Diurnal variation in gas exchange: the balance between carbon fixation and water loss

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ABSTRACT

Stomatal control of transpiration is critical for maintaining important processes, such as plant water status, leaf temperature, as well as permitting sufficient CO₂ diffusion into the leaf to maintain photosynthetic rates (A). Stomatal conductance (gₛ) often closely correlates with A and is thought to control the balance between water loss and carbon gain. It has been suggested that a mesophyll driven signal co-ordinates A and gₛ responses to maintain this relationship, however the signal has yet to be fully elucidated. Despite this correlation under stable environmental conditions, the responses of both parameters vary spatially and temporally and are dependent on species, environment and plant water status. Most current models neglect these aspects of gas exchange though it is clear they play a vital role in the balance of carbon fixation and water loss. Future efforts should consider the dynamic nature of whole plant gas exchange and how it represents much more than the sum of its individual leaf level components, as well as taking into consideration the long term effect on gas exchange over time.
INTRODUCTION

As the waxy surface of most leaves make them virtually impermeable to CO₂ and H₂O, nearly all CO₂ absorbed by the plant and water lost passes through the stomatal pores (Cowan and Troughton, 1971; Caird, 2007; Jones 2013). Although these pores represent only a small fraction of the leaf surface, stomatal behaviour has major consequences for photosynthetic CO₂ fixation and H₂O loss from leaf to canopy levels, therefore influencing carbon and hydrological cycles at global scales (Keenan et al, 2013; Hetherington and Woodward, 2003). Guard cells that surround the stomatal pore, open and close in response to environmental stimuli, controlling the flux of gas between the leaf interior and the bulk atmosphere. Stomatal conductance (gₛ) appears closely linked with mesophyll demands for CO₂ and a strong correlation between A and gₛ is often observed (Wong et al, 1979; Farquhar & Sharkey, 1982; Mansfield et al, 1990; Buckley & Mott, 2013), and although conserved is not always constant (Lawson & Morison 2004; Bonan et al, 2014). However, the photosynthetic rates and properties of each leaf may not be identical and depend on acclimation to the surrounding microclimatic conditions, and therefore each leaf could be considered unique (Niinemets, 2016). In order to maintain an appropriate water status, plants must balance water loss between leaves with different properties depending on the availability of soil water, which raises the question about the regulation of gₛ at whole plant level. Early experiments by Meinzer and Grantz (1990) showed that the balance between water loss and water transport capacity enables maintenance of a constant leaf water status over a wide range of plant sizes and growing conditions. Therefore, plants regulate transpiration of each leaf independently in response to variations in microclimate by constantly adjusting stomatal aperture. Stomatal control of transpiration rate is also an important component of the leaf energy balance, and can be of great importance for maintaining an optimal or appropriate leaf temperature for photosynthesis, particularly under conditions of increasing or high light intensity that are observed over a typical diurnal period. The diurnal gₛ response could be an emergent property of different regulatory processes (e.g. maintenance of leaf water status) and limitations (e.g. water availability) (Hills et al, 2012), or the optimization of carbon fixation as a function of water loss (Buckley, 2017). Transpiration is often seen as a cost for carbon fixation at leaf level but it is important to take into consideration its roles in the transport of solutes in the different parts of the plant, or for leaf cooling. For example, nocturnal transpiration does not come with any carbon fixation but has been shown to have an important role in nutrient uptake and ultimately growth (Caird, 2007).

The close correlation between A and gₛ is thought to help maximize A as a function of E over a diurnal period and there is evidence to suggest this relationship is driven by a signal produced by the mesophyll to which guard cells sense and respond (see Section 2). However, it could also be the result of covariation in response to environmental factors, such as light intensity, with gₛ often limiting A irrespective of whether A is the main optimisation target. For example, it has been shown that the maintenance of leaf water status under drought conditions is more important than carbon fixation and as such is a priority signal to which the plant will respond (Lawson & Morison 2004; Lawson et al, 2010; Aasamaa and Sober, 2011). Cowan and Farquhar (1977) inferred that the coordination between A and gₛ can be seen as a plant response to control the trajectory of gₛ to maximize A and minimize E over a typical diurnal sinusoidal light pattern (Buckley, 2017). However, observations of gₛ in response to variations in light intensity revealed that in general stomatal responses do not mimic these simulations. Instead, gₛ responses are an order of magnitude slower
than $A$, and can continue to increase even when $A$ reaches steady-state, resulting in a limitation of $A$
during the initial part of the response, followed by an unnecessary increase in $E$ (Vico et al, 2011;
resulting in more water loss than is necessary for the gain in CO$_2$ (Lawson & Blatt, 2014). In general,
the diversity of coordination between $A$ and $g_s$ observed in steady state across species, suggests that
there is not strong selective pressure for this trait in the field, and therefore highlights room for
potential improvement in plant performance (McAusland, 2016).

In order to understand how plants balance carbon fixation and water loss, gas exchange needs to be
considered at the plant or canopy scale, and for that reason it is important to recognise the spatial
and temporal aspect of stomatal response over a diurnal period. A number of current models
(Damour et al, 2010) predict the diurnal time course of $g_s$ and $A$ based on equations developed by
Ball et al (1987) and Farquhar et al (1980, hereafter “FvCB”) respectively. These models predict $g_s$
and $A$ in steady state and do not include any temporal or long term effect, as well as how the
relationship between $A$ and $g_s$ may vary across the leaf surface. The Ball model used the apparent
coordination of $A$ and $g_s$ as a basis to predict $g_s$, but does not consider the slow temporal response of
stomata, which lead to inaccurate predictions of the diurnal time course of $g_s$ (Viala-Chabrand et al,
2013; Vialet-Chabrand et al, 2017). The FvCB has been successful in describing the kinetic of the
Calvin cycle but does not take into consideration external feedbacks such as those induced by the
accumulation of photosynthesis products over the course of the day. Possible improvements of
current models through the integration of diurnal effects on $g_s$ and $A$ will be discussed here. This
review will examine spatio-temporal (diurnal) responses of $g_s$ and $A$ using examples of diurnal
variations in $g_s$ and $A$ in herbaceous crop species to highlight the implications for plant carbon
assimilation and water use efficiency. We have focussed on topics that we consider of greatest
relevance for future research in this area and begin by briefly reviewing the possible mechanisms
and processes that have been proposed to be responsible for the coordination between $A$ and $g_s$.
Although we recognise the importance of mesophyll conductance ($g_m$) in the balance of CO$_2$ to H$_2$O
diffusion, this will not be covered here (Flexas et al. 2012).

2. MECHANISMS OF CO-ORDINATION BETWEEN STOMATAL BEHAVIOUR AND MESOPHYLL PHOTOSYNTHESIS.
For many years internal CO$_2$ concentration ($C_i$) was considered to link stomatal
responses to photosynthetic demands for CO$_2$ (Mott, 1988; Ball & Berry, 1982). For example, when
photosynthetic rate ($A$) increases due to an increase in irradiance, $C_i$ is reduced and stomata respond
to the increased demand for CO$_2$ by increasing aperture, and conversely when the demand for CO$_2$
decreases high $C_i$ results in stomatal closure. However, relatively recent research from several
laboratories has suggested that $C_i$ is not the only determinant of the coordination between $A$ and $g_s$.
Von Caemmerer et al (2004) suggested that guard cells may not sense $C_i$ but instead sense external
[CO$_2$], whilst other reports have suggested that stomatal responses to $C_i$ are too small to account for
the observed change in $g_s$ in response to light (Raschke, 1975; Farquhar & Raschke, 1978; Sharkey &
Raschke, 1981; Farquhar & Sharkey, 1982). More recently studies on transgenic plants have shown
that $g_s$ increases with photosynthetic photon flux density (PPFD) even in plants with reduced $A$ and
higher $C_i$ values (von Caemmerer et al, 2004; Baroli et al, 2008; Lawson et al, 2008), which agrees
with reports that $g_s$ responds to various stimuli even when $C_i$ is held constant (Messinger et al, 2006;
Lawson et al, 2008; Wang & Song, 2008). This has led to the suggestion that an unknown signal
produced by the mesophyll is sensed by the guard cells triggering a stomatal response. Early
research suggested an aqueous signal (Lee & Bowling, 1992; 1995) with candidates including photosynthetic metabolites such as ATP, NADPH and ribulose 1, 5-bisphosphate (RuBP) (Wong et al., 1979; Farquhar & Wong, 1984; Lee & Bowling, 1992; Zeiger & Zhu, 1998; Tominaga et al., 2001; Buckley et al., 2003) as well as malate and sugar (Hedrich & Marten, 1993; Hedrich et al., 1994; Lee et al. 2008). Mott et al (2008) used a novel epidermal-mesophyll transfer experiment and showed that stomata in the isolated epidermis of *Tradescantia* only responded to light and CO₂ when the epidermis was transplanted back onto the mesophyll tissue from which the peel had been taken or from that of another leaf. Sibbernsen & Mott (2010) suggested that the signal must be gaseous as after injecting leaves with liquid and reducing the air spaces, they observed a decline in stomatal response and later work inferred that the mesophyll signal was a vapour phase ion (Mott and Peak, 2013). However, Fujita et al (2013) further tested this hypothesis, by using different combinations of cellophane and polyethylene films between an epidermal peel and a gel based substance used to mimic a leaf, with aqueous substances able to pass through the cellophane but not the polyethylene films but only gaseous substances able to pass through the polyethylene film. No stomatal response to CO₂ was observed when using polyethylene film, however a response was found when using cellophane film leading the authors to conclude that the signal must be aqueous. A number of alternative suggestions have been put forward including guard cell photosynthesis itself (Lawson et al, 2003; Lawson and Morison, 2004; Lawson, 2009), however the exact mechanism has yet to be elucidated. Most of these experiments have been performed on herbaceous angiosperms and there is evidence to suggest that the responses described above differ in both non-herbaceous angiosperms and non-angiosperms (Chater et al, 2011; Ruszala et al, 2011; McAdam and Brodribb, 2012). This will include evolutionary differences in the way stomata perceive signals such as: CO₂, Abscisic acid (Brodribb, 2017), leaf to air vapour pressure deficit (VPD, Martins et al. 2015; McAdam and Brodribb, 2015) and the intensity and quality of light (Doi et al, 2015). Difference in the stomatal response to these signals will influence the diffusion of CO₂ to mesophyll tissues and therefore impact the coordination between A and gs.

The signalling pathways and mechanisms described above mainly refer to short-term responses (seconds to minutes) and are not sufficient to explain the diurnal effect influencing A and gs. Sucrose metabolism has been proposed to play a role in the longer-term co-ordination (over the diurnal period) of A and gs (see review Lawson et al, 2014). Initially proposed by Outlaw and co-workers (Outlaw and Manchester, 1979; Lu et al, 1995, 1997; Ewert et al, 2000; Outlaw and De Vlieghere-He, 2001; Kang et al, 2007) sucrose generated by mesophyll photosynthesis is uploaded to the phloem and transported away from sources to sinks driven by transpiration (Outlaw and De Vlieghere-He, 2001). Excess sucrose (when photosynthesis is high) is carried towards the stomata by the apoplast, stimulating stomatal closure either through some signalling mechanism or by acting as an osmoticum (Lu et al, 1997; Outlaw and William, 2003; Kang et al, 2007; Kelly et al, 2013). Such a process could only occur over longer timescales as high rates of photosynthesis are not associated with low gs, however decreases in gs are often seen towards the end of the diurnal period despite environmental conditions being similar to morning conditions (Lawson et al, 2014). In most species, the synchronized decrease over the course of the day of A and gs is potentially under the control of the same negative feedback (Vialet-Chabrand et al, *Submitted this issue*), which could be explained by the slow catabolism of ABA toward the end of the diurnal period (Tallman, 2004). Figure 1 illustrates the relative co-ordination between A and gs, as well as the decreases in gs, and A towards the end of the diurnal period. Although gs, in *Phaseolus vulgaris* decreased along with A (after only 3h
in the light), interestingly \( \text{gs} \) in \textit{Vicia faba} was not synchronized with \( A \) (Fig. 1A and 1B), resulting in a different pattern of intrinsic water use efficiency (\( W_i \)) (Fig. 1C).

In the field, environmental conditions are rarely stable and influence \( A \) and \( \text{gs} \) responses continuously through the day leading to complex kinetic patterns. Therefore, increasing the speed of

*Figure 1*: Interspecific diversity of stomatal conductance (\( \text{gs} \)), carbon assimilation (\( A \)), and water use efficiency (\( W_i \)) of \textit{Phaseolus vulgaris}, \textit{Vicia faba}, \textit{Triticum aestivum} and \textit{Nicotiana tabacum} in response to a diurnal (8 hour) sinusoidal variation of light intensity (from 0 to 2000 \( \mu \)mol m\(^{-2} \) s\(^{-1} \)). Gas exchange parameters (\( \text{gs} \), \( A \) and \( W_i \)) were recorded at 10s intervals, leaf temperature maintained at 25°C, and leaf VPD at 1.3 KPa. A representative plant of each species was grown in the greenhouse at University of Essex and maintained under well-watered conditions. Under the same pattern of light, the diversity of the temporal response of \( \text{gs} \) and \( A \) between species resulted in large differences in the pattern and magnitude of \( W_i \) over the course of the diurnal period, highlighting the importance of processes that may determine the slow decrease of \( A \) and \( \text{gs} \) through the day.
stomatal response and/or improving the coordination between mesophyll and stomatal responses represent an unexploited potential avenue to improve photosynthetic rates and plant water use efficiency (Lawson et al, 2010). Improving sugar export from the leaf to other parts of the plant could help to maintain $A$ at its maximum level through the day but maybe at the expense of a higher $E$.

3. INFLUENCE OF STOMATAL PATTERNING ON LEAF LEVEL GAS EXCHANGE

It is generally well known that significant variation exists between and within species in the number, size and distribution of stomata (Tichà, 1982) and that these numbers are influenced by environmental growth conditions (Weyers et al, 1997; Lawson & Weyers, 1999). However, it is less well established that considerable heterogeneity in stomatal characters and function exists over the leaf lamina. Stomatal density over the leaf lamina is determined by both cell differentiation as well as cell expansion (Poole et al, 1996; 2000; Lawson et al, 2002), however stomatal spacing generally follows the basic one-cell spacing rule that results in stomata being separated by at least one epidermal cell (Geisler, 2000; Torii & Bergmann, 2017; Gray, 2017) to ensure proper guard cell function (Sachs, 2005). Spatial patterns of stomatal density have been illustrated in a number of different species (Smith et al, 1989; Poole et al, 1996; Weyers et al, 1997; Lawson & Weyers, 1999) and the influence of environmental conditions on such patterning reported (Poole et al, 2000; Croxdale, 2000). Variation in anatomical features can result in considerable heterogeneity in functional characteristics over the leaf lamina that are often ignored although extremely important when considering sampling protocols. Smith et al (1989) was first to show spatial variation in stomatal aperture over the entire leaf surface of *Commelina communis*, whilst later studies illustrated that such variation also impacted on photosynthesis (Weyers et al, 1997; Weyers & Lawson, 1997), although the patterns of variation in $A$ and $g_s$ were not always coordinated (Lawson et al, 1998). An understanding and quantification of the nature of stomatal heterogeneity is important for scaling up from the leaf to the canopy level (Weyers et al, 1997), and highlights the functional advantage or disadvantage in terms of photosynthetic performance or water use efficiency (Mott and Peak, 2007). Figure 2 illustrates spatial and temporal differences in leaf gas exchange measured simultaneously with infra-red gas analysers (IRGAs) attached to three different areas of a single tobacco leaf (Fig. 2A). Despite all three areas receiving an identical light pattern under constant air temperature and relative humidity, distinctly different levels of $g_s$ (Fig. 2B) and $A$ (Fig. 2C) were observed in the different areas, which influenced $W_i$ (Fig. 2D). Another type of variation in stomatal aperture is ‘patchy’ stomatal behaviour (Mott et al, 1993; Cardon et al, 1994; Kaiser and Kappen, 2001; Peak et al, 2004; West et al, 2005), which was defined by Mott et al (1993) as “the non-random distribution of stomatal aperture over the leaf surface”. This received a great deal of attention in the late 80s and early 90s due to the impact of patchy stomatal behaviour on the calculation of internal CO2 concentration, $C_i$ (e.g. Mott and Parkhurst, 1991). This calculation assumes uniform $g_s$ over the measured surface and led to the erroneous conclusion that drought stress directly affected photosynthesis rather than via reduced $g_s$ and restricted CO2 diffusion (Terashima and Wong, 1988; Terashima, 1992). Using chlorophyll fluorescence imaging, Mott et al (1993) demonstrated patchy stomatal behavior in well-watered amphistomatous leaves of *Xanthium strumarium* by changes in air relative humidity. Interestingly there was asymmetry in patches from the two surfaces indicating that a general mesophyll signal was not entirely responsible for patchy stomatal behaviour, which questions the mechanisms that co-ordinate stomata and mesophyll. However, the large overlap in patches suggested some communication between the two surfaces.
(Mott et al. 1993). This patchy stomatal behavior results in heterogeneous measurements of \( g_s \) in different parts of the leaf, potentially impacting photosynthesis levels (see Fig. 2). More recently, Dow et al. (2014a) investigated the importance of stomatal density and spacing on photosynthesis using cluster mutants in which the one cell spacing rule was broken resulting in stomata occurring in groups of varying degrees depending on the mutation. Maximum stomatal conductance estimated
from gas exchange and anatomical measurements were comparable in genotypes with proper
stomatal spacing (< 5% of stomata occurring in clusters), whilst those with patterning defects (> 19%
of stomata in clusters) had lower $g_s$ and $A$, but an equivalent $W_s$. The reduced stomatal opening in
the genotypes with patterning defects was reportedly due to mechanical failure of the guard cells of
one or more of the following: 1) impaired guard cell function due to a lack of ions from epidermal
cells for osmotic function (e.g Outlaw and William, 1989); 2) competition between adjacent guard
cells through increasing turgor pressure creating opposing forces between the two guard cells; 3)
disruption to the signaling mechanism that determines the structure of the guard cells (Dow et al,
2014 a & b). Papanatsiou et al (2016) confirmed that incorrect stomatal patterning impacted on
guard cell dynamics in the cluster mutant ‘too many mouths’ ($tmm1$) and showed that this was
accompanied by a reduction in $K^+$ accumulation in the guard cells, but highlighted that this was not
due to reduced supply from the lower number of epidermal cells and that alternative mechanisms
must be responsible. Hydraulic limitation has been put forward as an alternative to mechanical
failure of the guard cells, for example if the hydraulic supply was insufficient to provide enough
water to numerous stomata close to each other, guard cell turgor pressure would be limited to
ensure complete stomatal opening (Dow et al 2014a). This mechanism is supported by the numerous
recent reports demonstrating a close correlation between variations in hydraulic supply, $g_s$ and $A$
recently supported the hydraulic limitation theory showing that uniformity of spatial patterning
demonstrates an organization of veins and stomata that ensures a constant mesophyll hydraulic
resistance throughout the leaf in woody angiosperm species, and agrees with functional models of
al (2016) did not report the same spatial heterogeneity that has been found previously for many
other species (Poole et al., 1996; Lawson et al, 1998; Weyers et al, 1997), which could be due to the
more heterogeneous organization of veins and stomata in non-woody species. Using the
measurements of Dow et al (2014a), Lehmann and Or (2015) developed a model to determine the
effect of clustering on gaseous diffusion that also took into account the effect of overlapping shells
of hydration of adjacent stomatal pores. Stomata in close proximity to each other resulted in
interactions between concentration shells that reduced diffusional fluxes by 5-15%. This predicted
reduction due to clustering, suggests that guard cell function was impaired, potentially limiting the
response of stomata to environmental cues. The spatial clustering reported by Dow et al (2014a)
could be considered similar to functional clustering as observed in patchy stomatal behavior, and
Lehmann & Or (2015) suggested that stomatal patchiness of a sufficient size (or cluster) could
reduce vapour losses from the leaf and heat exchange in between patches relative to homogeneous
stomatal behavior. It should be additionally noted that individual stoma have different temporal
behavior, leading to complex spatial and temporal patterns of stomatal movement that can lead to
local limitations in $CO_2$ supply for photosynthesis (Kaiser and Kappen, 2000; 2001).

4. DIURNAL IMPACT ON STOMATAL BEHAVIOUR AND IMPLICATIONS FOR FUTURE MODELS

Most of the models describing diurnal variations in gas exchange use predicted steady state values
of $g_s$, which suppose instantaneous variations of $g_s$ to a stable value ($G_s$) for each light intensity
(Damour et al, 2010). These models describe the response of $g_s$ to a series of light intensities but fail
to accurately predict transient variations in $g_s$, as they neglect the temporal aspect of stomatal
response (Violet-Chabrand et al, 2013). Using a steady state model, a sinusoidal pattern of light will
result in a similar symmetrical pattern of $g_s$, which when measured under these conditions may not
be the case, as observed in Figure 1. Over the diurnal period, a number of species display a decrease in $g_s$ and $A$ that is not driven by decreases in light intensity or the temporal response of $g_s$ (Mott and Parkhurst, 1991; Allen and Pearcy, 2000; Mencuccini et al, 2000; Moriana et al, 2002; Dodd et al, 2006; Resco de Dios et al, 2012), but the exact mechanism for this requires further investigation. As discussed earlier, sugar accumulation due to high photosynthetic rates are believed to provide a long-term photosynthetic feed-back on $g_s$ (Lu et al, 1995, 1997; Outlaw and William, 2003; Kang et al, 2007; Kelly et al, 2013), which also needs to be taken into account when considering the incorporation of temporal responses into models of stomatal behaviour. Noe and Giersh (2004) proposed a model based on the assumption that the pool of sugars, resulting from the difference between the rate of sugar production by photosynthesis and their rate of export, increasingly inhibited $A$ over the diurnal period. By analogy with this model, Vialet-Chabrand et al (2016) also described stomatal closure through the diurnal period as the size of the pool of sugar increased. These models agree with recent research that has focused on the role of sugars in the regulation of guard cell aperture and the co-ordination between stomatal behaviour and mesophyll photosynthesis (see reviews by Lugassi et al, 2015; Santelia and Lawson, 2016; Daloso et al, 2016; Santelia & Lunn, 2017). By the end of the day, the slow response of $g_s$ can result in the maintenance of high $g_s$, which leads to substantial water losses that are not accompanied by any carbon uptake (Blom-Zandstra et al, 1995). Improving rapidity of the response of $g_s$ to reduce the limitation of $A$ and prevent the slow decrease in $A$ and $g_s$ through the day, could maintain photosynthetic carbon assimilation for longer, influencing plant productivity and biomass.

It should also be kept in mind that the water status of the plant will affect temporal responses of $g_s$ (Lawson & Blatt, 2014), which will be species specific as the transduction of the light signal triggering stomatal opening (Kinoshita, 2017) could be modified or reduced to maintain leaf turgor (Aasamaa and Sober, 2011). As a consequence, the water status of the plant is an important determinant of $G_s$ that could result in a strong limitation on $A$ throughout the diurnal period (Tuzet et al, 2003; Yan et al, 2016). For example, in Figure 3a the temporal response of $g_s$ in *Vicia faba* was altered under drought, compared to well-watered conditions with a decrease in $g_s$ occurring earlier in the day as the soil water content decreased, and the effect of ABA increased (Brodribb, 2017). It is interesting to note that the strong stomatal limitation on CO$_2$ diffusion only appeared when soil water content was lower than 30% and resulted in reduced $A$ by approximately 30-50% (Fig. 3b). The slow decrease of $A$ over the diurnal period was observed irrespective of the soil water content and could result from the negative feedback of sugar accumulation on stomata as previously described. Diurnal variations of $W_t$ were not only higher under drought conditions but also followed a different pattern (Fig. 3c), highlighting the importance of the temporal variations of $g_s$ under these conditions. It should be noted that these observations could vary greatly between species with different vein and stomatal organisation (see section 3). However, as most studies have been carried out under well-watered conditions or using steady state approaches (Wolf et al, 2016; Sperry et al, 2016), there are very few data describing the influence of drought on the temporal response of $g_s$ (Lawson & Blatt, 2014), and even less on modelling it. The water available and its transport from roots to the stomata could be a limiting factor for the rapidity of $g_s$ response. Therefore, factors such as hydraulic conductance, leaf vein density and stomatal distributions are important in spatial and temporal stomatal responses (see Section 3).

5. CONCLUSION
Despite decades of stomatal research, there are still major gaps in our knowledge of stomatal behaviour and the mechanisms that drive the co-ordination between $A$ and $g_s$. In this review, using examples from herbaceous crop species, we have demonstrated that without a full understanding of how stomata integrate multiple signals and their hierarchical nature relative to photosynthesis and water balance at the whole plant level, it is impossible to predict the impact of current and future climate change.

Figure 3: Effect of progressive drought on the response of (a) stomatal conductance ($g_s$), (b) carbon assimilation ($A$) and (c) intrinsic water use efficiency ($W$) in *Vicia faba*, to a diurnal sinusoidal variation in light intensity (from 0 to 2000 μmol m$^{-2}$ s$^{-1}$, black line). Gas exchange parameters ($g_s$, $A$ and $W$) were recorded at 10s intervals, leaf temperature maintained at 25°C, and leaf VPD at 1.3 KPa. The plant was grown in the greenhouse at University of Essex. A well-watered plant was subjected to progressive soil drying in absence of re-watering and measured for 4 days consecutively. Soil water content was quantified via a gravimetric method and represented with different colours in the figure legend as a percentage of soil water content. The decrease in $g_s$ only limited $A$ when soil water content was lower than 30%, revealing the unnecessary water loss occurring with no further gain in $A$ under well-watered conditions.
environments on plant productivity and water use. The spatial and temporal aspect of the coordination between $A$ and $g_s$ has often been ignored, although there is renewed interest in this area with several recent studies exploring the impact of spatial variation in stomatal density on gas exchange and productivity. Currently, most models neglect temporal and spatial variation in $g_s$ on gas exchange and make the assumption of an instantaneous stomatal response. It is clear that temporal effects play a crucial role in the balance of carbon fixation and water loss, with increasing importance as water availability for the plant decreases and the limitation on $A$ by $g_s$ becomes greater. It is time to develop a common modelling platform capable of describing the plant-soil-atmosphere continuum, which integrates spatial and temporal $g_s$ behaviour to reflect the impact on carbon gain and water use.

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**Figure 1:** Interspecific diversity of stomatal conductance ($g_s$), carbon assimilation ($A$), and water use efficiency ($W_i$) of *Phaseolus vulgaris, Vicia faba, Triticum aestivum* and *Nicotiana tobaccum* in response to a diurnal (8 hour) sinusoidal variation of light intensity (from 0 to 2000 µmol m$^{-2}$ s$^{-1}$). Gas exchange parameters ($g_s$, $A$, and $W_i$) were recorded at 10s intervals, leaf temperature maintained at 25°C, and leaf VPD at 1.3 KPa. A representative plant of each species was grown in the greenhouse at University of Essex and maintained under well-watered conditions. Under the same pattern of light, the diversity of the temporal response of $g_s$ and $A$ between species resulted in large differences in the pattern and magnitude of $W_i$ over the course of the diurnal period, highlighting the importance of processes that may determine the slow decrease of $A$ and $g_s$ through the day.

**Figure 2:** Effect of stomatal patchiness on the spatial heterogeneity of stomatal conductance ($g_s$), carbon assimilation ($A$), and water use efficiency ($W_i$) in a *Nicotiana tobaccum* leaf subjected to a diurnal (8 hour) sinusoidal variation in light intensity (from 0 to 2000 µmol m$^{-2}$ s$^{-1}$). Three areas (a, b and c) of the leaf were measured simultaneously for $g_s$, $A$ and $W_i$. Gas exchange parameters were recorded at 10s intervals, leaf temperature was maintained at 25°C, and leaf VPD at 1.3 KPa. All plants were grown in the greenhouse at University of Essex and were maintained under well-watered conditions. Each leaf cuvette only covered 2cm$^2$ of the leaf surface (c.a 300 cm$^2$) providing an insight into the heterogeneous response of gas exchange over the leaf surface. Remarkably, despite the differences in $A$ and $g_s$, $W_i$ exhibited a similar trajectory at all sites, questioning how the balance between $A$ and $g_s$ is maintained over the leaf surface (see section 2).

**Figure 3:** Effect of progressive drought on the response of (a) stomatal conductance ($g_s$), (b) carbon assimilation ($A$) and (c) intrinsic water use efficiency ($W_i$) in *Vicia faba*, to a diurnal sinusoidal variation in light intensity (from 0 to 2000 µmol m$^{-2}$ s$^{-1}$, black line). Gas exchange parameters ($g_s$, $A$ and $W_i$) were recorded at 10s intervals, leaf temperature maintained at 25°C, and leaf VPD at 1.3 KPa. The plant was grown in the greenhouse at University of Essex. A well-watered plant was subjected to progressive soil drying in absence of re-watering and measured for 4 days consecutively. Soil water content was quantified via a gravimetric method and represented with different colours in the figure legend as a percentage of soil water content. The decrease in $g_s$ only limited $A$ when soil water content was lower than 30%, revealing the unnecessary water loss occurring with no further gain in $A$ under well-watered conditions.
• Diurnal $g_s$ and $A$ responses are not always synchronized, illustrated by different kinetics of $W_i$ with variation in diurnal gas exchange both species-specific and influenced by plant-water status.
• The slow decrease of both $A$ and $g_s$ through the day appears to be the result of light-driven accumulations of photosynthetic products.
• Considerable spatial heterogeneity in stomatal distribution, shape, and function exists both between and within leaves that does not necessarily match with photosynthesis with implications for the optimization of whole-plant gas exchange.
• Current models do not take into account spatial or temporal $g_s$ behavior, with implications for estimating leaf level gas exchange as well as predicting the influence of climate change on these processes at the canopy scale.
OUTSTANDING QUESTIONS

- The importance and benefits of spatial variation in \( g_s \) and the impact on \( A \) is largely unknown and ignored.
- What are the mechanisms that synchronize \( g_s \) responses with mesophyll demands for \( CO_2 \)? Is there a mesophyll signal coordinating these processes to achieve a target \( W_s \) and what is the nature of this signal?
- Further development in dynamic models of gas exchange will only be possible with more quantitative data on diurnal and temporal stomatal responses under different environmental conditions, as well as information on the spatial variation in gas exchange at the leaf level that can be scaled to the canopy.


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