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Short-term dynamics of stomatal response to sudden increase in CO_2 concentration in maize supplied with different amounts of water

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Abstract

Environmental factors that influence stomatal conductance (g_s) interact through a complex network of signal transduction and have therefore highly interdependent effect.

In the present study we examined how plant water status affects stomatal sensitivity to the change of CO_2 concentration ($[CO_2]$). We investigated the short-term dynamic of stomatal response to a sudden $[CO_2]$ increase (from 400 to 700 μmol(CO_2) mol^{-1}) in maize supplied with different amounts of water (resulting ψ_w = –0.35, –0.52 and –0.75 MPa). Gas exchange measurements were performed in short logging intervals and the response was monitored under two different levels of water vapour pressure deficit (VPD) of 1 and 2 kPa in order to observe the impact of air humidity. Generalized logistic curves were fitted to standardized stomatal response data, which enabled us to objectively estimate the level (relative decrease of g_s) and the dynamics of the response.

Soil water stress and high VPD significantly decreased relative stomatal closure in response to $[CO_2]$ rise, but simultaneously accelerated stomatal response to $[CO_2]$, as revealed by shorter half life ($t_{1/2}$). VPD significantly affected the response of well-watered plants. In contrast, a fast stomatal reaction of water-deprived plants was predetermined by a low xylem water potential (ψ_w) of the leaf and the influence of air humidity was minor.

Additional keywords: curve fitting; elevated CO_2; stomatal dynamics; water potential; water vapour pressure deficit; *Zea mays*.

Introduction

Conductivity of leaf stomata is regulated by external environmental stimuli, such as light, $[CO_2]$, humidity and temperature, *via* the complex signaling network (Schroeder *et al*. 2001, Li *et al*. 2006). As a consequence the influence of these factors on stomata is highly interdependent. The interplay of environmental factors governs not only the instantaneous response of stomata, but can also determine their response to subsequent changes of environmental conditions (Barradas *et al*. 1994, Pepin and Livingston 1997, Frechilla *et al*. 2002, Powles *et al*. 2006). It is well documented, for example, that the response of guard cells to light is strongly influenced by the water status of the plant. Low soil water potential and low air humidity both accelerate the response of stomata to changes of irradiation (Assmann and Grantz 1990, Barradas *et al*. 1994). Similar correlations have been reported for other factors as well (Bunce 1997), Maherali *et al*. 2003, Talbott *et al*. 2003).

Regarding $[CO_2]$, several studies suggest that environmental factors are able to modify stomatal sensitivity to changes of $[CO_2]$. This can explain the high variability in reported $[CO_2]$ response (Raschke *et al*. 1978, Mott 1988, Sharkey and Raschke 1981, Huxman and Monson 2003). The ABA-mediated enhancement of stomatal sensitivity to $[CO_2]$ in water-stressed plants is well established (Raschke 1975, Leymarie *et al*. 1998, Young *et al*. 2006). Long term growth under elevated $[CO_2]$ causes an acclimation in the stomatal $[CO_2]$ response when plants are well-watered (Šantrůček and Sage 1996, Del Pozo *et al*. 2005). Additionally, Frechilla *et al*. (2002) report reversible acclimation of stomatal reactivity to $[CO_2]$, which is induced by different levels of air humidity (Talbott *et al*. 2003). Differences in stomatal response to $[CO_2]$ can be attributed to the alterations in $[CO_2]$ sensing, or in $[CO_2]$-related osmoregulation of guard cells (*e.g*. Cousins *et al*. 2007).

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Abbreviations: $[CO_2]$ – CO_2 concentration; g_s – stomatal conductance; $t_{1/2}$ – half life; VPD – leaf to air vapour pressure deficit; ψ_w – plant xylem water potential.
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The majority of studies that deal with the effects of [CO_2] on stomatal conductance investigate a steady-state response to a given [CO_2]. Also the models that are predicting stomatal response to elevated [CO_2] and other environmental constrains, simulate it for steady-state conditions (Yu *et al* 2004, Ye and Yu 2008), rarely addressing short term dynamics. (Buckley *et al* 2003). Despite the fact that the response time of guard cells to the changes of environmental stimuli can be of general importance for plant performance (*e.g*. response of plants to variable light conditions), the information on the short-term dynamics of stomatal response to [CO_2] change is extremely scarce (Kamakura and Furukawa 2008). Although plants are not frequently exposed to variable [CO_2] conditions, the responsiveness of stomata to [CO_2] can be important in some environments (*e.g*. mofettes; Pfanz *et al*. 2004, dense understories; de Araujo *et al*. 2008). Furthermore, knowledge on the fast dynamics of stomatal response to [CO_2] can also add to the general understanding of stomatal regulation mechanisms.

In the present study, we investigated the short-term dynamic of stomatal response to [CO_2] in maize plants that differ in ψ_w. Plants were grown under ambient [CO_2] and exposed to sudden increases of [CO_2] during the gas-exchange measurements. We hypothesized that the water potential of the plants will affect not only the steady-state level of g_s, but also the short-term course of the stomatal response. In order to consider the influence of air humidity on the dynamics of g_s, measurements were performed at two different levels of VPD.

Materials and methods

Plants: The study was performed as a pot experiment in a greenhouse. Maize (*Zea mays* L. hybrid *PR37H24, Pioneer*) was sown on 16 June 2008 directly to pots (1.5 l) filled with 1.3 kg dry mass of silty-loam soil [pH 6.6 ($CaCl_2$ method), 1.88 mg g^{-1} P_2O_5, 0.257 mg g^{-1} K_2O, 1.9 % organic matter]. The soil was fertilized prior sowing in order to achieve an equivalent of 325 N ha^{-1} [0.5 g KAN (27 % N) kg^{-1} (DM)]. Altogether, 33 pots for gas exchange measurements were set, that were later separated into three irrigation treatments (11 pots per treatment).

In order to define different irrigation regimes the water-holding properties of the substrate were determined by ceramic pressure plate extraction (Klute 1986; model *1600* and *1500*, *Soilmoisture Equipment Corp*., Goleta, USA). The the water content was 25 % and 16.5 % (m/m) at 0.33 and 1 500 kPa, respectively.

At the beginning of the experiment, plants were well watered, keeping soil moisture of 24 %, until they attained the development stage with four fully-extended leaves (1st week of August). Thereafter, three different watering treatments were started. Soil water content was monitored gravimetrically and corrected daily to achieve water contents of 24, 15 and 10 % (m/m). Appropriate water contents were attended in 5 days and were kept for another four days before the measurements started. In this paper, we indicate plants from these three treatments as non-stressed, mildly-stressed and strongly-stressed.

Measurements: Gas exchange measurements were taken with the *Li-6400* (*LI-COR Biosciences*,, Lincoln, USA) measuring system. The first fully-developed leaf was enclosed in the chamber positioned with abaxial side downwards and left to achieve steady-state g_s at 400 µmol(CO_2) mol^{-1}, 25 °C, photosynthetic photon flux density of 1600 µmol m^{-2} s^{-1} (LED light source 6400-02B mounted on the top of chamber) controlling VPD at 1 kPa. g_s and photosynthesis were recorded in three-second intervals. A sudden rise of [CO_2] in the chamber from 400 to 700 µmol(CO_2) mol^{-1} was performed and data recording proceeded until a new stable level of g_s was reached. The measurements were then repeated at VPD of 2 kPa enclosing a nearby area of the same leaf blade.

In parallel to gas exchange measurements, leaf chlorophyll content was measured (*SPAD, Konica-Minolta Sensing Inc.,* Osaka, Japan, USA). Immediately after gas exchange examination, ψ_w was determined using the pressure chamber technique (Scholander *et al*. 1964; chamber *3005-1223*, *Soil Moisture Equipment Corp*., Goleta, USA). The aboveground plant parts and root system were harvested, oven dried (60 °C) to a constant mass and weighed for dry mass estimation.

Data analysis: To compare the dynamics of stomatal response to sudden [CO_2] rise, the five-parameter logistic curves (Richards equation) (Eq. 1) were fitted to standardized stomatal response data with time (x) as the independent variable. Firstly time variable of all g_s data sets was zeroed by using the inflection point of normal logistic model fitted to sample [CO_2] data. Thereby we arbitrarily standardized starting point of leaf exposure to changed [CO_2]. g_s at 400 µmol CO_2 mol^{-1} was set to 100 %, representing the upper asymptote T of the following asymmetric logistic curve:

$$y = B + \frac{T - B}{\left(1 + 10^{(C-x)\cdot D}\right)^S} \quad (1)$$

where T is the upper and B is the lower asymptote (representing steady-state response), C is the time at the curve inflection point, D is the Hill slope of the curve, and S is the curve asymmetry parameter.

To fit Eq. 1 to the stomatal response, the data parameter Hill slope (D) of this equation was constrained to –10 since it is the mathematical property of the fitted curve that the parameters D and S tend to be highly correlated (Motulsky and Christopoulos 2004). Additionally, the $t_{1/2}$ of the relative response was

computed using the following equation (Eq. 2):

$$t_{1/2} = C - \left(\frac{1}{D}\right) \cdot \log(2^{1/S} - 1) \quad (2)$$

Statistics: For the analysis of gas-exchange measurements data and modeled parameters, split-plot *ANOVA* design and simple main effects contrasts were used. For statistical comparison of other measurements (Table 1), one-way *ANOVA* and *LSD* multiple range tests were used. For nonlinear estimation, *GraphPad 5.0* (*GraphPad Software Inc*., La Jolla, USA) was used. Other tests were performed in the *SPSS 16* program 2006 (*SPSS Inc*., Chicago, USA).

Results

The watering treatments resulted in clearly ψ_w differences between three groups of the plants. After 10 days of reduced water supply, plant xylem water potential (ψ_w) reached –0.35 MPa in well-watered, –0.52 MPa in mildly-stressed and –0.75 MPa in strongly-stressed plants. Especially severe water supply restriction resulted in growth retardation as revealed by shoot and root biomass, and a typical drought plant response was reflected in the root-to-shoot ratio, which increased with water deprivation (Table 1). In contrast, there was no significant difference in leaf chlorophyll content (SPAD) between watering treatments.

As expected, the leaf photosynthetic rate clearly responded to water deprivation (Table 2), which can be explained by the stomatal inhibition of photosynthesis. In well-watered plants, the photosynthetic rate (A_{400}) was

Table 1. Biomass, water potential (ψ_w) and leaf chlorophyll content of maize (*Zea mays* L.) plants exposed to three different watering regimes. Means ± SE are presented, n = 10–11. Means of a single parameter, followed by different letters significantly differ at $p < 0.05$. SPAD – relative chlorophyll content units; DM – dry mass.

	Non-stressed	Mildly-stressed	Strongly-stressed	ANOVA p
ψ_w [MPa]	–0.35 ± 0.04 a	–0.52 ± 0.04 b	–0.75 ± 0.04 c	0.000
Shoot DM [g]	5.77 ± 0.28 a	5.02 ± 0.27 a	3.53 ± 0.27 b	0.000
Root DM [g]	2.07 ± 0.12 a	1.89 ± 0.11 ab	1.64 ± 0.11 b	0.040
Root : shoot ratio	0.37 ± 0.02 a	0.37 ± 0.02 a	0.46 ± 0.02 b	0.001
SPAD	39.40 ± 0.61	38.76 ± 0.58	38.08 ± 0.58	0.313

Table 2. Photosynthetic rates (A), intercellular CO_2 concentrations (C_i) and steady state stomatal conductance (g_s), measured at 400 and 700 µmol(CO_2) mol^{-1}, and model derived parameters B (lower asymptote of curve by Eq. 1) and half-life of stomatal response ($t_{1/2}$) of maize (*Zea mays* L.) plants exposed to a sudden increase of ambient CO_2 concentration [400 to 700 µmol(CO_2) mol^{-1}]. Plants were exposed to different watering regimes and VPD levels during the measurements. Means ± SE are presented. n = 9–11. Different letters that follow the means of a single parameter within one watering regime, indicate a significant difference at $p < 0.05$. Split plot *ANOVA* results (p-values) are shown beneath.

	A_{400} [µmol(CO_2) m^{-2} s^{-1}]	A_{700} [µmol(CO_2) m^{-2} s^{-1}]	C_{i400} [µmol(CO_2) mol^{-1}]	C_{i700} [µmol(CO_2) mol^{-1}]	g_{s400} [mol(H_2O) m^{-2} s^{-1}]	g_{s700} [mol(H_2O) m^{-2} s^{-1}]	B [% of initial]	$t_{1/2}$ [min]
Non-stressed								
VPD 1 kPa	22.8 ± 1.4a	22.5 ± 1.2a	202.1 ± 7.8a	331.9 ± 13.2a	0.28 ± 0.01a	0.14 ± 0.004a	44.6 ± 1.1a	1.99 ± 0.15a
VPD 2 kPa	24.8 ± 1.2b	24.3 ± 1.2a	172.4 ± 8.0b	277.1 ± 10.8b	0.22 ± 0.01b	0.11 ± 0.004b	52.1 ± 1.0b	1.15 ± 0.12b
Mildly-stressed								
VPD 1 kPa	19.9 ± 1.2a	20.0 ± 1.0a	144.9 ± 9.6a	244.4 ± 13.6a	0.16 ± 0.01a	0.09 ± 0.004a	52.5 ± 0.9a	0.85 ± 0.12a
VPD 2 kPa	20.1 ± 1.1a	20.2 ± 1.1a	124.4 ± 9.4b	214.2 ± 9.9b	0.14 ± 0.01b	0.08 ± 0.004a	55.6 ± 0.9b	0.67 ± 0.12b
Strongly-stressed								
VPD 1 kPa	14.9 ± 1 0a	15.0 ± 1.0a	128.7 ± 8.5a	237.3 ± 10.9a	0.11 ± 0.01a	0.06 ± 0.004a	53.9 ± 1.0a	0.59 ± 0.12a
VPD 2 kPa	16.0 ± 0.8a	16.5 ± 0.8a	113.2 ± 9.0a	207.1 ± 9.4b	0.11 ± 0.01a	0.06 ± 0.004a	58.7 ± 1.0b	0.53 ± 0.12a
ANOVA								
Water supply	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
VPD	0.06	0.07	0.00	0.00	0.00	0.01	0.00	0.00
Water supply × VPD	0.20	0.29	0.50	0.51	0.00	0.07	0.18	0.06

approximately 24 μmol m^{-2} s^{-1}, while it dropped to 20 and 15 μmol m^{-2} s^{-1} in mildly- and strongly-stressed plants, respectively. The differences between the photosynthetic rates measured at two levels of VPD (1 and 2 kPa) were negligible.

Initial g_s of well-watered plants, measured at 400 μmol(CO_2) mol^{-1}, reached 0.28 and 0.22 mol(H_2O) m^{-2} s^{-1} at VPD of 1 and 2 kPa, respectively. In plants with lower water potential (mildly-stressed, strongly-stressed), much lower g_s was detected. In strongly-stressed plants, it reached only the half of g_s of well-watered plants and was insensitive to VPD.

A sudden change of [CO_2] in the measuring chamber [400 to 700 μmol(CO_2) mol^{-1}] resulted in relatively rapid stomatal closure (Fig. 1). The extent of the relative stomatal closure depended on plant water status and VPD (Fig. 2). In the case of well-watered plants, g_s decreased to 44.7 % [g_s = 0.14 mol(H_2O) m^{-2} s^{-1}] and 52.1 % (g_s = 0.11 mol(H_2O) m^{-2} s^{-1}] of the initial conductance under VPD of 1 kPa and 2 kPa, respectively. In water-stressed plants, the relative decrease of g_s was smaller than in the well-watered control, especially when monitored under higher VPD. Strongly-stressed plants, for example, exhibited 53.9 % (VPD 1 kPa) and 58.7 % (VPD 2 kPa) of initial g_s when exposed to elevated [CO_2].

The rate of stomatal reaction to sudden [CO_2] increase was higher in stressed plants as revealed by $t_{1/2}$ (Table 2, Fig. 2). The slowest response ($t_{1/2}$= 1.9 min) was detected in well-watered plants at low VPD. On the contrary, the steady state of g_s was reached in the range of a minute in the most stressed plants. In these plants, the rate of stomatal response was insensitive to the VPD level, while the significant effect of VPD was found only in well-watered plants. There, the response was about 40 % faster at 2 kPa when compared to 1 kPa. A significant interaction of the two factors, watering treatment and VPD, was revealed by *ANOVA* (Table 2).

The initial fast decrease of g_s after a sudden increase of [CO_2] was frequently (20 % of measurements) followed by a fast rhythmic stomatal movement with a time period of 1.3–2 min (Fig. 1*D–F*). A descending amplitude of this oscillation led ordinary to a steady-state g_s within 4–10 min.

Discussion

The rate of reduction of g_s, induced by increase of [CO_2], is well in agreement with previous literature reports. Similar decreases of g_s can be found both when plants are for a longer time exposed for elevated [CO_2] in fumigation experiments (Ainsworth and Rogers, 2007) and also when transient exposure of plants to elevated [CO_2] is applied (Morison, 1987, Lawson *et al*. 2008) The range of stomatal response to [CO_2] is, however,

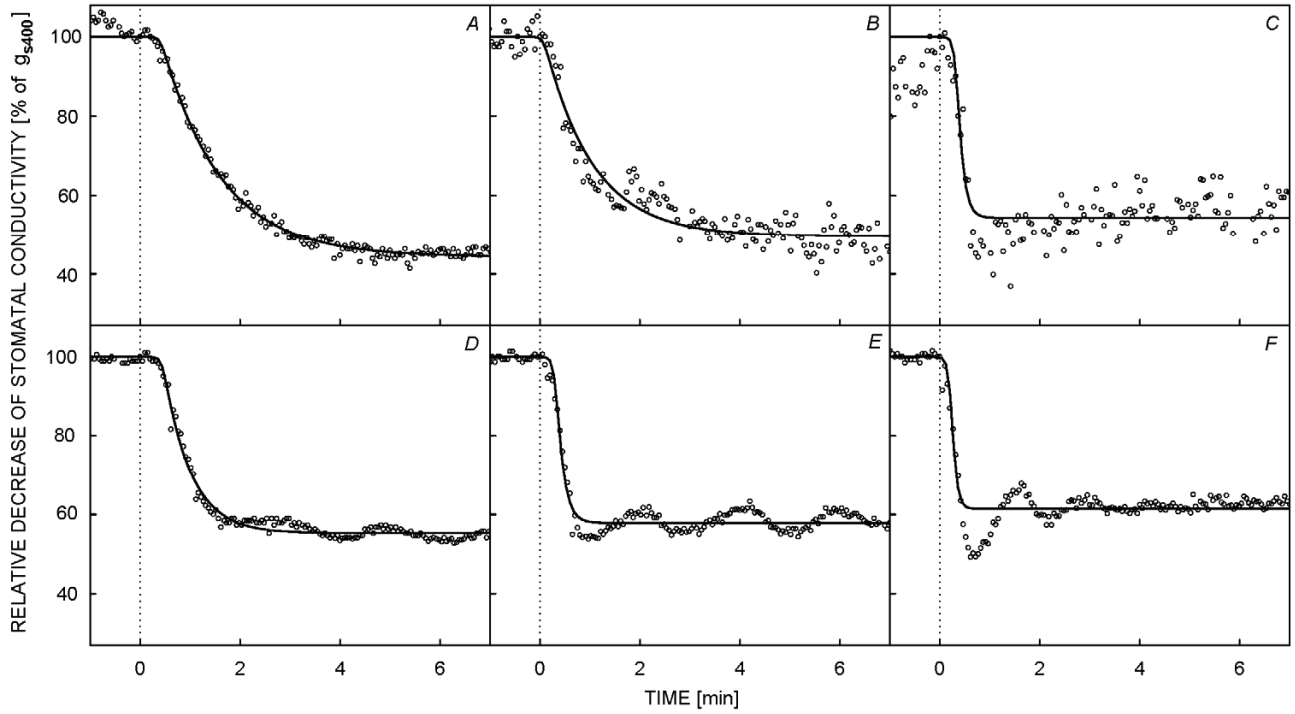

Fig. 1. Stomatal response of maize (*Zea mays* L.) plants exposed to a sudden increase of ambient [CO_2] [400 to 700 μmol(CO_2) mol^{-1}] in time 0. The most typical patterns of the change of stomatal conductance (g_s) plotted as a percentage decrease from the initial g_s, measured at 400 μmol(CO_2) mol^{-1} and accompanying asymmetric logistic model (Eq. 1) curve fits are presented.

widely depended on [CO_2] (Kamakura and Furukawa 2008), but also on other factors that govern the action of guard cells (*e.g*. humidity, Talbott *et al*. 2003). In our case, the response of stomata, measured as a relative decrease of initial g_s, clearly depended on plant water status and VPD. The lower the water potential and the higher the VPD, the lower the relative decrease in g_s was. Comparison of initial g_s, measured at 400 μmol(CO_2) mol^{-1}, revealed that the effect of higher VPD on stomatal closure ceases with decreasing water potential. The influence of VPD on g_s occurs through diffusion of water from evaporating sites in the leaf and is therefore diminished in water deprived plants where g_s is limited (Buckley *et al*. 2003). We can presume that in water stressed maize a water potential driven hydroactive regulation of stomata predetermined a rate of reaction, while it is suggested that hydropassive regulation is involved in the stomatal responses to VPD in plants that are not severely drought-stressed (Bunce 1997, Dzikiti *et al*. 2007).

On the other hand stomatal sensitivity of maize plants to [CO_2] increase was preserved at low water potentials, which reflects differences in regulation of guard cell response between VPD and [CO_2]. In comparison to the former, a sensing pathway of [CO_2] is poorly understood (Vavasseur and Raghavendra 2005). The input signal of CO_2-dependent regulation of guard cell movements is considered to be the substomatal intercellular [CO_2] (Mott, 1990), but it is still controversial what is the role of photosynthesis in signaling. Lawson *et al* (2008) recently reported that CO_2-dependent closing response of stomata is not dependent upon guard or mesophyll cell photosynthetic capacity, but that photosynthetic electron transport, or its end-products, regulate the control of stomatal response to [CO_2]. It was suggested that ATP (Buckley *et al*. 2003) or zeaxanthin (Zhu *et al*. 1998) could be involved in signaling intercellular [CO_2]. In response to elevated [CO_2] plasma membrane anion channels of guard cells are activated (Vavasseur and Raghavendra 2005). In *Vicia faba* an increase of apoplastic Cl^- was found to occur within 10 min after a rise in [CO_2] from 350 to 600 μmol(CO_2) mol^{-1} (Hanstein and Felle 2002). A shorter response time of anion fluxes was reported by Brearley *et al*. (1997 when epidermal strips of the same species were exposed to a rise in [CO_2] from 350 to 1000 μmol(CO_2) mol^{-1}. This discrepancy could result from the rate of [CO_2] increase. Similarly, reports on pore measurements in *Ipomea pes-caprae* clearly revealed a trend of increased rates of stomata closure with increasing [CO_2] [0 to 500, 0 to 700, 0 to 900 μmol(CO_2) mol^{-1}] (Kamakura and Furukawa 2008). In our case the final effect of the [CO_2] rise [400 to 700 μmol(CO_2) mol^{-1}], a steady-state of g_s, was achieved within several minutes. Much shorter time is, however, needed when plants are insufficiently watered and/or exposed to high evaporative demand. We were namely able to show that the air humidity, as indicated by different VPD levels, influences not only the level of steady state response of stomata to [CO_2], but also the dynamics of the response to sudden [CO_2] increase. In this case, drier air in a measuring chamber (VPD of 2 kPa) induced a faster reaction of stomata to sudden [CO_2] change, which was more pronounced in well-watered plants with high initial g_s. In the case of water-deprived plants, a fast reaction can be related to a low water potential of the leaf and is not influenced by VPD. Dependence of the stomatal response on the initial rates of g_s can lead to presumption that the stomatal dynamics is related to C_i levels and to the carbon requirements of photosynthesis, which are reduced under water deprivation.

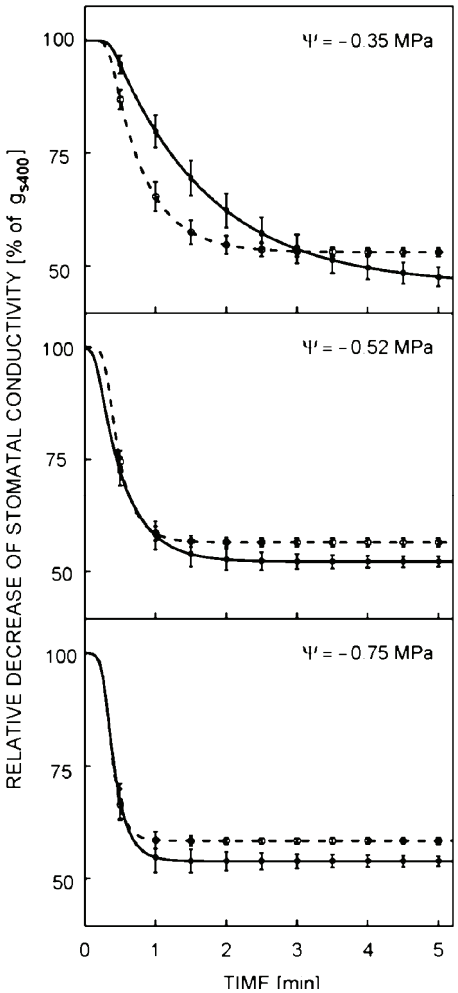

Fig. 2. Stomatal response of maize (*Zea mays* L.) plants exposed to a sudden increase of ambient [CO_2] [400 to 700 μmol(CO_2) mol^{-1}] in time 0. The change of stomatal conductance (g_s) is plotted as a percentage decrease from the initial g_s, measured at 400 μmol(CO_2) mol^{-1} and is derived from an asymmetric logistic model (Eq. 1). The results are presented as means and SE for non-stressed (*upper panel*), mildly-stressed (*middle panel*) and strongly-stressed plants (*lower panel*), VPD = 1 kPa (*solid line*) and VPD = 2 kPa (*dashed line*). n = 9–11.

Sudden changes of one environmental factor in otherwise stable environmental conditions often trigger stomatal oscillations (Kaiser and Kappen 2001). The period of the g_s oscillations that have been observed in some of our measurements (Fig. 1) is much shorter than the period of oscillations that are primarily controlled by plant water status (Barrs 1971, Steppe *et al*. 2006). A pattern of measured g_s was more scattered in plants exposed to low VPD, which indicates that stomatal response could be more synchronous under higher VPD, as suggested by Kaiser and Kappen (2001). As purposed by Barrs (1971), stomatal oscillations of short duration (<10 min) and small amplitude can also be controlled by external $[CO_2]$. They are believed to occur due to instability in the negative feedback loops (Barrs 1971, Herppich and von Willert 1995) caused by lags and/or overshooting responses to feedback signals (Kaiser and Kappen 2001). In our case, these oscillations were more frequently observed in high VPD exposed maize plants with a low initial g_s, which is in agreement with literature reports (Kaiser and Kappen 2001, West *et al*. 2005).

In conclusion, we have demonstrated a very fast response of maize stomata to sudden increases of $[CO_2]$. The dynamics of the response of maize were clearly dependent on water potential and on the VPD. These results reflect that the strength of a single environmental factors in the stomatal regulation increases or decreases according to the overall environmental conditions.

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