Update on Diurnal Variation in Gas Exchange

Diurnal Variation in Gas Exchange: The Balance between Carbon Fixation and Water Loss[1][OPEN]

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Stomatal control of transpiration is critical for maintaining important processes, such as plant water status, leaf temperature, as well as permitting sufficient CO_2 diffusion into the leaf to maintain photosynthetic rates (A). Stomatal conductance 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 coordinates A and stomatal conductance 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, although it is clear that 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, and they should take into consideration the long-term effect on gas exchange over time.

As the waxy surface of most leaves makes them virtually impermeable to CO_2 and water, nearly all CO_2 absorbed by the plant and water lost pass through the stomatal pores (Cowan and Troughton, 1971; Caird et al., 2007; Jones, 2013). Although these pores represent only a small fraction of the leaf surface, stomatal behavior has major consequences for photosynthetic CO_2 fixation and water loss from leaf to canopy levels, influencing carbon and hydrological cycles at global scales (Hetherington and Woodward, 2003; Keenan et al., 2013). 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 (g_s) appears to be closely linked with mesophyll demands for CO_2, and a strong correlation between photosynthetic rate (A) and g_s is often observed (Wong et al., 1979; Farquhar and Sharkey, 1982; Mansfield et al., 1990; Buckley and Mott, 2013), and although conserved, it is not always constant (Lawson and Morison, 2004; Bonan et al., 2014). However, the A and properties of each leaf may not be identical and depend on acclimation to the surrounding microclimatic conditions; 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 g_s at the whole-plant level. Early experiments by Meinzer and Grantz (1990) showed that the balance between water loss and water transport capacity enables the maintenance of a constant leaf water status over a wide range of plant sizes and growing conditions. Therefore, plants regulate the transpiration of each leaf independently in response to variations in microclimate by constantly adjusting stomatal aperture. The stomatal control of transpiration rate also is an important component of the leaf energy balance and can be of great importance for maintaining an optimal or appropriate leaf temperature for photosynthesis,

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ADVANCES

- 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.

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particularly under conditions of increasing or high light intensity that are observed over a typical diurnal period. The diurnal g_s 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 often is seen as a cost for carbon fixation at the 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 it has been shown to have an important role in nutrient uptake and, ultimately, growth (Caird et al., 2007).

The close correlation between A and g_s is thought to help maximize A as a function of E over a diurnal period, and there is evidence to suggest that this relationship is driven by a signal produced by the mesophyll that guard cells sense and respond to (see below). However, it also could be the result of covariation in response to environmental factors, such as light intensity, with g_s often limiting A irrespective of whether A is the main optimization 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 and Morison, 2004; Lawson et al., 2010; Aasamaa and Söber, 2011). Cowan and Farquhar (1977) inferred that the coordination between A and g_s can be seen as a plant response to control the trajectory of g_s to maximize A and minimize E over a typical diurnal sinusoidal light pattern (Buckley, 2017). However, observations of g_s in response to variations in light intensity revealed that, in general, stomatal responses do not mimic these simulations. Instead, g_s responses are 1 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; Lawson et al., 2012; Vialet-Chabrand et al., 2013; Lawson and Blatt, 2014; McAusland et al., 2016), which results in more water loss than is necessary for the gain in CO_2 (Lawson and Blatt, 2014). In general, the diversity of coordination between A and g_s observed in steady state across species suggests that there is no strong selective pressure for this trait in the field, which highlights the room for potential improvement in plant performance (McAusland et al., 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 recognize the spatial and temporal aspects of the 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), 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 model of Ball et al. (1987) 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 leads to inaccurate predictions of the diurnal time course of g_s (Vialet-Chabrand et al., 2013, 2017). The model of Farquhar et al. (1980) has been successful in describing the kinetics 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 spatiotemporal (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 focused 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 recognize the importance of mesophyll conductance in the balance of CO_2 to water diffusion, this will not be covered here (Flexas et al., 2012).

MECHANISMS OF COORDINATION BETWEEN STOMATAL BEHAVIOR AND MESOPHYLL PHOTOSYNTHESIS

For many years, internal CO_2 concentration (C_i) was considered to link stomatal responses to photosynthetic demands for CO_2 (Ball and Berry, 1982; Mott, 1988). For example, when 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; 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 may sense external $[CO_2]$, while 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 and Raschke, 1978; Sharkey and Raschke, 1981; Farquhar and Sharkey, 1982). More recent studies on transgenic plants have shown that g_s increases with photosynthetic photon flux density 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 and Song, 2008).

This 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 and Bowling, 1992, 1995),

with candidates including photosynthetic metabolites such as ATP, NADPH, and ribulose 1,5-bisphosphate (Wong et al., 1979; Farquhar and Wong, 1984; Lee and Bowling, 1992; Zeiger and Zhu, 1998; Tominaga et al., 2001; Buckley et al., 2003) as well as malate and sugar (Hedrich and 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 pallida* only responded to light and CO_2 when the epidermis was transplanted back onto the mesophyll tissue from which the peel had been taken or from that of another leaf. Sibbernsen and 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 suggested that the mesophyll signal was a vapor phase ion (Mott and Peak, 2013). However, Fujita et al. (2013) tested this hypothesis further 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 CO_2 was observed when using polyethylene film, although 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 were performed on herbaceous angiosperms, and there is evidence to suggest that the responses described above differ in both non-herbaceous angiosperms and nonangiosperms (Chater et al., 2011; Ruszala et al., 2011; McAdam and Brodribb, 2012). These include evolutionary differences in the way stomata perceive signals such as CO_2, abscisic acid (ABA; Brodribb and McAdam, 2017), leaf-to-air vapor pressure deficit (VPD; McAdam and Brodribb, 2015; Martins et al., 2016), and the intensity and quality of light (Doi et al., 2015). Differences in the stomatal response to these signals will influence the diffusion of CO_2 to mesophyll tissues and, therefore, impact the coordination between A and g_s.

The signaling 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 g_s. Suc metabolism has been proposed to play a role in the longer term coordination (over the diurnal period) of A and g_s (for review, see Lawson et al., 2014). Initially proposed by Outlaw and coworkers (Outlaw and Manchester, 1979; Lu et al., 1995, 1997; Ewert et al., 2000; Outlaw and De Vlieghere-He, 2001; Kang et al., 2007), Suc 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 Suc (when photosynthesis is high) is carried toward the stomata by the apoplast, stimulating stomatal closure either through some signaling mechanism or by acting as an osmoticum (Lu et al., 1997; Outlaw, 2003; Kang et al., 2007; Kelly et al., 2013). Such a process could only occur over longer time scales, as high rates of photosynthesis are not associated with low g_s; however, decreases in g_s often are seen toward 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 g_s is potentially under the control of the same negative feedback (Vialet-Chabrand et al., 2017), which could be explained by the slow catabolism of ABA toward the end of the diurnal period (Tallman, 2004). Figure 1 illustrates the relative coordination between A and g_s as well as the decreases in g_s and A toward the end of the diurnal period. Although g_s in *P. vulgaris* decreased along with A (after only 3 h in the light), interestingly, g_s in *V. faba* was not synchronized with A (Fig. 1, A and B), resulting in a different pattern of W_i (Fig. 1C).

In the field, environmental conditions are rarely stable and influence A and g_s responses continuously through the day, leading to complex kinetic patterns. Therefore, increasing the speed of the stomatal response and/or improving the coordination between mesophyll and stomatal responses represent an unexploited potential avenue to improve A 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.

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 (Ticha, 1982) and that these numbers are influenced by environmental growth conditions (Weyers et al., 1997; Lawson and Weyers, 1999). However, it is less well established that considerable heterogeneity exists in stomatal characters and function over the leaf lamina. Stomatal density over the leaf lamina is determined by both cell differentiation and 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 et al., 2000; Chater et al., 2017; Torii and Bergmann, 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 and Weyers, 1999), and the influence of environmental conditions on such patterning has been reported (Croxdale, 2000; Poole et al., 2000). Variation in anatomical features can result in considerable heterogeneity in functional characteristics over the leaf
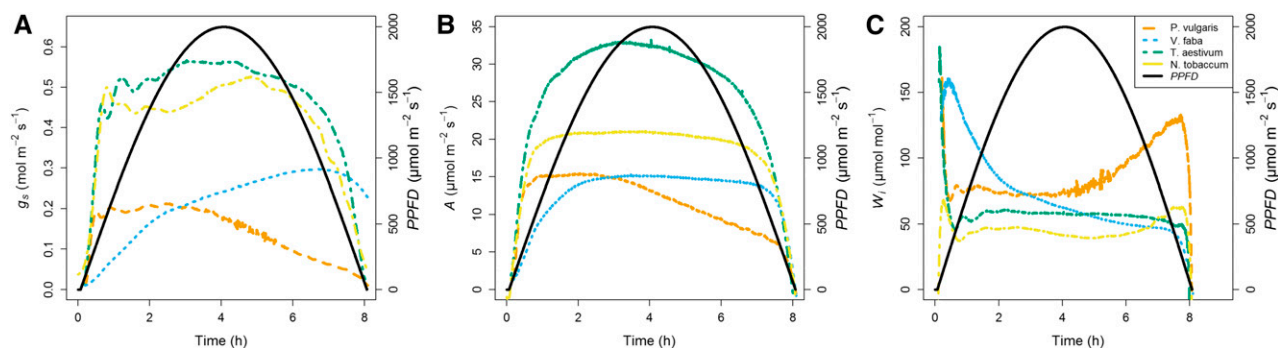
Figure 1. Interspecific diversity of (A) g_s, (B) A, and (C) intrinsic water use efficiency (W_i) of *Phaseolus vulgaris, Vicia faba, Triticum aestivum,* and *Nicotiana tabacum* in response to a diurnal (8-h) sinusoidal variation of light intensity (from 0 to 2,000 μmol m^{-2} s^{-1}). Gas-exchange parameters (g_s, A, and W_i) were recorded at 10-s intervals, leaf temperature was maintained at 25°C, and leaf VPD was maintained at 1.3 kPa. A representative plant of each species was grown in the greenhouse at the 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. PPFD, Photosynthetic photon flux density.

lamina that are often ignored although extremely important when considering sampling protocols. Smith et al. (1989) were the first to show spatial variation in stomatal aperture over the entire leaf surface of *Commelina communis*, while later studies illustrated that such variation also impacted photosynthesis (Weyers and Lawson, 1997; Weyers et al., 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 the spatial and temporal differences in leaf gas exchange measured simultaneously with infrared gas analyzers attached to three different areas of a single *N. tabacum* 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 behavior (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 1980s and early 1990s due to the impact of patchy stomatal behavior on the calculation of C_i (Mott and Parkhurst, 1991). This calculation assumes uniform g_s over the measured surface and led to the erroneous conclusion that drought stress affected photosynthesis directly rather than via reduced g_s and restricted CO_2 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 behavior, which questions the mechanisms that coordinate 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 heterogenous measurements of g_s in different parts of the leaf, potentially impacting photosynthesis levels (Fig. 2).

More recently, Dow et al. (2014) 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 g_s estimated from gas exchange and anatomical measurements was comparable in genotypes with proper stomatal spacing (less than 5% of stomata occurring in clusters), while those with patterning defects (greater than 19% of stomata in clusters) had lower g_s and A but an equivalent W_i. 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 (Outlaw, 1989); (2) competition between adjacent guard cells through increasing turgor pressure, creating opposing forces between the two guard cells; and (3) disruption to the signaling mechanism that determines the structure of the guard cells (Dow and Bergmann, 2014; Dow et al., 2014). Papanatsiou et al. (2016) confirmed that incorrect stomatal patterning impacted guard cell dynamics in the cluster mutant *too many mouths* and showed that this was accompanied by a reduction in K$^+$ accumulation in
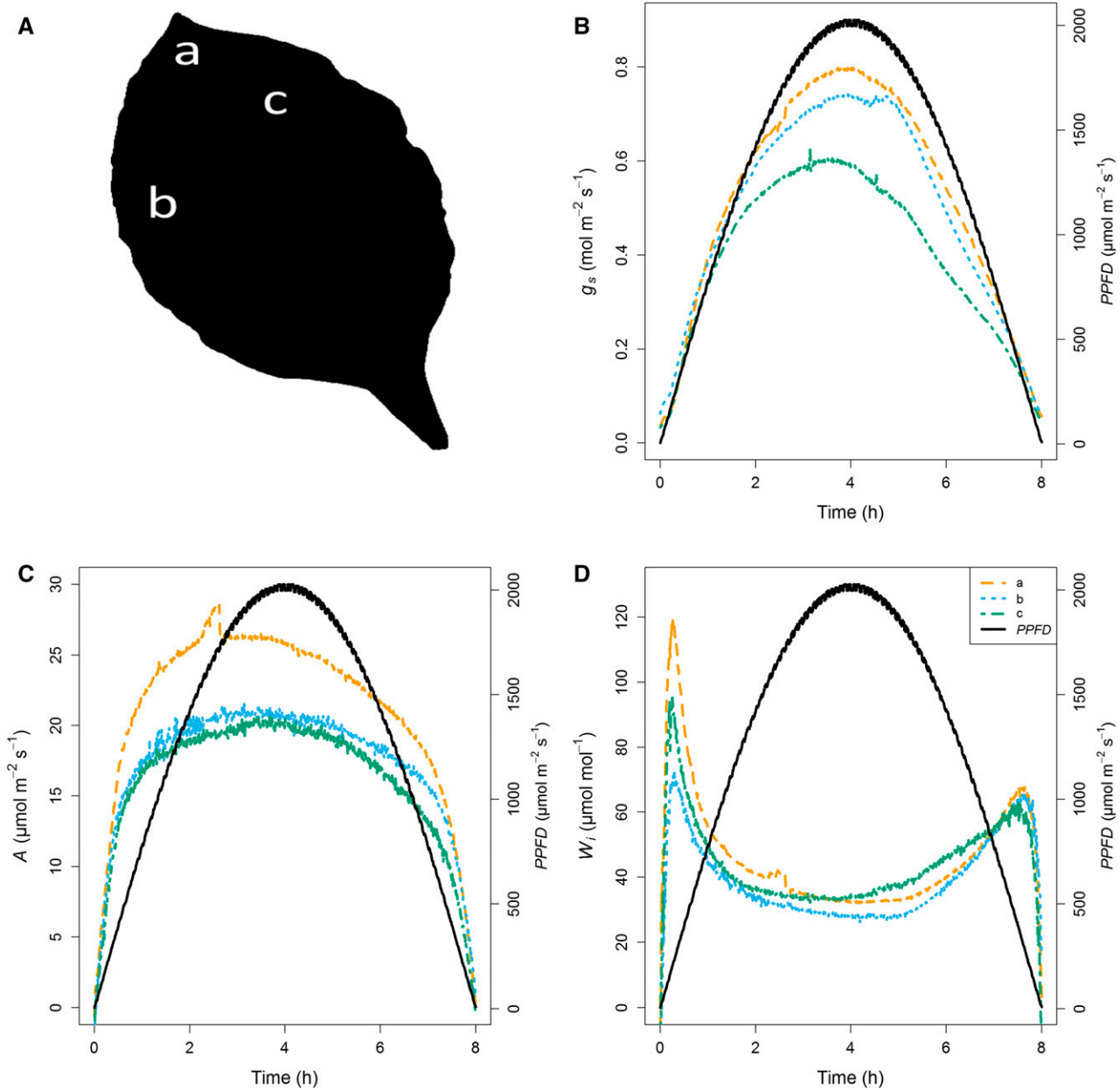
Figure 2. Effects of stomatal patchiness on the spatial heterogeneity of g_s, A, and W_i in an *N. tabacum* leaf subjected to a diurnal (8-h) sinusoidal variation in light intensity (from 0 to 2,000 μmol m^{-2} s^{-1}). (A) Three areas (a, b, and c) of the leaf were measured simultaneously for (B) g_s, (C) A, and (D) W_i. Gas-exchange parameters were recorded at 10-s intervals, leaf temperature was maintained at 25°C, and leaf VPD was maintained at 1.3 kPa. All plants were grown in the greenhouse at the University of Essex and were maintained under well-watered conditions. Each leaf cuvette only covered 2 cm^2 of the leaf surface (~300 cm^2), providing an insight into the heterogenous 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 text). PPFD, Photosynthetic photon flux density.

the guard cells, but they 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., 2014).

This mechanism is supported by the numerous recent reports demonstrating a close correlation between variations in hydraulic supply, g_s, and A (Brodribb et al.,

2005, 2007; de Boer et al., 2016; McElwain et al., 2016; McElwain and Steinthorsdottir, 2017). Fiorin et al. (2016) 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, which agrees with the functional models of leaf hydraulic supply of Brodribb et al. (2007) and Buckley et al. (2015). It should be noted that Fiorin et al. (2016) did not report the same spatial heterogeneity that was found previously for many other species (Poole et al., 1996; Weyers et al., 1997; Lawson et al., 1998), which could be due to the more heterogenous organization of veins and stomata in nonwoody species. Using the measurements of Dow and Bergmann (2014), 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% to 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 and Bergmann (2014) could be considered similar to the functional clustering observed in patchy stomatal behavior, and Lehmann and Or (2015) suggested that stomatal patchiness of a sufficient size (or cluster) could reduce vapor losses from the leaf and heat exchange between patches relative to homogenous stomatal behavior. It should be noted as well 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).

DIURNAL IMPACT ON STOMATAL BEHAVIOR 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 the stomatal response (Vialet-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; de Dios et al., 2012), but the exact mechanism for this requires further investigation. As discussed earlier, sugar accumulation due to high A is believed to provide a long-term photosynthetic feedback on g_s (Lu et al., 1995, 1997; Outlaw, 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 behavior. Noe and Giersch (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 coordination between stomatal behavior and mesophyll
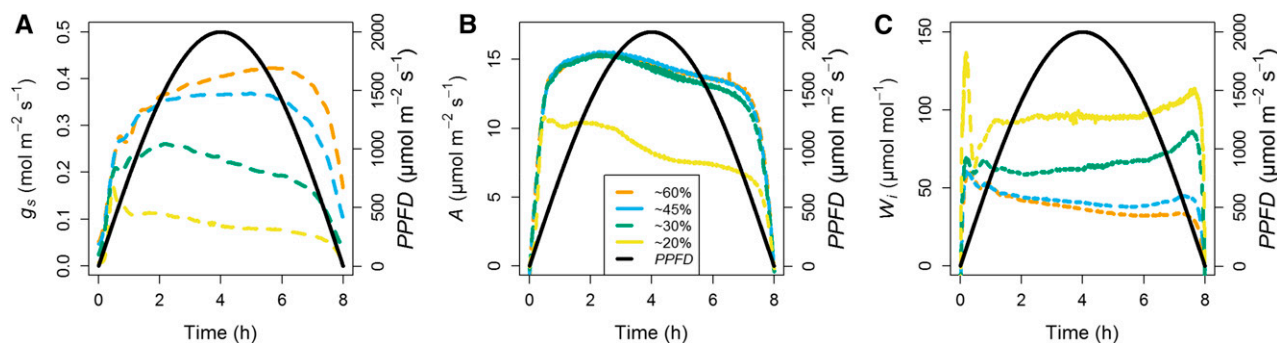

Figure 3. Effects of progressive drought on the response of g_s (A), A (B), and W_i (C) in *V. faba* to a diurnal sinusoidal variation in light intensity (from 0 to 2,000 μmol m^{-2} s^{-1}; black lines). Gas-exchange parameters (g_s, A, and W_i) were recorded at 10-s intervals, leaf temperature was maintained at 25°C, and leaf VPD was maintained at 1.3 kPa. The plant was grown in the greenhouse at the University of Essex. A well-watered plant was subjected to progressive soil drying in the absence of rewatering and measured for 4 d consecutively. Soil water content was quantified via a gravimetric method and is represented with different colors 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. PPFD, Photosynthetic photon flux density.

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_i 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.

photosynthesis (for review, see Lugassi et al., 2015; Daloso et al., 2016; Santelia and Lawson, 2016; Santelia and 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 the 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 and Blatt, 2014), which will be species specific, as the transduction of the light signal triggering stomatal opening (Inoue and Kinoshita, 2017) could be modified or reduced to maintain leaf turgor (Aasamaa and Söber, 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 *V. faba* was altered under drought compared with 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 and McAdam, 2017). It is interesting 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% to 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 described previously. Diurnal variations of W_i 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 organization (see above). However, as most studies have been carried out under well-watered conditions or using steady-state approaches (Sperry et al., 2016; Wolf et al., 2016), there are very few data describing the influence of drought on the temporal response of g_s (Lawson and Blatt, 2014) and even fewer on modeling it. The water available and its transport from roots to the stomata could be a limiting factor for the rapidity of the g_s response. Therefore, factors such as hydraulic conductance, leaf vein density, and stomatal distributions are important in spatial and temporal stomatal responses (see above).

CONCLUSION

Despite decades of stomatal research, there are still major gaps in our knowledge of stomatal behavior and the mechanisms that drive the coordination 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 environments on plant productivity and water use. The spatial and temporal aspects of the coordination between A and g_s have 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 modeling platform capable of describing the plant-soil-atmosphere continuum that integrates spatial and temporal g_s behavior to reflect the impact on carbon gain and water use.

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