S29-004

Measurements of mitochondrial respiration in the light and estimation of its refixation in C₃ and C₄ leaves

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Keywords: ¹³CO₂, CO₂ emission (refixation), gas-exchange, mitochondrial respiration in the light.

Introduction

There are infrared gas analysers that are insensitive to ${}^{13}\text{CO}_2$. We previously exploited this feature to detect the photorespiratory emission of CO₂ and to calculate the photorespiratory carbon recycled in C₃ plants (Loreto et al. 1999). Later on we demonstrated that the same method can be applied to measure the emission of CO₂ due to mitochondrial respiration in the light (R_d) in C₄ leaves (Loreto et al. 2001). Here we show that, based on gas exchange measurements and concurrent measurements of R_d, the refixation of mitochondrial respiration by C₃ and C₄ leaves can be calculated and apparently accounts for the missing amount of CO₂ with respect to the mitochondrial respiration in the dark.

Materials and Method

Plant material. Plants of a C₄ species (*Zea mays* L.), a C₃ herbaceous species (*Lycopersicon esculentum*), and a C₃ schlerophyllous tree (*Quercus ilex*) were cultivated in 2 L pots with commercial, nutrient-enriched soil. Plants were irrigated daily and were maintained at a day light intensity of 400-800 μ mol m⁻² s⁻¹, and at an air temperature (day/night) of 25/18°C. Some maize leaves were rapidly salt-stressed by feeding cut leaves with a solution containing 1% NaCl.

Conventional gas-exchange (${}^{12}CO_2$) on intact leaves. The gas-exchange system was similar to that described by Loreto et al. (1999). The last fully expanded leaf was inserted in a gas-exchange cuvette with a 4.9 cm² glass round window from which the leaf disc was exposed to 1000 µmol m⁻² s⁻¹ light intensity. The cuvette was thermostated at 25°C by water circulating inside its body. The leaf was exposed to a flow of 500 mL min⁻¹ of synthetic air formed by 80% N₂, 20% O₂ and 350 ppm ¹²CO₂, simulating the ambient conditions, or of O₂-free air to inhibit respiration. The absolute CO₂ concentration entering the cuvette was measured with an infrared gas analyser (Gas-hound, Li-Cor, Lincoln, USA) while CO₂ and H₂O exchanges between the leaf and air were measured with a differential infrared gas analyser (Li 6262, Li-Cor).



Fig.1 ¹²CO₂ reading in a chart recorder during the 4 steps of experiments performed with healthy maize (solid line) and tomato (dashed line) leaves. Arrows show when R_d (after correcting for the signal due to photosynthesis) and R_n were measured.

Measurements of R_d and R_n by ¹³CO₂ feeding. The method is based on the very low sensitivity of some infrared gas analysers to ¹³CO₂ (Loreto et al. 1999) The Gas-hound used in these experiments has a very low sensitivity to ${}^{13}CO_2$ (1.7% of the sensitivity to ${}^{12}CO_2$). The four steps composing each measurement are shown in Fig. 1. The first two steps are measurements made by passing the cuvette with the illuminated leaves, that is: 1) A CO_2 -free air entered the Gas-hound to ascertain the possible occurrence of instrumental zero drift. 2) Air containing 350 ppm 13 CO₂ entered the Gas-hound. 3) The air flowing over the illuminated leaf in the cuvette (containing 350 ppm of ¹²CO₂) was rapidly substituted with the air containing an equal concentration of ${}^{13}CO_2$ as shown in Loreto et al. (1999). After leaving the cuvette, the air with ${}^{13}CO_2$ entered the Gas-hound and the amount of ${}^{12}CO_2$ emitted by the leaf was measured as the difference between the emission calculated at step 3. This measurement was corrected for the photosynthetic CO₂ drawdown, i.e. 0.017 ppm for each ppm of ${}^{12}CO_2$ photosynthetic uptake. The release of ${}^{12}CO_2$ attributable to R_d was measured between 60 and 120 s when the CO_2 release due to leakage from bundle sheath cells (C_4 plants) and photorespiration (C_3 plants) was completely labelled. 4) After 120 s, the light was then switched off and the release of ${}^{12}CO_2$ from the leaf (the difference between the CO_2 recorded at step 4 and step 2) was measured after 120 more s.

Calculation of refixation of mitochondrial respiration in leaves maintained under ${}^{13}CO_2$.

The respiratory ¹²CO₂ refixed within the leaf mesophyll (R_{dr}) can be calculated determining the intercellular concentration of ¹²CO₂ produced by mitochondrial respiration (¹²C_i). Assuming that under the ¹³CO₂ atmosphere, the ambient concentration of ¹²CO₂ (¹²C_a) is zero, ¹²C_i = R_d / g_s where g_s is the stomatal conductance to CO₂. R_{dr} was then calculated R_{dr} = ¹²C_i / ¹³C_i * P_n, where ¹³C_i is the intercellular concentration of ¹³CO₂ calculated by gas exchange, and P_n is the photosynthetic rate.

Results and Discussion

As previously reported (Loreto et al. 1999, Haupt-Herting et al. 2001), and also shown in Fig. 1, it takes a few seconds before all processes releasing CO_2 are saturated with $^{13}CO_2$, with the exception of mitochondrial respiration. About 60 s are necessary to label the CO_2 emitted by photorespiration (Loreto et al. 1999). After this lag-time, the signal becomes steady and the emission due to mitochondrial respiration (R_d) can be calculated as explained in Materials and Methods.

We measured a R_d consistently lower than the emission in the dark measured after step 4, which is the mitochondrial respiration in the dark (R_n , Table 1), as confirmed by conventional gas-exchange in dark-adapted leaves, and as also previously shown by us (Loreto et al. 1999, 2001). This may indicate that the mitochondrial respiration in the light is inhibited. Such an inhibition, of a magnitude similar to that reported with our method, has been proposed on the basis of indirect measurements (Kok 1948, Laisk 1977). Alternatively, it is possible that, as for photorespiration in C_3 plants (Loreto et al. 1999), the missing CO_2 is refixed by illuminated leaves. Our calculations (Table 1) show that the sum of R_d and the amount of the CO_2 created by mitochondrial respiration and refixed before exiting the leaf (R_{dr}) is similar to R_n both in C_3 and C_4 plants. We therefore believe that mitochondrial respiration in the light is not inhibited but that the CO₂ emitted is low under these circumstances because of the occurrence of a substantial amount of CO_2 refixation, particularly in C_4 leaves. The only case on which the sum of R_d and R_{dr} is consistently lower than R_n is in salt-stressed maize leaves (Table 1). Thus, it is possible that the mitochondrial respiration is actually inhibited in the light in stressed leaves, perhaps because refixation is not needed to fuel high rates of photosynthesis.

Table 1. Measurements of photosynthesis (P_n), stomatal conductance (g_s), mitochondrial respiration in the dark (R_n) and in the light (R_d), and calculation of the intercellular concentration of ¹² and ¹³CO₂ (C_i) and of the refixed amount of mitochondrial respiration in the light (R_{dr}). R_d and R_{dr} are also expressed as % relative to their sum.

Plant species	P _n (µmol/m ² s)	g s (mmol/m ² s)	¹³ C _i (ppm)	¹² C _i (ppm)	1.1.1.1 R _d (μmol/m ² s) (%)	R _{dr} (µmol/m ² s) (%)	R _n (µmol/m ² s)
Lycopersicon	22	270	210	6.4	1.0	0.6	1.6
esculentum					(62)	(38)	
Quercus	11	120	228	3.3	0.4	0.2	0.6
ilex					(67)	(33)	
Zea mays	23	100	120	2.0	0.2	0.4	0.6
					(33)	(67)	
Zea mays	8	52	190	10	0.5	0.4	1.5
(salt-stress)							

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