

## **Stomatal response of some trees and shrubs from various tropical environments to variable CO<sub>2</sub> concentrations**

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**Abstract:** The stomatal conductance of leaves for water vapor as a function of the intercellular CO<sub>2</sub> concentration and irradiance intensity and soil moisture was investigated for three species of trees and shrubs at their natural habitats in South America: a seasonal tropical rainforest, a tropical dry forest, and a savanna of the Campo Cerrado type. The maximum stomatal conductance of the species from humid environments (rain forest and Campo Cerrado) was higher than that of the species from the dry forest. For all three species the stomata closed with increasing intercellular CO<sub>2</sub> concentration. At high levels of intercellular CO<sub>2</sub> the stomata did not close completely if the soil was water saturated and the irradiance was high. At ambient CO<sub>2</sub> concentrations the stomata were not fully open even under favorable light and soil water conditions. Lowering the external CO<sub>2</sub> concentration to values of the last glacial maximum increased the stomatal conductance beyond that for present-day values. The sensitivity of stomatal closure to an intercellular CO<sub>2</sub> increase was fairly similar for the two species from the rain forest and the dry forest, but was higher for the species from the Campo Cerrado.

**Resumen:** Se investigó la conductancia estomática del vapor de agua en las hojas como una función de la concentración intercelular de CO<sub>2</sub> y la intensidad de la radiación y la humedad del suelo en tres especies de árboles y arbustos en sus hábitats naturales en América del Sur: un bosque tropical estacional, un bosque tropical seco y una sabana tipo campo cerrado. La máxima conductancia estomática de las especies de ambientes húmedos (bosque lluvioso y campo cerrado) fue más alta que la de las especies propias del bosque seco. En las tres especies los estomas se cerraron al haber incrementos en la concentración intercelular de CO<sub>2</sub>. A niveles altos de CO<sub>2</sub> intercelular los estomas no cerraron completamente si el suelo estaba saturado con agua y la radiación era alta. A concentraciones ambientales de CO<sub>2</sub> los estomas no estaban completamente abiertos, aun si las condiciones lumínicas y de agua en el suelo eran adecuadas. Haciendo disminuir la concentración externa de CO<sub>2</sub> hasta llegar a los valores registrados para el último máximo glacial, se incrementó la conductancia estomática más allá de los valores registrados en el presente. La sensibilidad del cierre estomático a los incrementos de CO<sub>2</sub> intercelular fue muy semejante para las dos especies del bosque lluvioso y del bosque seco, pero fue más alta para las especies del campo cerrado.

**Resumo:** A condutância estomática das folhas para o vapor de água como uma função da concentração de CO<sub>2</sub> intercelular, e a intensidade de irradiação e humidade de terra foram investigadas em três espécies de árvores e arbustos nos seus habitats naturais na América do Sul: uma floresta tropical sazonal, uma floresta seca tropical, e uma savana do tipo Campo de Cerrado. A condutância máxima estomática das espécies de ambientes húmidos (floresta tropical e Campo Cerrado) era mais alta do que a das espécies da floresta seca. Para todas as três espécies os estomas fecharam com o aumento da concentração intercelular de CO<sub>2</sub>. A altos níveis de CO<sub>2</sub> intercelular, os estomas não fecharam completamente se o solo estava saturado

de água e a irradiação era alto. A concentrações de CO<sub>2</sub> ambientais os estomas não estavam completamente abertos mesmo sob condições favoráveis de luz e de água no solo. Diminuindo a concentração externa de CO<sub>2</sub> a valores do último máximo glacial verificou-se um aumento da condutância estomática para além dos valores actuais. A sensibilidade do encerramento estomático para um aumento intercelular de CO<sub>2</sub> foi bastante semelhante para as duas espécies da floresta tropical e da floresta seca, mas foi mais elevado para as espécies do Campo Cerrado.

**Key words:** elevated CO<sub>2</sub>, gas exchange, intercellular CO<sub>2</sub>, stomatal conductance,

## Introduction

The CO<sub>2</sub> concentration in the atmosphere has changed dramatically in the recent earth history. While it was low, eventually as low as 180 µl l<sup>-1</sup> (Barnola *et al.* 1999), during the last glacial maximum at 20000 BP, it increased rapidly to 280 µl l<sup>-1</sup> (Neftel *et al.* 1994) at the transition to the postglacial. In the last 150 years a further increase to 370 µl l<sup>-1</sup> (Keeling *et al.* 2001) occurred due to human emissions from fossil carbon sources and land-use changes.

It has been long speculated, that CO<sub>2</sub> has an effect on the stomatal conductance in plants (Freudenberger 1940; Scarth 1932). Mott (1988) demonstrated that the CO<sub>2</sub> concentration in the intercellular spaces,  $c_i$ , rather than the concentration in the free air,  $c_a$ , controls the movement of the stomatal guard cells. Recently Brearley *et al.* (1997) found that K<sup>+</sup> channels in the plasmalemma of the guard cells increase their permeability for K<sup>+</sup> if  $c_i$  rises. This results in a lower osmotic potential and thus hydrostatic pressure, in the guard cells, and causes a closing tendency of the stomata.

Since the stomatal conductance is a major feature in the water budget of plants, lower CO<sub>2</sub> will force the transpiration by opening the stomata, while higher CO<sub>2</sub> may reduce the conductance and thus save water for the plants. This may be especially important in the tropics, where a high saturation deficit of water vapor in air occurs during the day even in rainforest environments (Aoki *et al.* 1975; Kira & Yoda 1989). In order to better understand the potential impacts of low CO<sub>2</sub>, during the glacial maxima, and the high CO<sub>2</sub> to be expected in the near future, on tropical vegetation, it is important to know if different adaptations of the

stomatal regulation occur in different tropical environments.

We have carried out measurements of stomatal conductance of trees, shrubs and herbs at various levels of CO<sub>2</sub> in natural environments in the tropics and other parts of the world. We have used the stomatal conductance and the  $c_i$  data to parameterize our Giessen Global Generic Water Conductance Model (GIWACOM, Esser & Hoffstadt 2000). Here I compare results for three plant species from different tropical environments: *Luehea divaricata* Mez. of the evergreen seasonal rain forests of eastern Paraguay, *Mandevilla velutina* (Mart.) Woodson a shrub of the Campos Cerrados of northeastern Paraguay, and *Aspidosperma quebracho-blanco* Schl. a tree of the dry forests of the northwestern Chaco Boreal.

## Materials and methods

### *Plant species and experimental sites*

*Luehea divaricata* Mez. of the family Tiliaceae is a tall (up to 25 m) deciduous tree of the seasonal rain forests of the Alto Paraná region in eastern Paraguay and southern Brazil. It occurs on humid but well drained terra firme sites. The coordinates (GPS) of the experimental site are S 25° 42' 43" W 56° 12' 24". The soil is a sandy well drained orthic Acrisol. Climate data were extracted from the World Climate database, which is found under <http://www.worldclimate.com/index.htm>. The climate of the station Villarica, about 20 km west of the site, is: Mean annual temperature 22.1 °C, mean temperature of coldest month (June) 17.1 °C, mean temperature of warmest month (January) 27.0 °C. Annual precipitation 1575 mm, driest

month (July) 76 mm, wettest month (November) 157 mm, no arid month.

*Mandevilla velutina* (Mart.) Woodson of the family Apocynaceae is a low shrub of the Campos Cerrados in northeastern Paraguay. It survives the fires as a "shrubby geophyte" with a large lignotuber below ground while the (herbaceous) above-ground shoots are mostly less than 0.75 m tall. The experimental site was at S 23° 33' 44" W 56° 16' 22" on a deep sandy soil (Arenosol). The climate of the station Paso Barreto, about 90 km NW of the site, is: Mean annual temperature 23.4 °C, mean temperature of coldest month (June) 18.7 °C, mean temperature of warmest month (January) 27.5 °C. Annual precipitation 1439 mm, driest month (July) 39 mm, wettest month (November) 197 mm, no arid month.

*Aspidosperma quebracho-blanco* Schl. of the family Apocynaceae is an evergreen small-leaved tree of the dry forests of the central and western Chaco Boreal. The experimental site was at S 22° 18' 37", W 60° 03' 31" (dry season, September) and at S 22° 19' 19", W 60° 03' 17" (wet season, November) on a luvic Kastanozem. The climate of the station Mariscal Estigarribia, about 65 km NW of the site, is: Mean annual temperature 24.8 °C, mean temperature of coldest month (July) 19.5 °C, mean temperature of warmest month (January) 28.9 °C. Annual precipitation 780 mm, driest month (July) 14 mm, wettest month (December) 121 mm. Four arid month occur on average. A month was considered arid, according to Walter & Lieth (1967 ff.), if: (monthly precipitation / 20) < (monthly mean temperature / 10).

### Leaf anatomy

Lac prints were taken from the leaf surfaces to count the number of stomata. If this was not possible, due to pilose, tomentose, or squamose surfaces, stomata numbers were determined from microscopic sections.

A number of leaves was collected from the branch in the vicinity of the measured leaves. They were preserved in 80% ethanol with 3% propionic acid. The leaf anatomy was studied using microscopic techniques (surface sections, longitudinal and transversal sections).

To estimate the maximum aperture of the stomatal split parts of the leaf epidermis were preserved using the above solution. The fully infiltrated tissues were transferred into a hypotonic

medium (deionized water) under the microscope. After a few minutes the aperture was measured.

### Gas exchange measurements

The measurements were carried out at the natural habitat of the species. Experiments with *Luehea* were carried out in the last two weeks of October and those with *Mandevilla* in the first week of November. *Aspidosperma quebracho-blanco* of the dry forests was measured during an arid (beginning of September) and a humid (last two weeks of November) period.

Adult leaves in their natural position exposed to the full sunlight at the border of the crown were taken for the measurements. The results were calculated as means of the results of several different leaves of the same plant.

While the measurements in September in the Chaco Boreal were under dry conditions at the end of the arid season, the measurements in October and November in eastern Paraguay and in November in the Chaco Boreal were carried out under wet conditions with frequent rains and water-saturated soil. We, therefore, assumed that the soil water potential was close to zero during the measurements with *Luehea*, *Mandevilla* and with *Aspidosperma* in November.

The rain forest species, *Luehea divaricata*, was measured at three intensities of incident photosynthetically active radiation (PAR): 58, 216, 2260 [ $\mu\text{E m}^{-2} \text{s}^{-1}$ ]. The other species were measured at light saturation (PAR above 2000 [ $\mu\text{E m}^{-2} \text{s}^{-1}$ ]).

The leaves were fixed within a climatized leaf chamber (Walz GK 022) which was illuminated by a halogen light top (Walz LA-4). Light intensities were controlled using neutral density filters. The air flow through the leaf chamber was held at 1000 ml min<sup>-1</sup>. The very important leaves temperature was measured using a thermocouple (NiCr-Ni) with its thin (diameter 0.1 mm) wires attached to the lower surface of the leaf.

Gas exchange of the leaves was measured using a Walz CMS 400 Compact Minicuvette System equipped with a Rosemount Binos 100 4P CO<sub>2</sub>/H<sub>2</sub>O difference infrared analyzer (CO<sub>2</sub> ± 50  $\mu\text{l l}^{-1}$ , H<sub>2</sub>O ± 1%), leaf chamber temperature control, input humidity control, and light control.

The air mixture (2500 ml min<sup>-1</sup>) was prepared using a Walz CO<sub>2</sub>/O<sub>2</sub>/N<sub>2</sub> gas mixing unit GMA-3, equipped with a Rosemount Binos 100 for absolute CO<sub>2</sub> concentration monitoring.

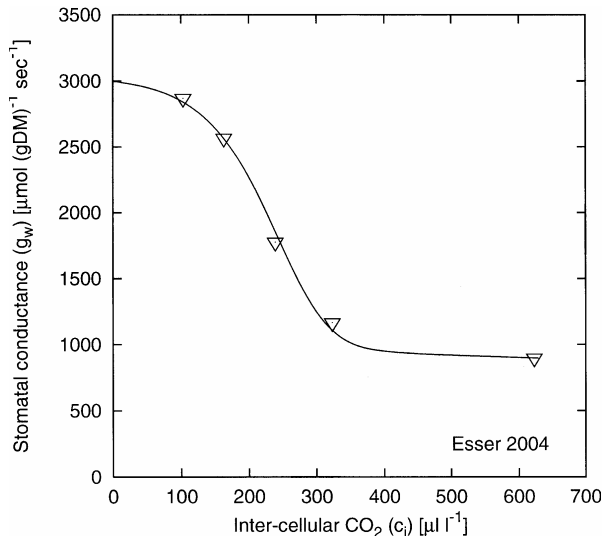
The input humidity was set so that the relative humidity of the air in the leaf chamber was within the limits 70-85%. The CO<sub>2</sub> concentration in the air mixture ( $c_a$ ) was modified during a measurement series according to the following scheme: 360, 100, 360, 200, 360, 600, 360, 1200, 360 [ $\mu\text{l l}^{-1}$ ]. The replication of 360 [ $\mu\text{l l}^{-1}$ ] (ambient CO<sub>2</sub>) was necessary in order to be able to detect and eliminate measurements which were biased by dynamic water demand/availability changes over the day.

Since we were only interested in the steady-state reaction of the stomatal apparatus to various CO<sub>2</sub> levels, the measurements at each of the above concentrations was extended until steady state conductance was achieved (about 30 minutes to one hour).

The relevant data were logged on a Toshiba Laptop computer under the operating system Linux using our own program. The program saved the data on hard disc and was able to graphically display the data in user definable resolution and combinations.

### Data evaluation

The primary data provided by the analyzing system are concentration changes of CO<sub>2</sub> and H<sub>2</sub>O in the air that has passed the leaf. Corrections of these raw data were necessary considering the



**Fig. 1.** The effect of increasing concentration of CO<sub>2</sub> in the intercellular spaces,  $c_i$  [ $\mu\text{l l}^{-1}$ ], on the stomatal conductance for water for the Campo Cerrado species *Mandevilla velutina*. The cspline is a hypothetical reaction derived from the measured points.

absolute concentration of the respective compound, the air pressure, and the co-diffusion of CO<sub>2</sub> and H<sub>2</sub>O in the leaf.

The derived data which were used in the analysis are "stomatal conductance for water vapor",  $g_w$  [ $\mu\text{mol g}^{-1} \text{s}^{-1}$ ], and "CO<sub>2</sub> concentration in the intercellular spaces",  $c_i$  [ $\mu\text{l l}^{-1}$ ]. They were calculated from the measured fluxes of H<sub>2</sub>O and CO<sub>2</sub> after correction for the atmospheric air pressure, the absolute concentration of H<sub>2</sub>O and CO<sub>2</sub>, and the small mass flow caused by the displacement of intercellular air by evaporating water. The theory is explained in detail by Pearcy *et al.* (1989) and Field *et al.* (1989).

The sigmoidal functions in the Fig. 1 through 3 are csplines which were fitted to the data points using the program "gnuplot".

We prefer to relate stomatal conductance to the leaf dry weight rather than to leaf area, if we are interested in the comparison of different species in varying environments. This is because we observed (Kattge 2002) that (1) a better correlation is found with environmental variables; (2) a better correlation is often found with leaf characteristics, for example nitrogen contents; (3) data scattering is reduced. As physiological background for this observation we assume that the diffusive transport through the stomata has been adjusted by evolution to the requirements of the mass-related physiological processes in a plant in a given environment.

## Results

### Leaf anatomy

The rainforest tree *Luehea* has the softest leaves and the lowest value of the leaf mass area (Table 1). *Mandevilla* is a Cerrado plant and has harder leaves, although it does by far not belong to the common Cerrado plants with very sclerophyllous leaves. The highest leaf mass area has the evergreen dry-forest tree *Aspidosperma*. While its leaves are small and hard, large and soft leaves are also frequently found in the dry forests of the Chaco Boreal, but only on dry-deciduous trees (*Chorisia*, *Astronium*, *Schinopsis*).

The number of stomata is highest at the leaves of the Cerrado plant *Mandevilla*. This is in agreement with the observation, that their leaves are very susceptible to drying out if separated from the plant. This characteristic may be also associated

**Table 1.** Anatomical characteristics of the stomatal apparatus of the plant species used in the experiments. The symbols mean: lma- leaf mass area, nst- number of stomata, sl- length of split, maxapert- maximum aperture of split, volg- volume of guard cells, vols- volume of subsidiary cells, vola volume of the apoplast of guard and subsidiary cells.

	<i>Mandevilla velutina</i>	<i>Luehea divaricata</i>	<i>Aspidosperma quebracho-blanco</i>
lma [gDM m <sup>-2</sup> ]	119.0	85.5	227.3
nst [m <sup>-2</sup> ]	4.3 * 10 <sup>8</sup>	1.4 * 10 <sup>8</sup>	1.0 * 10 <sup>8</sup>
type	amphistomatic	hypostomatic	amphistomatic
sl [m]	1.4 * 10 <sup>-5</sup>	1.0 * 10 <sup>-5</sup>	2.3 * 10 <sup>-5</sup>
maxapert [m]	9 * 10 <sup>-6</sup>	6 * 10 <sup>-6</sup>	9 * 10 <sup>-6</sup>
volg [m <sup>3</sup> ]	8.7 * 10 <sup>-15</sup>	3.3 * 10 <sup>-15</sup>	8.1 * 10 <sup>-15</sup>
vols [m <sup>3</sup> ]	75 * 10 <sup>-15</sup>	35 * 10 <sup>-15</sup>	130 * 10 <sup>-15</sup>
vola [m <sup>3</sup> ]	40 * 10 <sup>-15</sup>	10 * 10 <sup>-15</sup>	280 * 10 <sup>-15</sup>

with incomplete and/or slow stomata closure after detachment.

The largest stomatal apparatus has *Aspidosperma*. The volume of the subsidiary cells is more than twice as high as that of the other two species. The large volume of the apoplast is mainly due to the relatively thick cell walls.

### Gas exchange and stomatal conductance

In Table 2 the maximum stomatal conductance as measured under full light (PAR>2000 μE m<sup>-2</sup> s<sup>-1</sup>), water-saturated soil, and low CO<sub>2</sub> concentration (100 μl l<sup>-1</sup>), is given related to leaf dry weight and leaf area. The species *Mandevilla velutina* and *Luehea divaricata* of humid climates of the Campo Cerrado and the rain forest have higher values of maximum g<sub>w</sub> than *Aspidosperma quebracho-blanco* of the warmer and drier climate of the Chaco boreal with four arid month. The values of the two

**Table 2.** Maximum stomatal conductance for water vapor for the three species, related to leaf dry matter and related to leaf area.

	<i>Mandevilla velutina</i>	<i>Luehea divaricata</i>	<i>Aspidosperma quebracho-blanco</i>
g <sub>w</sub> [μmol (g DM) <sup>-1</sup> s <sup>-1</sup> ]	3000	3300	841
[mmol m <sup>-2</sup> s <sup>-1</sup> ]	358	282	193

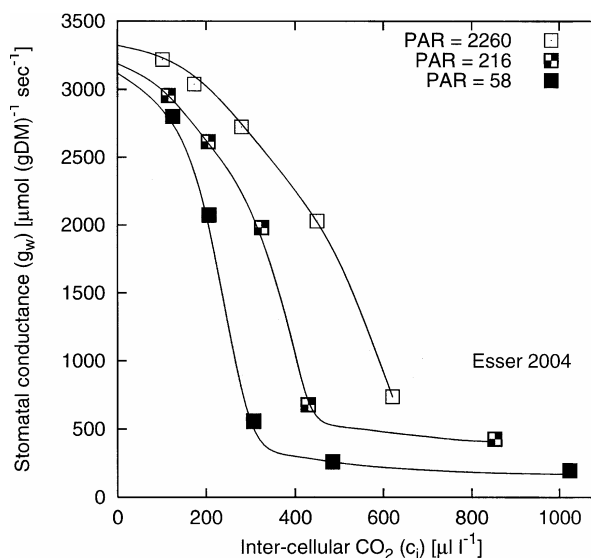
plants of the humid sites are closer together and differ more from that of the semi-arid site if the values are related to dry matter.

Generally, all three species show a decrease of g<sub>w</sub> as c<sub>i</sub> rises, independent of the soil water contents (see Fig. 3: *Aspidosperma*), and of the incident light (Fig. 2: *Luehea*).

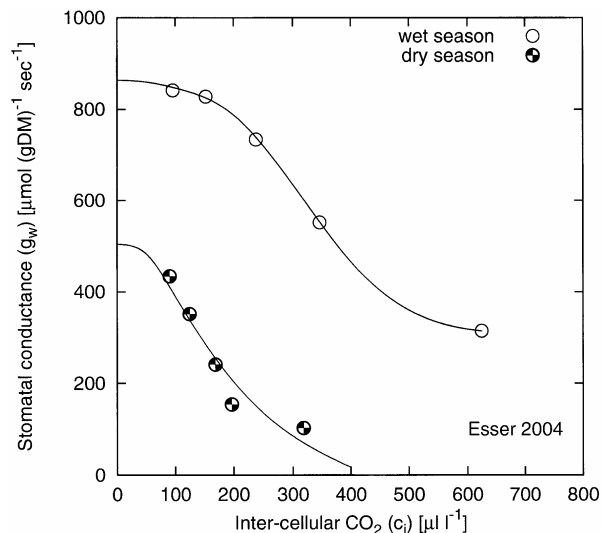
The data suggest that the shape of the function g<sub>w</sub> = f(c<sub>i</sub>) is probably a descending sigmoidal curve. This is particularly obvious for the high-c<sub>i</sub> end of the curves.

It is interesting that high c<sub>i</sub> concentrations alone are not able to close the stomata completely. There seems to be a minimum g<sub>w</sub> that is not reduced in spite of continuous increases in c<sub>i</sub>. This minimum is about 1000 μmol (gDM)<sup>-1</sup> s<sup>-1</sup>, that is 30% of the maximum conductivity, for *Mandevilla*. For *Aspidosperma* it is about 300 μmol (gDM)<sup>-1</sup> s<sup>-1</sup>, which is again 30% of the maximum conductivity.

For *Luehea* the minimum could not be found at full light because it was not reached at c<sub>i</sub> of 620 μl l<sup>-1</sup>, the highest c<sub>i</sub> that could be achieved with c<sub>a</sub> of 1200 μl l<sup>-1</sup>. At lower light level a minimum level for g<sub>w</sub> could be found. It was about 500 μmol (gDM)<sup>-1</sup> s<sup>-1</sup> for the light level 216 μE m<sup>-2</sup> s<sup>-1</sup>, and about 250 μmol (gDM)<sup>-1</sup> s<sup>-1</sup> for 58 μE m<sup>-2</sup> s<sup>-1</sup>.



**Fig. 2.** The effect of increasing concentration of CO<sub>2</sub> in the intercellular spaces, c<sub>i</sub> [μl l<sup>-1</sup>], on the stomatal conductance for water for the rain forest species *Luehea divaricata*, at three levels of incident photosynthetically active radiation (PAR). The radiation is in μE m<sup>-2</sup> s<sup>-1</sup>. The csplines are hypothetical stomatal reactions derived from the measured points.



**Fig. 3.** The effect of increasing concentration of CO<sub>2</sub> in the intercellular spaces,  $c_i$  [ $\mu\text{l l}^{-1}$ ], on the stomatal conductance for water for the dry forest species *Aspidosperma quebracho-blanco*, in the wet and in the dry season. The csplines are hypothetical stomatal reactions derived from the measured points.

The experiment with *Aspidosperma quebracho-blanco* in the dry season, where a negative soil water potential is to be expected, shows that  $c_i$  may close the stomata completely, together with the soil signal (Fig. 3).

The  $c_i$  threshold above which no further closing of the stomata is observed is different for the plant species and the light levels. This threshold is about  $350 \mu\text{l l}^{-1}$  for *Mandevilla*, about  $600 \mu\text{l l}^{-1}$  for *Aspidosperma*, and above  $600 \mu\text{l l}^{-1}$  for *Luehea*. With reduced light level this threshold lowers: from above  $600 \mu\text{l l}^{-1}$  at full light ( $2260 \mu\text{E m}^{-2} \text{s}^{-1}$ ) to  $400 \mu\text{l l}^{-1}$  at  $216 \mu\text{E m}^{-2} \text{s}^{-1}$  and further to  $300 \mu\text{l l}^{-1}$  at  $58 \mu\text{E m}^{-2} \text{s}^{-1}$ , for *Luehea* (Fig. 2).

The slope of the functions in the Figs. 1 to 3 defines how strong the influence of a change in  $c_i$  is on the conductance  $g_w$ . The maximum slope, i.e. the slope of the function at the turning point, given in  $\mu\text{mol (gDM)}^{-1} \text{sec}^{-1} \text{l } \mu\text{l}^{-1}$ , is  $-1.9$  for *Aspidosperma* in the wet season and  $-3.8$  for this species in the dry season. For *Luehea* it is  $-7.5$  for full light,  $-12.7$  for  $216$  and  $-15.7$  for  $58 \mu\text{E m}^{-2} \text{s}^{-1}$ . For *Mandevilla* it is  $-10.3$ .

Expressed as a fraction of maximum conductance it is, at full light and wet soil,  $-0.23 \%$   $\text{l } \mu\text{l}^{-1}$  for *Aspidosperma*, also  $-0.23$  for *Luehea*, and  $-0.34$  for *Mandevilla*. This result suggests that the dry

forest species and the rain forest species are equally sensitive for increasing CO<sub>2</sub> while the reaction of the Cerrado species seems to be stronger.

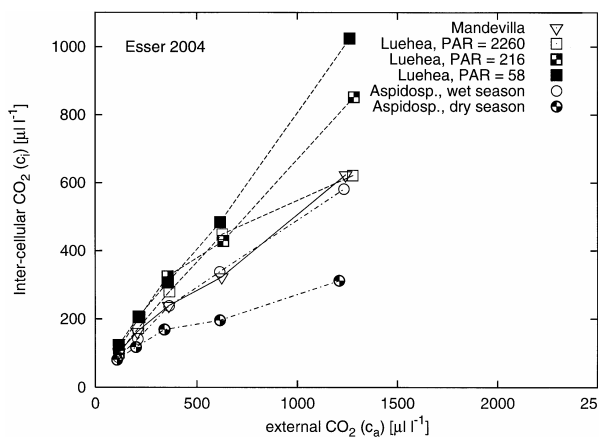
In Fig. 4 the relationship of external ( $c_a$ ) and internal ( $c_i$ ) CO<sub>2</sub> concentration is shown. It is more or less linear for the three species. The internal concentration is still increasing above  $300 - 600 \mu\text{l l}^{-1}$ , although the stomatal conductance is at its lower level and does not further decrease (Figs. 1 to 3). For *Luehea*, the three levels of incident PAR result in a spreading of  $c_i$  values at  $c_a=1200$ , due to the different consumption fluxes of photosynthesis.

## Discussion and conclusions

The reason to carry out these experiments was to get information for the parameterization of a global conductivity model. The experiments reported here were part of more than 500 series of similar measurements in many ecosystems of the world from the polar regions to the tropics (Esser, unpublished data).

We compared species from three important and common tropical environments: A rainforest tree, a dry forest tree and a savanna shrub of "geophytic" habit on the humid savanna type of Campo Cerrado. We found common but also very different features for the three species.

The three species have in common that the stomata are not completely open under ambient air conditions. The  $360 \mu\text{l l}^{-1}$  of present day's atmospheric CO<sub>2</sub> concentration lead to a  $c_i$  of  $170-325 \mu\text{l l}^{-1}$  in the plant (Fig. 4), depending on the species



**Fig. 4.** The relationship of external ( $c_a$ ) and internal ( $c_i$ ) CO<sub>2</sub> concentration for the three species and the different experimental conditions.

and conditions, which is not sufficiently low to fully open the stomata. At full sunlight and well watered soil the stomatal aperture is only 60% for *Mandevilla*, 80% for *Luehea* and 87% for *Aspidosperma*. At the CO<sub>2</sub> concentration of the last glacial maximum, 180 µl l<sup>-1</sup>, the relative opening is 87% for *Mandevilla*, 94% for *Luehea*, and about 100% for *Aspidosperma*. The plants have some excess capacity of stomata aperture which may be interpreted as adaptation to the conditions of the glacial maximum. But, under present CO<sub>2</sub> concentration, faster reactions to rapid changes of c<sub>i</sub> during PAR changes (i.e. due to scattered clouds) are also enabled and may be important.

At higher c<sub>i</sub> there seems to be a minimum level of the conductance so that the stomata never close completely due to CO<sub>2</sub> alone. Although we consider that the determination of c<sub>i</sub> at high c<sub>a</sub> and low stomatal conductance is uncertain, due to the problems to measure the leaf temperature, this effect is found in nearly all measurements. An exception is *Aspidosperma* at high PAR, where the lower level of the conductance has obviously not (yet) been reached.

The descending sigmoid shaped function  $g_w=f(c_i)$  is a consequence of the influence of c<sub>i</sub> on the mechanism of turgor regulation in the guard cells. According to the ion theory (MacRobbie 1987) K<sup>+</sup> ions together with Cl<sup>-</sup> ions and Malate are major osmotica to regulate the turgor. Besides that, Saccharose seems to play a role in turgor regulation of guard cells (Talbot & Zeiger 1998). Recently it was shown that ion channels in the plasmalemma of guard cells change their gating characteristics with increasing concentrations of CO<sub>2</sub> (Brearley *et al.* 1997), so that the K<sup>+</sup> efflux is increased. Considering this we may interpret the three phases of the sigmoid curve. At maximum conductivity, the ion channels are at their lowest permeability for osmotica. Together with the light dependant ion pumps (Willmer & Fricker 1996) this determines the turgor at full opening, which is translated into maximum conductivity for water vapor by morphologic and anatomic characteristics of the stomatal apparatus and of the entire leaf. The minimum level of conductance at high c<sub>i</sub> would mean that ion channels are at their highest permeability, so that the osmotic potential is balanced by the ion pumping and the losses through the channels, at a low value of conductance.

The "window" in which the stomata react to changes of the c<sub>i</sub> ranges 200-500 µl l<sup>-1</sup> and is rather narrow. One may speculate that these species have never seen atmospheric CO<sub>2</sub> concentrations which cause c<sub>i</sub> out of the range of that "window", throughout their time of evolution. They have probably adapted to the low CO<sub>2</sub> concentrations of the glacial periods. But the expected doubling of CO<sub>2</sub> will bring the stomatal conductance close to the minimum level.

Investigations of the influence of the CO<sub>2</sub> concentration on stomatal conductance which were made at the habitat are rare. Experiments were mostly made with potted plants. Field *et al.* (1995) collected 23 data sets of seedlings or saplings of tree species from various environments including a few from the tropics. For doubling of the CO<sub>2</sub> concentration the mean reduction of stomatal conductance was -23% with the extremes -87% and +36%. Morison & Gifford (1984), using 16 crop species, observed a reduction of the stomatal conductance of -36% on average for a CO<sub>2</sub> concentration increase from 360 to 750 µl l<sup>-1</sup>. With negative soil water potential and/or decreasing light intensity a stronger reaction of the stomata to elevated CO<sub>2</sub> is often found (Bunce 2000; Eamus & Jarvis 1989; Morison & Gifford 1984).

Due to the number of external variables influencing the stomatal aperture as well as the maximum stomatal conductance besides CO<sub>2</sub>, a high variability of stomatal reactions to elevated CO<sub>2</sub> is found in experiments, even for a single species: For doubling of CO<sub>2</sub> the change in stomatal conductance for *Liriodendron tulipifera* was between +36% (Norby & O'Neill 1991) and -44% (Williams *et al.* 1986), for *Quercus rubra* between +22% (Dixon *et al.* 1995) and -34% (Williams *et al.* 1986). Curtis *et al.* (1995) could not identify an uniform effect on trees of elevated CO<sub>2</sub>.

One still open question is whether stomata may adapt to changed CO<sub>2</sub> levels if grown under these levels for extended time (Bunce 2001; Chen *et al.* 1995; Heath 1998; Medlyn *et al.* 2001; Rey & Jarvis 1998; Santrucek & Sage 1996; Talbot *et al.* 1996; Tognetti *et al.* 2000). One reason for the difficulties may be based on the experimental problems to separate the different control circuits which determine the stomatal opening and the leaf morphology which is responsible for the maximum conductance. Recently it could be shown the nutrient status of the plant, especially nitrogen,

strongly influences the stomatal conductance (Kattge 2002). This may be the reason why we found less variability of the stomatal reaction to CO<sub>2</sub> for the three tropical species: Plants at their habitat in a natural ecosystem may have a better balanced nitrogen status than plants in pots. This has to be further investigated.

The steepest part of the descending function in our experiments is in the range of  $c_i$  which results from preindustrial to future doubling of CO<sub>2</sub> in the atmosphere. This underlines the relative importance of the persistent increase of atmospheric CO<sub>2</sub> for the water balance of ecosystems. Reduced transpiration per leaf weight must be expected.

It is still not clear, whether or not an adaptation to persistent high CO<sub>2</sub> concentrations in the atmosphere of the morphology and anatomy or of the function of the stomatal apparatus is possible in the rather short time of about 50-100 years until doubling of CO<sub>2</sub> is expected.

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