}\ s^{-1}$ with the PLIB measured after decreasing the light to 150 $\mu E\ m^{-2}\ s^{-1}$.

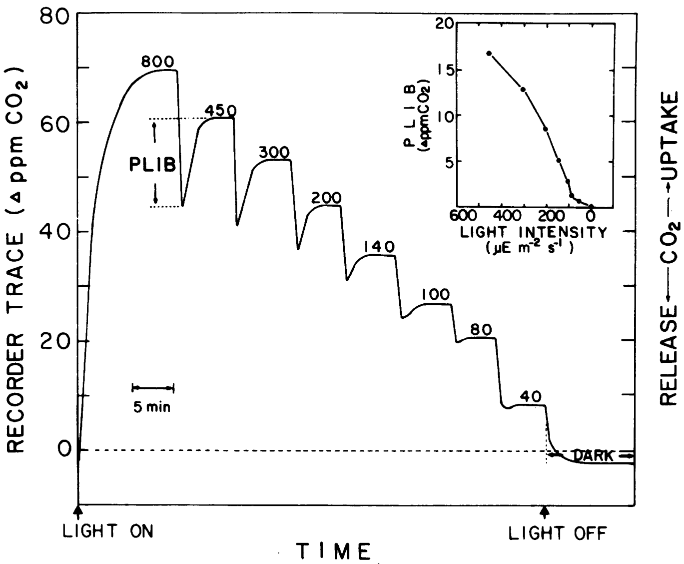

FIG. 1. Graph of an original recorder trace showing the influence of lowering the irradiance intensity on CO_2 exchange with intact geranium leaves. Note the CO_2 bursts following each lowering of irradiance. The irradiance intensity value on each curve is in $\mu E\ m^{-2}\ s^{-1}$. The method of measuring the amplitude of the PLIB is illustrated at the first irradiance reduction. The insert graphs the amplitude of each CO_2 burst (or the PLIB) at the various irradiance intensities.

number of apparent CO_2 bursts occurred, each following a progressive irradiance intensity reduction. Figure 1 illustrates the original observation showing the transient CO_2 release observed as the irradiance was decreased. Then CO_2 uptake occurred, and a new steady-state rate of photosynthesis was attained at each irradiance intensity. The initial kinetics of the light CO_2 burst were somewhat similar to the previously reported PIB which is measured quickly in the dark prior to attaining steady-state dark respiration (2–5). The relative amplitude of the PLIB following each irradiance intensity reduction appeared visually to be a function of the previous irradiance intensity. For easier visualization and quantification, the PLIB amplitudes were measured (as illustrated on Fig. 1) and plotted in the insert of Figure 1. For example, an irradiance decrease from 800 to 450 $\mu E\ m^{-2}\ s^{-1}$ in Figure 1 produced a PLIB amplitude of 17 before returning to a rate of steady-state photosynthesis at 450 $\mu E\ m^{-2}\ s^{-1}$. Detailed investigations now in progress with geranium leaves show that the amplitude of the light CO_2 burst varies with time and intensity of irradiation, with temperature, and is inhibited by CO_2 levels above ambient of O_2 levels below 21%. To date, our geranium data indicate a similar response of the PLIB to these environmental components as does photorespiration.

To ascertain if the PLIB is peculiar to the geranium plant or if it is a more widely distributed phenomenon, a number of other plants were surveyed. The PLIB also was present in attached leaves of the following C_3 plants: sweet potato, cotton, potato, broad bean, soybean, begonia, coleus, and *Brassica rapa* (Table I). No PLIB was detected in the C_4 plants: corn or crabgrass. Another measure of photorespiration (the inhibition of photosynthesis in 21% *versus* 2% O_2) also was determined, and for these C_3 plants it ranged from 26% to 33% with essentially zero inhibition observed for C_4 plants (Table I).

In Table I, the photosynthetic rate of intact geranium leaves was optimized with the chamber volume, irradiation intensity, gas volumes, and flow rates (1). The photosynthesis rate in mg CO_2 $dm^{-2}\ h^{-1}$ was 13.3 in 21% O_2 and ~300 $\mu l/l$ CO_2 and 19.3 in 2% O_2 giving an enhancement of 6 mg CO_2 $dm^{-2}\ h^{-1}$. The estimated photorespiration by this method is 31%. Under the same conditions in air, the PLIB was 4.6 mg CO_2 $dm^{-2}\ h^{-1}$ with an irradiance intensity decreased from 600 to 150 $\mu E\ m^{-2}\ s^{-1}$, or 34% of leaf photosynthesis. Clearly 31% and 34% are quite comparable values (Table I). However, if similar values are calculated for the other C_3 plants in Table I, much lower and quite variable PLIB/photosynthesis values are obtained. This simply is a function of the physical volumes, gas flow, etc., of the CO_2 measuring system and the fact that we did not try to maximize photosynthesis in these plants. As already stated, we have worked extensively with geranium and wish to recommend that each new plant be studied to maximize the sensitivity of the system for detecting the PLIB. Finally, the PLIB is a transient phenomenon (Fig. 1) and may slightly underestimate the total CO_2 released.

DISCUSSION

Attempts to measure photorespiration accurately have been underway since its original detection (3). Perhaps the most widely used estimation of photorespiration is the increase in photosynthetic rate measured near 2% O_2 *versus* 21% O_2 (6, 7). The light-to-dark PIB has been used to detect and qualitatively estimate photorespiration. Some other methods of estimating photorespiration which have been used include the CO_2 compensation concentration, light $^{14}CO_2$ release, biochemical analyses of photorespiration intermediates such as serine and glycine or peroxisomal metabolism, and mass spectrophotometric analyses usually employing isotopes of oxygen and carbon (6, 7). In general, these methods require either special equipment or have some limitation in widespread usage to measure photorespiration although all have been useful in contributing to our current understanding of photorespiration.

We are led to propose that the PLIB can be used as a direct, easy, and quantitative measure of photorespiration which involves the use of fairly common and inexpensive instrumentation. Though we are proposing that the PLIB can be used as a direct quantitative measurement of photorespiration specifically in geranium (Table I), we wish to express some cautions. First, we have worked extensively with attached geranium leaves and only surveyed other leaves. Second, inasmuch as the PLIB is not a steady-state rate of CO_2 release but simply as the peak height of a transient CO_2 burst (Fig. 1), the leaf area, chamber of system volume, gas flow rate, temperature, irradiance intensity, and other physical as well as plant physiological states, *e.g.* leaf age, should be optimized and/or studied for each species.

The PLIB as presented here in geranium is proportional to irradiance intensity, is sensitive to changes in concentration of O_2 and CO_2 as is photorespiration, is relatively simple to measure, is a measurement of photorespiration at various irradiance intensities, and also appears to be a ubiquitous phenomenon in other C_3 leaves. Thus, we propose that the PLIB has potential usefulness as a direct measurement of photorespiration in attached leaves.

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