

Responses of stomatal conductance to CO₂ and vapor pressure deficit in silver birch (*Betula pendula*)



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Responses of stomatal conductance to CO₂ and vapor pressure deficit in silver birch (*Betula pendula*)

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Abstract

Stomatal conductance (g_s) and photosynthesis (A_n) to CO₂ and vapor pressure deficit (VPD) of silver birch were measured in silver birch (*Betula pendula*) in order to assess stomatal behavior of this species. Stomatal conductance decreased notably after an increase in [CO₂] or VPD and increased in response to a decrease in [CO₂]. VPD had a clear dominance over CO₂, since stomata could reopen after CO₂ induced closure at low VPD but not at high VPD. Stomata closure by high VPD was irreversible, suggesting a significant influence of Abscisic acid (ABA) remaining in the leaves on stomatal responses. VPD had also a significant influence in the photosynthetic capacities, since J_{max} showed lower values under high VPD.

1. Introduction

Stomata are small pores on the surfaces of leaves and stems, gated by highly specialized cells called guard cells. Guard cells are responsible of the exchange of gases between the interior of the leaf and the atmosphere (Hetherington & Woodward, 2003). They regulate their concentration of osmotically active solutes in order to control the volume and turgor pressure and they are the reason of the variation in stomatal conductance (g_s) (Mott & Buckley 2000, Vavasseur & Raghavendra 2004). They have been proposed as the main centers of stomatal CO₂ sensing. The main function of these cells is to reduce the water losses and at the same time facilitate the diffusion of CO₂ to the mesophyll cells (Vavasseur & Raghavendra, 2004). In order to reach this aim in a changing environment, guard cells have developed complex metabolism and signal transduction pathways, allowing the rapid control and modulation of the stomata to environmental signals. It is well known that environmental signals can control the aperture and development of the stomata because of the high sensitivity of the guard cells to environmental variables (Jones, 1992).

g_s responds to changes of different environmental variables (VPD, CO₂, light, temperature) by increasing or decreasing the aperture of the stomata, in order to balance CO₂ uptake and H₂O losses by transpiration, increasing their survival in a changing environment (Aasama & Söber, 2011). Many studies have focused on the stomatal response to [CO₂] since researchers have been very interested in this topic because of two reasons: (i) The evidence that stomata response to intercellular CO₂ concentration (C_i) and (ii) the increase in the CO₂ of the atmosphere since the industrial revolution, that may have affected the stomata performance nowadays (Morrison, 1998). CO₂ is the most important anthropogenic greenhouse gas because of the increasing anthropogenic emissions from fossil fuels and the change in land use. According to previous researches, plants respond to increasing [CO₂] by increasing photosynthesis (A) and decreasing

their g_s . A lot of papers have been based on the stomatal response to the current $[CO_2]$, so their responses are well known so far. According to these studies the increase of $[CO_2]$ induced a decrease in the g_s . Results from [Morison \(1998\)](#) suggest an average decrease of 40% with twice than the current $[CO_2]$ as a direct physiological response.

This direct response may not translate into a similar long-term effect of growing in elevated CO_2 on g_s , since the stomatal response can depend on a lot of different factors as the different species (Heath and Kerstiens, 1997) or the growing conditions (Talbot *et al*, 1996). For instance, g_s reduction was about 21 % when plants were grown under controlled conditions, being this a long-term period stomatal response (Ainsworth *et al*, 2007). [Drake *et al* \(1997\)](#) got similar stomatal responses since g_s was reduced by 20% across in many species during long term high $[CO_2]$ exposure.

Many studies have defined the stomatal responses to individual environmental factors, but environmental factors may change in combination. Most of the researches have been focused on the stomatal response to the change of one factor at the time and some data is also available on stomatal responses to sequential changes in different environmental factors, as air humidity and CO_2 (Aasama & Söber, 2011). However, stomatal responses to different factors can interact, leading to different results (Aasama & Söber, 2011). Many papers have shown that the effect of different environmental factors on g_s (eg CO_2 , VPD) (Morison & Gifford 1983, Talbot *et al* 2003). Importantly, [Aasama & Söber \(2011\)](#) showed that hydraulic factors (as VPD) had a stronger effect on stomatal responses than photosynthetic factors (as $[CO_2]$) when they changed both at the same time. Hence, stomata response to simultaneous factors is necessary in order to understand the stomata functioning more accurately. However, despite the importance of this topic there have been little research on the stomatal responses to simultaneous changes in a controlled environment (Aasama & Söber, 2011).

Different plant hormones have also been proposed to affect the response of the guard cells and thereby, to change the g_s (Archaya & Assmann, 2009) such as the abscisic acid (ABA) concentration (Morrison, 1998). Abscisic acid (ABA) has been supposed to play an important role in the stomatal conductance, responding directly to the air humidity and soil moisture. ABA is a stress hormone, so at high VPD or low soil moisture, when the water potential of the plant is low the $[ABA]$ increases, leading to a decrease in the g_s in order to avoid the loss of water (Aasama & Söber 2011, Morison 1998).

Photosynthesis has been shown to be also affected by environmental factors. While responses to radiation, CO_2 and temperature are fairly well known, the possible influence of environmental factors affecting leaf water potential (i.e., VPD, soil moisture) is poorly known. Since photosynthesis is the main physiological process that increase the plant growth and crop productivity, studying it is very important in order to assess the effect of the climate change on the crop's production (Yin & Struik, 2009). Photosynthesis is one of the most studied and best understood processes in plant physiology, representing the conversion of light energy into chemical energy through the reduction of CO_2 to triose phosphate (Beadle & Long, 1985)

There are several models based on the photosynthetic response to environmental factors, as the biochemical model published by [Farquar \(1980\)](#). According to this model, the leaf carbon assimilation (A) of a C_3 leaf under any conditions, is limited by the rubisco carboxylation and by the RuBP regeneration by the electron transport (Bernacchi *et al* 2001, Medlyn *et al* 2002). By parameterizing this model is possible to see if there is an influence of the environmental factors on the photosynthetic capacities: the maximum rate of rubisco

carboxylation (V_{cmax}) and the maximum rate of electron transport rate (J_{max}).

The aims of the current study are:

- (i) Determine the effect of CO₂ and VPD alone and in combination on the g_s of the silver birch (*Betula pendula*).
- (ii) Assess if there is a dominance of the VPD over CO₂ or vice versa.
- (iii) Find out if there is a dominance of the closure response over the opening response or vice versa.
- (iii) Study the influence of VPD on the photosynthetic capacities.

2. Material and methods

2.1 Plants

In February 2013, 12 two year old seedlings of the silver birch (*Betula pendula*) were transported to the laboratory and germinated in an ambient (400 $\mu\text{mol mol}^{-1}$) atmospheric CO₂ concentration and grown in pots in a controlled environmental chamber in Gothenburg University of Environmental Sciences for three months.

Trees were watered three times a week (Monday, Wednesday and Friday) from February to the beginning of May, when the measurements were finished. Trees were fertilized often in order to allow for a relative growth rate of 3-5 % per week (falling from 5% to 3% as self-shading progressed)

The conditions of the chamber were typically: temperature (25°C), [CO₂] (400 $\mu\text{mol mol}^{-1}$), relative humidity (40-60%) and VPD (1.3-1.9 kPa). Relative humidity changed in the beginning and in the end of the experiment, falling to 40% as the lowest value. Photosynthetic photon flux density (PPFD) was between 400-500 $\mu\text{mol}^{-2} \text{s}^{-1}$ during 5.30 to 21.30.

2.2 Gas exchange measurements

The short-term stomatal response was measured with a Li-6400 (LICOR Inc., Lincoln, NE, USA). Each leaf was clamped in the standard leaf chamber of the Li-6400, where the conditions (VPD or CO₂) were changed. Measurements were done on 20 days within the three months period measuring two or three days in a row each week (4 days in February, 12 days in March and 6 days in April, respectively). Trees were measured from 8:00 in the morning at the earliest to 20:00 at the latest.

During the gas exchange measurements, leaf chamber conditions were the following: 500 $\mu\text{mol}^{-2}\text{s}^{-1}$ PPFD, 25°C leaf temperature, 1 or 3 kPa VPD and 400 or 800 $\mu\text{mol mol}^{-1}$. CO₂ or VPD values were different depending on the treatment. After a minimum period of 15-20 minutes of acclimation to the chamber conditions the steady state was awaited ($\leq 3\%$ change during 4 minutes). When the steady state was reached, the g_s was recorded. After recording the steady state g_s , VPD or CO₂ were immediately changed in the leaf chamber.

Two different measurement campaigns were conducted during the experiment, with the change of two environmental factors with two levels of each: high [CO₂] (800 $\mu\text{mol mol}^{-1}$) and high VPD (3 KPa) and low [CO₂] (400 $\mu\text{mol mol}^{-1}$) and low VPD (1 KPa).

Depending on the measurement campaign, only one factor was changed or both were changed together. The set of changes were the following: An increase in CO₂ (from 400 to 800 μmol mol⁻¹) a decrease in CO₂ (from 800 to 400 μmol mol⁻¹) an increase of VPD (from 1 to 3 KPa), a decrease of VPD (from 3 to 1 KPa) and the simultaneous change of VPD and CO₂:

1st measure campaign: a) 800/1 → 400/1 → 400/3 → 800/1 → 400/3
b) 400/3 → 800/3 → 800/1 → 400/3 → 800/1

2nd measure campaign a) 400/1 → 800/1 → 400/1
b) 400/3 → 800/3 → 400/3

a) 400/1 → 400/3 → 400/1
b) 400/1 → 800/1 → 400/1
c) 400/3 → 800/3 → 400/3

2.3. Photosynthetic model

The photosynthetic response to different intercellular [CO₂] (C_i) under high light (A) was also measured during the current experiment. Several A/C_i curves were measured during the beginning and the end of the treatment in order to not disturb the stomatal response measurements. The [CO₂] used during the A/C_i curves were 400, 300, 225, 125, 60, 400, 800, 1200, 1600, 2000 and 400 μmol mol⁻¹. According to Farquar *et al* photosynthetic model, it is possible to model gross photosynthesis (A_g) from the minimum of two limiting rates:

$$A_g = \min(A_c, A_j) - R_d$$

Where A_c is the photosynthesis when Rubisco carboxylation is limiting, A_j is the photosynthesis when RuBP regeneration is limiting and R_d is the maximum rate of dark-respiration.

From the A/C_i curves, the Farquar *et al* (1980) model was used in order to parameterize the maximum rate of rubisco carboxylation (V_{Cmax}) and the maximum rate of maximum electron transport rate (driving RuBP regeneration) (J_{max}). The parameterization was conducted in excel and V_{Cmax} and J_{max} were obtained from A_c and A_j after applying the solver with the error of the predicted photosynthesis, A_{min} (A_c.A_j).

2.4. Statistical Analysis

Paired samples t-test analysis were done with excel in order to evaluate the significance of the differences between the stomatal conductances after the changes of environmental factors and to predict the different values of V_{Cmax} and J_{max} at high VPD (3 kPa) and low VPD (1 kPa). Moreover, SAS tests were applied in order to assess the interaction between the different measurement protocols and the VPD effect on V_{Cmax} and J_{max}.

3. Results

The effect of changing CO₂ or VPD separately (fixing the other factor)

When a low [CO₂] was fixed, the increase of VPD induced a significant decrease of stomatal conductance ($P < 0.01$; Figure 1). Just after, when the VPD was decreased, stomata did not reopen again, namely, there was not a significant increase in the stomatal conductance ($P > 0.05$). Even if waiting between 30-50 minutes the opening wasn't still significant.

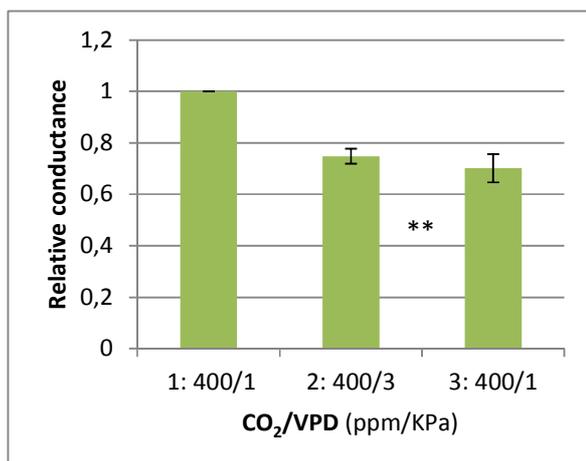


Figure 1. Change in relative stomatal conductance to the change of VPD (when a low [CO₂] was fixed). The initial change is an increase in the VPD (from 1 to 3 kPa), followed by a subsequent decrease in VPD (from 3 to 1 kPa). The order in which different environmental conditions were applied were from left to right (Figure 1, 2a-b, 3a-b, 4, 5). Asterisks denote significant changes in *g_s* when changing VPD. ** $P < 0.01$ * $P < 0.05$. Error bars represent standard error (StErr) (Figure 1, 2a-b, 3a-b, 4, 5).

At high humidity (VPD = 1 kPa) stomata closed in response to an increase of the [CO₂] from 400 to 800 $\mu\text{mol mol}^{-1}$ and the stomata could reopen again when the [CO₂] was decreased from 800 to 400 $\mu\text{mol mol}^{-1}$ (Figure 2 and 3) so there was a significant opening and closure of stomata induced by CO₂ ($P < 0.05$) in the low VPD treatment.

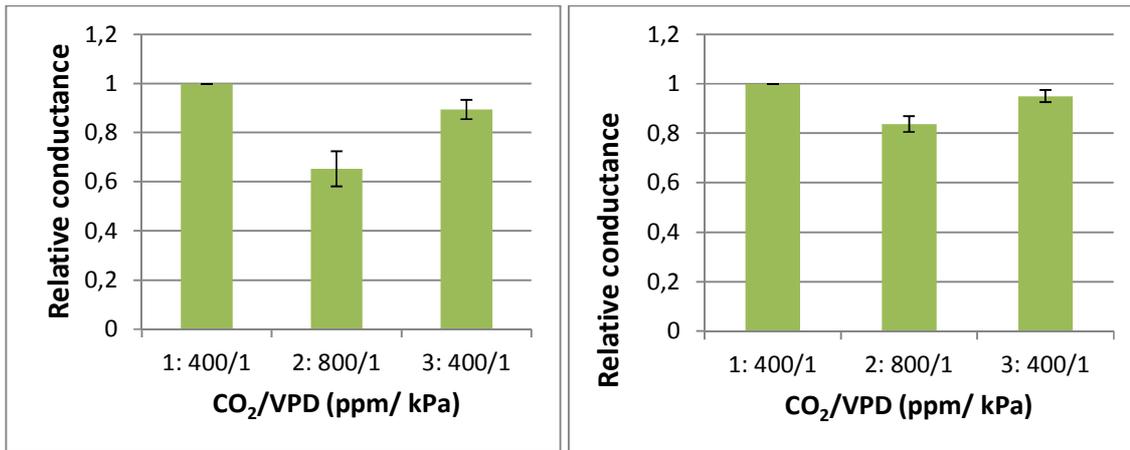


Figure 2a-b. Change in relative stomatal conductance to the change of [CO₂] when high humidity (VPD 1) was fixed. The [CO₂] was increased (from 400 to 800 $\mu\text{mol mol}^{-1}$ in the beginning and decreased (from 800 to 400 $\mu\text{mol mol}^{-1}$) again in the last step. Two protocol measurements were done in the beginning and the end of this measurement campaign (Figure 2a-b). Both figures correspond to the same measurement campaign, but at different moments of the experiment.

When high VPD was fixed, there wasn't a significant opening with the decrease of [CO₂] ($P > 0,05$). However, the stomata responded with a significant closure ($P < 0,05$) to the increasing [CO₂] (Figure 4 and 5).

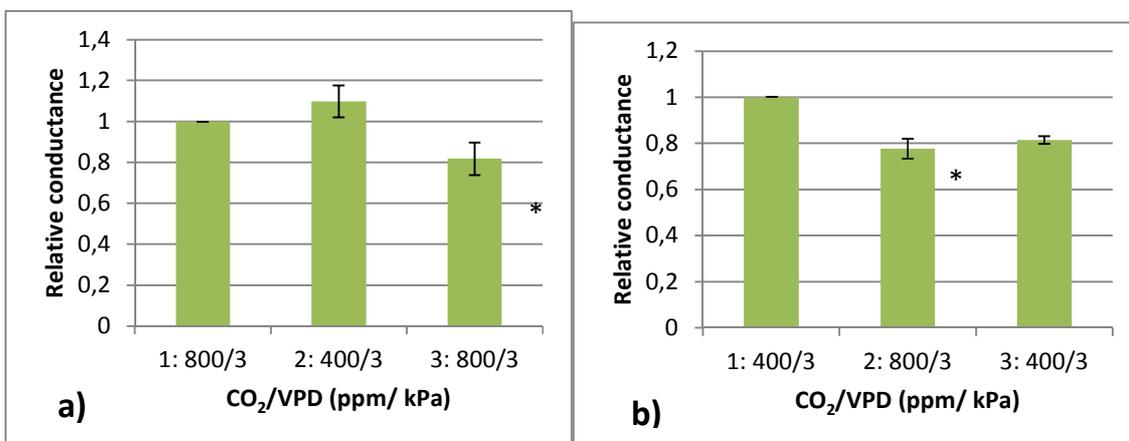


Figure 3a-b. Change in relative conductance changing CO₂ and fixing high VPD. In the figure a) [CO₂] was decreased (from 800 to 400 $\mu\text{mol mol}^{-1}$ in the beginning and was followed by a subsequent increase in [CO₂] (from 400 to 800 $\mu\text{mol mol}^{-1}$). In the figure b) the initial change was an increase in the [CO₂] (from 400 to 800 $\mu\text{mol mol}^{-1}$) and the last step was a decrease (from 800 to 400 $\mu\text{mol mol}^{-1}$). Both figures correspond to the second measurement campaign, but at different moments of the experiment (In the beginning and in the end)

The effect of changing both factors at the same time

At low VPD, the decrease of $[CO_2]$ (from 800 to 400 $\mu\text{mol mol}^{-1}$) didn't induce a significant opening ($P>0.05$). However, there was a significant closure of the stomata following an increase in VPD (from 1 to 3 KPa) (Figure 4). On the other hand, the simultaneous change of both factors (VPD and CO_2) didn't have a clear effect in the stomata response, since the stomatal conductance didn't show significant changes (Figure 4, Figure 5).

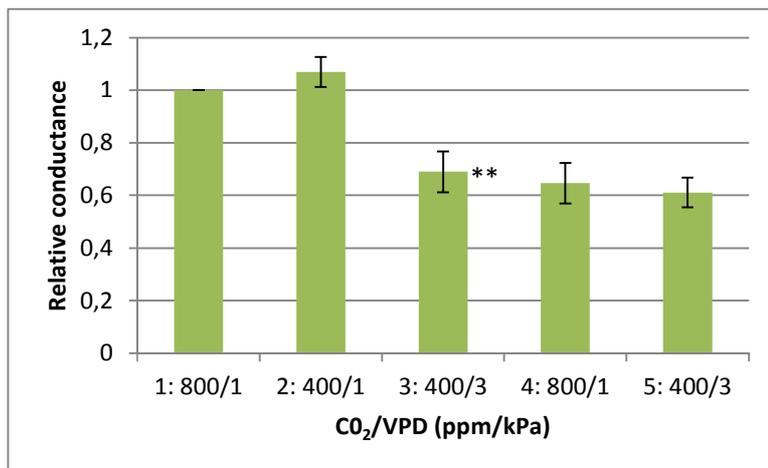


Figure 4. Change in relative conductance to the change of both factors (CO_2 or VPD) at the same time. In the beginning there was a decrease of the CO_2 (from 800 to 400 $\mu\text{mol mol}^{-1}$) at low VPD. Afterwards, there was an increase in the VPD (from 1 to 3 KPa) at low $[CO_2]$ and in the end there was a change of VPD and CO_2 simultaneously. Asterisks denote significant differences between the different treatments, ** $P<0.01$ * $P<0.05$.

At high VPD, following an increase in $[CO_2]$ (from 400 to 800 $\mu\text{mol mol}^{-1}$) the stomatal conductance decreased significantly ($P<0.01$) and this decrease was higher at high VPD (50%) than at low VPD (35% and 18%) (Figure 2 and 3). After the closure, the stomata didn't reopen even after a decrease in the VPD (from 3 to 1 KPa). Neither did the change of both factors have a significant effect ($P>0.05$) on the stomatal conductance (Figure 5).

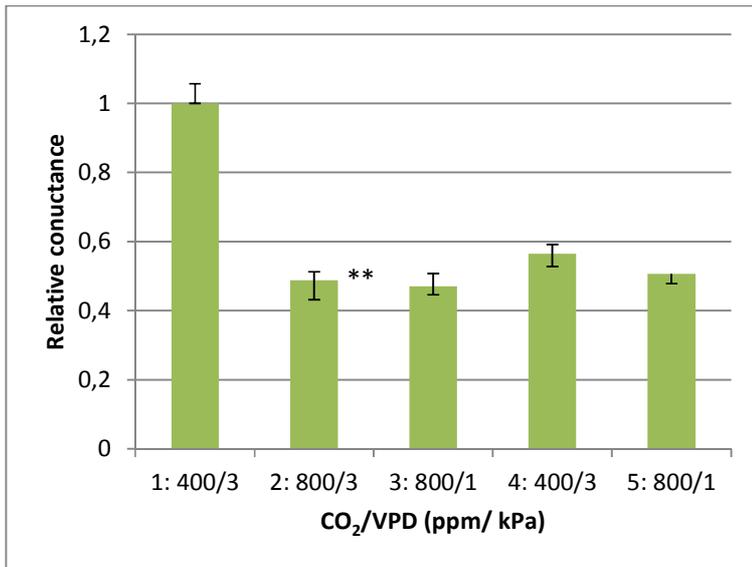


Figure 5. Rate of change in relative conductance to the change of both factors (CO₂ or VPD) at the same time. In the beginning there was an increase of the CO₂ (from 400 to 800 μmol mol⁻¹) at high VPD. In the next step there was a decrease in the VPD (from 3 to 1 KPa) at high [CO₂] and in the end there was a change of VPD and CO₂ simultaneously. Asterisks denote significant differences between the different treatments, **P<0.01 * P<0.05.

The response of *V*_{cmax} and *J*_{max} to the different humidity (VPD).

When analyzing the photosynthetic parameters, a highly significant interaction (P=0.0136) was found between the measurement protocol and the VPD (P< 0,05), namely, the VPD effect was different for the two protocols. There was a strong effect of the VPD on the *J*_{max} in protocol 2, since there were significant differences between *J*_{max} at low (1KPa) and high (3 KPa) VPD (P<0.05) while there weren't significant differences in protocol 1 (Figure 6). Although *V*_{cmax} was higher at low VPD (1 KPa) than at high VPD (3KPa) in both protocols, there wasn't a significant different at different humidity in protocol 1 and protocol 2 (Figure 6).

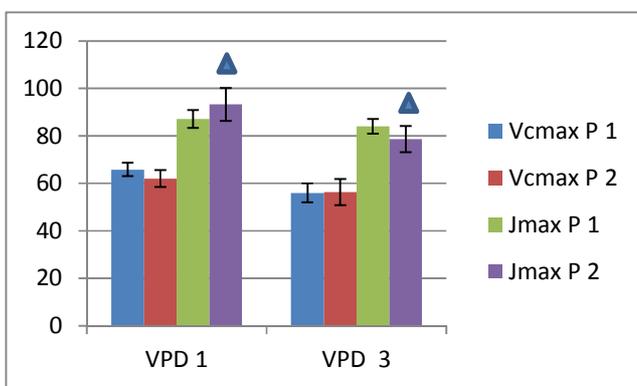


Figure 6. *V*_{cmax} and *J*_{max} values at low (1 KPa) and high (3 KPa) VPD. The figure shows the different values of *V*_{cmax} and *J*_{max} in two different protocols: protocol 1 (P1) and protocol 2 (P2). Triangles denote significant differences at different VPD. Error bars

represent the standard error (StErr).

4. Discussion

Stomatal responses to VPD.

Silver birch (*Betula pendula*) g_s showed a strong stomatal closure induced to high $[CO_2]$ ($800 \mu\text{mol mol}^{-1}$) at high VPD. On the other hand, opening responses when VPD was decreased were totally absent, suggesting a strong stress effect of high VPD on the stomata performance (Figure 7).

Many explanations have been found in order to explain this marked decrease in g_s during high VPD. It is well known that in response to water stress, plants regulate their transpiration by the decrease of stomatal conductance in order to avoid loss of water and dehydration, so they can protect their cells and tissues from being damaged (Sperry, 2000, Bond 1997).

In *Betula occidentalis* the decrease in g_s was a strategy in response to the water stress in order to avoid the death of the roots and the plant because of the cavitation, that is a very common response of mesic plants in xeric habitats in order to be more resistant to very low water potentials (Sperry *et al* 1993, Sperry 2000).

ABA could have played an important role in stomatal responses of our experiment, since after an increase of VPD all the samples showed a notably decrease in g_s . Moreover, even after improving the humidity conditions (decrease of VPD) or after a decrease of $[CO_2]$ stomata didn't reopen again. This stress hormone is one of the main causes of the stomatal closure responses to water stress, since in response to a decrease in water potential in a plant, $[ABA]$ increases. The increase of this stress hormone induce a closure of the stomata, so the conductance decrease strongly (Aasama & Söber 2011, Assmann, 2010). ABA has an important influence in the regulation of guard cells, which surround and define the width and size of the stomata. Stomata are essential in order to take up CO_2 for photosynthesis but inevitably they can also lose water through transpiration. Hence, guard cells respond to the increase in $[ABA]$ by promotion of stomatal closure or by the inhibition of stomatal opening in order to reduce the evaporative water loss (Assmann, 2010). What is more surprising, ABA can have an interesting effect on stomata response, since the increase in ABA prevailing in the leaves can inhibit the stomatal opening by the increase of Ca^{2+} in the guard cells (Assmann 2010, De Silva 1985). Figure 7 shows the expected effect of ABA on stomatal opening and closure when ABA is increased or decreased, which matched with the results of our research.

After the closure of stomata because of high VPD, a longer period of time (between fifty and sixty minutes) was waited after the subsequent decrease of VPD in order to assess if the lack of sensitivity of the stomata to the humidity was a matter of time, but the results were the same, since stomata remained closed. These results match with the results of Uddling *et al* (2004), where they showed a clear and strong influence of the time of the day with high VPD on g_s of silver birch. They found that stomata closed during afternoon because of the increase of VPD and afterwards they didn't reopen even if VPD was decreased again. The sensitivity of stomata to high VPD can prevent the plant from suffering xylem cavitation, that is a typical negative effect because of water stress in plants and trees that leads to dehydrative damage to cells and tissues (Uddling *et al* 2004, Sperry 2000, Bond 1997) since as water deficit develop, the transport characteristics of the hydraulic pipeline between soil and leaf impose physical limits on the supply of water to the leaves and thereby, on the

potential transpiration allowed by stomata (Tyree and Sperry, 1989). This isohydric behavior in order to avoid cavitation is a common response to water stress in fast-growing species and has been observed in more species than *Betula pendula*, such as *Betula occidentalis* and *Populus euramericana* (Saliendra et al 1995, Tardieu and Simonneau 1998).

In order to describe the stomatal responses to different environmental factors, empirical models of g_s have been used (Ball *et al* 1987, Leuning 1995). In these models, g_s is supposed to change directly with humidity and $[CO_2]$ (besides other factors), but these models only include direct responses to current conditions and otherwise, they don't include the effects of plant hydraulic and metabolic memory because of the effect of ABA so the development of a new model would be needed in order to get a more successful description of stomatal responses (Leuning, 1995).

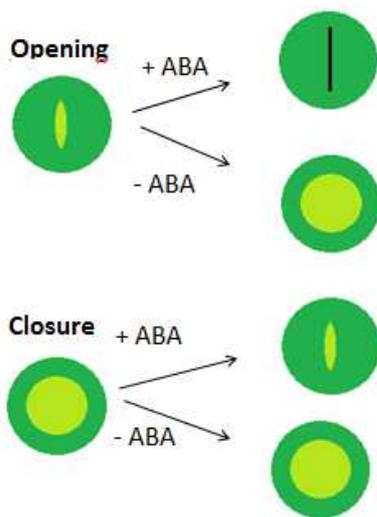


Figure 7. Influence of ABA on stomata responses. ABA inhibit stomatal opening and promotes stomatal closure.

The dominance of closure to VPD over CO_2 responses.

A dominance of the VPD closure over CO_2 responses was observed during our experiment. At high VPD (3 KPa) the stomata always closed, suggesting a strong effect of VPD on the closure, regardless of the other factor. In accordance with the results of this study, Aasama *et al* (2011) revealed that stomatal response is more sensitive to hydraulic factors (as VPD) than to photosynthetic factors (as CO_2) in temperate deciduous trees. This dominance of closure at high VPD can have been developed as a strategy of the stomata in order to avoid the loss of water during water stress, by reducing the transpiration (Aasama & Söber 2011, Sperry 2000, Hacke & Sauter 1995). These responses are not uncommon, as many papers have shown so far water availability play a very important role in g_s , since g_s decrease notably when the water potential, soil humidity or air humidity decrease (Bond & Kavanagh, 1997). The relation between stomatal behavior and the hydraulic properties of the plant (water potential, xylem conductance) or the soil and atmosphere humidity has been discussed previously by many researchers (Williams *et al*, 1996)

The effect of CO₂ on the stomatal performance.

Despite VPD closure was dominant over CO₂ responses, [CO₂] had a strong effect on the stomata performance, showing a clear sensitivity of silver birch to this environmental factor. In our experiment, stomata responded to the increase in [CO₂] with a clear decrease in the conductance, at high and low VPD. This physiological response is very common in nature, since when stomata of several species are exposed to high [CO₂], the aperture of stomata usually decline (Morison 1993, Morison 1998, Aasama & Söber 2011). According to Morison (1998) this closure can vary between the species and according to the different environmental factors and growing conditions or leaf age. During our experiment, reduction percentages varied between 20 and 50 %, reaching the maximum closure at high VPD (3kPa) and high [CO₂] (800 μmol mol⁻¹) (50 %).

Matching with our results, [CO₂] twice as high as current atmosphere resulted in a reduction of g_s in previous studies. For instance, Morison showed a decrease of g_s between 40 and 60 % as a short term stomatal response (Morison 1998, Morison 1987) and a decrease of only 20% was observed in many long term response experiments (Ainsworth *et al* 2007, Drake *et al* 1997), although this lower response could happen due to in most of the studies the samples were trees which were grown under controlled conditions and because of trees have lower sensitivity to CO₂ (Curtis 1996). In fact, a less common but probable response is that stomatal response remain unchanged under high [CO₂]. In several studies related to long-term response, g_s did not decrease at all or had a very slight no significant opening, which could be because of those plants grew under stress conditions or they showed a responsiveness to CO₂ because they had been previously exposed to high [CO₂] during short periods (Curtis 1996, Sage 1994, Rey & Jarvis 1997). Importantly, a more likely explanation could be that the effect of elevated CO₂ on g_s is highly dependent on the stage and the stand development of the plant, so early stages of the plant could be more sensitive to high CO₂ than mature trees (Uddling, 2009).

In our experiment, the opposite response was observed with low [CO₂] concentration (400 μmol mol⁻¹) resulting in a stomata aperture (increase in stomatal conductance), in accordance with our results, an increase in g_s has been showed in many studies (Morison 1993, Morison 1998) because of the need to increase C_i and CO₂ uptake and in order to avoid the damage of cells because of the excess of photoreductive power when there is not enough CO₂ substrate (Brodibb *et al*, 2009).

Stomatal sensitivities of fast growers and their competitive success during drought conditions.

Stomatal sensitivities differ remarkably in fast-growers and slow-growers tree species, and these differences are essential in order to their survival and development in different habitats (Aasama & Söber, 2011). Silver birch (*Betula pendula*) is a fast-growing tree species and is characterized by a high sensitivity to VPD and water potential of the plant, showing very high conductance during low VPD conditions but this response is notably affected by the influence of ABA during drought (Aasama & Söber 2011). Our results demonstrated a high sensitivity of *Betula pendula* to VPD, since although the g_s was high at high humidity (1 KPa) the conductance decreased sharply when we applied a high VPD (3 KPa), so we suggest that there was also a strong influence of ABA mediated by high VPD in the stomatal closure.

The dominance of these species in riparian habitats (Jones et al,2009) is due to their ability to reach high g_s and aperture under high humidity, so they can uptake more CO_2 in order to make photosynthesis but they can also show an efficient decrease of the aperture during water stress periods (Aasama & Söber,2011). This could be another possible explanation for our results, since *Betula pendula* could uptake more CO_2 and increase photosynthesis during low VPD and after the increase in VPD and the closure in stomata the opening wasn't necessary.

This strategy gives an important competitive advantage to the fast-growers, since they can perform an efficient consumption of water during high humidity periods and they can react rapidly by decreasing their stomatal conductance leading to a fast water use and consequently, to a higher water use efficiency (WUE) during short water stress periods. Because of these features, this kind of plants have fast and high growth rates, so they can spread efficiently in a short time, being some invasive species a very good example of fast growers in riparian areas (Larcher 2003, Eldalf & Luukkanen 2006).

The influence of VPD (vapour pressure deficit) on photosynthetic capacities.

According to our results, VPD had an important effect on the photosynthetic electron transport capacity (J_{max}). Many papers have found an inhibition of the photosynthetic capacities during water-deficient experiments, showing that carboxylation and RuBP regeneration had lower activity. High VPD leads to a low water potential in the leaf, causing the inhibition of photosynthetic processes in silver birch in an earlier study (Uddling *et al*,2005). Moreover, Mayoral *et al* (1981) discovered that photophosphorylation was the photosynthetic activity that was more strongly inhibited, like we saw in our experiment. During the experiment there was a higher influence of VPD on protocol 2 than on protocol 1 but these discrepancies can be explained if we account that silver birch was suffering high VPD during the double of time in protocol 2, since the time for stomata to stabilization was longer in the beginning of the experiment, just after clamping onto the leaf.

5. Conclusions

Following conclusions were obtained during this research:

- There was a clear dominance of VPD closure over CO_2 responses
- The closure response to high VPD dominated over opening responses to low VPD, which was probably related to the increase of the hormone ABA.
- High VPD affected the photosynthetic capacities, decreasing the J_{max} .
- Increase of CO_2 and VPD in the future are supposed to have a strong influence in stomatal performance, so g_s will likely decrease markedly, thereby reducing the water use and also the photosynthetic capacity.

6. Acknowledgements

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