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Differing substomatal and chloroplastic CO_2 concentrations in water-stressed wheat

Jean-Louis Renou[1,*], Alain Gerbaud[2], Daniel Just[1], and Marcel André[1]

[1] Service de Radioagronomie, Département de Biologie, CEN Cadarache, F-13108 Saint Paul Lez Durance Cedex, and
[2] Laboratoire d'Etude des Symbiotes des Racines, INRA, Place Viala, F-34000 Montpellier, France

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Abstract. Gas exchanges of wheat (*Triticum aestivum* L. cv. Courtot) shoots were measured before and during a water stress. While photosynthesis, transpiration and dark respiration decreased because of the stress, photorespiration increased initially, up to a maximum of 50% above its initial value. The CO_2 concentration in the intercellular space was calculated from gas-diffusion resistances, and remained approximately constant before and during the stress. On the other hand, the CO_2 concentration in the chloroplast, in the vicinity of Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), was evaluated from the ratio of CO_2 to O_2 uptake, using the known kinetic constants of the oxygenation and carboxylation reactions which compete for Rubisco. In the well-watered plants, the calculated chloroplastic concentration was slightly smaller than the substomatal concentration. During water stress, this concentration decreased while the substomatal CO_2 concentration remained constant. Hypotheses to explain this difference between substomatal and chloroplastic CO_2 concentrations are discussed.

Key words: Carbon dioxide (internal concentration) – Gas exchange (CO_2, $^{18}O_2$) – Mesophyll resistance (CO_2 diffusion) – Photorespiration – Stomata – *Triticum* (water stress) – Water stress

Introduction

Carbon dioxide is one of the main factors limiting the growth of plants. Although its ambiant concentration is very stable, the concentration inside plants depends on stomatal aperture and may vary with conditions, thereby affecting plant growth. The internal CO_2 concentration is thus an important parameter to study. However, only the external concentration can be measured directly.

The most frequently used method for the estimation of internal CO_2 is based on the application of Fick's Law for gas diffusion to the exchange of CO_2 and water vapor between the plant and the atmosphere (Jarvis 1971; Jarman 1974). The validity of this method has been confirmed by several authors who estimated the mean internal CO_2 concentration inside leaves by using two-sided gas-exchange chambers and recycling the gas flow through one of the chambers until the CO_2 concentration was equilibrated with that of the leaf (Sharkey et al. 1982; Mott and O'Leary 1984; Parkhurst et al. 1988).

Farquhar et al. (1982) and Evans et al. (1986) have determined the CO_2 concentration in the vicinity of Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) from a model of carbon-isotope discrimination. The concentrations estimated were lower than those estimated by the diffusion model. The difference was interpreted as being caused by the existence of a mesophyll resistance to CO_2 diffusion between the air spaces and the chloroplasts.

There has been controversy over the evolution of internal CO_2 during water stress (Kriedemann and Downton 1981). Some authors ascribed the decrease of CO_2 assimilation during stress to stomatal closure leading to a decrease in the internal CO_2 level, while others found that the internal CO_2 level was stable, and that the decrease of CO_2 assimilation had other causes, among them changes in chloroplast ultrastructure and inhibition of the photosynthetic electron-transport capacity (Polyakoff-Mayber 1981). The latter opinion has recently gained support from experiments showing that in a variety of natural conditions the stomata act only to regulate the internal CO_2 to an approximately constant level (Tenhunen et al. 1984; Wong 1985a, b, c; Raschke and Resemann 1986; Downton et al. 1987).

However, these results seemed inconsistent with the increase of photorespiration relative to photosynthesis, which has been observed in several plants by Krampitz et al. (1984) and by Thomas and André (1982, 1987).

* To whom correspondence should be addressed

Abbreviations: Rubisco = Ribulose-1,5-bisphosphate carboxylase/oxygenase, EC 4.1.1.39

According to Farquhar et al. (1980), the ratio of oxygenase to carboxylase activities of the Rubisco molecule depends only on the O_2/CO_2 concentration ratio at the enzyme. Hence, we thought it should be possible to evaluate the chloroplastic CO_2 concentration from the measurement of photorespiratory oxygen uptake. The use of the ^{18}O isotope of oxygen, measured with a mass spectrometer, allows the determination of the O_2-uptake flux. In this study, we have compared the two evaluations of the internal CO_2 level, calculated either from the gas-diffusion or from the Rubisco model, in the case of water-stressed wheat.

Material and methods

Seeds of wheat (*Triticum vulgare* L. cv. Courtot; INRA) were germinated on sterile sand with deionized water. Seedlings were transferred to pots where the roots were immersed in an aerated Arnon-Hoagland nutrient solution. They were grown in a controlled-environment growth cabinet as described by André et al. (1979). The environmental conditions in the cabinet were: irradiance 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400–700 nm), photoperiod (light/dark) 14/10 h, thermoperiod 22/17° C with a constant dew point of 14° C. Two months after sowing, the plants were placed in an assimilation chamber (Gerbaud and André 1979) and the gas exchanges of the shoots measured. The external CO_2 concentration was regulated at 340 (± 10) $\mu l \cdot l^{-1}$ and O_2 concentration at 21% ($\pm 1\%$). Photosynthesis and respiration were monitored by a computer. The methods and calculations used were as described by André et al. (1979). Transpiration water was condensed by the air-cooling system and weighed. Photorespiration was measured through the decrease in the concentration of the $^{18}O^{18}O$ isotope of O_2 compared with that of an inert reference gas (neon) and was obtained by the equation (Gerbaud and André 1979) (Cv = chamber volume):

$$U_0 = Cv[^{16}O_2]\frac{d}{dt}\text{Log}\frac{[^{18}O_2]}{[Ne]} \qquad \text{(Eq. (1)}$$

Plants were water-stressed by adding 250 $g \cdot l^{-1}$ of polyethyleneglycol 6000 (PEG) to the nutrient solution, giving a water potential of about -12 bar (-12200 hPa), according to Steuter et al. (1981).

Evalution of the substomatal CO_2 concentration. For calculation of the substomatal CO_2 concentration, we used the classical method described by Jarvis (1971). In the first step, the stomatal resistance to water-vapor diffusion is calculated by Fick's Law relating the rate of evaporation to the water-vapour pressure gradient. The internal water-vapour pressure is calculated from the leaf temperature (approximated by air temperature), assuming that the internal air space is vapour-saturated. In the second step, the CO_2 concentration gradient is calculated by multiplying the net CO_2-exchange rate by the CO_2 diffusion resistance, assumed to be 0.625 times the resistance for water vapour.

The temperature of the leaves was assumed to be equal to ambient air temperature. Because many leaves are involved in the gas exchange of a single wheat plant, it would not have been feasible to measure the temperature of even a few leaves in the small, closed chambers without disturbing the plant.

Evaluation of the chloroplastic CO_2 concentration. The oxygenation and carboxylation of ribulose-1,5-biphosphate (RuBP) is catalysed competitively by Rubisco (Laing et al. 1974). The carboxylation of RuBP constitutes the gross CO_2 photosynthesis, whereas RuBP oxygenation by 1 mol O_2 produces phosphoglycolate entering the glycolate pathway, leading to the further uptake of 0.5 mol O_2 and the evolution of 0.5 mol CO_2 (Fig. 1). If we call vc the rate of carboxylation catalysed by Rubisco, vo the oxygenation rate, P the net light-dependent CO_2-uptake rate of the shoot (photosynthesis) and U_0 the total light-dependent O_2 uptake (photorespiration), we may write the following equations:

$$P = vc - 0.5\,vo - Rl \qquad \text{Eq. (2)}$$

$$U_0 = vo + 0.5\,vo + Rl \qquad \text{Eq. (3)}$$

where Rl denotes either the O_2 uptake or the CO_2 evolution resulting from mitochondrial respiration, taken as being equal. It has been assumed in the calculation that there is no Mehler reaction (direct O_2 reduction in the chloroplasts). This assumption will be discussed later. Equations 2 and 3 give:

$$vc = P + 1/3\,U_0 + 2/3\,Rl \qquad \text{Eq. (4)}$$

$$vo = 2/3\,U_0 - 2/3\,Rl \qquad \text{Eq. (5)}$$

As Rl is not known, calculations of the chloroplastic CO_2 concentration have been made using two different assumptions: $Rl = 0$ and $Rl = Rn$.

On the other hand it has been shown with purified Rubisco that the ratio of carboxylation to oxidation depends only on and is proportional to the ratio of O_2 to CO_2 concentrations at the Rubisco site (Laing et al. 1974):

$$vc/vo = Sf[CO_2]/[O_2] \qquad \text{Eq. (6)}$$

where Sf is the specificity factor of the enzyme. Specificity factors have been determined by Jordan and Ogren (1984) relative to O_2 and CO_2 concentrations in solution. The internal O_2 partial pres-

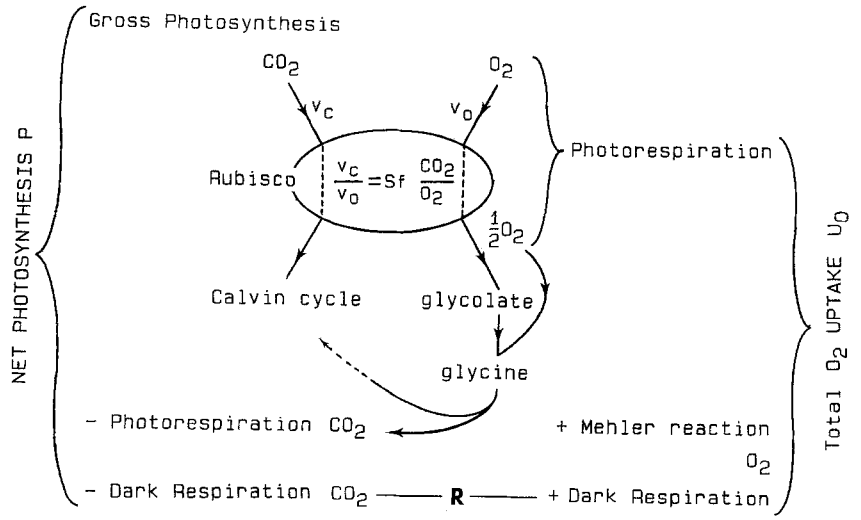

Fig. 1. The model and notations used for the calculation of chloroplastic CO_2 concentrations. vo, vc = rates of oxygenation and carboxylation, respectively, catalysed by Rubisco; R = mitochondrial respiration; sf = Rubisco specificity factor

sure can be taken as equal to 21% (Gerbaud and André 1987). A conversion factor has to be applied when gas partial pressures are entered in the formula, because of the differential solubilities of O_2 and CO_2 (Ku and Edwards 1977), so we come to the final equation:

$$vc/vo = 0.0139\,[CO_2] \quad \text{or} \quad [CO_2] = 72\,vc/vo \qquad \text{Eq. (7)}$$

with $[CO_2]$ in $\mu l \cdot l^{-1}$. As vc and vo can be calculated from gas exchanges (Eqs. 4 and 5), this equation allows us to calculate the concentration of $[CO_2]$ inside chloroplasts. Brooks and Farquhar (1985) have demonstrated that the coefficient in the equation, here 72, is equal to two times Γ^*, the $[CO_2]$ concentration at which photorespiration equilibrates with carboxylation. Their measurements would give a value of 75 for $2 \cdot \Gamma^*$ at 22° C, nearly confirming our calculation.

Possible errors owing to leaf-temperature uncertainty. Leaf temperature was not measured in the experiments presented. In another experiment, under similar conditions, leaf and air temperatures were measured with a small (4 mm^2) thermoresistance. It was found that temperatures of wheat leaves were 0.5–1° C higher than the surrounding air temperature in a well-watered plant, and that this difference could rise to a maximum of 1.5° C after a severe water stress. We estimated the effect of approximating leaf temperature by using air temperature on the results of our calculations: (i) An error in leaf temperature affects the calculation of substomatal CO_2 (8 $\mu l \cdot l^{-1}$ per °C), but the main point here is the constancy of the concentration during stress, which is confirmed by several authors (Sharkey et al. 1982; Mott and O'Leary 1984; Wong et al. 1985c). (ii) Temperature influences the specificity factor of Rubisco: 1° C of leaf-temperature underestimation leads to an underestimation of 6 $\mu l \cdot l^{-1}$ of the chloroplastic CO_2 concentration. It appears that these errors are negligible compared with the effects observed.

Results

Figure 2 shows the daily averages for net photosynthesis (P), photorespiration (U_0), night respiration (Rn) and transpiration (T) for two experiments. During the period before the water stress, P, U_0, Rn and T slowly increased as a result of plant growth. Upon application of the water stress, P and T decreased by 70% in 2 d, but Rn decreased by only 10%; U_0 increased initially up to a maximum of 50% above its initial value, then it decreased. During treatment, leaves progressively withered, rolled themselves and turned yellow. During the first day of stress the sum $P + U_0$ remained almost constant, which means that the amount of reducing equivalents produced by the photosystems and used for CO_2 and O_2 reduction remained almost constant.

Substomatal and chloroplastic CO_2 concentrations were calculated as explained above, assuming either that dark respiration was completely inhibited during the day, or that it was the same as during the night (Fig. 3). Using the latter, continuing-respiration hypothesis, the chloroplastic CO_2 concentration was calculated to be almost equal to the substomatal CO_2 concentration, whereas using the zero-respiration hypothesis it was 130 $\mu l \cdot l^{-1}$ lower. After stress, however, the calculated chloroplastic CO_2 concentration decreased to about 100 $\mu l \cdot l^{-1}$ regardless of the assumption for dark respiration, while the substomatal CO_2 concentration remained nearly constant.

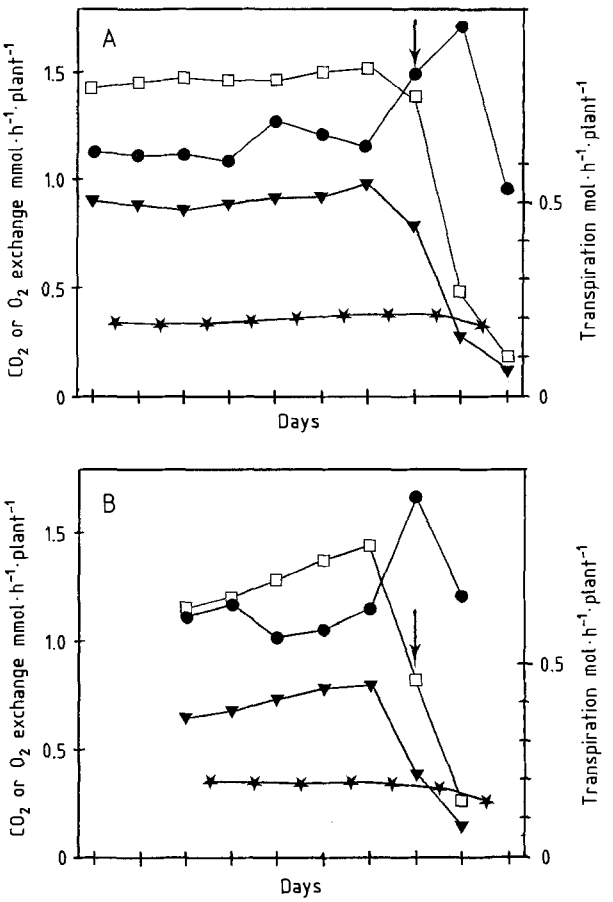

Fig. 2A, B. Daily course of gas exchange of a wheat plant before and after the application of water stress. In experiment **A** Polyethyleneglycol (PEG) treatment of the nutrient solution started 4 h after the beginning of the daily light period. In experiment **B**, PEG treatment started just after the beginning of the light period. □—□, Net photosynthesis; ●—●, O_2 uptake; ▼—▼, transpiration; ★—★, night respiration. The *arrow* indicates the start of the stress period

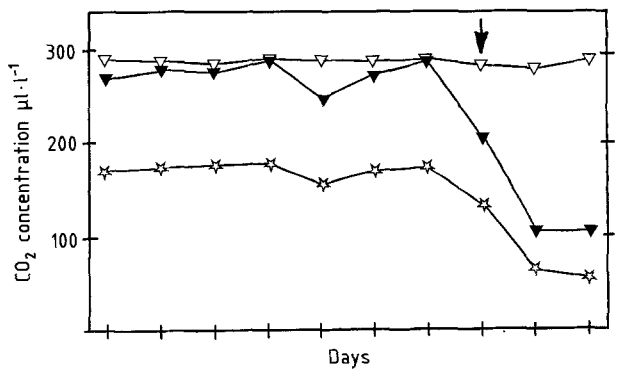

Fig. 3. The internal CO_2 concentration of a wheat plant calculated by three methods for experiment A of Fig. 2 (experiment B would give similar results). ▽—▽, Substomatal CO_2 concentration calculated by the gas-diffusion model; ▼—▼, chloroplastic CO_2 concentration calculated from the model of Rubisco kinetics with the assumption of continuing dark respiration; ☆—☆, chloroplastic CO_2 concentration calculated by the Rubisco-kinetics model with the assumption of nul dark respiration. The *arrow* indicates the start of the stress period

Discussion

Precise measurement of the exchange of gases by wheat leaves during water stress indicates that photorespiration is stimulated while photosynthesis is inhibited. According to the model of Farquhar et al. (1980), we interpret this to be caused by a decrease in the internal CO_2 concentration, which we calculate to be as low as 100 $\mu l \cdot l^{-1}$. By contrast, the substomatal CO_2 concentration calculated from water-vapor exchange remained constant at 290 $\mu l \cdot l^{-1}$. The following criticisms of the calculations should be considered:

(i) The stability of Rubisco activities during water stress could be questioned; however, Rubisco appears to be much less sensitive to water stress than other components of the photosynthetic apparatus (Kriedemann and Downton 1981).

(ii) An increase of mitochondrial respiration during the stress could explain the increased total oxygen uptake, as well as the supposed decrease in CO_2 concentration. However, a fourfold increase in the rate of dark respiration would be necessary to explain the observed increase in O_2 uptake. This does not seem likely as, on the contrary, night respiration decreases after the stress (Fig. 2).

(iii) The possibility of the occurrence of the Mehler reaction, i.e. the direct transfer of electrons to oxygen at the reducing end of photosystem I, has not been taken into account in our model. According to the review of Badger (1985), the Mehler reaction has been shown to occur in C_3 chloroplasts and cells, but its existence in intact leaves is still hypothetical. Cornic et al. (1989) have shown that it can be active in water-stressed leaves in which photosynthesis is almost zero. The Mehler reaction probably occurs mainly when electron transport is acceptor-limited, which could be the case during water stress, so it would increase in parallel with photorespiration when the internal $[CO_2]$ decreases. In that situation the occurrence of the Mehler reaction would not substantially bias our calculation of chloroplastic CO_2 concentration.

(iv) It has been known for some time that stomata may be unequally open in leaves treated with abscisic acid (Laisk 1983; Downton et al. 1988; Terashima et al. 1988) or submitted to water stress (Sharkey and Seemann 1989), causing zones of the leaves to have differing internal CO_2 concentrations. When this occurs, the calculations of substomatal CO_2 concentrations are misleading: leaf regions under closed stomata are as if "dead" with respect to transpiration and CO_2 fixation, but they would have an active photorespiration, the greater so because the internal CO_2 is at the compensation point. The substomatal CO_2 concentration that we calculated corresponds to the parts of the leaves which are active with respect to water and CO_2 exchange, whereas the O_2 uptake includes all the leaf area. It follows that observed excess of O_2 uptake over and above what would be expected from the Rubisco kinetics applied to the substomatal CO_2 concentration could be caused by the occurrence of photorespiration and- or the Mehler reaction in those leaf regions under closed stomata.

Although stomatal patchiness has not been demonstrated in wheat leaves, there are arguments in favor of its occurrence here: during moderate water stress, photosynthetic electron transport and the Calvin cycle have been reported to be unaffected, as CO_2 assimilation can be restored by high CO_2 levels (Cornic et al. 1989; Kaiser 1987). This appears to be contradictory to the approximate constancy of the apparent sub-stomatal CO_2 concentration. Patchiness is one explanation for the decrease of photosynthesis (Downton et al. 1988).

Another hypothesis is the existence of an increased mesophyll resistance that could be caused by cell collapse in water-stressed leaves, as has been recently suggested (Cornic et al. 1989). The mean chloroplastic CO_2 concentration required to explain the observed O_2 uptake on the second day of stress is around 100 $\mu l \cdot l^{-1}$. At the same time, the apparent substomatal CO_2 concentration remains constant aroung 290 $\mu l \cdot l^{-1}$, for an external CO_2 concentration of 340 $\mu l \cdot l^{-1}$ CO_2. Interpreted in terms of uniform resistances, such a gradient of CO_2 would indicate a mesophyll resistance nearly four times higher than the stomatal resistance, corresponding to a CO_2 gradient of 190 $\mu l \cdot l^{-1}$ from the intercellular space to the chloroplasts, against 50 $\mu l \cdot l^{-1}$ across the stomata.

In any case the decrease in photosynthesis and the increase of O_2 uptake during the stress can be attributed to the decreased chloroplastic CO_2 concentration, although it cannot be ascertained whether this decrease is homogeneous or not.

An increase of the O_2 uptake/photosynthesis ratio, without an absolute increase of photorespiration (U_0), has been reported during water stress in soja and Crassulacean acid metabolism (CAM) plants (Thomas and André 1982, 1987). This phenomenon was not explained because the internal CO_2, as calculated by the stomatal-diffusion model (Wong et al. 1985c), was supposed to be constant. The replacement of CO_2 uptake by O_2 uptake in the case of CO_2 limitation (mirror effect) had been observed (Gerbaud and André 1980), but it was not known whether this was only a laboratory artifact or if CO_2 limitation could also occur in natural conditions. The demonstration that it is actually so gives considerable importance to the mirror effect and confirms speculations about the protective role of photorespiration in stress situations (André 1986).

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