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Carbon dioxide signalling in plant leaves ☆

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Abstract

The role of carbon dioxide (CO_2) as a signal in biochemical regulation networks of plants is fathomed. Transport mechanisms of CO_2 and HCO_3^- are surveyed, which are the prerequisite for signalling. A CO_2 sensor is not known to date, but any reaction where CO_2/HCO_3^- is a substrate can be a candidate. Carbon concentrating mechanisms, e.g., in higher plants C_4-photosynthesis and crassulacean acid metabolism (CAM), generate high internal CO_2 concentrations, important for photosynthesis, but also as a basis for signalling via diffusion of CO_2. Spatiotemporal dynamics of desynchronization/synchronization of photosynthetic activity over leaves can be followed by chlorophyll fluorescence imaging. One example of desynchronization is based on patchiness of stomatal opening/closing in heterobaric leaves due to anatomic constraints of lateral CO_2 diffusion. During CAM, largely different internal CO_2 concentrations prevail in the leaves, offering opportunities to study the effect of lateral diffusion of CO_2 in synchronizing photosynthetic activity over the entire leaves. ***To cite this article: U. Lüttge, C. R. Biologies 330 (2007).***

Résumé

Signalisation par le dioxyde de carbone dans les feuilles des végétaux. On détermine le rôle que joue le dioxyde de carbone (CO_2) dans le réseau des régulations biochimiques chez les plantes. On procède à l'étude des mécanismes de transport de CO_2 et HCO_3^-, mécanismes qui sont à la base de la signalisation. On ne connaît actuellement aucun senseur de CO_2, mais toute réaction admettant CO_2/HCO_3^- comme substrat est un candidat potentiel. Chez les plantes supérieures, les mécanismes de concentration du carbone tels que la photosynthèse en C_4 et le métabolisme acide des crassulacées (CAM) génèrent de fortes concentrations internes de CO_2, lesquelles non seulement jouent un rôle important dans le processus de photosynthèse, mais interviennent également dans la signalisation par le biais de la diffusion de CO_2. Par l'imagerie par fluorescence de la chlorophylle, on peut suivre la dynamique spatiotemporelle de désynchronisation/synchronisation de l'activité photosynthétique des feuilles. Un exemple de désynchronisation est fourni par la répartition en patches de l'ouverture/fermeture des stomates dans les feuilles hétérobares, en raison des contraintes anatomiques qui s'exercent sur la diffusion latérale du CO_2. Dans le cas du CAM on peut trouver des concentrations très diverses de CO_2 dans les feuilles, ce qui permet d'étudier les effets de la diffusion latérale de CO_2 sur la synchronisation de l'activité photosynthétique dans la feuille entière. ***Pour citer cet article : U. Lüttge, C. R. Biologies 330 (2007).***

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Mots-clés : Mécanismes de concentration du carbone (CCM) ; Imagerie par fluorescence de la chlorophylle ; Rythmes circadiens ; Diffusion du CO_2 ; Métabolisme acide des crassulacées (CAM) ; Feuille hétérobare ; Feuille homobare

1. Carbon dioxide in relation to general aspects of signalling in plants

Regulatory networks in systems biology of plants respond to various signalling elements such as:

- electromagnetic radiation of
  - ultraviolet radiation with tetra-hydrofolic acid as receptor,
  - blue light with the receptors cryptochrome and phototropine,
  - red light with the phytochromes as receptors;
- electric signals, with local and action potentials at membranes for short-distance signalling and action potentials transmitted over long distances via the sieve tubes of the phloem [1,2];
- primary phytohormonal messengers;
- secondary messengers, particularly Ca^{2+} ions, cyclic AMP (cAMP), and possibly also pH;
- metabolic messengers, particularly substrates in carbon and nitrogen metabolism, but also in oxygen metabolism with reactive oxygen species (ROS), where H_2O_2 is often seen as a messenger.

It has long been recognized that the transport of metabolites is carrying information for regulatory processes [3] and a large amount of literature is accumulating about this. With this background, it appears plausible to consider also carbon dioxide (CO_2) as a potential powerful signal molecule in metabolism. Currently CO_2 receives much attention as an environmental cue due to dramatic anthropogenic increases of atmospheric CO_2 concentrations and the potential dangers related to the non-homeostasis of this global climatic factor. Within plants, CO_2 is a central element of biochemical networks.

2. CO_2 in biochemical networks

2.1. Transport

Carbon dioxide is transported in the plants via various ways. It diffuses in the gas phase and in the liquid phase. In the latter, diffusive resistance is high and diffusion mostly occurs after equilibration with water in the form of bicarbonate, an important reaction catalysed by the enzyme carbonic anhydrase:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

In the gas phase, transport from the atmosphere into the internal leaf air spaces is restricted by cuticular and controlled by stomatal resistance. Transport to the photosynthetic carboxylation sites is restricted by internal diffusion resistances made up of resistance in the gas phase of the intercellular spaces, liquid-phase resistance in the cell walls and within the cells, and membrane resistance of the chloroplasts. Transport of both CO_2 and HCO_3^- across various membranes can be mediated by facilitated or active transport [4,5]. HCO_3^- transporters are known in membranes [6–10]. CO_2 is moving across membranes via water channels, i.e. the well-known aquaporins [11–14]. Lateral CO_2 diffusion over larger distances in the air spaces within leaves is determined by the homobaric or heterobaric nature of the leaves [15–17]. In homobaric leaves at steady state, no conspicuous partial pressure differences of gases including CO_2 are expected over larger lateral distances. When part of a leaf is darkened, it can be shown that respiratory CO_2 can laterally diffuse over distances as large as 8 mm from the darkened parts into illuminated parts where it may be used as a substrate in photosynthesis [18–22]. Conversely, in heterobaric leaves, due to anatomical constraints of lateral diffusion mainly given by the arrangement of vascular bundles, patchiness of internal gas partial pressure may build up [18,22].

2.2. Carboxylation reactions

CO_2 sensors in metabolism potentially can be any carboxylation reaction, such as:

- ribulose-bis-phosphate carboxylase/oxygenase (RubisCO), the CO_2-fixing enzyme in photosynthesis,
- phospho*enol*pyruvate carboxylase (PEPC) forming oxaloacetate and in a subsequent step of reduction malate from phospho*enol*pyruvate (PEP) and CO_2, which is important in anaplerotic reactions of basic metabolism as well as in primary CO_2 fixation in the modifications of photosynthetic metabolism C_4-photosynthesis and crassulacean acid metabolism (CAM), respectively,

- acetyl-CoA-carboxylase, which forms malonyl-CoA from acetyl-CoA and CO_2 in a two-step reaction of biotin carboxylase and a carboxyl transferase,
- RubisCO activase, which activates RubisCO by carbamylation binding of CO_2 to the enzyme molecule,
- formation of carboamyl-phosphate from CO_2, glutamine and ATP, which is essential in pyrimidine synthesis and in the urea cycle,
- carbonic anhydrase catalysing the CO_2/bicarbonate equilibrium (see Section 2.1).

RubisCO-activase is a regulatory enzyme and could be involved in signalling systems at least at the level of CO_2 fixation in the chloroplast stroma. Pyrimidine biosynthesis is important for formation of nucleic acids and there might be a connection to regulation at the molecular level. Chloroplastic carbonic anhydrase, as recently suggested, may be the CO_2-sensor of a marine diatom (*Phaeodactylon tricornutum*) for perception of CO_2 at the ocean surface [23]. Carbonic anhydrase is a key enzyme in various internal CO_2-concentrating mechanisms (see Section 3).

3. Inorganic carbon concentrating mechanisms (CCMs)

CCMs are important because RubisCO has a remarkably low affinity for CO_2 and at atmospheric CO_2 concentration it operates well below substrate saturation. By affecting CO_2/HCO_3^- equilibria, carbonic anhydrase may modulate concentration gradients important for diffusion. Thus, it has been proposed that the enzyme plays an important role in CCMs in algae and also in chloroplasts [24,25]. At a pH of 8.0 in the chloroplast stroma, it would shift the equilibrium towards HCO_3^-, so that the diffusion gradient for CO_2 across the stroma would be increased [26]. However, there is no clear evidence that this enhances photosynthesis. It was also shown that a reduction in chloroplastic carbonic anhydrase activity of two orders of magnitude did not produce a major limitation on photosynthesis at atmospheric CO_2 levels [27] and high levels of RubisCO activity may be an alternative to CCM [28].

In the CCM of cyanobacteria, inorganic carbon is accumulated in the cells in the form of HCO_3^- after diffusive uptake of CO_2 and/or HCO_3^- transport. At the cytoplasmic face of the thylakoid membranes, a CO_2-hydrating complex, which is part of a proton channel and driven by cyclic electron transport of photosystem I, converts CO_2 to HCO_3^-, which is then taken up by HCO_3^- transporters. HCO_3^- enters the RubisCO-containing carboxysomes, where carbonic anhydrase catalyses conversion to CO_2, which, together with a restricted CO_2 leakage from the carboxysomes, results in elevated CO_2 concentration at the CO_2-fixing enzyme RubisCO [5].

CCMs in leaves of higher plants are the modifications of photosynthesis of C4-photosynthesis [29] and crassulacean acid metabolism (CAM) [30]. In both cases, primary CO_2 fixation is not via RubisCO, but via PEPC, which has an about 60 times higher affinity to CO_2 than RubisCO. For the function of PEPC, carbonic anhydrase is important [31], because the actual substrate of PEPC is HCO_3^- and not CO_2.

In the C_4-plants, the organic acids formed (malate and in some cases aspartate) are transported from a peripheral green 'mesophyll tissue' into green bundle-sheath cells, where they are decarboxylated, making the CO_2-available for refixation and assimilation via RubisCO. The process leads to an about 6-fold higher CO_2 concentration in the bundle sheath cells as compared to atmospheric concentration.

In CAM plants, we distinguish four diurnal phases. In the dark period, phase I, atmospheric CO_2 is fixed via PEPC. Organic acids, i.e. malate and in some cases citrate, are stored in the cell sap vacuoles. After a transition phase (phase II) in the early morning, organic acids are remobilized from the vacuoles and decarboxylated behind closed stomata for provision of CO_2 to be fixed and assimilated via RubisCO (phase III). This leads to the very considerable internal CO_2 concentrating of 0.08–2.5% or 2.0–62.5 times the atmospheric concentration in different species of CAM plants (see [30]). This phenomenon provides an excellent opportunity to study lateral diffusion of CO_2 in leaves and CO_2 signalling functions (see Section 4). Environmental conditions permitting stomata may open in the later afternoon in phase IV for uptake of atmospheric CO_2 and fixation via RubisCO.

4. Heterogeneity of photosynthesis in leaves: desynchronizations and synchronizations

Spatiotemporal dynamics of photosynthesis over entire leaves can be followed by chlorophyll fluorescence imaging [32–36]. When a picture of chlorophyll *a* fluorescence of photosystem II (PSII) is taken at a low irradiance (LOW) of measuring light, this corresponds to the ground fluorescence of the light adapted leaf (F'), and when then a picture is taken at a light saturating flush of high irradiance (HIGH), this corresponds to the maximum fluorescence of the light-adapted leaf

(F'_m). Thus, in direct analogy to the calculation of apparent quantum yield of PSII, i.e. $\Delta F/F'_m = (F'_m - F')/F'_m$, relative quantum use of PSII can be obtained as ΦPSII = (HIGH − LOW)/HIGH. Taken over time, such pictures allow assessment of spatiotemporal dynamics of ΦPSII, providing a means of assessment of the desynchronization/synchronization of photosynthesis in patches of leaves under various conditions and allowing one to ask the question to which extent lateral diffusion of CO_2 is regulating the spatiotemporal dynamics.

4.1. Stomatal patchiness

A pertinent phenomenon is stomatal patchiness, which is observed in heterobaric leaves [37,38]. It is based on the fact that internal CO_2 concentration in the air spaces of leaves is the signal for the movements of stomatal guard cells, where high CO_2 concentrations lead to stomatal closure and lower concentrations to stomatal opening [39–41]. Little is known about the signalling pathway [38,41,42], and the CO_2 sensor is not known (see Section 2.2). It is possible that gradients between atmospheric and leaf internal CO_2 concentration are sensed [43]. The phytohormone abscisic acid, cytoplasmic concentration of the secondary messenger Ca^{2+}, as well as blue and red lights modulate sensitivity of stomatal guard cells to internal CO_2 [41]. Activation of ion channels operating in regulation of osmotic potentials in stomatal guard cells may also be involved [42,44]. In any event, it is evident that when CO_2 concentration differs in isolated internal air spaces, and thus, in sub-stomatal cavities in the leaves of heterobaric plant species also, patches of different stomatal opening ('stomatal patchiness') may occur over the leaves.

4.2. Spatiotemporal dynamics of ΦPSII in relation to CO_2 and O_2 competition at the substrate-binding site of RubisCO

RubisCO has dual substrate reactivity with either CO_2 or O_2. The former, i.e. the carboxylase function, leads to inorganic carbon assimilation. The latter, i.e. the oxygenase function, leads into photorespiration. Photorespiration can be measured on line with gas exchange when regularly short-term pulses of air with 1% O_2 are applied. This increases the CO_2/O_2 ratio in the air to such an extent that non-photorespiratory conditions are established. At 1% O_2, CO_2-gas exchange then corresponds to the maximum carboxylation capacity of RubisCO and at 21% O_2 in normal air to the actual carboxylation rate. The difference between the two represents the rate of photorespiration [45,46]. Studies were made with plants of the C_3/CAM-intermediate species *Clusia minor* acclimatized to perform C_3-photosynthesis and CAM, respectively.

In the C_3-mode of photosynthesis, photorespiration was rather constant over the entire light period. In the CAM mode, it depended on the CAM phases. In phase II, during the onset of the internal CO_2 concentrating process, with increasing internal CO_2 concentration, it was less than in the C_3-mode. In phase III, it could not be measured, as with stomatal closure the impact of changes of O_2 concentration in the external gas phase was much reduced or eliminated. In phase IV, photorespiration was higher than in the C_3-mode. This may be explained by a high internal O_2 concentration still remaining in the leaves from phase III, because, in this phase, when CO_2 concentration is highly raised and photosynthetic CO_2 assimilation operates at high rates with substrate saturation of RubisCO, high internal oxygen concentrations up to 40% are also building up [30,47,48].

With respect to patchiness or heterogeneity over the leaves and putative desynchronization/synchronization mechanisms, concomitant determinations of ΦPSII are most informative. ΦPSII is a measure of the photosynthetic use of irradiance and excitation energy. From the chlorophyll fluorescence images, its degree of heterogeneity can be calculated using the nearest-neighbour matrix concept [49]. In the C_3-mode, ΦPSII was constant under 21% O_2 throughout the light period and it was strongly reduced under 1% O_2 when photorespiration was eliminated. This is due to the higher energy demand of photorespiration as compared to CO_2 assimilation [50–52]. Heterogeneity was generally low and constant under 21% O_2. However, it increased drastically in the non-photorespiratory conditions under 1% O_2. This means that the high photorespiratory energy demand has a stabilizing effect on the overall energy use of the leaves and synchronizes energy use over the entire leaves. In the CAM-mode, ΦPSII was highest in phase III when photosynthesis was substrate saturated. In phases II and IV, a reduction by 1% O_2 was observed as in the C_3-mode. Heterogeneity, however, was much more dependent on the CAM phases than on the application of 1% O_2. This indicates that the heterogeneity observed under 21% O_2 in the CAM-mode is a particular feature of CAM and due to desynchronization processes when dramatic changes of internal CO_2 concentration and hence CO_2 signalling occur in the transitions between phases (see Section 4.3).

4.3. Crassulacean acid metabolism

4.3.1. Day/night cycles

In the obligate CAM plant, *Kalanchoë daigremontiana* heterogeneity of ΦPSII was always very high in phases II and IV when internal CO_2 concentration was low. It declined between phases II and III when a high internal CO_2 concentration built up and increased again between phases III and IV. When ΦPSII was followed in three separated patches as it declined from maximum to minimum, the patches became desynchronized and when ΦPSII rose again to maximum values, they were resynchronized. Heterogeneity was constantly low in phase III [35,36]. This strongly suggests that internal CO_2 is the signalling element in synchronizing photosynthetic activity over the leaves in phase III. In many cases, during the transition from phase III to phase IV, wave fronts of high ΦPSII that were initiated at different spots on the leaves were seen to run in opposite directions and proceeded to meet each other when they extinguished rather than to superimpose on each other [36]. This is another hint for the suggestion that diffusive processes must be the underlying mechanism.

Structurally, with respect to leaf-vein anatomy, *K. daigremontiana* appears to have homobaric leaves. However, the leaf cells are very densely packed and the entire internal air spaces make up only 3% of the whole leaf volume. Constraints for lateral CO_2 diffusion in the leaves have been demonstrated and it was concluded that *K. daigremontiana* leaves are functionally heterobaric [53]. This is confirmed by the heterogeneity of ΦPSII in relation to CAM phases. Internal constraints to CO_2 diffusion in CAM plants are also demonstrated by studies of carbon isotope ratios ($\Delta^{13}C$), showing isotope effects of diffusion [54].

4.3.2. Endogenous circadian rhythmicity

In the C_3/CAM intermediate species *C. minor* in both modes of photosynthesis, there are endogenous free running circadian oscillations of CO_2 and water vapour gas exchange and photorespiration in continuous illumination. ΦPSII only oscillated in the non-photorespiratory conditions under 1% O_2, a value under which the degree of heterogeneity also oscillated [55]. These observations confirm the findings and conclusions gained for the normal day/night cycles (Section 4.2), i.e. that under varying energy demand, photorespiration has a compensating effect on ΦPSII and stabilizes and synchronizes the energy use in the whole leaf.

Some facets of endogenous circadian oscillations of CAM in constant conditions, including continuous illumination in the obligate CAM-species *K. daigremontiana*, however, provided more definitive evidence for the synchronizing role of lateral CO_2 diffusion in leaves. It was found in this plant that there is an upper temperature threshold, above which regular endogenous rhythmicity is lost and gas-exchange patterns become arrhythmic. This is reversible when temperature is lowered again [56–58]. It was seen then, however, that reversibility only occurs when temperature is lowered abruptly from above the threshold back into the rhythmic domain. When temperature is reduced gradually, rhythmicity is not recovered [59]. The interpretation of this phenomenon was that a rather strong signal was required to synchronize individual oscillators present in all green CAM performing cells of the leaves. It could be supported by theoretical model simulations with coupled oscillators reproducing the experimental observations [60]. The individual oscillators are functionally based on the synthesis and breakdown of malate and the biophysics of malate compartmentation, viz., vacuolar accumulation, and remobilization dynamics [61].

A rather simple experimental approach then led to clear evidence of the involvement of lateral CO_2 diffusion in signalling for synchronization [62]. The leaves of *K. daigremontiana* are amphistomatic, having stomata on both sides. In localized places on a leaf, both surfaces were covered with inert transparent silicon grease, creating an artificial patch where stomata were occluded and CO_2 uptake from the atmosphere was prevented at any time. The leaf was then kept in continuous illumination above the temperature threshold, i.e. in the arrhythmic domain. Gas exchange integrated over the whole leaf and ΦPSII recorded by chlorophyll fluorescence imaging remained more or less constant, and ΦPSII was much lower in the greased part, where external CO_2 was not available, than in the adjacent non-greased leaf tissue. Then temperature was abruptly lowered in the rhythmic domain. Whole-leaf gas exchange immediately started its first period of endogenous oscillations, with increased stomatal opening and CO_2 uptake. In the non-greased parts of the leaf, ΦPSII increased somewhat and then remained rather constant, as stomata opened further and atmospheric CO_2 uptake increased. In the greased patch, where atmospheric CO_2 could not be taken up, ΦPSII declined. Then, during the first period of the oscillations, stomata began to close and CO_2 uptake was reduced until it reached its lowest level. ΦPSII remained high in the non-greased parts and low in the greased part and did not change very much until the lowest point of gas exchange was reached. This point indicated that, in the endogenous oscillation, the system had changed from assimilating atmospheric CO_2 to the use of CO_2 derived from vacuolar malate and to internal CO_2 concentrating (Section 3). The latter,

of course, was only possible in the non-greased parts, because the greased parts had not been able to take up CO_2 before and to synthesise malate and store it in the vacuoles. Nevertheless, at this very point, ΦPSII in the greased patches increased abruptly and reached a level similar to that of the non-greased parts. This can only be explained by lateral CO_2 diffusion from the non-greased parts, where it was produced from malate, to the greased parts, supplying substrate for RubisCO and the use of photosynthetic energy, as indicated by ΦPSII. The events described were repeated in subsequent periods of the endogenous oscillations.

5. Conclusions and perspectives

Well-known gaseous messengers in regulation networks of plants are ethylene and nitric oxide. Carbon dioxide may be an additional one, functioning as a signal molecule in synchronizing metabolic processes especially related to photosynthesis in leaves. Information on transport processes being a prerequisite for a signalling function is accumulating, and more is to be expected from intensive current aquaporin research. The quest for a possible central CO_2 sensor remains open. Alternatively, CO_2 sensing may be decentralized in various carboxylation reactions. Interactions via pH effects, especially in CO_2-concentrating mechanisms, e.g., in crassulacean acid metabolism [61], have so far received not enough attention. Regulatory responses at the gene level remain to be tackled.

References

[1] J. Fromm, S. Lautner, Electrical signals and their physiological significance in plants, Plant Cell Environ. 30 (2007) 249–257.
[2] T.E.E. Grams, C. Kozoiolek, R. Lautner, R. Matyssek, J. Fromm, Distinct roles of electric and hydraulic signals on the reaction of leaf gas exchange upon re-irrigation in *Zea mays* L., Plant Cell Environ. 30 (2007) 79–84.
[3] U. Lüttge, N. Higinbotham, Transport in Plants, Springer-Verlag, New York, 1979.
[4] T. Mimura, R. Müller, W.M. Kaiser, T. Shimmen, K.-J. Dietz, ATP-dependent carbon transport in perfused *Chara* cells, Plant Cell Environ. 16 (1993) 653–661.
[5] G.D. Price, S.-I. Maeda, T. Omata, M.R. Badger, Modes of active inorganic carbon uptake in the cyanobacterium, *Synechococcus* sp. PCC7942, Funct. Plant Biol. 29 (2002) 131–149.
[6] T.T. Eighmy, L.S. Jahnke, W.R. Fagerberg, Studies of *Elodea nuttallii* grown under photorespiratory conditions. II. Evidence for bicarbonate active transport, Plant Cell Environ. 14 (1991) 157–165.
[7] W.R. Fagerberg, T.T. Eighmy, L.S. Jahnke, Studies of *Elodea nuttallii* grown under photorespiratory conditions. III. Quantitative cytological characteristics, Plant Cell Environ. 14 (1991) 167–173.
[8] Z. Drechsler, R. Sharkia, Z.T. Cabantchik, S. Beer, Bicarbonate uptake in the marine macroalga *Ulva* sp. is inhibited by classical probes of anion exchange by red blood cells, Planta 191 (1993) 34–40.
[9] Z. Drechsler, R. Sharkia, Z.T. Cabantchik, S. Beer, The relationship of arginine groups to photosynthetic HCO_3^- uptake in *Ulva* sp. mediated by a putative anion exchanger, Planta 194 (1994) 250–255.
[10] R. Sharkia, S. Beer, Z.I. Cabantchik, A membrane-located polypeptide of *Ulva* sp. which may be involved in HCO_3^- uptake is recognized by antibodies raised against the human red-blood-cell anion exchange protein, Planta 194 (1994) 247–249.
[11] N. Uehlein, C. Lovisolo, F. Siefrietz, R. Kaldenhoff, The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological function, Nature 425 (2003) 734–737.
[12] Y.T. Hanba, M. Shibasaka, Y. Hahashi, T. Hayakawa, K. Kasamo, I. Terashima, M. Katsuhara, Overexpression of the barley aquaporin HvPIP2;1 increases internal CO_2 conductance and CO_2 assimilation in the leaves of transgenic rice plants, Plant Cell Physiol. 45 (2004) 521–529.
[13] R. Kaldenhoff, Besides water: Functions of plant membrane intrinsic proteins and aquaporins, Prog. Bot. 67 (2005) 206–218.
[14] J. Flexas, M. Ribas-Carbo, D.T. Hanson, J. Bota, B. Otto, J. Cifre, N. McDowell, H. Medrano, R. Kaldenhoff, Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 in vivo, Plant J. 48 (2006) 427–439.
[15] F.W. Neger, Spaltöffnungsschluß und künstliche Turgorsteigerung, Ber. Dt. Bot. Ges. 30 (1912) 179–194.
[16] F.W. Neger, Wegsamkeit der Laubblätter für Gase, Flora 111 (1918) 152–161.
[17] I. Terashima, Anatomy of non-uniform leaf photosynthesis, Photosynth. Res. 31 (1992) 195–212.
[18] S. Jahnke, M. Krewitt, Atmospheric CO_2 concentration may directly affect leaf respiration measurement in tobacco, but not respiration itself, Plant Cell Environ. 25 (2002) 641–651.
[19] R. Pieruschka, U. Schurr, S. Jahnke, Lateral gas diffusion inside leaves, J. Exp. Bot. 56 (2005) 857–864.
[20] S. Jahnke, R. Pieruschka, Air pressure in clamp-on leaf chambers: a neglected issue in gas exchange measurements, J. Exp. Bot. 57 (2006) 2553–2561.
[21] T. Lawson, J. Morison, Visualising patterns of CO_2 diffusion in leaves, New Phytol. 169 (2006) 641–643.
[22] R. Pieruschka, U. Schurr, M. Jensen, W.F. Wolff, S. Jahnke, Lateral diffusion of CO_2 from shaded to illuminated leaf parts affects photosynthesis inside homobaric leaves, New Phytol. 169 (2006) 779–788.
[23] H. Harada, K. Nakajima, K. Sakane, Y. Matsuada, CO_2 sensing at ocean surface mediated by cAMP in a marine diatom, Plant Physiol. 142 (2006) 1318–1328.
[24] K. Palmquist, J.-W. Yu, M.R. Badger, Carbonic anhydrase and inorganic carbon fluxes in low- and high-C_i cells of *Chlamodomonas reinhardtii* and *Scenedesmus obliquus*, Physiol. Plant. 90 (1994) 537–547.
[25] F.M.M. Morel, E.H. Cox, A.M.L. Kraepiel, T.W. Lane, A.J. Milligan, I. Schaperdoth, J.R. Reinfelder, P.D. Tortell, Acquisition of inorganic carbon by the marine diatom *Thalassiosira weissflogii*, Funct. Plant. Biol. 29 (2002) 301–308.
[26] H.-W. Heldt, Plant Biochemistry, third ed., Elsevier Academic Press, Amsterdam, 2005.
[27] G.D. Price, S. von Caemmerer, J.R. Evans, J.-W. Yu, J. Lloyd, V. Oja, P. Kell, K. Harrison, A. Gallagher, M.R. Badger, Specific reduction of chloroplast carbonic anhydrase activity by antisense RNA in transgenic tobacco plants has a minor effect on photosynthetic CO_2 assimilation, Planta 193 (1994) 331–340.

[28] K. Palmquist, E. Ögren, U. Lernmark, The CO_2-concentrating mechanism is absent in the green alga *Coccomyxa*: a comparative study of photosynthetic CO_2 and light responses of *Coccomyxa, Chlamydomonas reinhardtii* and barley protoplasts, Plant Cell Environ. 17 (1994) 65–72.
[29] R.C. Leegood, C_4 photosynthesis: principles of CO_2 concentration and prospects for its introduction into C_3 plants, J. Exp. Bot. 53 (2002) 581–590.
[30] U. Lüttge, CO_2-concentrating: consequences in crassulacean acid metabolism, J. Exp. Bot. 53 (2002) 2131–2142.
[31] A.B. Cousins, M.R. Badger, S. von Caemmerer, Carbonic anhydrase and its influence on carbon isotope discrimination during C_4 photosynthesis. Insights from antisense RNA in *Flaveria bidentis*, Plant Physiol. 141 (2006) 232–242.
[32] K. Siebke, E. Weis, Assimilation images of leaves of *Glechoma hederacea*: analysis of non-synchronous stomata related oscillations, Planta 196 (1995) 155–165.
[33] K. Siebke, E. Weis, Imaging of chlorophyll-a-fluorescence in leaves: topography of photosynthetic oscillations in leaves of *Glechoma hederacea*, Photosynth. Res. 45 (1995) 225–237.
[34] T. Maddess, U. Rascher, K. Siebke, U. Lüttge, B. Osmond, Definition and evaluation of the spatiotemporal variations in chlorophyll fluorescence during the phases of CAM and during endogenous rhythms in continuous light, in thick leaves of *Kalanchoë daigremontiana*, Plant Biol. 4 (2002) 446–455.
[35] U. Rascher, M.-T. Hütt, K. Siebke, B. Osmond, F. Beck, U. Lüttge, Spatiotemporal verification of metabolism in a plant circadian rhythm: The biological clock as an assembly of coupled individual oscillators, Proc. Natl Acad. Sci. USA 98 (2001) 11801–11805.
[36] U. Rascher, U. Lüttge, High-resolution chlorophyll fluorescence imaging serves as a non-invasive indicator to monitor the spatiotemporal variations of metabolism during the day–night cycle and during the endogenous rhythm in continuous light in the CAM plant *Kalanchoë daigremontiana*, Plant Biol. 4 (2002) 671–681.
[37] W. Beyschlag, J. Eckstein, Stomatal patchiness, Prog. Bot. 59 (1977) 283–298.
[38] U. Lüttge, M.-T. Hütt, Spatiotemporal patterns and distributed computation – A formal link between CO_2 signalling, diffusion and stomatal regulation, Prog. Bot. 68 (2007) 242–260.
[39] K.A. Mott, Sensing of atmospheric CO_2 by plants, Plant Cell Environ. 13 (1990) 731–737.
[40] J.I. Schroeder, G.I. Allen, V. Hugouvieux, J.M. Kwak, D. Waner, Guard cell signal transduction, Annu. Rev. Plant Physiol. Plant Mol. Biol. 52 (2001) 627–658.
[41] A. Vavasseur, A.S. Raghavendra, Guard cell metabolism and CO_2 sensing, New Phytol. 165 (2005) 665–682.
[42] S.M. Hanstein, H.H. Felle, CO_2-triggered chloride release from guard cells in intact fava bean leaves. Kinetics of the onset of stomatal closure, Plant Physiol. 130 (2002) 940–950.
[43] S.M. Assmann, The cellular basis of guard cell sensing of rising CO_2, Plant Cell Environ. 22 (1999) 629–637.
[44] J. Brearley, M.A. Venis, M.R. Blatt, The effect of elevated CO_2 concentrations on K^+ and anion channels of *Vicia faba* L. guard cells, Planta 203 (1997) 145–154.
[45] H.M. Duarte, Chronobiologie von *Clusia minor*: circadianer Rhythmus in einer Pflanze mit C_3/CAM-intermediärem photosynthetischen Verhalten, Dr. rer. nat. Dissertation, Darmstadt, Germany, 2006.
[46] U. Lüttge, Photosynthesis, in: U. Lüttge (Ed.), *Clusia*: A woody neotropical genus of remarkable plasticity and diversity, Ecol. Stud. 194 (2007) 135–186.
[47] M.H. Spalding, D.K. Stumpf, M.S.B. Ku, R.H. Burris, G.E. Edwards, Crassulacean acid metabolism and diurnal variations of internal CO_2 and O_2 concentrations in *Sedum praealtum* DC, Aust. J. Plant Physiol. 6 (1979) 557–567.
[48] U. Lüttge, Historical recollections, in: U. Lüttge (Ed.), *Clusia*: A woody neotropical genus of remarkable plasticity and diversity, Ecol. Stud. 194 (2007) 3–9.
[49] M.-T. Hütt, R. Neff, Quantification of spatiotemporal phenomena by means of cellular automata techniques, Physica A 289 (2001) 498–516.
[50] C.B. Osmond, C.E. Grace, Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? J. Exp. Bot. 46 (1995) 1351–1362.
[51] U. Heber, N.G. Bukhov, V.A. Shuvalov, Y. Kobayashi, O.L. Lange, Protection of the photosynthetic apparatus against damage by excessive illumination in homoiohydric leaves and poikilohydric mosses and lichens, J. Exp. Bot. 52 (2001) 1999–2006.
[52] U. Heber, Irrungen und Wirrungen? The Mehler reaction in relation to cyclic electron transport in C_3 plants, Photosynth. Res. 73 (2002) 223–231.
[53] K. Maxwell, S. von Caemmerer, J.R. Evans, Is a low internal conductance to CO_2 diffusion a consequence of succulence in plants with crassulacean acid metabolism? Aust. J. Plant Physiol. 24 (1997) 777–786.
[54] H. Griffiths, A.B. Cousins, M.R. Badger, S. von Caemmerer, Discrimination in the dark. Resolving the interplay between metabolic and physical constraints to phospho*enol*pyruvate carboxylase activity during the Crassulacean acid metabolism cycle, Plant Physiol. 143 (2007) 1055–1067.
[55] H.M. Duarte, U. Lüttge, Circadian rhythmicity, in: U. Lüttge (Ed.), *Clusia*: A woody neotropical genus of remarkable plasticity and diversity, Ecol. Stud. 194 (2007) 245–256.
[56] U. Lüttge, F. Beck, Endogenous rhythms and chaos in crassulacean acid metabolism, Planta 188 (1992) 28–38.
[57] T.E.E. Grams, F. Beck, U. Lüttge, Generation of rhythmic and arrhythmic behaviour of Crassulacean acid metabolism in *Kalanchoë daigremontiana* under continuous light by varying the irradiance or temperature: Measurements in vivo and model simulations, Planta 198 (1996) 110–117.
[58] T.E.E. Grams, A.M. Borland, A. Roberts, H. Griffiths, F. Beck, U. Lüttge, On the mechanism of reinitiation of endogenous crassulacean acid metabolism rhythm by temperature changes, Plant Physiol. 113 (1997) 1309–1317.
[59] U. Rascher, B. Blasius, F. Beck, U. Lüttge, Temperature profiles for the expression of endogenous rhythmicity and arrythmicity of CO_2 exchange in the CAM plant *Kalanchoë daigremontiana* can be shifted by slow temperature changes, Planta 207 (1998) 76–82.
[60] F. Beck, B. Blasius, U. Lüttge, R. Neff, U. Rascher, Stochastic noise interferes coherently with a model biological clock and produces specific dynamic behaviour, Proc. R. Soc. Lond. B Biol. Sci. 268 (2001) 1307–1313.
[61] U. Lüttge, The tonoplast functioning as the master switch for circadian regulation of crassulacean acid metabolism (CAM), Planta 211 (2000) 761–769.
[62] H.M. Duarte, I. Jakovljevic, F. Kaiser, U. Lüttge, Lateral diffusion of CO_2 in leaves of the crassulacean acid metabolism plant *Kalanchoë daigremontiana* Hamet et Perrier, Planta 220 (2005) 809–816.