

Methodology
to study g_m

MEASUREMENT OF MESOPHYLL CONDUCTANCE BY MEANS OF GAS-EXCHANGE IN COMBINATION WITH CHLOROPHYLL FLUORESCENCE: EVALUATION OF POTENTIAL ERRORS.

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Abstract

The combination of gas-exchange measured on a leaf with yield measurements by means of chlorophyll fluorescence is a widely used approach for the measurement of mesophyll conductance for CO₂ transport in leaves (g_m). Two variants of this approach exist: the constant J and the variable J methods. In the constant J method, gas-exchange is measured across a range of CO₂ concentrations where electron transport (J) is constant as verified with chlorophyll fluorescence. The decrease in net CO₂ assimilation (A_n) with decreasing CO₂ concentration as caused by increasing proportion of photorespiration is used for an estimate of the chloroplastic CO₂ concentration (C_c), which in combination with the intercellular CO₂ concentration (C_i) gives an estimate of g_m . In the variable J method, gas-exchange and electron transport by chlorophyll fluorescence (J_F) are measured under the conditions of interest. C_c is calculated from the proportion of photorespiration and g_m is calculated from A_n , C_i and C_c . Both methods require an estimate of the CO₂ compensation point of Rubisco in the chloroplast (Γ^*) and an estimate of other decarboxylation processes than photorespiration ongoing during photosynthesis in the light (R_{light}). As has been pointed out by several authors, both methods are sensitive to measurement errors and model assumptions. Errors in the calculation of g_m can be due to errors in 1) the measurement of gas-exchange, 2) the measurement of chlorophyll fluorescence, 3) model parameter values and in 4) underlying model assumptions.

Gas-exchange measurements are necessarily carried out on a small piece of leaf because chlorophyll fluorescence can not be easily done on larger areas. Instrument noise is typically larger when measuring small leaf area. More seriously, border effects can cause systematic errors when a small area of a larger leaf is enclosed in a leaf chamber by clamping gaskets onto the leaf surface. These are due to ongoing respiration under the gasket and escape of CO₂ into the chamber, transport of CO₂ through intercellular spaces in homobaric leaves, and diffusion of CO₂ and H₂O along the leaf -

gasket interface. The magnitude of these errors is in a range that significant effects on g_m calculations can be expected, and corrections are difficult to make.

From measurements of yield from chlorophyll fluorescence, irradiance and absorptance, J_F can be calculated. However, there can be several reasons for a discrepancy between J_F and electron transport calculated from gas-exchange (J_A). J_F measurements are thus best considered as relative electron transport rates. The two need to be matched for the g_m calculations in the variable J method. This matching is typically done at low O_2 , assuming that the partitioning of total J between electron acceptors is independent of O_2 concentration. With the constant J method it is assumed that the partitioning is independent of CO_2 concentration.

An evaluation of sources of error is given on the basis of measurements carried out on leaves of *Arabidopsis thaliana*. Both methods were used to estimate g_m on the same 6 leaves. The variable J method gave a somewhat higher value for g_m compared to the constant J method. The variable J method became inaccurate at high $[CO_2]$ where the proportion of photorespiration is low. The means of the gas-exchange and fluorescence measurements were used to evaluate the effect of measurement error at ambient $[CO_2]$ and variation in model parameter values on g_m . The sensitivity to error of the constant J method was about half that of the variable J method. The largest change in the calculations was caused by realistic changes in Γ^* . That was not surprising, because Γ^* determines the proportion of photorespiration, but it causes concern because the measurement of Γ^* is complicated and there is evidence for species specific variation in this key parameter of Rubisco. A good estimate of R_{light} is also important, since an overestimation by using measured apparent CO_2 release in the dark resulted in a substantial overestimation of g_m . Effects of the gasket and the reduction of decarboxylation in the light should be taken into account. The same is true for A_n . Here an additional error is introduced when the band broadening caused by O_2 in the IRGA is not taken into account. Realistic errors in R_{light} and A_n caused changes in g_m of about 15% (constant J) and 35% (variable J). Similar changes in g_m were caused by small variations (2%) in J_F , which could be caused by instrument error or a small change in partitioning to electron acceptors at different $[O_2]$ and $[CO_2]$. The effect of changes in C_i as caused by possible errors in the measurement of leaf temperature or transpiration rate were small.

It is concluded that both methods based on a combination of gas-exchange with chlorophyll fluorescence ask for the highest possible accuracy of particularly the gas-exchange data. Instrument noise should be reduced to a minimum, and systematic errors

be avoided by applying appropriate corrections. Given the high sensitivity of the calculation of g_m for variation in Γ^* , a good estimate of this parameter remains crucial.

ISOTOPIC METHODS FOR g_m MEASUREMENTS

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Abstract

Mesophyll conductance (g_m) induces a decrease on CO₂ concentration from the intercellular spaces (C_i) to the chloroplast (C_c). For a long time, g_m was considered infinite and C_i equal to C_c . It is now well known that g_m is not only finite but also quite variable. There are several methods presently available to measure mesophyll conductance (g_m). A combination of gas exchange measurements with fluorescence or carbon isotope fractionation are the most frequently used (Warren, 2006).

The most common stable isotopes of carbon in carbon dioxide are ¹²CO₂ and ¹³CO₂ with the former being the most abundant. Due to their different physical properties, ¹³CO₂ diffuses and reacts more slowly than ¹²CO₂, causing “isotopic discrimination” (Farquhar et al., 1982). On-line carbon isotope fractionation, introduced by Evans et al. (1986), measures the change in carbon isotopic composition of CO₂ passing over the leaf. Mesophyll conductance can be measured from the differential fractionation between the theoretical fractionation expected if g_m would be infinite (Δ_i) and the measured fractionation (Δ_{leaf}):

$$\Delta_i - \Delta_{leaf} = \frac{A_N(b - a_i)}{g_m C_a} + \frac{(eR_d / k + f\Gamma^*)}{C_a}$$

There are two methods to determine g_m with carbon isotopic discrimination. The slope of the relationship between A_N/C_a and $(\Delta_i - \Delta_{leaf})$ would give an estimation of $1/g_m$ (Evans et al., 1986). This method assumes that g_m is constant under the different light or CO₂ conditions induced to change A_N/C_a . Recent data shows that g_m can be quite variable under these conditions (Flexas et al., 2008). On the other hand, g_m can be directly calculated by ignoring the respiration and photorespiration term (Evans et al., 1986).

$$g_m = \frac{A_N(b - a_i)}{C_a(\Delta_i - \Delta_{leaf})}$$

There are several methods to measure the isotopic composition of carbon in CO₂ with the Isotope Ratio Mass Spectrometry (IRMS) being the most frequently used under both continuous flow (CF-IRMS) and dual-inlet (DI-IRMS) while Tunable-Diode Absorption Laser Spectrometry (TDLAS), introduced by Becker et al. (1992), is increasingly being used for carbon isotope composition analysis (Bowling et al., 2003).

This presentation aims to open a discussion on a) the validity of the slope method when considering the possibilities that g_m might change under the different established conditions needed to create the slope and b) the limits of isotopic uncertainty and their effects on the calculation of mesophyll conductance.

The precision of measurements for $^{13}\delta$ depends on the method used. Dual-inlet IRMS presents the lowest standard deviation, ranging from 0.01‰ to 0.03‰ while continuous-flow IRMS and Tunable-Diode Absorption Laser Spectrometry give a SD not lower than 0.2‰. The uncertainty in the isotopic composition of $^{13}\text{CO}_2$ is translated in errors in the measurement of g_m .

Analysis of g_m on *Arabidopsis thaliana* combined gas exchange measurements performed in a 2 cm² cuvette (LI-64000) with a fluorescence chamber (LI-6400-40) and an off-line system where the entering and outgoing gas were collected and further analyzed in a dual-inlet IRMS. With a photosynthesis rate of 12 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and a stomatal conductance of 0.280 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, the draw-down in CO₂ concentration was of 18.2 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air. The precision (standard deviation) of the dual-inlet system was of 0.02‰. The value of g_m obtained from these results was 0.100 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ with an uncertainty of 0.005 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ or a 5% error. Had these measurements being made with a CF-IRMS or a TDALS with an associated error of 0.20‰, the deviation of the calculation of g_m would have been of 0.075 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ or a 75% error.

A sensitivity analysis has been performed where the different standard deviations of measurements are converted into deviations of the calculated g_m in different tissues and total CO₂ draw-dawns. The results of this analysis will be presented and discussion about technical details and errors will be encouraged.

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THE APPLICATION OF CARBON ISOTOPES IN RECENTLY ASSIMILATED CARBOHYDRATES TO ESTIMATE MESOPHYLL CONDUCTANCE IN ECOPHYSIOLOGICAL STUDIES

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Abstract

In nature, photosynthesis is dynamically regulated to respond to changing environmental conditions. Irradiance, temperature, air humidity and soil water availability are subjected to frequent changes in the short-term. In addition, global climate change is causing a long-term increase in temperature and exacerbating drought in large regions of the Earth. Regulation of photosynthesis in such conditions may involve diffusive and biochemical limitations. However, it is now recognized that often, stress-induced changes in photosynthesis involve mostly regulatory processes rather than “damage”.

One of such regulatory mechanisms involves mesophyll conductance (g_m) and its acclimation during development or in response to changes in environmental conditions. Carbon isotope discrimination has proven to be one of the most reliable methods to estimate g_m , and it has been widely applied to a large number of species and environmental conditions. We have developed a method based on measurements of the C isotopic discrimination (Δ) in leaf sucrose and, therefore, reflecting g_m during a day or few hours before sampling, in contrast to the on-line Δ measurements, providing an almost instantaneous time scale.

The aim of the present work was to compare g_m in different species growing under a wide range of environmental conditions to understand the extent of mesophyll limitations to photosynthesis and its variations induced for example, by drought, salinity and high irradiance.

The results obtained demonstrated that the foliar sugar Δ method provides reliable results very close to those obtained using the most common on-line measurements. It is also showed that photosynthetic response to “stresses” implies

dynamic variations in mesophyll conductance, even during short-term stress imposition of one or two days.

The ecophysiological significance of such variations in mesophyll conductance and their consequences on photosynthetic assimilation and on water use efficiency are discussed.

ON THE EFFECT OF HEAVY WATER (D₂O) ON CARBON ISOTOPE FRACTIONATION IN PHOTOSYNTHESIS

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Abstract

Although it is a key aspect of leaf photosynthesis, internal conductance to carbon dioxide is still not well understood. It is thought that it comprises two components, namely, a gas phase component (diffusion from intercellular spaces to cell walls) and a liquid phase component (dissolution, diffusion in water, hydration equilibrium). Here we use heavy water (D₂O), which is known to slow down CO₂ hydration by a factor of nearly 3. Using ¹²C/¹³C stable isotope techniques and *Xanthium strumarium* leaves, we show that the on-line carbon isotope discrimination ($\Delta^{13}\text{C}$, or Δ_{obs}) associated with photosynthesis is not significantly decreased by heavy water, and that the internal conductance, estimated with relationships involving the deviation of $\Delta^{13}\text{C}$, decreased by 8-40% in 21% O₂. It is concluded that in typical conditions, the CO₂-hydration equilibrium does not exert an effect on CO₂ assimilation larger than 9%. The carbon isotope discrimination associated with CO₂ addition to ribulose-1,5,bisphosphate by Rubisco is slightly decreased by heavy water. This effect is proposed to originate from the use of solvent-derived proton/deuteron during the last step of the catalytic cycle of the enzyme (hydration/cleavage).

USING HIGH-FREQUENCY STABLE ISOTOPE LASER SPECTROMETRY TO INVESTIGATE THE INFLUENCE OF DROUGHT ON THE MESOPHYLL CONDUCTANCE TO CO₂

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Abstract

The recent development of continuous, high-frequency (10 Hz) laser spectrometry offers a unique opportunity to measure continuously carbon isotope discrimination at the leaf level. With the possibility to measure stable isotopes continuously at high time resolution I aim in my PhD project to measure mesophyll conductance during transitional stages of drought and identify its environmental controls. The experimental setup and the applied laser spectrometers will be introduced here.

In this study we use a Quantum Cascade Laser Spectrometer (Aerodyne Research Inc.) for the simultaneous measurement of CO₂ isotopologues [¹⁶O¹²C¹⁶O; ¹⁸O¹²C¹⁶O; ¹⁶O¹³C¹⁶O] as well as an Off-Axis Integrated Cavity Output Laser Spectrometer (Los Gatos Research Inc.) for the measurement of isotopologues in water vapour [¹H¹⁶O¹H; ²H¹⁶O¹H; ¹H¹⁸O¹H]. The entire experiment will be carried out in the laboratory under defined light, humidity, and temperature conditions [climate chambers], measuring a total of six European beech saplings (*Fagus sylvatica* L.; 3 treatment / 3 control) in alternating intervals continuously over a 20 day period with progressing drought. For that, the laser spectrometers will be coupled to self-designed open flow-through chambers which enclose the entire canopy of the plants. Concurrently, air, soil, and leaf temperature as well as relative humidity and soil moisture will be monitored at each flow-through chamber. In addition, we intend to perform conventional gas-exchange measurements (LI-6400, LI-COR Biosciences Inc.) and fluorescence measurements to observe plant ecophysiological response in terms of maximal rate of carboxylation and PSII electron transport.

In general, the results of this project intend to provide process understanding on the response of ecosystems to drought. If we understand these responses mechanistically we are able to implement this knowledge into climate models helping to ensure an accurate prediction of future climate scenarios.

INVESTIGATING INTRINSIC WATER-USE-EFFICIENCY IN TREE SPECIES WITH COMBINED GAS EXCHANGE AND ON-LINE ^{13}C ISOTOPE DISCRIMINATION WITH A TUNABLE DIODE LASER: SYSTEM DESCRIPTION AND FIRST RESULTS.

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Abstract

Large inter and intra-specific differences of $\Delta^{13}\text{C}$ have been recorded in several tree species (poplars, oaks, chestnuts, ...) and found to be in many cases under tight genetic control. There is a demonstrated correlation between intrinsic water use efficiency (i.e., the ratio net CO_2 assimilation to stomatal conductance, W_i) and $\Delta^{13}\text{C}$ as predicted by the model developed by Farquhar and Richards (1984). Nevertheless, the model depends on a range of physiological functions that might differ among genotypes, like for instance internal conductance to CO_2 . As a result, there is a need to confirm that genetic variability of $\Delta^{13}\text{C}$ fully reflects that of W_i without confounding effects of a potential variability in model parameters. We therefore are currently developing a measurement technique combining gas exchange measurements with a custom made whole-leaf chamber and on-line ^{13}C discrimination measurements with a tunable diode laser method recording concurrently the concentration of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ at the inlet and outlet of the measurement chamber. The technique allows repeated measurements without the inconvenience of gas sampling and measurements with an IRMS. This system would allow us to estimate internal conductance by discrimination against $^{13}\text{CO}_2$ as described by Evans et al. in 1996. The talk will present the measurement system as well as the first results obtained with poplars.

Mechanisms
regulating g_m

CO₂ TRANSFER IN THE MESOPHYLL: PHYSICAL AND BIOCHEMICAL COMPONENTS

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Abstract

By combining various assays using carbonic anhydrase (CA) and Rubisco to probe CO₂ transfer *in vivo*, we attempted to unravel the determinants of CO₂ transfer in the mesophyll of various C₃ plants. Firstly, the contribution of the gaseous phase diffusion in the intercellular air spaces versus transfer into the liquid phase of the cells to CA and Rubisco was investigated. Further, by using *Arabidopsis thaliana* mutants depleted in the main CA isoforms (see abstract by Reiter & *al.*), the role of CA activity in CO₂ transfer in the liquid phase has been addressed.

CHLOROPLAST SIZE AND LOCATION EFFECTS ON MESOPHYLL CONDUCTANCE

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Abstract

This talk will cover three major issues: 1. How do we determine what limits photosynthesis when fitting models to data, 2. How does chloroplast location affect mesophyll conductance, and 3. Is there an advantage in terms of mesophyll conductance of having many small chloroplasts versus one large chloroplast? Estimation of mesophyll conductance is difficult but great progress has been made in using modeling approaches. These depend on knowing what controls photosynthetic responses, in essence what “limits” photosynthesis. It shall be argued that this is much more variable than is often assumed and that it is dangerous to abandon this determination to an algorithm. This will be demonstrated by tobacco plants engineered to express extra phytochrome. These plants had high levels of chloroplast constituents but this caused them to curve away for the cell wall, decreasing mesophyll conductance to CO₂. The final topic will address the question of why land plants have many small chloroplasts in preference to one large chloroplast found in earlier diverging clades (e.g. hornworts). The *arc6* mutant of *Arabidopsis* which has a single large chloroplast was studied and found to have a *higher* mesophyll conductance, not lower. Further examination revealed that this large chloroplast is thin and pressed against the cell wall. It is concluded that mesophyll conductance cannot explain why land plants consistently have many small chloroplasts since it is possible to arrange a single large chloroplast in a way to allow a high mesophyll conductance.

THE CMSII MUTATION AND THE MESOPHYLL CONDUCTANCE.

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Abstract

It is well known that mitochondrial activity interacts with photosynthesis. This was investigated in mutants presenting some defects in respiratory complex I activity (NADH: ubiquinone oxidoreductase). We particularly studied a male sterile mutant (CMSII mutant) of *Nicotiana sylvestris* where the mtDNA lacks the *nad7* gene coding for the complex I subunit. As a result, the complex I is not detectable. Compared to the wild type, (1) the oxidation of exogenous NADH and the capacity of the cyanide-resistant respiration (AOX) are enhanced while the ratio of normal and alternative respiration remained unchanged, more over (2) when expressed on a leaf area basis the respiration rate is higher while the net CO₂ uptake measured in normal air is lower and, (3) the adjustment of photosynthetic carbon assimilation to elevated growth irradiances is impaired.

Some evidences such as the increase of electron transport to O₂ and the increase in the glycine pool, suggest that photorespiration is increased in the mutant. By using isotopic mass spectrometry and concomitant measurements of net CO₂ uptake and chlorophyll fluorescence emission we showed that mesophyll conductance to CO₂ diffusion (g_m) was half as high in the mutant as compared to the wild type. Similar observations were made in another Complex I mutant of *N. sylvestris* (NMS1 nuclear mutant) defective in processing the *nad4* transcript. In NMS1 the g_m value was similar to that measured in the CMS mutant and approximately half as high as stomatal conductance to CO₂ diffusion.

The same observation was made also in an *Arabidopsis thaliana* (Columbia strain) CMSII mutant, lacking the 23 kDa subunit of mitochondrial complex 1.

How can a mitochondrial mutation affect g_m ? Both the CMSII and the NMS1 mutants of *N. sylvestris* lack the γ CAs which are usually associated with complex I (presently no information for the *A. thaliana* 23 kDa mutant are available). Can these γ CAs be involved in setting a high g_m ? Alternatively, it is known that the CMSII and

NMS1 mutations change the redox equilibrium of the photosynthesizing cells. Can g_m value be linked to some kind of regulation in aqaporins activity?

PHYSIOLOGICAL CONSEQUENCES OF PLANT AQUAPORIN CO₂ CONDUCTIVITY

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Abstract

Over the past 25 years, speculation about the magnitude and variability of CO₂ diffusion resistance within leaves has ranged from assumptions, that it is insignificant and invariant to large and variable. Currently, it is recognized that the resistance is too large to be ignored, but the source of variation remains controversial. CO₂ coming from the atmosphere has to cross different membranes to reach the chloroplast stroma. The PIP1 aquaporin NtAQP1 was shown to function as a CO₂ conducting membrane channel in different heterologous expression systems. Furthermore NtAQP1 fulfills a physiologically significant role in the intact plant, as the photosynthetic performance of tobacco plants directly correlates with the expression level of NtAQP1. Recent observations showed that NtAQP1 is also a component of the chloroplast envelope. Reduction of NtAQP1 expression in the plant increases the resistance to CO₂ movement within leaves as a result of functional changes in the chloroplast inner membrane, leaving diffusion through the plasma membrane unaltered. This demonstrates that the protein composition of the chloroplast inner envelope membrane, namely presence of NtAQP1, effects on the diffusion of CO₂ within leaves. In addition it directly shows a physiologically relevant function of an aquaporin located in an organelle.

MOLECULAR BASIS OF PLANT AQUAPORIN CO₂ CONDUCTIVITY

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Abstract

The molecular basis of membrane gas transport is currently highly debated. The main controversy arose from the discussion if membrane gas transport is facilitated by proteins or if the membrane permeability for gases is so high that resistances in other phases than the membrane would determine gas transport velocity. Consequently, any change in membrane resistance like integration of a protein would be completely ineffective. The presentation will take up this discussion and provide data obtained on heterologous expression of plant aquaporins in yeast and its effect on CO₂ and NH₃ transport. A model describing aquaporin facilitated membrane gas transport at the molecular level will be presented. It will be critically reviewed with regard to the above mentioned debate.

THE EFFECT OF CHLOROPLAST MOVEMENTS ON MESOPHYLL CONDUCTANCE IN *ARABIDOPSIS THALIANA*

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Abstract

It has long been speculated that chloroplast movement may affect carbon assimilation by changing the internal conductance to CO₂ diffusion between the intercellular airspaces and the chloroplast stroma. To address this hypothesis, we examined the relationship between chloroplast arrangement and mesophyll conductance in *Arabidopsis thaliana*. Chloroplast position was manipulated by varying the amount of blue light, and by cytochalasin D treatment. In addition, we investigated two chloroplast positioning mutants.

Chloroplast arrangement was assessed by the surface area of chloroplasts adjacent to intercellular airspaces (S_c). Although it has previously been shown that long-term acclimation to high light is linked with a large S_c , we found that short-term exposure to high light induces a chloroplast avoidance response that can reduce S_c . This effect was not apparent in the blue-light insensitive *phot2* mutant, which did not show the avoidance response.

From Fick's first law it follows that the flux of CO₂ is dependant on the surface area available for gas-exchange. Indeed, the smaller S_c induced by the avoidance response was coupled to a similar decrease in internal conductance. Moreover, this reduction in internal conductance resulted in a increased limitation of the rate of photosynthesis. The limiting effect of S_c on internal conductance and photosynthesis was also observed in *chup1*, a mutant with a constant small S_c as the result of an unusual chloroplast arrangement.

We conclude that light induced chloroplast movements can rapidly alter leaf morphological parameters and this has significant consequences for the diffusion of CO₂ through the mesophyll. In contrast to earlier assumptions, the short-term chloroplast avoidance response decreases internal conductance in *A. thaliana*. This suggests that the protection of the photosynthetic machinery from rapidly increasing light intensities is preferred over maximizing internal conductance.

ARABIDOPSIS LINES DEFICIENT IN MAJOR LEAF CARBONIC ANHYDRASES SHOW REDUCED GROWTH IN LOW CO₂

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Abstract:

By using *Arabidopsis thaliana* T-DNA insertion lines depleted in the main carbonic anhydrase (CA) isoforms in the chloroplast (β CA1), the cytosol (β CA2) and in both, the role of CA activity with respect to their cellular compartments has been addressed. We show that β CA1 and β CA2 together represent up to 99 % of the CO₂ hydration activity in the leaf, and that up to 25 % of total CO₂ hydration is catalysed by cytosolic (β CA2) activity. In ambient or elevated CO₂ (980 ppm) conditions, the chloroplastic CA mutant (Δ CA1), the cytosolic CA mutant (Δ CA2), the double mutant (Δ CA1:2) and the wildtype (WT) show no difference in growth. In low CO₂ (150 ppm) the growth of the Δ CA1 mutant was reduced (about 75 % of the WT biomass, after 55 days). The growth was even more reduced in the Δ CA1:2 mutant (about 50 % of the WT biomass), whereas the Δ CA2 mutant had shown no difference in growth as compared to the WT. Instantaneous measurements of photosynthesis, stomatal and mesophyll conductance revealed no detectable difference between the CA mutants (Δ CA1, Δ CA2, Δ CA1:2) and the WT grown in the three CO₂ conditions used in the experiments. A simple growth and carbon balance model shows, that the difference in growth between the CA deficient lines and the WT, can be explained by the accumulation of a small difference in the rate of CO₂ fixation. The difference corresponds to up to 1 % in photosynthetic rate, which is indeed below detection limits of instantaneous measurements. The extend on changes that can be expected for mesophyll conductance are estimated and discussed in the context of a role of CAs in their respective compartment for the transfer of CO₂ in the mesophyll.

Environmental effects on g_m

ENVIRONMENTAL VARIATION IN MESOPHYLL CONDUCTANCE

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Abstract

Mesophyll conductance is a large and dynamic limitation of photosynthesis. It used to be thought that mesophyll conductance was a (more or less) simple function of anatomical traits of leaves (e.g. areas of chloroplasts as a function of intercellular air spaces). A range of recent studies have shown that while mesophyll conductance is often related to anatomical traits, it is also highly dynamic and affected by various environmental variables. This talk will examine the dynamics of mesophyll conductance at long and short time scales. I will show that on a scale of months to years mesophyll conductance acclimates to light intensity (e.g. due to gradients in light through the canopy), growth temperature and is affected by foliage age. On shorter time scales of hours or even minutes, mesophyll conductance is affected by many environmental variables such as soil water deficits, salinity, leaf temperatures, CO₂ concentration. This talk will highlight our largely empirical understanding of the dynamics of mesophyll conductance, and identify key challenges that remain to be solved.

MESOPHYLL CO₂ LIMITATIONS ON CARBON ASSIMILATION IN NATURAL VEGETATION DURING DROUGHT STRESS AND RECOVERY

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Abstract

Whether photosynthesis is limited during water stress and recovery due to diffusive or biochemical factors is still open to debate, and apparent contradictions appear when various studies on species with different growth forms are compared. Among diffusive limitations, mesophyll conductance (g_m) has been pointed out as a main factor limiting CO₂ transport to Rubisco sites and, therefore, carbon assimilation (A_N).

Mediterranean natural vegetation represents an excellent target to study the relative importance of diffusive or biochemical limitations on A_N under drought conditions, because of their long-term standing with natural fluctuations in water availability. Moreover, the existing large variability on leaf morphological and physiological traits naturally found among Mediterranean species ensures the analysis of widely different models.

The objective of the present work was to perform an analysis of photosynthetic limitations during drought stress imposition and after re-watering. Ten Mediterranean species representing different leaf habits were subjected to different levels of water stress, the most severe followed by re-watering. A quantitative limitation analysis was applied to estimate the effects of water stress on stomatal (S_L), mesophyll conductance (MC_L) and biochemical limitations (B_L).

The results confirmed that a general pattern of photosynthetic response to water stress exists among C₃ plants when stomatal conductance (g_s) is used as a reference parameter. As g_s values decreased from a maximum to about 0.05 mol H₂O m⁻² s⁻¹, the total photosynthetic limitation rose from 0 to about 70%, and this was due to a progressive increase of both S_L and MC_L limitations, while B_L remained negligible. When lower values of g_s were achieved (i.e., total photosynthetic limitation increased

from 70% to 100%), the contribution of S_L declined, while MC_L still increased and B_L contributed significantly (20% to 50%) to the total limitation.

As expected, the extent of photosynthetic recovery of severely stressed plants after re-watering was dependent on the leaf habit, with herbs showing the largest recovery, semi-deciduous species an intermediate recovery, and evergreens the lowest recovery. The limitation analysis revealed that, contrary to what is usually assumed, the recovery of B_L after severe water stress was generally large. On the contrary, in the ten species studied here and irrespective of the degree of A_N recovery, limited recovery of g_m was the most important factor limiting A_N recovery.

Overall, the present results highlight the role of g_m in controlling photosynthesis, not only in response to drought imposition, but also during recovery.

NON-STOMATAL LIMITATIONS OF PHOTOSYNTHESIS IN DIFFERENT CULTIVARS OF *VITIS VINIFERA* SUBJECTED TO DEFICIT IRRIGATION

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Abstract

In Mediterranean regions, crops like grapevine (*Vitis vinifera* L.) are subjected during summer to soil water deficit, high temperatures and irradiance which reduces photosynthesis, growth, yield and berry quality. Under moderate water deficits stomatal regulation is probably the most important factor influencing carbon uptake and water use efficiency but diffusive limitations due to mesophyll conductance (g_m) have also been pointed out to limit CO₂ transport to Rubisco sites and, therefore, limit photosynthetic assimilation (A_n). These type of limitation will vary with the severity of the water stress and may also be specific of a cultivar.

In this study one of our aims was to estimate mesophyll conductance for a group of grapevine cultivars currently grown in Portugal: Aragonez (the most cultivated), Touriga Nacional, Trincadeira, Cabernet Sauvignon and Syrah. In addition, plants of the cultivar Aragonez were subjected to different deficit irrigation regimes and compared to fully (FI) and non-irrigated (NI) plants at different moments of the summer season.

For Aragonez, stomatal conductance (g_s) and g_m decreased in response to water deficit and to the increased severity of stress. In general, values of both g_s and g_m were lowest for NI plants, intermediate for mild stressed plants and highest for FI plants. This suggests that g_s and g_m may be somehow coupled in what concerns the response of leaves to water stress. The decrease of stomatal conductance in NI and mild stressed plants relatively to FI plants was more pronounced than that of g_m . Maximum quantum yield did not vary along the season showing that the photosynthetic apparatus was not damaged. Nevertheless, Rubisco carboxylation (V_{cmax}) and maximum electron transport capacity at saturating light (J_{max}) decreased under water stress conditions.

The comparison of the five cultivars growing under identical conditions showed that there are significant differences in both g_s and g_m and consequently on CO₂ diffusion. Maximum values of g_s and g_m were found for Touriga Nacional. Syrah in

turn, showed the lowest stomatal conductance but at same time one of the highest mesophyll conductances. There were also differences among cultivars for biochemical characteristics (J_{max} and V_{cmax}) although these were less pronounced than diffusive limitations. The fact that differences found for g_s were not parallel to those of g_m suggests that other physiological and/or morphological traits (e.g. photosynthetic potential, leaf morpho-anatomy) are responsible for the specific response of cultivars.

PHOTOSYNTHESIS LIMITATION IN RESPONSE TO DROUGHT STRESS: SOME INSIGHTS ON ACCLIMATION AND RECOVERY

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Abstract

In the context of climate change, the number of extreme drought events will increase within the next decades in Europe, while limited water availability will impair plant productivity and growth. Thus, the capacity of stress-adaptation, recovery and survival are important aspects for plants across Europe.

Responses of different plant species growing under various climatic conditions were studied to investigate the limiting factors in their carbon balance, in particular changes of photosynthetic and respiratory traits during drought and recovery. Here, drought-recovery-experiments were carried out with tobacco (*Nicotiana sylvestris*) during spring and summer in the field and inside a growth chamber, using in addition a respiration mutant (CMSII). In all three experiments water was withheld until $g_s \leq 50$ $\text{mmol m}^{-2} \text{s}^{-1}$ (considered as severe drought), then these plants were maintained for several days at this level by adding the amount of daily lost water and thereafter they were re-watered to field capacity every day.

Under progressing drought g_s and net photosynthesis (A_N) declined in parallel under all three conditions. During severe drought ($g_s \leq 50$) photosynthetic electron transport rate (ETR) and the number of open PSII reaction centers (qP) were markedly reduced, while maximum carboxylation rate (V_{cmax}) did not change and values of A_N remained low but still positive. In parallel, respiration rates decreased by 30%, mainly due to reduced v_{cyt} , except for stressed tobacco outside, which maintained its respiratory activities. Alterations in photosynthetic pigments, soluble proteins and in global lipid-peroxidation were negligible.

The rate of recovery of photosynthetic traits (A_N , ETR, qP) differed among the three experiments, where plants under growth-chamber conditions recovered the most rapid and plants outside during summer the least rapid. Increased limitation of internal diffusion of CO_2 (g_m) seemed to contribute to the delayed recovery of stressed tobacco plants outside in summer, as g_m remained markedly lower than in the other cases.

Furthermore, with regard to the incident irradiance, recovery might also be affected by the differences in solar radiation (including photo-oxidative stress).

In addition to the effects of varying climatic conditions (i.e. irradiance, temperature) during the experimental period in one species, a comparison of different species under varying drought intensity and subsequent recovery was conducted. In these experiments *Nicotiana sylvestris*, *Vitis vinifera*, and *Cistus albidus* plants, growing under field conditions were subjected to moderate and severe drought by withholding water, using as a reference stomatal conductances for water vapour (g_s) of ≤ 100 and $\leq 50 \text{ mmol m}^{-2} \text{ s}^{-1}$, respectively. After having reached the desired drought-intensity, plants were maintained at this level for a week to let them acclimate and thereafter they were re-watered to field capacity every day. Stomatal and non-stomatal limitations of photosynthetic recovery varied due to the severity of stress and the species analyzed, suggesting different strategies for adaptation/tolerance among the different growth forms.

In conclusion, recovery of photosynthetic traits after severe drought seem to be mainly limited by diffusion components of the leaf (g_s , g_m), while also climatic conditions (high irradiance, temperature) may affect the rate of recovery.

RESPONSE OF PHOTOSYNTHESIS, CARBON ISOTOPE DISCRIMINATION AND MESOPHYLL CONDUCTANCE TO WATER DEFICITS DURING REPRODUCTIVE STAGE IN RICE

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Abstract

Drought stress is the largest constraint to rice production in the rainfed systems, affecting 19 million ha of upland rice and over 14 million ha of rainfed lowland rice. At the whole plant level, soil water deficit is an important environmental constraint influencing all the physiological processes involved in plant growth and development. Most of the carbon stored in the mature rice grains originates from CO₂ assimilation during the grain-filling period, the flag leaf performing the highest inflows of photosynthetic carbon. All factors that lower the photosynthesis rate of the flag leaf during this period could potentially limit grain yield.

The objectives of this study were: i) to identify the possible causes of the different sensitivity of both photosynthesis and WUE to different levels and intensities of water deficit in rice varieties, ii) to investigate possible intra-specific variability of mesophyll conductance in limiting photosynthesis under different drought stress intensities, iii) whether there was a close relationship between intrinsic transpiration efficiency (A/g_s) and whole-plant WUE. This information has important implications for developing drought screening tools for breeding programs, and to provide much-needed insights into the biological effects of drought stress at both the cellular and whole-plant levels.

Contrasting rice genotypes were evaluated in two field experiments under upland conditions in the dry season of 2006 on the IRRI Experiment Station at Los Banos, Philippines. A set of seven rice cultivars (i.e. Apo, IR55419-04, IR64, IR71525-19-1-1, Moroberekan, PSBRc80, Vandana) was evaluated in experiment 1 (drip irrigation). The same set plus one additional genotype (i.e. DK98) were used in experiment 2 (line-source sprinkler irrigation).

We found that varieties were significantly different for their inherent photosynthesis, that is, for the photosynthetic rate in non-stressed (control) conditions. Although plants in the two irrigation experiments were in different phenological stages, consistently IR71525-19-1-1 and Moroberekan showed the highest A . Similarly, in both irrigation experiments, A was consistently more inhibited relative to controls in Apo, IR64, and PSBRc80, indicating a relatively higher sensitivity to water deficit in these genotypes. Furthermore, IR71525-19-1-1 and Moroberekan showed significantly reduced photosynthesis rates in response to water deficit. Nevertheless these rates resulted higher than those of all other water-stressed genotypes. Generally, g_s and g_m were higher in the genotypes with inherent higher photosynthesis in both control and stressed conditions. There were good linear correlations between photosynthesis and stomatal conductance pooling together controls and water-stressed leaves. Similar correlations were found between photosynthesis and mesophyll conductance. However, better correlations were found between photosynthesis and total leaf conductance. The lack of consistent increase in both C_i/C_a and C_s/C_a in water-stressed genotypes indicates that stomatal limitations overcame possible biochemical limitations. In particular the lower C_s/C_a ratios in stressed relative to control leaves indicate that A was primarily limited by the combination of stomatal and mesophyll resistances to CO_2 diffusion. There were also no significant differences between the initial slope of the A/C_c curves of control and water-stressed plants, indicating that the activity of the chloroplastic soluble proteins was not impaired by water deficit. Moreover, the A/C_c curves of water-stressed plants were not saturated by C_a values of about $2200 \mu\text{mol } \mu\text{mol}^{-1}$, and this is a further evidence that A was limited by diffusional limitations more than by biochemical ones.

Finally, when gas exchanges were measured following an early morning irrigation, A of water-stressed genotypes recovered to well-watered rates, and this was mirrored by a parallel increase in both g_s and g_m that also recovered to well-watered values in all genotypes. To our knowledge, this is one of the very few studies that show that a reduction of mesophyll conductance brought about by environmental stress can be reversed, and that this in turn may influence photosynthesis

EFFECT OF AIR VAPOUR PRESSURE DEFICIT ON MESOPHYLL CONDUCTANCE

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Abstract

Mesophyll conductance to CO₂ (g_m) in leaves is currently considered of great importance in the determination of diffusional limitation to photosynthesis. Recent studies are highlighting that g_m is not at all constant, but affected by environmental variables, in many cases as stomatal conductance (g_s) is. For example, there are evidences of the effect of leaf temperature on g_m in several species, soil water deficit, light and internal CO₂. All these effects suggest that g_m can be considered pretty dynamic during the day, even coevolving with g_s . In fact, it has been reported that changes of g_m in response to environmental changes can be faster than those of g_s .

However, there is not much information of the effect of vapour pressure deficit (VPD) on g_m , despite of being one of the environmental variables which has a larger impact on the leaf gas exchange. The effect of VPD has been widely studied on g_s , and it has been reported in many species, especially woody plants, as the main variable affecting diurnal evolution of transpiration and g_s . Nevertheless, there are only two studies that have studied the effect of VPD on g_m with different results. In one of them it was found a negative effect, with g_m tracking the stomata response, and in the other no effect at all was found.

The objective of the present work was to study the effect of VPD on two woody species with a great agronomical importance in the Mediterranean basin: grapevine (*Vitis vinifera*) and olive (*Olea europaea*). In order to do so, two different experiments were carried out. In one of them the ambient VPD where the plants were grown was decreased by the use of a fog system which was activated since VPD went over 1.5 kPa, and then was able to maintain VPD in the chambers 50-60% lower than ambient (C_{wet}). Control open-side chambers were made to mimic the observed 20% reduction in PAR

due to coverage, without modifying ambient VPD (C_{amb}). At the same time, the soil water stress effect was studied imposing two water treatments which kept soil water content about field capacity (C_I) or 50-60% field capacity (C_D). The second experiment consisted on the analysis of the response of olive leaves to VPD, while the rest of the variables were kept fixed. VPD was increased up to values of 4.5 kPa. In both experiments g_m was estimated with the variable J method from concurrent measurements of gas exchange and fluorescence.

The results of the response of g_m to ambient VPD were different in both species. In grapevine, g_m was affected both by VPD and soil water deficit in early summer (not detected later probably due to a lower range of g_s), being g_m higher at low ambient VPD. However, VPD did not affect g_s , although soil water deficit did. In olive, g_s was high in both C_{wet} treatments, with values similar to $C_{amb,I}$. However, the effect of VPD on g_m was different depending on the soil water treatment. A lower ambient VPD decreased g_m in $C_{wet,I}$ respect to $C_{amb,I}$, but increased g_m in $C_{wet,D}$ respect to $C_{amb,D}$, finding no differences between water treatments in C_{wet} .

The results of the response to VPD in olive leaves under controlled conditions showed that while g_s responded negatively to increasing VPD, g_m did not. This meant that the ratio g_m/g_s was increased following increases in VPD, which modified the linear relationship usually found between g_s and g_m . This was true for all the measurements made at saturating PPFD ($1600 \mu\text{mol m}^{-2} \text{s}^{-1}$), but when PPFD was decreased to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ a positive response of g_m to VPD was observed. However, this result waits for further confirmation over a wider range of PPFDs.

Overall, the present results highlight the complexity in the response of g_m to VPD, which, apparently, can be modulated depending on the response to other environmental conditions like light or soil water stress. This suggests that, in the cases where a response of g_m to VPD has been observed, this could be not a direct response to VPD, but to other signal generated in the leaf and related to it.

MESOPHYLL CONDUCTANCE TO CO₂ IN HEAVY METAL TREATED PLANTS

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Abstract

Photosynthesis is one of the processes most affected in plants exposed to excess of heavy metals. There is much evidence that trace metals interfere with the functioning of the photosynthetic apparatus in higher plants and algae and several direct and indirect mechanisms are known to lead to nonspecific inhibition of photosynthesis. The diminished rate of photosynthesis has been related mainly to disrupted chloroplast structure, blocked chlorophyll synthesis, disordered electron transport, inhibited activities of the Calvin cycle enzymes, and CO₂ deficit caused by stomatal closure.

It is now generally accepted that the mesophyll conductance to CO₂ between the intercellular spaces and the chloroplasts (g_m) is an important factor limiting photosynthesis, often being as important as stomatal conductance. There is evidence that g_m can change in response to leaf temperature, drought and salt stress, ozone, CO₂ concentration, etc. However, the information about the response of mesophyll conductance to stress imposed by heavy metals is very scarce.

The objectives of this work were to examine the response of mesophyll conductance and photosynthetic parameters to Cd treatment in cauliflower (*Brassica oleracea*) and radish (*Raphanus sativus*) plants.

The plants were grown in a controlled greenhouse using Hoagland's nutrient solution. Two-weeks-old plants were divided in 3 groups according to the treatment: Control, 25 and 125 μ M Cd and the heavy metal was added as CdSO₄ in the nutrient media. The measurements were performed 7 and 14 days after the treatment. The mesophyll conductance to CO₂ (g_m) was calculated using both the 'variable' and the 'constant J method' [1-3].

The results showed that the mesophyll conductance to CO₂ changed in response to elevated concentration of Cd in both examined species thus affecting the CO₂

concentration at the sites of carboxylation (C_c). In cauliflower plants treated with 25 and 125 μM Cd g_m decreased respectively to about 70 and 30% of the values of g_m of non-treated plants. In radish, g_m decreased to about 55% of the control at 25 μM Cd and to about 40% at 25 μM Cd. Slightly lower values of g_m were obtained by using the 'constant J method' (applicable at higher values of internal CO_2) than at 'variable J' method (used at lower CO_2) and this fact could be in support of the suggestion that elevated CO_2 diminishes g_m .

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DOES INTERNAL CONDUCTANCE VARY WITH IRRADIANCE?

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Abstract

The drawdown in partial pressure of CO₂ from substomatal cavities to the sites of carboxylation within chloroplasts reflects the existence of a limiting conductance to CO₂ diffusion along this path. The partial pressure of CO₂ in substomatal cavities, usually referred to as intercellular CO₂ partial pressure (C_i), is routinely calculated from conventional gas exchange measurements. By combining the additional measurement of either carbon isotope discrimination or chlorophyll fluorescence, it is possible to calculate the CO₂ partial pressure at the sites of carboxylation (C_c). Although CO₂ assimilation is distributed throughout the mesophyll of the leaf, subsequent analysis usually approximates the system as a big chloroplast and applies Fick's Law to calculate internal conductance to CO₂, by analogy with stomatal conductance.

Although internal conductance can in theory be calculated from a single measurement, initially it was derived from multiple measurements. In the case of isotope discrimination, different irradiances generated linear relationships between the drawdown C_i – C_c and the rate of CO₂ assimilation. Deriving internal conductance from the slope of linear relationships also avoided uncertainty associated with respiration, photorespiration and fractionation due to Rubisco. If chlorophyll fluorescence was used, then either multiple irradiances or different CO₂ concentrations were used for the analysis. However, as the acceptance of the concept and magnitude of internal conductance gained wider support, together with improved instruments, there has been a tendency to calculate internal conductance from individual measurements. This revealed that internal conductance varied with CO₂ and light.

If internal conductance varies with CO₂ or light, then interpreting linear relationships created by varying CO₂ and light is problematic. Consequently, we have

revisited the methodology and made extensive analysis of carbon isotope discrimination in wheat under varying light and CO₂ conditions. We have also used the fluorescence method for a range of *Banksia* species. The two approaches have yielded different results.

When measuring carbon isotope discrimination by wheat, it was necessary to use 2% oxygen to minimise the production of compounds that interfered with the instrument zero. This also reduced the possible contribution of fractionation associated with photorespiration. We found that internal conductance was independent of both irradiance and CO₂.

We measured chlorophyll fluorescence from *Banksia* leaves under two irradiances across a range of CO₂ concentrations under 21% O₂. The fluorescence was calibrated for each leaf and irradiance under 2% O₂. Internal conductance generally declined as C_i increased and was 20% lower under 500 compared to 1500 μmol quanta m⁻² s⁻¹.

These results will be discussed in relation to possible sources of error associated with each method and compared to other published data.

THE IMPACT OF BLUE LIGHT ON MESOPHYLL CONDUCTANCE

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Abstract

Blue light impacts on many physiological processes. A moderate percent of blue light has a positive effect on photosynthesis, stimulating stomatal opening and CO₂ entry in the leaves. However, we observed a strong negative influence of higher levels of blue light on photosynthesis, being rates of CO₂ fixation reduced by up to 50% when blue light was 80% of the total incident light. The effect was fast (within minutes) and reversible. The reduction of photosynthesis was not associated to a reduced stomatal conductance. However, a concurrent reduction of mesophyll conductance was observed, which could reduce the concentration of CO₂ at the chloroplasts and, consequently, photosynthesis. Changes of mesophyll conductance could reflect chloroplast re-positioning under blue light, reducing the surface of chloroplasts exposed to intercellular spaces. However, as carotenoid absorption of blue light may reduce the efficiency of electron transport, mesophyll conductance calculation by chlorophyll fluorescence may not be accurate. Simulations are presented showing that, reducing the efficiency of electron transport, e.g. by assuming only 50% efficiency in terms of oxygen evolution for the blue light absorbed by carotenoids, the reduction of mesophyll conductance may not be as large as estimated by methods that have been developed using the entire light spectrum.

BOTTOM UP MESOPHYLL CONDUCTANCE UNDER DIVERSE LIGHT LEVELS IN *BRASSICA CARINATA*

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Abstract

Since its first publication the model by Farquhar et al. (1980) has triggered debates whether substomatal CO_2 concentration can be significantly higher than that at the carboxylation site and, as such, the photosynthetic parameters V_{cmax} and J_{max} calculated by the model might be biased. A number of studies were undertaken on this topic giving evidence that mesophyll conductance (g_m) can significantly decrease the CO_2 concentration at the Rubisco site to an extent depending on water and salinity stress, leaf age, mineral nutrition etc.. Irradiance significantly affects leaf anatomy and mesophyll architecture (Nobel, 1991; Parkhurst, 1994), hence it can be expected to influence g_m as well. However, few studies have documented this aspect (see Flexas et al., 2008, for a recent review), and therefore this research addressed the influence of light upon g_m in a plant profile of *Brassica carinata* (Fig. 1).



Fig. 1. Growth chamber equipped with two light levels: T1, conventional light source (from the top); T2, top and lateral light sources (left figure). *Brassica c.* subjected to two different radiation treatments (inner figure). Considered plant layers (right figure).

Each plants was properly divided in six layers (Fig. 1) including leaves (F1-F3) and siliqua (S1-S3) of different age and position. Chlorophyll pigments, carotenoids, photosynthetic parameters (A , g_s , V_{cmax} , J_{max}), fluorescence were measured in each layer in leaves, siliqua and seeds. Carbon isotope fractionation, which was taken to calculate

g_m (Brugnoli & Lauteri, 1991), was determined on soluble sugars in the three organs (Δ_{leaf} , Δ_{sil} , Δ_{seed} , respectively), and on erucic (Δ_{er}) and oleic (Δ_{ol}) acids in seeds only.

The results showed g_m to be surprisingly negatively related to C_i/C_a . The latter was inversely related to A , while stomatal conductance (g_s) unchanged appreciably. As expected, C_i/C_a was instead positively related to (Δ_{leaf}). Therefore, an hypothesis might be that the rise of C_i at the carboxylation site was due to a lower carboxylation efficiency of aged leaves, and not to an increase of g_m , which is in fact commonly associated with higher photosynthetic rates. A conjecture that seems to be confirmed by analysing g_m and photosynthesis parameters at different layers: F1 showed in fact the lowest g_m values and F3 the highest ones. Therefore, with respect to young leaves, aged leaves had lower g_m but higher C_i/C_a likely due to lower A values. The effect of leaf age appeared even stronger than that of irradiance. Two-way irradiated leaves showed lower g_m than leaves irradiated from the top only, and this was increasingly clear from top to bottom in the plant profile, i.e. from younger to older leaves.

To summarize, the present results showed that g_m was always trivial in young leaves, irrespective of light levels. Conversely, when leaves aged, g_m drastically decreased, with irradiance being unimportant compared to the effects of leaf age on g_m . Therefore, the surprising negative consequences of irradiance on g_m are likely spurious being biased by the preponderant ageing effects. It derives that, two-way irradiated plants showed clearly lower g_m values simply because of leaf ageing that occurred earlier under higher light conditions.

Ecological
implications of g_m

VARIATION OF MESOPHYLL CONDUCTANCE DEPENDING ON PLANT FUNCTIONAL TYPE: ROLES OF CHLOROPLAST SURFACE AREA, CELL WALL THICKNESS AND COOPORINS

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Abstract

Conductance for CO₂ diffusion from the substomatal cavity to the chloroplast stroma (mesophyll conductance, g_m) is roughly proportional to chloroplast surface area (S_c) and decreases with the increase in thickness of the mesophyll cell wall. Involvement of cooporins (aquaporins that permeate CO₂) in the plasma membrane and in the chloroplast envelope has been also reported. These three factors, namely, S_c , cell wall thickness, and abundance and permeability of cooporins, would explain variations of g_m among leaves of different plant functional types and those observed in response to environmental variables at various time scales, at least to a considerable extent.

S_c dynamically changes in response to changes in light environment. Using several mutants of *Arabidopsis thaliana* and photobiological/cell biological techniques, Tholen et al. (2008) found that changes in S_c due to chloroplast movement were always accompanied by proportional changes in g_m . In strong light containing a substantial amount of blue light, chloroplasts took positions to avoid strong light. In the *A. thaliana* plants grown in moderate light, palisade tissue cells were round-shaped, and such avoidance movement of chloroplasts caused a decrease in S_c . In sun leaves having elongated palisade tissue cells, the avoidance movement of chloroplasts would probably increase S_c .

The importance of S_c in acclimation to light environment has been clearly shown by Oguchi et al. (2003, 2005, 2006). In plant species that showed increases in the photosynthetic rate after transfer from shade to sun conditions, S_c values increased proportional to the increases in the photosynthetic rate.

The wall resistance for the unit chloroplast surface area can be written as $g_w = (p D_w K_{CO_2}) / (\tau \delta_w)$, where p is porosity, D_w is the weighed diffusion coefficient of inorganic carbon species in water, K_{CO_2} is a partitioning coefficient (the ratio of the concentration of inorganic carbon species in the liquid phase to that of CO_2 in the gas phase), τ is tortuosity, and δ_w is cell wall thickness. Terashima et al. (2006) postulated that p/τ is around 0.1, which is lower than the value (0.3) proposed by Nobel (1999). If p/τ is indeed around 0.1, the differences in g_m among annual herbs, deciduous broad-leaved trees and ever-green broad-leaved trees can be mostly attributed to a difference in δ_w . Experimental verification is awaited.

Miyazawa et al. (2008) compared g_m between tobacco leaves acclimated to long-term drought (DA) and those grown under sufficient irrigation (WW). g_m , estimated by combined analyses of CO_2 gas exchange with chlorophyll fluorescence, in the DA plants was approximately half that in the WW plants. S_c values were not significantly different between the WW and DA plants. The amounts of plasma membrane aquaporins (PIPs) were almost identical. When the detached leaves were fed with 0.5 mM $HgCl_2$, g_m in WW decreased to the level similar to that in DA. Water permeability also decreased. In contrast, both water permeability and g_m were insensitive to $HgCl_2$ in DA plants. These results suggest that deactivation of aquaporins was responsible for the significant reduction in g_m observed in DA plants. The study clearly indicates that abundance of active PIP2 plays an important role in regulation of g_m .

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STRUCTURAL CONTROLS OF INTERNAL DIFFUSION CONDUCTANCE: SCALING FROM LEAF TO GLOBE

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Abstract

Plant species widely differ in integral leaf structural traits such as leaf dry mass per unit area (M_A) and its components, leaf density (D) and leaf thickness (T). Species with large M_A and densely packed mesophyll cells with thick cell walls generally possess lower internal diffusion conductances per unit area (g_{area}) under optimal environmental conditions. Due to overall greater fraction of biomass in support structures, species with larger M_A and greater D also often have lower photosynthetic potentials per unit area (A_{area}). As the result of negative scaling of both g_{area} and A_{area} with M_A and leaf density, the correlation between g_{area} and A_{area} is commonly positive. Because of this co-variation, it has been suggested that the drawdown in CO_2 mole fraction between sub-stomatal cavities (C_i) and chloroplasts (C_c) due to internal conductance ($C_c = C_i - A_{\text{area}}/g_{\text{area}}$) is similar among species with differing leaf structure, and thus, g_{area} limits photosynthesis to a similar degree in species with differing foliage structure. However, data demonstrating variations in C_c/C_i ratio and g_{area} for an extensive range of species with varying M_A and photosynthetic capacity have been limited so far. In addition, it is conceptually further important that for three-dimensional structures, such as plant leaves, C_c/C_i ratio is a volume-weighted average estimate, and should accordingly scale better with g_{mass} (g_{area}/M_A) and A_{mass} (A_{area}/M_A) than with the area-based estimates. This means that a positive correlation between A_{area} and g_{area} does not necessarily imply that photosynthesis is limited to the similar degree in leaves differing in structure. A compilation of published data for g_{area} and A_{area} , and g_{mass} and A_{mass} demonstrated a strong coordination between both g_{area} and A_{area} and g_{mass} and A_{mass} , but also that the relationships saturated at higher values of internal conductance. The C_c/C_i ratios increased with increasing both g_{area} and g_{mass} , indicating that the leaves with more robust structure had stronger internal diffusion limitations of photosynthesis. The

relationship between C_c/C_i and g_{mass} was stronger, in line with the circumstance that C_c/C_i is a volume-weighted average. These correlations were further tested among European, Australian and Hawaiian datasets representing a broad range of climates spanning from temperate to tropical ecosystems. In all cases, stronger control of photosynthesis by limited diffusion conductance was observed in structurally more robust leaves. This broad general structural control observed across species from different biomes suggests that mesophyll internal conductance is a key photosynthetic characteristic modulating the variation in photosynthetic productivity among species at given photosynthetic capacity, and among earth ecosystems at given resource availability.

HOW DO LEAF STRUCTURE, CO₂ AND IRRADIANCE INFLUENCE MESOPHYLL CONDUCTANCE IN SCLEROPHYLLS?

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Abstract

Mesophyll conductance (g_m), the conductance to diffusion of CO₂ in the mesophyll, is an important factor that limits photosynthesis (A). During the last two decades, research has shown that g_m is affected by both the structure of the mesophyll and the environment. A number of studies have indicated a close association of g_m , and/or its two components (g_{ias} , the conductance to diffusion in the intercellular air spaces, and g_{liq} , the conductance in the liquid phase), with leaf anatomy. Temperature, water stress, and more recently CO₂ concentration and light intensity, have also been shown to influence g_m .

g_m varies considerably among species and functional groups. Mesophytic species (low leaf dry mass per area, LMA) generally have greater g_m than sclerophyllous (high-LMA) species. The upper bound for g_m declines as LMA increases, extrapolating to zero at an LMA of about 240 g m⁻² (Flexas et al. 2008, PCE 31:602–621). Given that LMA's of *Banksia* species are between 130 to 500 g m⁻², these leaves provide a good model to examine g_m across a broad range of the sclerophylly scale, as well as extending it at the high LMA end. The relationships between g_m and LMA as well as

other leaf structural variables were compared between seven *Banksia* species, along with the response of g_m to two key environmental factors, namely CO_2 and irradiance.

g_m declined significantly with increasing LMA. The most sclerophyllous species studied, *Banksia elderiana* (LMA=478 $g\ m^{-2}$), had the lowest g_m (0.087 $mol\ m^{-2}\ s^{-1}$ at ambient CO_2 and 1500 $\mu mol\ PAR\ m^{-2}\ s^{-1}$). g_m also declined in response to increasing CO_2 concentration and to decreased irradiance. At high irradiance (1500 $\mu mol\ m^{-2}\ s^{-1}$), g_m was reduced by 35-60% in response to increasing CO_2 concentration, with a more variable response (0-60%) at low irradiance (500 $\mu mol\ m^{-2}\ s^{-1}$). At ambient CO_2 , g_m was 22% lower at low than at high irradiance, on average.

A (on a leaf area basis) decreased with increasing LMA, similar to g_m . The drawdowns in the CO_2 concentration from the atmosphere (C_a) to the substomatal cavities (C_i) and to the chloroplasts (C_c) did not change with sclerophylly. Consequently, C_i and C_c appeared remarkably stable across a range of A.

PHOTOSYNTHETIC CAPACITY AS DEPENDING ON LEAF ANATOMICAL STRUCTURE AND INTERNAL GAS EXCHANGE RESISTANCE IN *POPULUS TREMULA*.

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Abstract

Leaf anatomical parameters are strongly modified by various environmental factors, even though the general developmental pattern is maintained also in stressed plants. The environmental stresses modify the leaf photosynthetic potentials due to changes in leaf inner structure and enhanced internal diffusional conductance. The marked developmental changes in leaf anatomy and morphology also contribute to changes in, mesophyll conductance (g_m) and photosynthesis.

The objective of present work was to analyse the leaf anatomical limitations to g_m and how g_m limits carbon assimilation (A_n) during the leaf ontogeny. We studied the effects of stress conditions to development of leaf anatomy and showed which are the key mechanisms to cause the variability of g_m within species. Also the mechanisms how leaves can change g_m after they are fully expanded.

Experiment was carried out with *Populus tremula* clones. Trees were grown in the induced environmental stress conditions: low light with waterstress; low light without waterstress; high light with waterstress and control plants. Photosynthetic and anatomical parameters for gaseous and liquid phase were analysed for leaves in different ontogenetic stages.

Our study indicates that g_m increases during the ontogeny and reaches the maximum when leaf is fully expanded. After g_m has reached the maximum it starts to decline again. This tendency was correlated with thickening of cell walls and changes in the chloroplast area exposed to intercellular airspace. These results show that the effective utilization of leaf photosynthetic potential (A_n versus the cost of resources invested into the photosynthetic apparatus) depends on leaf age and vary within species.

g_m in mature leaves was smaller for leaves which have grown under the stress condition than it was for leaves grown in normal environmental conditions. our results also show that g_m limits photosynthesis more when plants grow in environmental stress conditions.

PHOTOSYNTHESIS MODELING IN OLIVE: EFFECTS OF MESOPHYLL CONDUCTANCE

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Abstract

The important role of mesophyll conductance (g_m) in the determination of final rates of net assimilation (A_N) is acquiring great relevance in the light of the increasing knowledge about this variable in the last few years. Recent studies are highlighting that g_m is finite and not constant, but affected by environmental variables (Flexas et al., 2008; Warren, 2007). There are evidences of the effect of leaf temperature on g_m in several species, soil water deficit, light and internal CO_2 . All these effects suggest that g_m can be considered highly dynamic during the day. As a consequence of this, the incorporation of g_m in models at the leaf level, whole plant, ecosystem and global ecophysiology is of vital importance.

In this work it is evaluated the impact of g_m on the photosynthesis model of Farquhar-von Caemmerer-Berry. The study was performed in olive (*Olea europaea*), in which there was previous information about the parameterisation of FvCB model (Diaz-Espejo et al., 2006), as well as, the effect of temperature on g_m (Diaz-Espejo et al., 2007). In addition to this, the effect of a variable g_m , echoing the findings by Flexas et al. (2007), will be evaluated in the analysis of A_N-C_i / A_N-C_c curves. Finally, in view of what is known about the coregulation among A_N , g_s , and g_m (/the response of g_m to environmental variables), as well as, novel data of diurnal evolution of g_m in olive leaves, it is made a preliminary attempt to model g_m , and include it on leaf level models.

Results showed that the incorporation of g_m to the response of V_{cmax} and J_{max} to temperature in olive modified the shape of the response. This modification did not affect significantly the output of the model, but it allowed to identify the causes of increase or decrease in A_N under natural conditions, i.e. changes in photosynthetic capacity or g_m . The effect of temperature on the ratio $J_{max}:V_{cmax}$ was also modified, especially at high temperature. Concerning to the effect of a variable g_m in response to changes in CO_2 , a consequence of an increase of C_i is that it is less frequent to find photosynthesis limitation by utilisation of triose phosphate (TPU). The large decrease in

g_m at high CO_2 concentrations yields lower C_c values, and it is seldom obtained data on the TPU region.

Finally, an approach based on the apparent coregulation among A_N , g_s , and g_m was evaluated as a model of g_m . This approach is based on the close relationship found by Flexas et al. present workshop in several species between A_N/g_s and g_m/g_s . This relationship can be fit to an equation in which the parameter obtained is C_a-C_c . This parameter has been found to be relatively constant, at light saturating conditions for g_m , which usually happens at values above $500\text{--}600 \mu\text{mol m}^{-2} \text{s}^{-1}$ in olive. A first attempt to use this finding in modelling g_m is presented.

Overall, the present results highlight the fact that g_m should be taken into account in photosynthesis models in the following years. Although, the necessity of incorporating g_m in models is obvious, we still do not know much about the regulation of this variable in response to environmental variables and its equilibrium with photosynthetic capacity and stomata. Therefore, further research in this area should be a priority in the near future.

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ESTIMATING INTRINSIC WATER-USE EFFICIENCY IN TREES: INTERFERENCES BETWEEN THE ^{13}C ISOTOPIC SIGNAL AND INTERNAL CONDUCTANCE TO CO_2 ?

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Abstract

The isotopic discrimination against $^{13}\text{CO}_2$ during photosynthesis (Δ) is frequently recorded from the isotopic composition of different metabolic pools (bulk leaf matter, soluble sugars, cellulose in leaves and wood). Δ is a largely used indicator for short term (interannual) changes in water availability and climate constraints. There is also a large range of evidence that in addition to this phenotypic plasticity, there is also a very tight genetic control over Δ in trees like in many other species. This was evidenced with populations from different origins, within populations on ecological clines, and among clones. Furthermore, quantitative genetics approaches identified a few highly significant QTLs for Δ in full-sib offsprings of oaks, maritime pine, chestnut, poplars, and identified a few genomic regions that are active in the control of Δ . The range of genotypic values identified in these approaches may encompass 3-4 ‰ which would translate into 30-50% difference in intrinsic water-use efficiency (W_i , net CO_2 assimilation rate vs. stomatal conductance, A/g_s).

Unfortunately, much less evidence is available about the genotypic stability of the model relating Δ and W_i . Several parameters of the model may display some degree of genotypic variability. Such parameters include among others the fraction of leaf C fixed by PEPcarboxylase as compared to that fixed by rubisco, the internal conductance to CO_2 (g_i), or ^{13}C discrimination during respiration and subsequent CO_2 recycling. The use of on-line discrimination measurements with a Tunable Diode Laser Spectrometer to assess $^{13}\text{CO}_2/^{12}\text{CO}_2$ in gas exchange chambers could to some extent allow to screen genotypes for the instant relationships between W_i and Δ .

INVOLVEMENT OF MESOPHYLL CONDUCTANCE TO CO₂ IN LEAF-LEVEL WATER-USE-EFFICIENCY

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Abstract

Theoretically, from Fick's first law of diffusion, net photosynthesis A_N can be expressed, under steady-state, as:

$$A_N = g_s (C_a - C_i), \quad [1]$$

$$A_N = g_m (C_i - C_c), \quad [2]$$

where, g_s and g_m are the stomatal and mesophyll conductance to CO₂ diffusion respectively, and C_a , C_i and C_c are the CO₂ concentrations in the atmosphere, in the substomatal internal cavity and in the chloroplast stroma, respectively. Dividing both terms in Eq. [1] by g_s , or combining equations 1 and 2, the following expressions are obtained:

$$A_N / g_s = g_m / g_s (C_i - C_c) \quad [3]$$

$$A_N / g_s = \frac{g_m / g_s}{1 + g_m / g_s} (C_a - C_c), \quad [4]$$

A_N/g_s , often called the “intrinsic water-use-efficiency”, is used as a proxy for leaf level water-use-efficiency (WUE). Both equations suggest a direct, positive relationship between A_N/g_s and g_m/g_s , therefore suggesting that improving the ratio g_m/g_s can be a potential target to improve plant WUE. However, these *apparently direct* relationships may not necessarily hold because in both equations the second term may not be a constant. C_a can vary with long-term changes in atmospheric composition, while changes on C_i and C_c are more complex, and depend on C_a , g_s , g_m and the photosynthetic activity, which in turn depends mostly on light intensity, leaf temperature and Rubisco kinetics.

The aim of this presentation is to test, based on experimental data, whether the above relationships actually holds in nature. A large number of studies have been published over the last decades showing variability of g_m among species, genotypes, or its responses to environmental conditions. This has allowed the compilation of a database composed of 194 paired measurements for different species and genotypes. The data set compiled do not fit with the apparent linearity expected from equation [3], because $(C_i - C_c)$ decreases exponentially with increasing g_m/g_s . However, a positive hyperbolic relationship between A_N/g_s and g_m/g_s is actually found in nature, regardless of whether variations in g_m/g_s are due to genotypic differences or imposed by water stress, that fits significantly ($r^2 = 0.59$) to equation [4]. This occurs regardless of the fact that $C_a - C_c$ is actually not constant, but it ranges from ca. 100 to 300 $\mu\text{mol mol}^{-1}$ without any clear relation with g_m/g_s .

These results suggest that there are possibilities of improving WUE by means of genetically manipulating g_m/g_s . These possibilities are explored by analyzing data from genetically-modified plants known to have an altered g_m . Contrary to what was expected, most genetic manipulations leading to modified g_m do not result in proportional changes in A_N/g_s . This can be due to several reasons, such as simultaneous variations of g_s rendering a constant g_m/g_s ratio (which occurs, for instance, when increasing the amounts of CO_2 -transporting aquaporins) or counteracting variations of $C_a - C_c$ (which occur, for instance, when decreasing the amounts of chloroplast carbonic anhydrase). Of all natural or induced genetic alterations explored, only a natural mitochondrial re-arrangement (MSC16 in cucumber) resulted in 22% increase in g_m/g_s accompanied by 31% increase in A_N/g_s , but this is at the expense of a decreased A_N (9%) and reduced growth due to alterations in respiration. Therefore, it seems that all the genetic manipulations presently known to modify g_m have resulted in plant acclimation responses counteracting the expected repercussion in WUE. On the contrary, some non-genetic manipulations such as exogenous application of abscisic acid, induction of water stress or increased atmospheric vapor pressure deficit result in variations of A_N/g_s and g_m/g_s that fit the general model. The fact that this can be reproduced by adding HgCl_2 suggests that perhaps altering the gating of aquaporins, rather than their total amount, could be a target to improve WUE.