Relationship between Photosynthesis and Respiration

THE EFFECT OF CARBOHYDRATE STATUS ON THE RATE OF CO2 PRODUCTION BY RESPIRATION IN DARKENED AND ILLUMINATED WHEAT LEAVES

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ABSTRACT

The rate of dark CO2 efflux from mature wheat (Triticum aestivum cv Gabo) leaves at the end of the night is less than that found after a period of photosynthesis. After photosynthesis, the dark CO2 efflux shows complex dependence on time and temperature. For about 30 minutes after darkening, CO2 efflux includes a large component which can be abolished by transferring illuminated leaves to 3% O2 and 330 microbar CO2 before darkening. After 30 minutes of darkness, a relatively steady rate of CO2 efflux was obtained. The temperature dependence of steady-state dark CO2 efflux at the end of the night differs from that after a period of photosynthesis. The higher rate of dark CO2 efflux following photosynthesis is correlated with accumulated net CO2 assimilation and with an increase in several carbohydrate fractions in the leaf. It also correlates with an increase in the CO2 compensation point in 21% O2 and an increase in the light compensation point. The interactions between CO2 efflux from carbohydrate oxidation and photorespiration are discussed. It is concluded that the rate of CO2 efflux by respiration is comparable in darkened and illuminated wheat leaves.

The rate of CO2 efflux by respiration from single leaves and whole plants in the dark is linearly related to the rate of previous photosynthesis when the latter is varied by changing the light level or CO2 concentration (18, 21). McCree (21) fitted an empirical equation in which the rate of dark CO2 efflux is proportional to photosynthesis and dry weight of living material on the plant. This served as a basis for the development of the theoretical concepts of growth and maintenance respiration by Penning de Vries (24). Both of these components of respiration are thought to involve, principally, carbohydrate oxidation through glycolysis, the pentose phosphate pathway and the tricarboxylic acid cycle. Growth respiration appears to be less sensitive to temperature than maintenance respiration (22). Explanations of the complex interactions between photosynthesis, temperature, and dark respiration are uncertain, although it is probable that the interaction is mediated by carbohydrate level (6, 8).

The extent to which tricarboxylic acid cycle respiration continues in the light in green leaves is uncertain. Graham (12) concluded that biochemical evidence suggests the tricarboxylic acid cycle continues to operate in illuminated leaves at about the same rate as it does in darkness. Physiological evidence is contradictory; some experiments are best explained in terms of significant CO2 efflux in illuminated leaves from sources other than photorespiration (3, 12) whereas others suggest that these other sources are negligible (7, 19, 23). The lack of methods for direct measurement of the rate of respiration during photosynthesis greatly complicates the resolution of this question. A new experimental approach is attempted in this paper.

The experiments described here investigate the relationship between photosynthesis in mature wheat leaves, its products (particularly carbohydrates), temperature, and CO2 efflux by respiration in the light (Rd) in the dark (Rn) (see Ref. 3 for terminology). We conclude that the increase in dark CO2 efflux (Rn) after a period of photosynthesis is correlated with the amount of carbohydrate synthesized, and that the temperature dependence of dark CO2 efflux varies with leaf carbohydrate concentration. We also conclude that the rate of respiration in the light (Rd) is comparable to Rn and that it makes a significant contribution to total CO2 efflux in illuminated wheat leaves.

MATERIALS AND METHODS

Plant Material. Triticum aestivum cv Gabo, Vicia faba, Eucalyptus grandis, and Lolium perenne plants were grown from seed in a cabinet in 12-cm pots of soil. They were watered twice a day, and were fertilized every other day with nitrate-type Hewitt solution. Total nitrate concentration was 12 mm. Quantum flux (400–700 nm) was 500 to 600 μE·m⁻²·s⁻¹. The day/night temperature regime was 25/20°C with a daylength of 13 h. RH was between 60 and 80%. Mature leaves of 30-d-old plants were selected at the end of the night period.

Gas Exchange Techniques. CO2 and water exchanges were measured in leaves using an open system gas analysis apparatus, utilizing an IR CO2 analyzer (model 865, Beckman Instruments) which was operated in both differential and absolute modes, and a dew point hygrometer (model 880; Cambridge Systems, Waltham, MA).

One or two attached intact leaves were inserted in a well-ventilated aluminum leaf chamber (boundary layer conductance to diffusion of water vapor was 2.2 mol·m⁻²·s⁻¹). Illumination was provided by a 2.5 kw water-cooled, high-pressure, xenon-arc lamp (model XBF 2500, Osram), the UV and IR components being removed with a Schott KG 2B filter. Quantum flux (400–700 nm) incident on the leaf was 1000 μE·m⁻²·s⁻¹, and it was measured with a quantum sensor (model LI-190 SR, Lambda Instruments). Leaf temperature, which was controlled by circulating water through a jacket, was measured with two copper-constantan thermocouples (0.1 mm diameter) in contact with the lower surface.

Air with the desired partial pressure of CO2 was obtained by injection of 5% CO2 in air into CO2-free air through a stainless steel capillary tubing. A self-ventilating pressure regulator (model MAR-1P, Clippard Minimatic, Cincinnati, OH) and a pressure gauge were used to control the injection rate. CO2-free air with different CO2 concentrations was obtained by mixing compressed ambient air with N2 from a cylinder, and then by passing the
resulting gas through two columns of soda lime (Carbosorb, self-indicating; BDH Chemicals Ltd, Poole, England). The O₂ concentration was measured with an O₂ electrode (model 5331, YSI). The gas was then humidified in a gas washing bottle with a scintillated disc. The dew point of the gas was maintained by passing the gas through a glass condenser, the temperature of the latter being controlled by circulating the water from a temperature-controlled water bath. Air flow through the leaf chamber was monitored with a mass flowmeter (model AFSC-10K; Hastings, Hampton, VA). Flowmeters with needle valves were used to distribute gas flow throughout the system. Copper tubing was used in the circuit.

The outputs of all sensors were registered on a digital voltmeter, and the outputs from the CO₂ analyzer and dew point hygrometer were continuously recorded. The outputs from the sensors allowed calculation of the rate of net CO₂ assimilation, stomatal conductance, intercellular CO₂ partial pressure, and dark CO₂ efflux in air. All these parameters were calculated according to the equations given in (30). Leaf area was measured with an electronic planimeter (model LI-3050A, Lambda Instruments).

**Measurement of the CO₂ and Light Compensation Points.** The CO₂ compensation point, Γ, was either measured by using a closed system and allowing the leaf to equilibrate with its CO₂ atmosphere, or by interpolation of a curve of ‘net CO₂ assimilation versus intercellular CO₂ partial pressure’ to zero assimilation. The gas exchange apparatus was modified by the inclusion of a closed system. A metal bellows pump (model MB-21E; Metal Bellows Corp., Sharon, MA) circulated the air through the system. Plug valves (model B-4P4T; Nupro Co., Cleveland, OH) were used to manually switch from open to closed system or vice versa. Both methods were compared in the same leaf of wheat at two O₂ concentrations (21% and 3%) yielding identical results (not shown).

When Γ was measured in closed system, its value was taken after 60 min in order to obtain steady-state values; then, the rate of dark CO₂ efflux was measured 30 min after the light was switched off.

The light compensation point was measured by interpolation of a curve of ‘net CO₂ assimilation versus quantum flux’ to zero assimilation. Light intensity was changed by interposing copper screens.

**Effect of a Period of Photosynthesis on the Rate of CO₂ Efflux in the Dark.** A pair of mature wheat leaves from the same plant was enclosed in the photosynthetic chamber and the rate of dark CO₂ efflux in ambient air was monitored for 2 h at the end of the night and after a period of photosynthesis of 6.25 h at ambient CO₂ and O₂ levels. Leaf temperatures were 13.5, 20, 24, 27, and 30°C in darkness. Leaf temperatures during the light period were 2 to 4°C higher than in the dark period. This experiment was repeated three times at every leaf temperature. A different plant was used every time.

In a similar experiment, the rate of dark CO₂ efflux was monitored for 1 h at the end of the night and after a period of photosynthesis of 6.25 h in which the O₂ concentration in the air in the last 20 min was 3%. In this experiment, temperature was kept constant in the light and in the dark. In the experiments performed at 30°C, the O₂ concentration in the dark period was 21% or 3%. Three replicates were done at every O₂ concentration, but no difference was found in the time course of dark CO₂ efflux after the light period (not shown). This experiment was also performed at 20°C in leaves selected from six plants, but the O₂ concentration in the dark period was 21%.

The results of these experiments are shown in Figures 1 and 2. The curves representing the time course of dark CO₂ efflux are averages of three or six individual curves. The statistical variation of the data was very small and it is not shown. The standard errors were less than 5% of the absolute values and ranged between 0.01 and 0.08 μmol CO₂·m⁻²·s⁻¹, the lower values being more common specially at lower temperatures.

**Relationship between Dark CO₂ Efflux and Leaf Carbohydrate Status.** Mature wheat leaves were allowed to photosynthesize for variable periods of time up to 7 h, at ambient and high (800 μbar) external CO₂ partial pressures; then, the rate of dark CO₂ efflux was measured 30 min after the termination of the photosynthetic period. Leaves were immediately killed in liquid N₂ and stored frozen for carbohydrate determination (see below). Leaf temperature was 21°C in darkness and 23.5°C in the light.

**Study of the Temperature Dependence of Dark CO₂ Efflux.** The rate of dark CO₂ efflux of mature wheat leaves selected at the end of the night was measured at different temperatures up to 40°C. The first measurement was made at 11°C. Other leaves were allowed to photosynthesize for 6.25 h at 22°C, at ambient CO₂ and O₂ levels. Then, dark CO₂ efflux was measured at 20°C, 30 min after the light was switched off; leaf temperature was steeply raised to 42°C in some experiments or decreased to about 8°C in other experiments.

**Carbohydrate Determination.** Leaves killed in liquid N₂ were freeze-dried. Carbohydrates were extracted in boiling water for 15 min and analyzed using an enzymic method. Free glucose plus fructose were measured from the leaf extract using a glucose-specific assay (Calbiochem-Behring Glucose s.v.r. No. 870104), after converting fructose to glucose with P-glucosidase (Sigma P-5381). Glucose was converted to glucose 6-P by hexokinase, and then oxidized by glucose 6-P dehydrogenase, reducing a molar equivalent of NADP. The change in A at 340 nm is proportional.
When the temperature of the atmosphere was lowered from 21% to 3% during the last 20 min of the light period and the rate of dark CO₂ efflux measured in 21% O₂, a different pattern was obtained (Fig. 2). The rate of dark CO₂ efflux was initially low and increased within 30 min to the level of leaves kept in 21% O₂ throughout the photosynthetic period. If the O₂ concentration during the measurement period in the dark was lowered to 3%, the result was the same.

These experiments suggest that the initially high rates of dark CO₂ efflux are in part due to O₂-dependent processes in the light period. Presumably, large pools of photorespiratory intermediates continue to be decarboxylated in the dark, for about 30 min at 20°C and 30°C.

The rate of dark CO₂ efflux 30 min after the termination of the photosynthetic period increased in proportion with the total net CO₂ assimilation which had occurred during this period (Fig. 3). Dark CO₂ efflux was also positively correlated with specific leaf weight which greatly increased during the light period due to the accumulation of products derived from photosynthesis, mostly carbohydrates (not shown). Dark CO₂ efflux was correlated with several leaf carbohydrate fractions (Fig. 4). The relationship between dark CO₂ efflux and fructosans was not investigated here.

The temperature dependence of dark CO₂ efflux at the end of the night period was compared with that of leaves after 6.25 h of photosynthesis in air. At the end of the night, dark CO₂ efflux showed an exponential relationship with temperature, with a single apparent activation energy, Eₐ, of 12.9 kcal·mol⁻¹ in the range from 11 to 40°C (Fig. 5). However, CO₂ efflux after a long period of photosynthesis presented a very different pattern in response to temperature. Its rate was higher at all temperatures, but Eₐ declined in the range from 20°C to 40°C while it increased in the range from 10°C to 20°C.

**RESULTS**

**Properties and Temperature Dependence of Dark CO₂ Efflux.** Dark CO₂ efflux measured after a period of photosynthesis was much higher than at the end of the preceding night period (Fig. 1). This effect occurred at all temperatures studied. However, the increase in total dark CO₂ efflux due to the effect of photosynthetic activity was relatively higher at lower temperatures (e.g. 20°C). At higher temperatures (e.g. 30°C), the rate of dark CO₂ efflux returned to the level at the end of the night within 2 h. At lower temperatures, it took longer (e.g. 5 h at 20°C). That is, the effect of the photosynthetic activity on dark CO₂ efflux was more accentuated and lasted longer at lower temperatures.

It was commonly found that the rate of dark CO₂ efflux was higher in the first 30 min after illumination and did not attain a steady slow rate of change until after about 60 min of darkness. When the O₂ concentration of the atmosphere was lowered from 21% to 3% during the last 20 min of the light period and the rate of dark CO₂ efflux measured in 21% O₂, a different pattern was obtained (Fig. 2). The rate of dark CO₂ efflux was initially low and increased within 30 min to the level of leaves kept in 21% O₂ throughout the photosynthetic period. If the O₂ concentration during the measurement period in the dark was lowered to 3%, the result was the same.

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**Relationship between Dark CO₂ Efflux, Carbohydrate Status, and CO₂ and Light Compensation Points.** Coincident with the increase in the rate of respiration following a period of photosynthesis, Γ in 21% O₂ also increased in the same leaf (Table I; Fig. 6). Interestingly, the values for Γ showed the largest increases after treatments in which photorespiration would have been least, but the rate of carbohydrate formation would have been maximal (800 μbar CO₂, 2% O₂). These correlations were confirmed in another set of experiments in which a leaf was initially illuminated for 4 h in air containing 750 μbar CO₂, 21% O₂ (low photorespiration), then in air containing low CO₂ pressures (slightly above Γ) for a second period of 4 h (high photorespiration). Table II shows again that Γ was higher after the period in which the rates of photorespiration were lower and the rates of carbohydrate formation were higher.

Measurements of Γ from many experiments in which dark CO₂
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Carbohydrate concentration (g glucose equiv m⁻²)

FIG. 4. Relationship between dark CO₂ efflux and several carbohydrate fractions in mature wheat leaves.

Leaf temperature (°C)

FIG. 5. Arrhenius plots for dark CO₂ efflux of mature wheat leaves selected at the end of the night period (○) or at the end of a period of photosynthesis of 6.25 h at ambient CO₂ and O₂ pressures (△). Apparent activation energies (Eₐ) are expressed in kcal·mol⁻¹. They can be converted to Q₁₀ values by using the formula 'log Q₁₀ = 2190.Eₐ/T₂/T₁', where T₁ − T₂ = 10 K.

efflux (Rₐ) was varied by varying CO₂ and O₂ partial pressures during the period of photosynthesis. The correlation between an increase in Ρ and Rₐ following a period of photosynthesis was also observed at temperatures other than 21°C (e.g., 15°C and 30°C) (data not shown) and in other species (Table III).

The increase in Ρ following a period of photosynthesis was reflected in a decrease in net rate of photosynthesis over a range of CO₂ partial pressures and was not due to a change in the slope of the curve of net CO₂ assimilation versus intercellular CO₂ partial pressure (Fig. 6). The displacement of this curve was 1.0 ± 0.2 μmol CO₂·m⁻²·s⁻¹, which is an average value obtained in four experiments including that shown in Figure 6. This value compared well with the increase in the rate of dark CO₂ efflux observed after a period of photosynthesis. The rate of CO₂ efflux into CO₂-free air in the light was also higher following a period of photosynthesis (Fig. 6).

The light compensation point also increased in the same leaf after a period of active photosynthesis. Figure 8 shows the correlation between the light compensation point and dark CO₂ efflux which was varied by the period of prior photosynthesis under different conditions of temperature and CO₂ partial pressure.
Table I. $\Gamma$ and Dark CO$_2$ Efflux, $R_a$. Measured at the End of the Night and after a Period of Photosynthesis of 5 Hours in the Same Leaf of Wheat.

$\Gamma$ was measured in closed system (see "Material and Methods"). $\Gamma$ and $R_a$ were measured in 21% O$_2$. Leaf temperature was 21°C in the light and in the dark. Net CO$_2$ assimilation rates were about 24 (A) and 30 (B) $\mu$mol CO$_2$·m$^{-2}$·s$^{-1}$. The values shown are means ± SE of three to four independent experiments.

<table>
<thead>
<tr>
<th>CO$_2$ and O$_2$ Levels during the Photosynthetic Period</th>
<th>At the End of the Night</th>
<th>After 5 h Light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Gamma$</td>
<td>$R_a$ ((\mu\text{bar}))</td>
</tr>
<tr>
<td>A. 370 $\mu$bar CO$_2$, 21% O$_2$</td>
<td>35 ± 1.5</td>
<td>0.65 ± 0.12</td>
</tr>
<tr>
<td>B. 800 $\mu$bar CO$_2$, 21% O$_2$ or 800 $\mu$bar CO$_2$, 2% O$_2$</td>
<td>36.5 ± 2.5</td>
<td>0.62 ± 0.03</td>
</tr>
</tbody>
</table>

Fig. 6. Curve of net CO$_2$ assimilation versus intercellular CO$_2$ partial pressure measured at the end of the night and after a period of photosynthesis of 3 h at 800 $\mu$bar CO$_2$ in the same leaf of wheat. Leaf temperature was 21°C. Measurements proceeded from high to low CO$_2$ partial pressures, as indicated by the arrows.

Table II. $\Gamma$ and $R_a$ of a Mature Wheat Leaf Measured after Two Consecutive Periods of Photosynthesis of 4 Hours at High and Low CO$_2$ Partial Pressures

O$_2$ concentration was 21%. The rates of net CO$_2$ assimilation during the first (A) and second (B) periods were about 23 and 2 $\mu$mol CO$_2$·m$^{-2}$·s$^{-1}$, respectively. Two independent experiments were performed. The rest of conditions were the same as in Table I.

<table>
<thead>
<tr>
<th>CO$_2$ Partial Pressure during the Photosynthetic Period</th>
<th>$\Gamma$</th>
<th>$R_a$ ((\mu\text{bar}))</th>
<th>$\Gamma$</th>
<th>$R_a$ ((\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 750 $\mu$bar CO$_2$</td>
<td>43 ± 1</td>
<td>1.48 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. 50–75 $\mu$bar CO$_2$</td>
<td>37 ± 2</td>
<td>0.80 ± 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Properties and Temperature Dependence of Dark CO$_2$ Efflux. Dark CO$_2$ efflux ($R_a$) of mature wheat leaves increased considerably after a long period of photosynthesis, as did that of tomato leaves (18). At least two groups of substrates contributed to the CO$_2$ efflux. Because 15% to 20% of the CO$_2$ evolved in the first 30 min of darkness was abolished if leaves were kept in low O$_2$ during the latter part of the photosynthetic period, we conclude that this CO$_2$ arose from photorespiratory substrates. In this sense, the levels of glycine measured in wheat leaves during the light period, 1.5 to 2 mmol·m$^{-2}$·h$^{-1}$ (M. Berger, personal communication), are high enough to sustain glycine decarboxylation in the dark for about 30 min at the rates observed in our experiments. This observation contrasts with the idea that the photorespiratory postillumination burst in leaves is restricted to the first 2 to 5 min in darkness, as commonly found in many species including wheat (11). However, this discrepancy may be explained by the short length of the preceding light period utilized in previous studies (often only a few min) compared to the present experiments. The remaining CO$_2$ efflux, was closely correlated with several carbohydrate fractions. This CO$_2$ presumably arose from tricarboxylic acid cycle and pentose phosphate pathway oxidation of carbohydrate derived substrates. A similar correlation between dark CO$_2$ efflux and carbohydrates has also been found in leaves of Cucumis sativa (8).

The linear relationship between the rate of photosynthesis and the subsequent rate of dark CO$_2$ efflux (see also Ref. 18) can be explained in terms of quantitative changes in carbohydrates, common metabolites to both processes.

The enhancement of leaf respiration by carbohydrates cannot be primarily related to growth requirements since mature leaves were used. Alternatively, excess respiration may be used for synthesis of compounds (e.g., amino acids) in the leaf which can be utilized for growth in other parts of the plant and for providing energy for transport of assimilates (15). However, when the rate of sugar export from the leaf is reduced by e.g., lowering sink demand, carbohydrates accumulate in this organ and the rate of
respiration increases (2, 14). This suggests that carbohydrate may be wastefully oxidized in some conditions in the absence of any apparent major requirement.

The rate of respiration extrapolated to a positive value at zero carbohydrate (Fig. 4), which may represent maintenance respiration (24). Respiration at the end of the night, when carbohydrate content of wheat leaves was very low, may be principally associated with maintenance processes. As other authors have found (6, 22), it increased exponentially with temperature. However, when carbohydrates accumulated in the leaf as a result of the photosynthetic activity, the rate of dark CO₂ efflux increased, and the shape of its temperature dependence changed dramatically, showing different activation energies (Eₐ) above and below 20°C. It is unlikely that this break in the Arrhenius plot for dark CO₂ efflux at high leaf sugar level could be attributed to membrane phase transitions (26) because mitochondrial respiration is presumably involved in both instances. The mechanism underlying this response may involve the effect of substrate concentration on the temperature dependence of enzymatic reactions. The apparent Eₐ of enzymatic reaction decreases at low substrates availability since the Kₘ of enzymes for their substrates generally increases with temperature (10). Therefore, a fixed substrate concentration could be saturating or limiting depending on temperature, and Eₐ should

be consequently affected. Considered as a multienzyme system, respiration could be saturated by substrates at low temperature after a period of photosynthesis, and its Eₐ should be very high. However, Eₐ would decline at higher temperatures as soon as substrates are present at concentrations close or below the Kₘ of key enzymes.

These explanations of the interaction between carbohydrate levels and temperature on respiratory CO₂ efflux assume that there is a direct regulation of respiration by substrate availability. The data suggest that glycolysis and mitochondrial reactions in leaves are not necessarily limited by the energy charge in a very narrow range, at least when substrate levels are low (cf. Beever, 4). A similar conclusion was reached by Saglio and Pradet (27) who have shown that O₂ uptake of maize root tips varied widely in response to sugars while the energy charge remained constant. However, these results do not exclude the possibility that respiration is regulated in a complex way. As will be reported later, the wheat leaves studied here show a large cyanide resistant component of respiration when carbohydrate levels are high (J. Azcón-Bieto, H. Lambers, and D. A. Day, unpublished). The Arrhenius plot of the cyanide-resistant respiration of wheat coleoptile mitochondria also shows a discontinuity at 17.5°C (20).

**Relationship between Dark CO₂ Efflux and the CO₂ and Light**

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**Table III. Rₐ and Rₐ of Mature Leaves of Several Plant Species Measured at the End of the Night and after a Period of Photosynthesis of 5 Hours**

<table>
<thead>
<tr>
<th>Species</th>
<th>CO₂ Pressures during the Photosynthetic Period</th>
<th>A</th>
<th>At the End of the Night</th>
<th>After 5 h Light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µbar</td>
<td>µmol CO₂·m⁻²·s⁻¹</td>
<td>µbar</td>
<td>µmol CO₂·m⁻²·s⁻¹</td>
</tr>
<tr>
<td>Eucalyptus gran-dis</td>
<td>340</td>
<td>13</td>
<td>45</td>
<td>0.07</td>
</tr>
<tr>
<td>Vicia faba</td>
<td>800</td>
<td>17</td>
<td>21</td>
<td>0.09</td>
</tr>
<tr>
<td>Lolium perenne</td>
<td>800</td>
<td>22</td>
<td>22</td>
<td>0.08</td>
</tr>
</tbody>
</table>

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**Fig. 7. Relationship between the CO₂ compensation point and dark CO₂ efflux of mature wheat leaves at 21°C.**

**Fig. 8. Relationship between the light compensation point and dark CO₂ efflux in mature wheat leaves. (●), Leaf temperature, 20°C; external CO₂ partial pressure, 330 µbar. (□), 30°C, 330 µbar CO₂. (△), 20°C, 640 µbar CO₂. (○), 30°C, 640 µbar CO₂.**

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**Dark CO₂ efflux, Rₐ (µmol m⁻² s⁻¹)**

\[ y = 30 + 9.4x \]

\[ r = 0.83 \]

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**Light compensation point (µmol m⁻² s⁻¹)**

\[ y = 13.4x \]

\[ r = 0.99 \]
Compensation Points. $\Gamma$ of mature leaves of wheat and other C₃ species varied during the photoperiod, its value being low at the end of the night period, but increasing during the day period. Similar changes of $\Gamma$ after periods of light or darkness have been reported in leaves of wheat (23) and Rumex acetosa (16). In contrast to these results, $\Gamma$ did not vary after prolonged exposure to darkness, leading to starvation in leaves of Nicotiana tabacum (13).

To investigate the nature of the changes in $\Gamma$ in wheat leaves, we varied the CO₂ and O₂ partial pressures in the atmosphere during the photosynthetic period to produce different rates of photorespiration and photosynthetic carbohydrate formation. We concluded that variations in $\Gamma$ during the photoperiod were principally related to processes other than photorespiration, and presumably were associated with respiration, because $\Gamma$ and $R_a$ increased maximally after periods in which the gas composition of the air favored high rates of photosynthetic carbohydrate formation and minimal rates of photorespiration (low O₂ and high CO₂ pressures). Conversely, $\Gamma$ and $R_a$ were lower following a period in which the rate of photorespiration was maximal and the rate of carbohydrate synthesis was reduced (low CO₂ and ambient O₂ pressures). This conclusion is further supported by the strong correlation found between $\Gamma$ and $R_a$ (Fig. 7). From this relationship, $\Gamma$ has a positive value when $R_a$ is zero, which presumably represents the photorespiratory component. Assuming an average value of 1 mmol CO₂·m⁻²·s⁻¹ (at 21°C) for $R_a$, we estimate that $R_a$ the CO₂ efflux due to respiration, contributes about 25% of the CO₂ efflux measured at $\Gamma$. This value for wheat leaves is similar to that found in L. perenne (3). However, the contribution of $R_a$ to $\Gamma$ is variable, and it is correlated with the carbohydrate level. This conclusion is consistent with the fact that externally added sugars increase the CO₂ compensation point and the rate of respiration of leaves (28, 29, and unpublished results).

The light compensation point of mature wheat leaves also increased during the day, being correlated with the rate of respiration. This relationship extrapolated to the origin suggesting that respiration is a major component of the light compensation point.

The rate of respiration in the light ($R_a$) can be estimated from the displacement on the curve of net CO₂ assimilation versus intercellular CO₂ partial pressure by varying the rate of respiration in the dark ($R_d$) through changes in the leaf carbohydrate concentration. It can be concluded that the rates of $R_d$ and $R_a$ are comparable in wheat leaves.

Peisker and Apel (23) also analyzed the responses of $\Gamma$, its O₂ dependence, and respiration in the dark in wheat leaves after a dark period and after an extended light period (18 h) at high CO₂ concentrations. They found that respiration increased following the extended period of photosynthesis, that the O₂ dependency of the CO₂ compensation point increased, but that the latter increased by only 30% of the value expected on the basis of their model. Our data confirm their observations, but the different analysis of our data does not support Peisker and Apel's (23) conclusion that respiration in the light is inhibited by 76%. Whether the expectations of their model, or technical discrepancies, are relevant to our different conclusions remains to be resolved.

Graham (12) reviewed the literature and concluded that glycolysis and tricarboxylic acid cycle can operate in illuminated green cells although some modifications probably occur in relation to the dark pattern. This is suggested by the increase in the malate/aspartate ratio and the different labeling patterns after administration of radioactive carbon compounds (e.g. CO₂, tricarboxylic acid cycle intermediates, amino acids, sugars) into citrate and other tricarboxylic acid cycle intermediates and related compounds, such as glutamate, glutamine, etc. (5, 12). The evidence is consistent with the suggestion that glycolysis and tricarboxylic acid cycle are modified in the light to allow a continuous anaplerotic carbon flow for supplying α-ketocids which the chloroplast is unable to make (17). These compounds can be used for a variety of synthetic reactions including amino acid and lipid formation. Important features of this anaplerotic flow are the probable operation of P-enolpyruvate carboxylase in the cytosol and malic enzyme and the mitochondrion to replenish carbon loss from the tricarboxylic acid cycle (1, 9). It is not known if the tricarboxylic acid cycle operates beyond succinate oxidation, and the operation of the mitochondrial electron chain in the light is a more uncertain aspect of the problem. If, however, respiration in leaves in the light is cyanide insensitive, control of electron transport via energy charge is likely to be less effective. The CO₂ arising from the above mentioned reactions (e.g. 1 mol CO₂ released/mmol of glutamine formed) could well be responsible for most of the rate of respiration in illuminated leaves observed in our experiments. Non-green cells in the leaf also contribute to $R_a$, but it is not known whether photosynthesis exerts the same influence on their respiratory metabolism as in green cells.

The effect of the photosynthetic activity on the rate of respiration in the light may be mediated by the supply of P-enolpyruvate from recently synthesized triose-P or from sugars. The latter alternative seems more unlikely inasmuch as the exogenous glucose is not metabolized through glycolysis in illuminated leaves including wheat (5, 12). High CO₂ concentration enhances the carbon traffic through the tricarboxylic acid cycle and related compounds, presumably by increasing the supply of substrates for P-enolpyruvate carboxylase (25). This may help to explain why some workers have failed to find significant CO₂ influx by respiration in illuminated leaves into CO₂-free air conditions (19). This also suggests that respiration in daytime ($R_a$) may be underestimated at the CO₂ compensation concentration.

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