Temporal dynamics of stomatal behaviour: modelling, and implications for photosynthesis and water use

Vialet-Chabrand S¹#, Matthews, J.S.A¹#, McAusland L², Blatt, M³, Griffiths, H⁴, Lawson T¹*.

¹School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, UK
²Current address: Division of Crop and Plant Science, School of Biosciences, University of Nottingham, LE12 5RD.
³Laboratory of Plant Physiology and Biophysics, University of Glasgow, Bower Building, Glasgow G12 8QQ UK
⁴Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA UK

*Corresponding Author.

tlawson@essex.ac.uk

#Joint first author.

Keywords: speed of response, stomatal size, stomatal density, dynamic model, fluctuating light, temporal

Running title: Temporal dynamics of stomatal behaviour

One Sentence Summary: Importance of modelling slow stomatal responses and the impacts on photosynthesis under dynamic light environments.
Advances

- Stomatal responses to changing environmental conditions can be an order of magnitude slower than photosynthetic responses, leading to a disconnection between $g_s$ and $A$, influencing $W_i$. This is particularly important considering the dynamic conditions in a field environment.
- Stomatal density is not the only target for manipulating $g_s$, as the speed of stomatal responses to environmental fluctuations is critical when assessing carbon uptake and water use efficiency, which is often determined by both guard cell anatomical characteristics and biochemistry.
- Current models calculate $g_s$ in steady-state or rely on estimating steady-state $g_s$ that may not be realised in the field and therefore do not take into account temporal (and spatial) heterogeneity in $g_s$ observed in the natural environment, limiting the predictive power of such models at ecosystem and global scales as well as the possible impact of future climate change.

Outstanding questions

- The importance of the temporal response of $g_s$ is largely unknown and underestimated, and there is currently no ‘standard method’ to estimate temporal responses to single or multiple environmental signals.
- What are the mechanisms that control or determine the speed of stomatal responses and the magnitude of change in order to exploit the rapidity of stomatal movements as a previously unexplored target for improving plant productivity and water use?
- Further development in dynamic models of guard cell and $g_s$ behaviour is limited by a lack of quantitative data on the rapidity of stomatal response under different environmental conditions, as well as an understanding of the mechanisms that link guard cell biochemistry with $g_s$, $A$ and $W_i$.

ABSTRACT

Stomata control gaseous exchange between the leaf and bulk atmosphere limiting CO$_2$ uptake for photosynthesis and water loss by transpiration, and therefore determine plant productivity and water use efficiency. In order to function efficiently, stomata must respond to internal and external signals to balance these two diffusional processes. However, stomatal responses are an order of magnitude slower than photosynthetic responses, which lead to a disconnection between $g_s$ and $A$. Here we discuss the influence of anatomical features on the rapidity of stomatal movement, and explore the temporal relationship between $A$ and $g_s$ responses. We describe how these mechanisms have been included into recent modelling efforts, increasing the accuracy and predictive power under dynamic environmental conditions, such as those experienced in the field.

1. INTRODUCTION
Stomatal anatomical characteristics and behaviour control gaseous fluxes between the internal leaf environment and the external atmosphere with major implications for photosynthesis, plant water status, evaporative cooling and nutrient uptake. The capacity of stomata to allow CO₂ into the leaf or lose water is known as stomatal conductance (gₛ) measured as a mole flux per unit area (mol m⁻² s⁻¹). Stomatal conductance is the reciprocal of stomatal resistance and primarily determined by stomatal density, distribution and pore area. Global water usage is predicted to double before 2030 (UNESCO, 2009) due to the rising global population, increasing the need for greater crop yields but with reductions in the amount of water available for their growth. This along with more erratic precipitation episodes is putting increasing pressure on breeders and scientists to find new crop varieties or breeding targets that would result in sustained or increased crops yields with less inputs of water. Most crop species are not indigenous to where they are currently cultivated and are often not fully adapted to the environmental conditions, potentially increasing the level of stress that the plant experiences. For decades, breeders focused mainly on selecting varieties for increased yield, decreasing the diversity of other traits of interest (e.g. stomatal behaviour) and potential targets for selection. As stomata are key to plant photosynthesis and water use this makes them attractive targets for manipulation to improve carbon uptake, optimise water use and reduce drought stress. Earlier work used stable carbon isotopic discrimination as a proxy for time integrated water use efficiency and revealed that higher stomatal conductance in wheat resulted in a lower level of limitation of net CO₂ assimilation (A) and higher yield (Fischer et al., 1998). For this reason, previous research explored improving gas exchange via specific manipulation of steady-state gₛ (for example by manipulating stomatal density), whilst we have taken a less obvious approach and are exploring the rapidity of stomatal responses that synchronize gₛ with mesophyll demands for CO₂ (Lawson et al., 2010; Lawson and Blatt, 2014; Raven, 2014) for improving A, water use efficiency (WUE) and leaf temperature.

Stomata balance CO₂ uptake and water loss by adjusting pore aperture to changing environmental and internal cues. In general stomata of C₃ and C₄ plants open with increasing or high light, low [CO₂] and low vapour pressure deficit (VPD), whilst closure is driven by the reverse, low light, high [CO₂] and high VPD (Raschke, 1975; Outlaw, 2003). However, it should be kept in mind that these environmental stimuli are rarely experienced by the plant in isolation, and therefore stomata must respond to multiple signals in a hierarchical manner (Lawson and Morison 2004; Lawson et al., 2010; Aasamaa and Sober, 2011). Although stomatal conductance is closely linked with mesophyll demands for CO₂ (Wong et al., 1979; Farquhar and Sharkey, 1982; Mansfield et al., 1990; Buckley and Mott, 2013), stomatal responses to changing conditions can be an order of magnitude and more slower than photosynthetic responses. Reports of correlations between photosynthetic CO₂ assimilation (A) and gₛ, often refer to steady state measurements or long term observations that do not reflect the reality of field conditions, as short-term fluctuations in environmental conditions can lead to a temporal disconnection between A and gₛ (Kirschbaum and Pearcy, 1988; Tinoco-Ojanguren and Pearcy, 1993; Lawson and Weyers, 1999; Lawson et al., 2010; McAusland et al., 2016). The lack in temporal synchronicity between A and gₛ under natural fluctuating light conditions, has important implications for photosynthetic carbon gain and for the ratio of CO₂ gained through photosynthesis to water lost by transpiration, known as water use efficiency (WUE), as well as resulting in heterogeneity in gas exchange over individual leaves (Lawson and Weyers, 1999; McAusland et al 2013) and within canopies (Weyers and Lawson, 1997). In this review we will explore the temporal relationship between A and gₛ responses, the impact on WUE and the
influence of anatomical characters on stomatal responses. Although we recognise the impact of environmental variables such as [CO₂], relative humidity and soil water content on the temporal response of gs, here we will only focus on changes in light intensity. As part of describing temporal responses in g, we will explore the use of models to better describe and allow a comparison of responses between different species. Many current and early models calculate g in steady state and although useful as a predictive tool for assessing the role of g on gaseous fluxes at the local and regional scale, fail to incorporate temporal (and spatial) heterogeneity in g, observed in the natural environment due to the ever-changing environmental conditions.

2. IMPACT OF THE TEMPORAL RESPONSE OF STOMATAL CONDUCTANCE ON PHOTOSYNTHESIS

Temporal response of stomatal conductance

Due to technical considerations, most studies regarding stomatal behaviour on intact leaves have used g as a proxy to investigate stomatal movements instead of directly measuring pore area. Despite this being a useful tool for understanding stomatal dynamics, it should be kept in mind that the relationship between g and pore area is not linear, as the influence of pore area on g decreases rapidly with the magnitude of stomatal opening (Kaiser et al., 2001). Nevertheless, Kaiser and co-workers (1997, 2000, 2001) showed that g and pore area measurements, although on different scales, generally lead to the same conclusion regarding limitations of photosynthesis (A) and water loss. It is well known that a low g, or slow stomatal opening can restrict the uptake of CO₂ and therefore A (Farquhar and Sharkey, 1982; Barradas et al., 1994; Barradas and Jones, 1996; McAusland et al, 2016), whilst high g, facilitates higher rates of A, but inevitably at the ‘cost’ of greater water loss through transpiration (E) (Barradas et al. 1994; Naumburg and Ellsworth, 2000; Lebaudy et al, 2008; Lawson et al, 2010; Lawson and Blatt, 2014; McAusland et al, 2013; 2016). In response to fluctuations in environmental parameters, it is commonly assumed that plants try to synchronize stomatal opening with the mesophyll demand for CO₂, and stomatal closure with the need to minimize water loss through transpiration (Cowen and Farquhar, 1977; Farquhar et al 1980; Mott, 2009; Drake et al, 2013; Lawson et al, 2012; Jones, 2013). However, slow g kinetics (e.g. McAusland et al, 2016) means that stomatal aperture lags behind the steady state target (Kaiser et al, 2000).

Light is the greatest environmental driver of photosynthesis, and stomatal response to light is one of the most well researched stomatal behaviours (Shimazaki et al, 2007). Numerous studies have investigated steady-state stomatal responses to light, however as these responses are measured under constant conditions they represent situations that are rarely found in nature (Jones, 2013). Measurements of g, collected under field conditions are highly variable and therefore correlate poorly with those measured under steady-state conditions in the laboratory (Poorter et al, 2016), usually due to slow g kinetics (e.g. McAusland et al, 2016) meaning that when measured, stomatal have not yet reached the new steady state target (Kaiser et al, 2000; Whitehead and Teskey, 1995; Lawson et al, 2010).

Stomatal response to dynamic light

Several studies have investigated the dynamics of stomatal response and photosynthesis to fluctuations in environmental variables, especially light (Knapp and Smith, 1987; Kirschbaum et al, 1988; Tinoco-Ojanguren and Pearcy, 1993; Barradas et al, 1994; Lawson et al, 2010; Wong et al,
However, the majority of these have concentrated on the influence of sun and shade flecks on carbon gain in understory forest dwelling species (Chazdon, 1988; Chazdon and Pearcy, 1991; Tinoco-Ojanguren and Pearcy, 1993; Pearcy, 1994; Leakey et al, 2005) and for plants that have developmentally acclimated to shaded or exposed conditions (Knapp and Smith 1987, 1988), often ignoring dynamic stomatal response and the potential limitation on carbon gain or water loss. Over the diurnal period, these fluctuations in light (sun/shade flecks) drive temporal and spatial dynamics of carbon gain and water loss (Lawson and Blatt 2014). It is often the speed of stomatal response to environmental fluctuations that is critical when assessing carbon uptake and water use efficiency (WUE) (Raschke, 1975; Kirschbaum and Pearcy, 1988; Lawson and Morison, 2004; Lawson et al, 2010). In the field the response of $A$ and $g_s$ is largely dominated by fluctuations in photosynthetic photon flux density (PPFD) (Pearcy, 1990; Way and Pearcy, 2012), which can vary on a scale of seconds, minutes, days, and even seasons (Assmann and Wang, 2001), and is driven by sun angle, cloud cover, and shading from overlapping leaves (Pearcy, 1990; Chazdon and Pearcy, 1991; Way and Pearcy, 2012), as a consequence leaves are subjected to varying spectral qualities and light intensities. It is noteworthy that such rapid changes in PPFD will result in rapid intense modifications to leaf temperature, with greater $g_s$ providing enhanced evaporative cooling and possible protection against heat damage (Schymanski et al, 2013).

In the 1980s to early 1990s, Pearcy and colleagues investigated the impacts of sun flecks, primarily on carbon gain and later on stomatal dynamics. They dissected the temporal photosynthetic and $g_s$ response into different phases, to explain the period of response associated with limitations in $A$ and overshoots of $g_s$ leading to excess water loss. The initial phase was termed the induction and represents periods of up to 10 minutes where biochemical processes rather than CO$_2$ supply limit carbon assimilation (Barradas and Jones, 1996). The second phase, dominated by stomatal limitation, describes slow $g_s$ responses that constrain CO$_2$ diffusion and $A$ (Lawson et al, 2010, 2012; Vialet-Chabrand et al, 2013; McAusland et al, 2016); the third phase explains the period in which $g_s$ remains high, exceeding the amount of $g_s$ required for maximum rates of carbon assimilation (Kirschbaum et al, 1988; Tinoco-Ojanguren and Pearcy, 1993; Lawson et al, 2010), leading to excess water loss (relative to carbon gained) and effectively a drop in water use efficiency (McAusland et al, 2016). Studies mainly on forest understory species have reported that sun flecks may contribute between 10 to 60% of the total daily carbon gain (Way and Pearcy, 2012), depending on forest type and plant age. Stomatal limitations on $A$ have been estimated at up to 30%, with significant implications for carbon sequestration and crop yields (Fischer et al 1998; Lawson and Blatt, 2014).

Indeed Kirschbaum et al (1988) found that if initial $g_s$ values were high, $A$ could be six times higher one minute after an increase in PPFD than if initial $g_s$ was low, an 82% $g_s$ limitation on $A$, illustrating the importance of $g_s$ in natural dynamic conditions such as those found in the field. Continued increases in $g_s$ after $A$ has reached light saturation, have also been reported which led to a decrease in intrinsic water use efficiency ($W_i$) with higher water loss for no CO$_2$ gain (Kirschbaum et al, 1988; Tinoco-Ojanguren and Pearcy, 1993; Lawson et al, 2010).

Differences in the speed of stomatal opening and closing and the magnitude of change in $g_s$ in response to sun and shade flecks, are known to exist between species and within individual plants (Assmann and Grantz, 1990; Ooba and Takahashi, 2003; Franks and Farquhar, 2007; Vico et al, 2011; Drake et al, 2013; Vialet-Chabrand et al, 2013). Response times are also dependent upon the plant water status (Lawson and Blatt, 2014), leaf age (Urban et al, 2008), the history of stress (Pearcy and
Way, 2012; Porcar-Castell and Palmroth, 2012; Wong et al, 2012; Zhang et al, 2012), and the duration and magnitude of changes in PPFD (Weyers and Lawson, 1997; Lawson et al, 1998; Lawson et al, 2012; Lawson and Blatt, 2014). There is also evidence to suggest changes in growth environment during stomatal development influences the speed of response in mature leaves (Arve et al, 2017). The speeds of opening and closing in response to changing PPFD in many species are not correlated (Ooba and Takahashi 2003); however, Vico et al (2011) compared 60 published gas exchange data sets on stomatal response to PPFD, to determine the impact of stomatal delays on photosynthesis and found a general parallel relationship in the rates of stomatal response, concluding that rates of stomatal opening were essentially correlated with the rate of closure. If we assumed there is no delay in stomatal opening or closing, optimal leaf gas exchange would be achievable (Cowan and Farquhar, 1977; Lawson and Blatt, 2014), but it is important to consider the fact that specific delays in stomatal movement may be indicators of the current needs of the plant (Ooba and Takahashi, 2003; Manzoni et al, 2011; Vico et al, 2011; Drake et al, 2013). The response of $g_s$ is thought to reflect this priority; where under well-watered conditions in the canopy, stomata will remain open (particularly lower down in the canopy where VPD will be lower) in order to utilise light energy from sunflecks to maximize $CO_2$ diffusion into the leaf (Way and Pearcy 2012; Lawson et al 2012), even at the cost of further water loss (Allen and Pearcy, 2000), whilst under drought or water-limited conditions stomata will often close to conserve water at the expense of carbon gain (Knapp and Smith, 1988).

**Influence of anatomy on stomatal response**

Stomatal anatomical features such as stomatal density and size are known to determine steady-state $g_s$ (Franks and Farquhar, 2001), and are a key component for determining the maximum theoretical stomatal conductance ($g_{s_{max}}$) of the plant (Dow et al, 2014). Stomatal size and density vary greatly between plant species, and are influenced by the growth environment (Willmer and Fricker, 1996; Hetherington and Woodward, 2003; Franks and Beerling, 2009). Stomatal density has often been negatively correlated with stomatal size (Hetherington and Woodward, 2003; Franks and Beerling, 2009). Recently a great deal of consideration has been given to the impact of stomatal anatomical features on stomatal function and gas exchange, particularly to the morphological and mechanical diversity of stomata with reference to performance and plasticity (Franks and Farquhar, 2007). Recent studies and reviews have implied that stomatal response times to environmental perturbations are affected by physical attributes such as size and density (Drake et al 2013; Raven, 2014), the presence or absence of subsidiary cells (Franks and Farquhar, 2001) as well as the shape of the guard cells (McAusland et al 2016) and their clustering (Papanatsiou et al, 2016), and that manipulation of these features could have positive effects for carbon gain and water use efficiency (Lawson et al 2012; Doheny-Adams et al 2012; Tanaka et al, 2013; Franks et al, 2015).

Hetherington and Woodward (2003) first suggested that dumb-bell shaped stomata could open and close faster than kidney shaped stomata in response to environmental perturbations, as even small changes in volume in the smaller dumbbell shaped guard cells would lead to greater stomatal opening compared with the larger kidney shaped guard cells. Franks and Farquhar (2007) took this further by advocating other factors that may influence the speed of response, such as guard cell geometry, mechanical advantage, osmotic or turgor pressures, and the energetic cost of guard cell movements (as previously mentioned). A mechanical advantage of dumb-bell shaped stomata was suggested to be associated with reciprocal coupling of guard and subsidiary cell osmotic and turgor
pressures leading to more rapid stomatal movements (Franks and Farquhar, 2007; Raven 2014). These findings underlie the potential of dumb-bell shaped stomata to track changes in environmental conditions, and maximise the efficiency of photosynthesis and water use through increased stomatal response times (Hetherington and Woodward, 2003; McAusland et al. 2016), a point also highlighted by Chen et al., (2017) in their analysis of stomatal evolution. Drake et al., (2013) investigated the correlation between stomatal anatomy, specifically density and size, with stomatal opening speeds and found that the maximum rate of stomatal opening was driven by size and density. Although the work of Drake et al. (2013) and review from Raven (2014) made significant progress in linking stomatal size to function, including speed of response to light and associated implications, the size of stomata is not the only and main determinant of the speed of response. For example, Papanatsiou et al, (2016) note that stomatal clustering can affect $g_s$ kinetics independent of stomatal dimensions and the available pool of osmotic solutes available for driving aperture changes. The results of Drake et al., (2013) could have been skewed also by the experimental condition as step changes in light from a state of darkness will not only incur biochemical limitations on stomatal movement and assimilation, but represent a state that is rarely seen in the natural environment except prior to dawn. Recent work from Kaiser et al., (2016) using similar experimental conditions, could have overestimated the biochemical limitation and underestimated the diffusional limitation on $A$ due to the slow response of $g_s$ from dark. Producing a step change from low to high light is more representative of the conditions experienced in the field during a diurnal period from passing clouds and overlapping leaves (McAusland et al, 2016; Vialet-Chabrand et al, submitted), and therefore more relevant information can be gained regarding the speed of stomatal response and the implications this may have for carbon assimilation and water use efficiency. In a recent study, McAusland et al. (2016) compared the speed of stomatal responses to a step change in light, in both dumbbell and elliptical-shaped guard cells in a range of species, including model species and crops. These authors found that guard cell shape (dumbbell or elliptical) and potentially photosynthetic type ($C_3/C_4$) played a key role in determining the speed of stomatal response, with dumbbell shaped guard cells exhibiting faster responses than those with elliptical guard cells. Slow stomatal opening in response to increasing light was demonstrated to limit carbon assimilation by ca. 10%, which would equate to substantial losses in carbon gain over the course of the day, potentially negatively impacting productivity and yield. Whereas, slow stomatal closure when PPFD decreased resulted in a significant decrease in water use efficiency, as overshoots in $g_s$ by up to 80% were observed with only a negligible 5% gain in $A$. Closer coupling of $A$ and $g_s$ therefore has the potential to enhance carbon gain and $W_w$, and in turn improve performance, productivity and yield (Lawson et al, 2010; Lawson and Blatt 2014; McAusland et al, 2016; Li et al, 2016; Qu et al, 2016).

3. MODELLING THE TEMPORAL RESPONSE OF STOMATA

As mentioned above dynamic stomatal behaviour plays a key role in regulating the flux of carbon and water through the soil-plant-atmosphere continuum, and is an important determinant for scaling leaf level measurements of water use efficiency and photosynthesis to the canopy level (Weyers et al, 1997). Modelling is generally considered the most effective tool for simulating stomatal responses to environmental conditions (Damour et al, 2010), and the importance of integrating stomatal behaviour into scaling models recognised (Weyers et al, 1997; Bernacchi et al, 2007; Lawson et al, 2010; Bonan et al, 2014; Barman et al, 2014; De Kauwe et al, 2015). Many
current models calculate steady state $g_s$ and have become successful tools for predicting the impact of $g_s$ on water and carbon fluxes at ecosystem and regional scales. However, heterogeneity in the spatial and temporal response of stomata are often overlooked (Weyers et al, 1997; Lawson and Weyers, 1999), therefore limiting the confidence with which these current models can predict larger scale responses or the impact of predicted climate change (Buckley et al, 2003; Dewar et al, 2009; Baldocchi 2014). The addition of stomatal dynamics to existing models has the potential to reveal the extent to which $g_s$ has been inaccurately predicted by steady-state models. As stomata are continuously responding to fluxes in environmental conditions and therefore $g_s$ is rarely in steady-state, this reinforces the need for improved mechanistic models of $g_s$ (Damour et al, 2010; Vialet-Chabrand et al, 2016). Greater focus in future modelling efforts attempting to scale from the leaf to canopy level should be given to the inclusion and integration of temporal stomatal dynamics to fluctuations in environmental signals (Vico et al, 2011; Vialet-Chabrand et al, 2013), to predict the impact of large-scale heterogeneity in stomatal traits on water and CO$_2$ fluxes through the canopy, ecosystem and global scales. Furthermore, as stomata are exposed to constant fluctuations over the diurnal period, it is often the speed of stomatal response that are critical in determining CO$_2$ uptake and transpiration dynamics over the course of the day (McAusland et al, 2016; Vialet-Chabrand et al, 2016), rather than the steady state values that are often the basis of many existing models. Here we will review the existing dynamic models and the advantages and disadvantages of their use and predictive power, whilst also discussing the incorporation of dynamic models for greater accuracy in predicting stomatal impacts on $A$, $g_s$, and $W$, in a natural environment.

**Modelling temporal response of stomatal conductance to changes in light intensity**

In the early seventies, temporal responses of stomatal conductance ($g_t$) were examined to determine the degree of limitation on $A$ and the regulation of water loss (Woods and Turner, 1971; Davies and Kozlowski, 1974; Horie, 1978). Most of these early studies were based on step increases and decreases in light intensity revealing a slow exponential or sigmoidal variation in $g_t$ with time (e.g. Fig. 1). The response of $g_t$ to a step change in light intensity was initially evaluated as the time for $g_t$ to reach the new steady state ($G_t$) at the new light level, or a percentage of this value as an estimator of the rapidity of response (Woods and Turner, 1971; Davies and Kozlowski, 1974; Grantz and Zeiger, 1986; Dumont et al, 2013). More recently, the rapidity of response has been estimated using a regression fit to the linear part of the $g_t$ response, providing an estimate of the maximum rate of $g_t$ increase (Tinoco-Ojanguren and Pearcy, 1992; Fay and Knapp, 1995; Naumburg et al, 2001; Drake et al, 2013). Temporal responses of $g_t$ assessed using these approaches are prone to errors as they are largely dependent on the estimation of $G_t$ that may never be reached and the linearity of the initial part of the curve. The lack of a ‘standard method’ to estimate the temporal response of $g_t$, (for example in the choice of the linear part of the curve), prevents a direct comparison of results from different studies. A more robust approach is to use normalised observations of $g_t$ between the initial and final $G_t$ (Laffray et al, 1982; lino et al, 1985; Barradas et al, 1994; Mencuccini et al, 2000; Drake et al, 2013). This approach not only provides a visual representation of the differences in temporal $g_t$ responses, but is also independent of the magnitude of the $g_t$ response, however it is unable to summarize the overall response in one descriptive statistic. Moreover, if a steady state is not reached during the measurement period, it is difficult to normalise data.
Dynamic models of stomatal conductance

An alternative to these earlier error prone approaches is to fit a model to the temporal response of $g_s$ following a step change in light intensity and determine a set of parameter values to describe and enable an evaluation of specific parts of the response curve. In general, such models require the following parameters; an initial and final value of $g_s$, and a time constant. These parameters are targets, which means that if $G_s$ is not reached during the response, the model can constrain the parameter value based on the shape of the response curve. Parameter values can be adjusted using a statistical method that provides the best set of values based on the comparison of the observations and the model outputs.

Typically, two empirical models based on the shape of the variation of $g_s$ are commonly used, an exponential and a sigmoidal model. For both models, a set of differential equations and associated analytical solutions are available. To date a large number of studies have used the analytical equations of the exponential response of $g_s$ (Horie, 1978; Knapp, 1993; Whitehead and Teskey, 1995; Naumburg and Ellsworth, 2000; Naumburg et al, 2001; Franks and Farquhar, 2001, 2007; Vico et al, 2011; Martins et al, 2016; Qu et al, 2016) that can be formulated for an increase (Eq. 1) or decrease (Eq. 2) in $g_s$:

$$g_s = G_{\text{max}} + (G_{\text{min}} - G_{\text{max}})e^{-t/\tau_i}$$  \hspace{1cm} (1)

$$g_s = G_{\text{min}} + (G_{\text{max}} - G_{\text{min}})e^{-t/\tau_d}$$  \hspace{1cm} (2)

where $G_{\text{min}}$ and $G_{\text{max}}$ represented the minimum and maximum steady state $g_s$, $\tau_i$ and $\tau_d$ the time constants for the increase and decrease in $g_s$, and $t$ the time at which $g_s$ is estimated starting from time 0. In this model, the time constants represent the time required to reach 63% of the total variation (when $\tau_d = \tau_i$, $\frac{G_{\text{max}} - G_{\text{min}}}{G_{\text{max}} - G_{\text{min}}} = 1 - e^{-1} \approx 0.63$). The large number of studies using the exponential model is due to its ease of use and the fact that most of the observed temporal responses of $g_s$ have an exponential shape.

A delay in the increase in $g_s$ responses after a step increase in light has been reported for several species (Barradas et al, 1994; Naumburg and Ellsworth,. 2000; Drake et al, 2013; McAusland et al, 2016; Elliot-Kingston et al, 2016) and the shape of this type of response can be described by a sigmoidal equation:

$$g_s = (G_{\text{max}} - G_{\text{min}})e^{-e^{\frac{t}{k_i+1}}} + G_{\text{min}}$$  \hspace{1cm} (3)

$$g_s = (G_{\text{min}} - G_{\text{max}})e^{-e^{\frac{t}{k_d+1}}} + G_{\text{max}}$$  \hspace{1cm} (4)

where $k_i$ and $k_d$ represent the time constants for the increase (Eq. 3) or decrease (Eq. 4) of $g_s$ and $\lambda$ the initial lag time. Time constants $k_i$ and $k_d$ do not directly represent a time to reach a percentage of $G$, but also depend on $\lambda$. However, the time to reach any value of $g_s$ can be calculated by solving the previous equation as a function of time:

$$t = \lambda - k_i \cdot \ln \left( -\ln \left( \frac{g_s - G_{\text{min}}}{G_{\text{max}} - G_{\text{min}}} \right) - 1 \right)$$  \hspace{1cm} (5)
Using equation 5, the equivalence between the exponential and sigmoidal time constants can be written as:

$$\tau_i = \lambda - k_i \cdot [\ln(-\ln(1 - e^{-1})) - 1]$$

(6)

where \( \tau \) represents the time to reach 63% of the total \( g_s \) variation including the initial lag time.

Another interesting property that has been used in numerous studies to describe the “speed of stomatal response” is the maximum slope of \( g_s \) increase, which is calculated based on the previously described parameters:

$$S_{lmax} = k \cdot \frac{G_{max} - G_{min}}{e}$$

(7)

Equation 7 relates the effect on \( g_s \) of stomatal density (approximated by \( G \)) and the speed of response of stomata (estimated by \( k \)), highlighting the importance of differences in SD when drawing conclusions from differences in \( S_{lmax} \). It should be kept in mind that as previously mentioned, the scaling up from stoma to leaf level is not a linear process and caution should be taken when interpreting temporal response of \( g_s \) in term of stomatal behaviour (Kaiser et al, 2001; Vialet-Chabrand et al, 2016).

Both the exponential (see Fig. 1A) and sigmoidal (see Fig. 1B) models can be fitted on data collected using a generic protocol that consists of a step increase in light intensity from 100 to 1000 µmol m\(^{-2}\) s\(^{-1}\) whilst other environmental variables are held constant (e.g. relative humidity). This generic protocol has been used in numerous publications and can be adapted depending on the species. Although a step changes in light intensity is often used as the standard method to assess temporal responses in \( g_s \), this approach is not fully representative of natural environmental variation, but is close to what a plant may experience during a sun-fleck in the field. We provide a curve fitting routine in Microsoft Excel to illustrate the use of the exponential and sigmoidal models described above, in an accessible format (see supplementary GS_Fit.xlsm). Despite differences in timing or light intensities, the parameters derived from this protocol can be compared to characterize the differences in temporal response of \( g_s \). Under a continuously changing light environment, the analytical models presented above can be biased as they assume a constant \( G_s \) between each calculated time point. In the case of a dynamic light environment, differential equations would be preferred for their accurate and continuous descriptions of the \( g_s \) response. A differential equation describing an exponential response of \( g_s \) has been described previously (Horie, 1978; Noe and Giersch, 2004; Vico et al, 2011) but requires a larger number of steps to be solved and has therefore rarely been used (Kirshbaum et al, 1988; Noe and Giersch, 2003; Vialet-Chabrand et al, 2016):

$$\frac{dg_s}{dt} = \frac{(G_s - g_0)}{\tau}$$

(8)

Alternatively, a differential equation for a sigmoidal variation of \( g_s \) can be used (Vialet-Chabrand et al, 2013; Moualeu-Ngangue et al, 2016) providing a control on the initial lag experienced by stomata after a change in light intensity:

$$\frac{dg_s}{dt} = k \cdot \ln\left(\frac{G - g_0}{g_t - g_0}\right) \cdot (g_t - g_0)$$

(9)
Alternative more complex equations than equation 8 have been proposed by Kirschbaum et al., (1988), but can be more difficult to parameterize due to their large number of parameters. The use of a differential equation required the calculation of the steady state target $G_s$ at any point of time, Vialet-chabrand et al., (2013) proposed the use of a spline function to estimate the light intensity (or any environmental variable) continuously and then use these values to predict $G_s$ using any already available steady-state model. Therefore, this approach to model the temporal response of $g_s$ can be used in existing steady state $g_s$ models to predict the transient states of $g_s$, resulting from the previous variations in light intensity.

In many studies, the temporal response of $g_s$ has been associated with stomatal behaviour and focused on the rapidity of stomatal movements (Franks and Farquhar, 2007; Drake et al., 2013; Raven, 2014). However, it is important to note that the rapidity of stomatal movements is not necessarily correlated to the rapidity of the variations of $g_s$, (Vialet-Chabrand et al., 2016). For example, a higher stomatal density can result in a higher rate of $g_s$ increase ($S_{slax}$) without changes in stomatal behaviour (McAusland et al., 2016). Both anatomical (e.g. stomatal density and size) and biochemical traits (e.g. number and regulation of ion channels) describing stomatal behaviour need to be considered to fully understand the kinetic of $g_s$ responses following a change in light intensity or any other environmental parameter. To this extent, empirical analysis of stomatal conductance may also be extracted from mechanistic models of guard cells, notably OnGuard that yields outputs in stomatal aperture that connect directly to the underlying processes of solute transport and metabolism (Hills et al., 2012), Chen et al., (2012), Wang et al, 2012). Indeed, Wang et al, (2014) have used this platform to undertake a study of stomatal kinetics, incorporating a first-order sensitivity analysis of the dependence on individual ion channels and pumps at the plasma membrane and tonoplast. Their study yielded a number of unexpected results as noted below.

**An example of dynamic modelling of stomatal conductance**

To illustrate the use of models to describe temporal $g_s$ responses and the effect of physical and functional stomatal attributes, we compared the rapidity of the temporal response of $g_s$ in two Arabidopsis genotypes to the ecotype Col-0; one with altered stomatal distribution (wer1-1; Lee and Schiefelbein, 1999) and the second with impaired stomatal closure (gork1-1; Hosy et al, 2003). Compared to Col-0, the ectopic stomata of wer1-1 resulted in a faster stomatal response as illustrated by the lower $G_s$ (Fig. 2A and 2B) and lower $\tau_i$ and $\tau_d$ (Fig. 2C and 2D). The ectopic anatomy of the wer1-1 stomata potentially allows faster pore opening as there is no back pressure from the surrounding epidermal cells because the stomatal guard cells are above and not in line with the epidermal cells, resulting in faster movements for the same energy cost. This change in stomatal anatomy also lead to a lower $G_s$ compared with the WT control, although the mechanism for this is unknown and needs further investigation. As previously shown by Hosy et al, (2003), plants with impaired outward K⁺ channels (gork1-1) have greater $\tau_i$ and $\tau_d$, and higher $G_s$, resulting in a large unnecessary water loss during stomatal closure, but little effect on stomatal limitation of $A$ due to the relatively high values of $g_s$. The strong reduction of the outwardly rectifying K⁺ channel activity in the guard cell membrane prevents K⁺ release and increases stomatal aperture by maintaining membrane depolarization at membrane potentials more positive than the K⁺ equilibrium potential. This imbalance in osmoregulation induced a slow stomatal response by potentially slowing down K⁺ uptake. Although there were small but significant differences in anatomical features such as
stomatal density (SD, Fig. 2E) and guard cell length (GC length, Fig. 2F) they cannot explain the different temporal response of gs in these plants, highlighting the importance of other parameters such as the biochemistry and mechanics of stomatal movement as previously describe in Section 2. The same conclusions can be drawn, for example, from studies of slc1 (Wang, et al, 2012), amy3 and bam1 (Horrer, et al, 2016), and other mutant and transgenic plants (see Jezek and Blatt 2017, this issue; De Angeli and Eisenach 2017, this issue; Lunn and Santelia 2017, this issue). These findings illustrate the plasticity of temporal gs responses and the potential impact that manipulating the speed of stomatal responses could have on A and WUE. For example, the fast gs response in the wer1-1 plants reduced gs limitation of A under an increase in light (Fig. 2A; Fig. 3), and reduced potential water loss when subjected to a decrease in light (Fig. 2B). These plants exhibited a potential for increased/greater synchronisation between A and gs (see Fig. 3), which may lead to higher water use efficiency over the course of the day (McAusland et al, 2016).

4. CONCLUSION

Despite stomatal behaviour occurring at the micro-scale, it is important to recognise the impact they have on cycles of carbon and water at large-scale global systems. Although stomata typically occupy only a small portion of the leaf surface (0.3 to 5%), they are known to control ca. 95% of all gas exchange between the leaf and environment, and estimations show that 98% of all water taken up through the roots may be transpired through stomatal pores (Morison, 2003), potentially translating to 60% of all terrestrial precipitation (Katul et al, 2012). Indeed, most crop plants will transpire over twice their fresh weight in water every day (Chaumont and Tyerman, 2014). With this in mind, stomata represent important targets, for manipulating crop photosynthetic productivity and water use which is particularly important considering that the allocation of fresh water resources which is becoming a significant global concern. As highlighted in this review, the importance in the temporal response of gs is largely unknown and underestimated, and understanding this variation will aid future scaling efforts from individual stoma to leaf and canopy levels. What is apparent, is the lack of quantitative data on the rapidity of stomatal response under different environmental conditions making it difficult to describe the mechanisms of guard cell movement and assess the impact of uncoordinated responses on leaf level gas exchange. By integrating the dynamic responses or stomatal to changing environmental conditions, and taking account of different stomatal morphology, as well as sensing and signalling systems, we may be able to maximise the benefit of photosynthesis (in terms of carbon gain) relative to the cost of water, and translate these findings into more sustainable crop production systems for the future.

Acknowledgements: This work was supported by BBSRC grants BB/L019205/1 and BB/L001276/1 to MRB and BB/I001187/1 to HG and TL.
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