The red and blue light dialogue of stomatal regulation for water-efficient crops

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Abstract

Stomatal responses to changing irradiance have significant impacts on photosynthetic carbon gain as well as on plant water use efficiency. These responses can be broken down into the red or mesophyll response which links stomatal conductance with photosynthesis and the specific blue light response that saturates at low fluence levels and is not linked to photosynthesis. Although the two pathways are thought to operate independently, there is growing evidence that the two are more closely linked than we first appreciated. Understanding both the independence and interplay between red light (RL) and blue light (BL) responses in stomatal behaviour could reveal unexploited but critical targets for sustaining or enhancing intrinsic water-use efficiency (W_i) and crop productivity. In this update review, we synthesise the recent advances in our understanding of the RL and BL

stomatal responses and propose future research directions to deepen our mechanistic understanding and inform crop improvement strategies.

Introduction

Stomata, microscopic pores found on most green tissues of higher terrestrial plants, mediate gas exchange between the leaf and the atmosphere governing CO_2 uptake for photosynthesis and water loss through transpiration. As such, stomata play a pivotal role in regulating leaf temperature, photosynthetic rates, whole-plant water status, nutrient uptake, and ecosystem-level evapotranspiration (Lawson and Matthews, 2020; Pelech *et al.*, 2021; Lawson and Leakey, 2024). Stomatal function is often measured and reported as changes in stomatal conductance (g_s), which is the maximum rate of gas exchange (usually H_2O vapour) between the leaf interior and the atmosphere. Stomatal conductance is determined by anatomical features, including stomatal size and density, as well as functional characteristics such as pore aperture (Lawson and Blatt, 2014). Highly specialised guard cells (GCs) surround each stomatal pore whose volume and turgor pressure dynamically regulate stomatal aperture in response to both internal and environmental cues. These changes are brought about through coordinated activity of the plasma membrane H+-ATPase, channels and transporters in the plasma membrane of GCs, which are activated via signalling cascades, triggering ion and solute fluxes that adjust the osmotic potential of GCs driving water movement (Kollist *et al.*, 2014; Jezek and Blatt, 2017; Lemonnier and Lawson, 2024). Environmental factors, including increasing light intensity (particularly red and/or blue wavelengths), increasing air temperature up to an optimum, low atmospheric CO_2 concentration [CO_2], and low vapour pressure deficit

(VPD), typically promote stomatal opening, while the inverse conditions drive closure (Matthews and Lawson, 2019). Internal mesophyll signals and plant hormones are also sensed by GCs to adjust stomatal aperture and optimise g_s (Matthews *et al.,* 2020).

GC responses are central to balancing the carbon demand for photosynthesis with the need to conserve water, making them a novel target for improving water-use efficiency (WUE, which is a measure of plant biomass relative to water use) of our major crops in a warming world (Lawson and Leakey, 2024). As may be expected, stomatal behaviour and photosynthetic carbon uptake (*A*) are closely correlated (Wong *et al.*, 1979), with increasing aperture removing diffusional constraints on CO_2 uptake but at the expense of increased water loss. However, stomatal responses are typically an order of magnitude slower than photosynthetic responses to changing environmental parameters (Lawson and Blatt, 2014; McAusland et al., 2016; Faralli *et al.*, 2019; Vialet-Chabrand and Lawson, 2019), and therefore the relationship between *A* and g_s is often not conserved with short term non-coordination evident between the two parameters (Lawson and Morison, 2004; Lawson *et al.*, 2012). In the natural environment, light intensity is one of the most dynamic environmental conditions that not only triggers changes in stomatal aperture, but is also a key determinant of photosynthetic carbon uptake rates (Kaiser *et al.*, 2015; Vialet-Chabrand *et al.*, 2016; Vialet-Chabrand *et al.*, 2017). Stomatal responses to light can be divided into two distinct pathways: the red light (RL) pathway and the blue light (BL) pathway. The RL response, also known as the mesophyll or photosynthetic response, operates at high fluence rates, saturates at light intensities similar to photosynthesis and is slower than the BL response. Photosynthesis in either mesophyll and/or GC chloroplasts is

involved in the RL response, with studies using photosynthetic inhibitors consistently reporting the eradication of the RL-induced stomatal opening response (Schwartz and Zeiger, 1984). Although, electron transport within GCs has been shown to be as much as 80% of the mesophyll levels (Lawson et al., 2002; Lawson et al., 2014) and it is accepted that Calvin cycle enzymes are present, the extent and contribution of GC photosynthesis to stomatal function is an ongoing subject of debate. Early studies reported that as little as 2% of the solute required for stomatal opening was provide by rubisco activity in *Pisum* (Reckmann et al., 1990), however, as discussed later in this review, GC photosynthetic processes have been shown to contribute to some aspects of stomatal function. In contrast, the BL response is GC-specific and is not inhibited by photosynthetic inhibitors. Typically, the BL response saturates at low fluence rates (~5-20 μ mol m^{-2} s^{-1}), although saturation is much higher in C_4 grasses at 120 μ mol m^{-2} s^{-1} (Zhen and Bugbee, 2020). The BL response is approximately 20 times more effective at stomatal opening than RL and is thought to play an important role in pore opening early in the diel period when the irradiance spectrum is enriched in blue wavelengths to facilitate photosynthetic carbon gain, and/or rapid stomatal responses (Matthews *et al.*, 2020; Vialet-Chabrand *et al.*, 2017a) to sun-flecks to maximise photosynthesis (Zeiger & Field, 1982). Both the red and blue light response contribute to stomatal opening, however the magnitude and the extent of each is species specific (Vialet-Chabrand et al., 2020), which may also play a role in the kinetics of stomatal responses.

While considerable research over the last several decades have elucidated the BL specific signal transduction pathway in GCs and made significant advancements in our

understanding of the RL light response, recent studies have identified previously unrecognised molecular and physiological links between these signalling networks, that will be the focus of this review. Specifically, shared regulation of the plasma membrane H+-ATPase and the dependence of BL responses on mesophyll driven changes in intercellular CO_2 concentration *(C_i)* and metabolic support challenge the long-held view that RL and BL operate independently.

The majority of today's crop canopies are monocultures with high plant density where leaves experience a high degree of self-shading and transient sun-flecking which is further impacted by the zenith angle of the sun, row orientation and cloud cover (Long *et al.*, 2022). These dynamics create pronounced fluctuations in irradiance and spectral distribution throughout the crop canopy profile, which impact the diurnal course of stomatal behaviour, carbon gain and ultimately intrinsic water use efficiency (W_i), a measure of A relative to water loss through g_s (Vialet-Chabrand *et al.*, 2016; Vialet-Chabrand *et al.*, 2017; Matthews *et al.*, 2020). Unravelling the exact mechanisms behind the coordination between g_s and net CO_2 assimilation (A_{net}) for the two distinct wavelength-dependent pathways of stomatal opening is a fundamental requirement to tackle any canopy-scale challenges to improve W_i. In addition, given that most crops have been domesticated from their wild ancestors adapted to relatively open habitats compared to today's monoculture canopies, variation may exist in the coordination of g_s and A_{net} and we may have inadvertently selected for differences in RL and BL stomatal responses.

Understanding both the independence and interplay between RL and BL responses in stomatal behaviour could reveal unexploited but critical targets for sustaining or enhancing

W_i and crop productivity. The available technologies for genetic manipulation, as well as the advancement of transcriptomics and metabolomics technologies can shed light on the effects of stomatal genes in response to red and blue light. In this update review, we synthesise the recent advances in our understanding of the RL and BL stomatal responses, how stomatal behaviour and species-specific differences are driven by both red and blue light, diurnal stomatal kinetics under dynamic light environments and technological advancements. We then propose future research directions to deepen our mechanistic understanding and inform crop improvement strategies.

Stomatal behaviour driven by red light

Red wavelengths (620-750 nm) initiate RL-induced stomatal opening response (Figure 1A) which is widely understood to be photosynthesis-dependent and has long been used to explain the tight coupling between A and g_s (Wong et al., 1971; Sharkey and Raschke, 1981). It has long been assumed that decreases in C_i following mesophyll photosynthesis is the main cue for RL-induced stomatal opening (Roelfsema et al. 2006), and the A/g_s relationship maintains a constant C_i. Research on GCs of albino patches of *Chlorophytum comosum* which do not contain chloroplasts but still respond to red light supported the idea that mesophyll driven changes in C_i drive stomatal opening (Olsen et al., 2002), rather than any direct red light perception in the GCs and there is not direct role for GC photosynthesis (Roelfsema et al., 2006).

However, several studies reported that stomatal responses to C_i were too low to account for the large light driven changes in g_s (Sharkey and Raschke, 1981; Wong et al. 1979)

providing support for an C_i – independent pathway. Several studies have shown that when C_i was kept constant, stomata continued to respond to increasing RL intensity (Messinger *et al.*, 2006; Lawson *et al.*, 2008), whilst in experiments using the CO_2-hyposensitive Arabidopsis mutant *ca1ca4* maintenance of RL-induced stomatal opening was observed (Matrosova *et al.*, 2015; Ando *et al.*, 2022). These findings have led to the hypothesis that C_i is not solely responsible for stomatal opening in response to red light and the coordination between A and g_s (see review by Lawson *et al.*, 2018). Several studies have postulated the idea of a direct 'mesophyll signal' to GCs (Olsen *et al.*, 2002; Messinger *et al.*, 2006; Wang *et al.*, 2011; Ando and Kinoshita, 2018), although the exact nature of this signal remains to be elucidated. Although, a recent study by Zait et al (2025) provides evidence for apoplastic sucrose is the mesophyll signal for regulating guard cell ion transport under red light (Zait *et al.*, 2025), which support earlier studies that suggested an important role of sucrose in stomatal regulations linked to photosynthesis (see Kang et al., 2007; Lawson *et al.*, 2014).

Recent work by Taylor *et al.* (2024) dissected and quantified the relative contributions of the C_i – dependent RL response and a C_i – independent pathway using wild-type Arabidopsis (Col-0) and CO_2-hyposensitive mutant *ca1ca4*. Their findings revealed that both mechanisms contributed equally to RL-induced stomatal opening. However, at high C_i concentrations, C_i – dependent closure dominated while C_i – independent opening was suppressed, suggesting molecular crosstalk between the two pathways. These authors quantified the contribution of both pathways by measuring g_s response to light when C_i was held constant at various concentrations, which supported the earlier findings (Messinger *et*

al., 2006; Lawson *et al.*, 2008). From these findings it was proposed that the C_i – dependent pathway functions as a rough baseline control of stomatal movements, while the C_i – independent pathway functions as a fine-tuning mechanism of stomatal aperture to enable a more direct relationship with photosynthetic rates (Taylor *et al.*, 2024). Their findings also provide support for an earlier hypothesis that the redox state of the chloroplast plastoquinone (PQ) pool (which reflects the dynamic balance between the light and dark reactions of photosynthesis, integrating excitation pressure at PSII with Calvin cycle activity), serves as a preliminary cue for coordinating C_i – independent stomatal responses. This could also provide the mesophyll photosynthesis-derived signal linking *A* and *gs* (Busch, 2014; Kromdijk *et al.*, 2019). Notably, these results complement the work presented by Ando *et al.* (2022) who concluded that RL-induced phosphorylation of the plasma membrane H^+-ATPases is a component of the C_i – independent pathway. However, chlorophyll fluorescence measurements used to infer PQ redox state predominantly represent mesophyll chloroplasts (Taylor *et al.*, 2024). Although GC chloroplasts exhibit comparable responses to light and [CO_2] stimuli (Lawson *et al.*, 2002; Lawson *et al.*, 2003), indicating that their redox states may be tightly coupled, their specific role in the signalling pathway remains speculative. . The current challenge lies in resolving the cellular origins of each pathway, i.e distinguishing mesophyll-derived signals from those originating within GCs (Figure 1A). Moreover, much of the current empirical evidence is correlative, complicating efforts to establish causality. Disentangling the molecular regulation behind the C_i – dependent and C_i – independent pathways is thus essential in future research.

Red light photoreceptors phytochromes (phys), in particular phyB and their downstream PHYTOCHROME INTERACTING FACTORS (PIFs)have also been implicated in stomatal light regulation, although current evidence points to a context dependent role rather than a single conserved effect on aperture. In Arabidopsis, over expression of phyB promoted red light induced stomatal opening, while pif3 and pif4 mutants display wider stomatal apertures than wild type, indicating that PIFs negative regulate light signalling, restraining opening via abscisic acid (ABA)-mediated stomatal closure (Figure 1B) (Wang et al., 2010). In rice and maize, PIF homologs such as OsPIL15, ZmPIF1, and ZmPIF3 negatively regulate stomatal opening by promoting ABA signalling through activation of key ABA pathway genes, thereby reducing stomatal aperture in response to red light and water-limited conditions (Li et al., 2022). More recent work extends PIF function to diurnal stomatal dynamics in *Arabidopsis*, showing nocturnal accumulation of PIFs which increases expression of the GC K+ channel KAT1, required for rapid blue light induced opening at dawn (Rovira et al., 2024). Emerging evidence has highlighted a role of PhyB and PIF4 in light quality integration, with low R:FR inhibits stomatal opening in Arabidopsis seedlings through a PIF4 and ABA dependent mechanism, with similar findings observed in cotyledon gas exchange in Chinese kale (Gustavsson et al., 2026). Although these studies together support PhyB as a RL photoreceptor that promotes stomatal opening and PIFs as central transcriptional regulators coordinating stomatal movement with other signals across monocots and eudicots, it is important note that the downstream mechanism and net effect on aperture depend on developmental stage and light environment rather than reflecting a single conserved stomatal module.

Stomatal behaviour driven by blue light

In contrast to the RL mediated stomatal opening pathway, more research has focused on understanding the signalling pathway of the specific BL response (Figure 1C) (Shimazaki *et al.*, 2007; Yang *et al.*, 2020; Lemonnier and Lawson, 2024; Ivsic et al., 2025). A GC-specific response to BL, independent of mesophyll photosynthesis, was first identified through physiological studies demonstrating that blue wavelengths directly stimulate ion transport and proton extrusion in GCs (Iino *et al.,* 1985; Assmann et al., 1985; Shimazaki *et al.,* 1986), with later molecular studies identifying phototropins as the primary BL receptors mediating this response (Kinoshita and Shimazaki, 1999; Kinoshita et al., 2001; Takemiya et al., 2013). This pathway induces a rapid GC specific response that is effective at operating at relatively low fluence levels to promote stomatal opening, although these levels may be higher than initially reported (Zhen & Bugbee, 2020) and species specific (Vialet-Chabrand *et al.*, 2021). The magnitude of the BL response is also species specific, with grasses showing a much greater magnitude than dicotyledons of which there is also considerable variation (Vialet-Chabrand et al., 2021). A recent study has illustrated that the BL response is essential for photosynthetic induction and removing diffusion constraints (Wang et al., 2026), although this was not observed across all species in the study by Vialet-Chabrand et al. (2021), who suggested that g_s was greater than required to remove diffusional constraints. However, the rapid BL stomatal opening maybe important for understory species which rely on carbon fixation during rapid sunflecks (Zeiger & Field, 1982).

Blue wavelengths (400–500 nm) are perceived by the phototropins (PHOT1 and PHOT2) within the GC's (Shimazaki *et al.*, 1986; Kinoshita and Shimazaki 1999; Briggs and Christie 2002; Doi *et al.*, 2004; Liu and van Iersel, 2021; Roeber *et al.*, 2021; Lin *et al.*, 2025), which triggers a signal cascade involving the phosphorylation of BLUE LIGHT SIGNALLING1 (BLUS1). BLUS1 subsequently activates the BLUE LIGHT DEPENDENT H+-ATPase PHOSPHORYLATION (BHP) kinase, promoting the phosphorylation activity of the plasma membrane H^+-ATPase (Kinoshita *et al.*, 2001; Takemiya *et al.*, 2013; Inoue and Kinoshita, 2017). The H^+-ATPase pump utilises ATP to export H^+ ions from the GC, leading to rapid hyperpolarisation of the plasma membrane, and activation of K^+ channels that drive stomatal opening via osmoregulation and changes to GC water potential (Assmann *et al.*, 1985, Schroeder *et al.*, 1987, Kinoshita *et al.*, 2001). Crytochromes (CRYs) also absorb BL (and UV) , although there are conflicting reports regarding their role in the BL response (Lascève *et al*. 1999; Eckert and Kaldenhoff, 2010). Experiments in isolated epidermal peels of Arabidopsis stomatal opening was impaired in the *cry1/cry2* mutant (Mao et al., 2005) suggesting a direct role in the GC specific BL response, and that these photoreceptors function under relatively high BL fluence rates compared with PHOTs (Mao et al., 2005). A direct role for CRYs in BL induced stomatal opening was questioned by Ohgishi et al. (2004), who explored *phot1-phot2-cry1-cry2* quadruple mutant and triple mutants containing either *cry1* or *cry2* and concluded that the CRYs were not involved in altered pore aperture in response to BL. These conclusions were supported by Boccalandro et al., 2012 who demonstrated that cry1 and cry 2 showed reduced aperture under both BL and RL illumination, and suggested that CRYs effects were on ABA levels and

were not GC specific. It has also been suggested that CRYs have an additive effect with phototropins on stomatal opening and that the two signals convert on CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) (Yang *et al.*, 2017). In Arabidopsis, CRYs interact with CONSTITUTIVE PHOTOMORPHOGENIC 1/SUPPRESSOR OF PHYA-105 (COP1/SPA) complex, leading to the inhibition of light-signalling repressors and the removal of downstream negative regulators (Ando *et al.*, 2013; Cha *et al.*, 2024). A similar COP1-SPA-CRY signalling module has been shown to function in the moss *Physcomitrium patens*, suggesting evolutionary conservation of this BL regulatory mechanism (Kreiss *et al.*, 2023). The resulting accumulation of the transcription factor CONSTANS (CO), which activates transcription of FLOWERING LOCUS T (FT) in the GC, induces stomatal opening via activation of the plasma membrane H^+-ATPase (Kinoshita *et al.*, 2011, Ivsic *et al.*, 2025). Additionally, COP1 promotes ABA closing and the associated signalling pathway. Recently it has been demonstrated that COP1 regulates GC pH via plasma membrane H^+-ATPase activity (Cha *et al.*, 2024). These studies highlight the complexity of BL triggered stomatal opening, with the majority of studies focusing on the role of phototropins, whilst CRY mediated responses have received much less attention (Figure 1C). Although the BL response is GC specific and not directly coupled to mesophyll photosynthesis, it can indirectly influence *A* by mitigating stomatal diffusional limitation. Under steady-state conditions, BL induced increases in g_s often exceed photosynthetic demand and may reduce intrinsic water use efficiency (Vialet Chabrand and Lawson, 2020; Zhen and Bugbee, 2020). In contrast, under fluctuating irradiance such as sun flecks, faster BL mediated stomatal opening can enhance total carbon gain by accelerating *A* induction,

with the magnitude of this response differing between species and dependent on environmental conditions (McAusland *et al.*, 2016; Vialet Chabrand *et al.*, 2017a; Bernardo *et al.*, 2023).

Stomatal behaviour driven by both red and blue light

Stomatal BL responses are greatly influenced by the intensity of RL (Suetsugu *et al.*, 2014) making direct comparisons between studies difficult. To determine the magnitude of the BL response alone, stomatal opening and photosynthesis must be saturated (with RL), as BL will also drive photosynthetic responses, and therefore sufficient RL must be applied in order to maximise mesophyll photosynthesis to determine the specific stomatal BL response. Furthermore, RL saturation is also thought to be needed to provide the ATP required for the BL light response (Suetsugu *et al.*, 2014).

Therefore, despite the independence from mesophyll photosynthesis, there is increasing evidence that suggests direct links between the two pathways (Figure 1D and Figure 2). Two kinases found in the GCs (CBC1 and CBC2 [CONVERGENCE OF BLUE LIGHT AND CO_2]) link the BL response to low [CO_2] (and therefore the RL C_i-dependent pathway). Recent studies have established CBC1/2 as central regulators of BL and [CO_2] signalling, bridging/balancing the demand for both stomatal opening pathways in GCs (Hiyama *et al.*, 2017; Dubeaux *et al.*, 2021; Jones *et al.*, 2022). Positioned downstream of HIGH LEAF TEMPERATURE 1 (HT1) and upstream of SLOW ANION CHANNEL ASSOCIATED 1 (SLAC1), CBC1/2 act as signal mediators of light and [CO_2] signalling (Takahashi *et al.*, 2025). This position allows them to integrate phototropin delivered BL signals with C_i (which is

associated with the mesophyll RL response) to regulate stomatal aperture according to the most important need of the plant. CBC1/2 contributes by suppressing SLAC1 activity under BL or low [CO_2] (or low C_i) which limits anion efflux associated with stomatal closure (Figure 1B). This facilitates hyperpolarization of the guard cell plasma membrane, membrane H^+-ATPase activity, which in turn is necessary to drive K+ uptake via K+-selective channels (Marten *et a*l., 2007; Hiyama *et al*., 2017; Hayashi *et al*., 2020; Jones *et al*., 2022; Takahashi *et al*., 2025).

The interlink between RL and BL in stomatal opening was further exemplified by Hosotani *et al*. 2021 who demonstrated using constitutive signalling variants of BLUS1 that the BL activation of H+-ATPase alone was insufficient for stomatal opening and that a simultaneous reduction of C_i was required. The reduction in C_i directly links the RL C_i-dependent mesophyll photosynthetic pathway with the BL specific pathway within the GCs. The role of starch degradation in BL induced stomatal opening (Horrer *et al*., 2016) also directly links the two pathways, as starch in the GCs is thought to be made primarily from sugars imported from the mesophyll and that this uptake may be linked with plasma membrane H+-ATPase activity (Flutsch *et al*., 2020), or from GC photosynthesis (Fuji et al., 2024), once again connecting RL mesophyll photosynthesis with GC requirements for BL driven responses. Whilst most studies have focused on BL phosphorylation of the plasma membrane H+-ATPase in GCs, Ando and Kinoshita 2018 first showed RL induced activation of this protein in stomatal opening, connecting RL and BL responses.

Progress to unravel the mechanisms underlying the crosstalk between both the BL and RL responses in GCs has recently been made. Using phosphoproteome analysis and site-

directed mutagenesis experiments in *Arabidopsis thaliana*, Fuji *et al*. 2024 and Hayashi *et al*. 2024 demonstrated H^+-ATPase is phosphorylated at several different position during light-induced stomatal opening. Phosphorylation of both Thr881 and Thr948 in the C-terminal autoinhibitory domain of the plasma membrane H^+-ATPase are essential for stomatal opening, and it appears that both RL and BL are involved (Figure 2). In GC protoplasts of *Vicia faba* and transgenic *Arabidopsis thaliana* phosphorylation of Thr-881 was evidenced under both BL and RL (Hayashi *et al.*, 2024), whilst Fuji et al. (2024) demonstrated that Thr-948 phosphorylation is a prerequisite for the BL phosphorylation of THR-881. In addition, Fuji *et al*. 2024 demonstrated that Thr-881 phosphorylation under RL in GC protoplasts was inhibited by DCMU suggesting that GC photosynthesis is required. However, phosphorylation of Thr-948 by RL did not occur in isolated GC protoplasts, and was only observed in intact leaves and suppressed by DCMU. These findings indicate that the involvement of mesophyll cell photosynthesis, (mostly likely through a diffusible signal) is essential for full activation of the plasma membrane H+-ATPase (Okumura *et al.*, 2016). Sucrose has been identified as a mesophyll derived signal, that enhances RL induced stomatal opening and links mesophyll photosynthesis with GC ion transport (Figure 2; Zait *et al.*, 2025). Together these studies clearly indicate that the specific signal transduction pathways first assigned to BL are not BL specific but can be moderated by RL demonstrating synergy of RL and BL induced stomatal opening. A very recent study by Yamauchi et al. (2026) has identified that phosphorylation of WDR48 (directly by PHOTs) is required for starch degradation and BL stomatal opening. This study identifies two separate independent pathways for starch degradation involving both WDR48 and BLUS1 and

demonstrates that the integration of red-light–driven starch synthesis with blue-light–dependent degradation via these pathways is crucial for stomatal opening in response to red and blue light, providing further support for the co-ordination between these two light signalling pathways.

Species specific differences in stomatal responses to red and blue light

Although the core BL signalling pathway is fairly conserved, recent studies reveal clear species-specific differences in the magnitude of the g_s response (Vialet-Chabrand *et al.*, 2021). Early studies established that several fern species within the Leptosporangiopsida (Doi *et al.*, 2006) and selected Polypodiopsida taxa (Doi *et al.*, 2015) lacked a GC specific BL response, although a subsequent paper has demonstrated a BL response in Polypodiales (Cai *et al.*, 2021). A recent study by McAdam *et al.* 2025 has shown that stomata in *Equisetum praealtum* and *E. diffusum* respond exclusively to BL, with no RL sensitivity even at high irradiance. A lack of a RL in these plants was postulated to be due to the absence of chlorophyll-containing-plastids in *Equisetum* GCs (Cullen and Rudall, 2016; Singh and Cullen, 2025).

Zhen and Bugbee (2020) examined differences in BL responses in C_3 and C_4 species and demonstrated that BL strongly promotes stomatal opening in C_3 crops (*Helianthus annus* and *Brassica napus*) but plays only a minor role in C_4 species (*Zea mays* and *Sorghum bicolor*) However, both C_4 species examined are grasses with dumbbell shaped stomata and specialised subsidiary cell mechanics, and the species compared belong to different

families, making it difficult to attribute these differences solely to photosynthetic pathway. Phylogenetically controlled comparisons that include C_4 dicots with kidney shaped stomata, such as *Gynandropsis gynandra* and *Flaveria bidentis*, have also reported weaker BL induced stomatal opening relative to C_3 relatives (Bernardo *et al.*, 2023), although differences in the RL response were not attributed directly to photosynthesis. It has been suggested that the diminished response in C_4 dicots was associated with lower GC transcript abundance of the BL photoreceptors PHOT1 and PHOT2, as well as CRY2, in the C_4 *G. gynandra* compared with C_3 *T. hassleriana*, proposing that the reduction in phototropin expression accounts for the weaker BL-induced g_s response (Bernardo *et al.*, 2023).

It has been postulated that the GC specific BL response which results in greater stomatal aperture than required for photosynthesis (Vialet-Chabrand and Lawson, 2020) may be important for leaf cooling (Lawson & Matthews, 2020), particularly in C_3 dicots, where photosynthetic carbon gain can be greatly reduced by enzymes kinetics (rubisco and rubisco activase) as well as photorespiration (Moore *et al.*, 2021). The carbon concentrating mechanism of C_4 species suppresses photorespiration and therefore the temperature photosynthetic optimum in these plants is generally much higher than C_3 species (Sage *et al.*, 2012). Therefore, the lack of BL response in C_4 dicots may help enhance W_i without the need to regulate leaf temperature (Bernado *et al.*, 2023). Stomatal BL responses were often thought to be absent in CAM species, however a recent study on Agave, revealed g_s responses to BL, although the effect was only observed in the afternoon and not in the morning. The lack of response in the morning was attributed to high internal

CO_2 concentration, from decarboxylation, which inhibits phosphorylation of HT1 and subsequent activation of CBC1/2 kinase, and integrates BL with CO_2 concentration (Sartori *et al.*, 2025).

A recent study by Bahadar *et al*. 2025 reported species specific differences in starch breakdown in response to BL, with no detectable starch degradation observed in *Arabidopsis*, cowpea and tobacco despite increased stomatal aperture. These findings contrast with the work of Horrer *et al*. (2016), who demonstrated rapid GC starch degradation, within 30 minutes of BL exposure in *Arabidopsis* and showed that this process contributes to efficient stomatal opening by quantifying the starch dynamics directly in GCs using microscopy-based approaches. In contrast, Bahadar *et al*. (2025) relied on GC-enriched material and bulk starch measurements, which may obscure rapid and cell-specific changes and acknowledged that differences in experimental design, growth conditions and starch quantification methods may account for the contrasting outcomes. These studies suggest that the contribution of starch degradation to BL induced stomatal opening is context dependent and may vary according to species, methodology and experimental conditions. Further GC specific analysis studies are required to fully elucidate the extent to which starch metabolism contributes to species-specific differences in BL sensitivity and the magnitude of the g_s response.

Guard Cell Photosynthesis

The role of GC chloroplasts in stomatal function and in the RL and BL responses has been the subject of intense debate over the last several decades (Lawson, 2009; Lawson *et al.*,

2014). Early work on RL-indued stomatal opening using epidermal peels led to the hypothesis that GC photosynthesis supplied sucrose required for osmoregulation and pore opening (Talbott and Zeiger, 1993; 1998). Other studies have demonstrated RL accumulation of GC potassium with activation of the plasma membrane H+-ATPase pump (Olsen *et al.*, 2002), with GC photophosphorylation thought to supply the ATP (Tominaga *et al.*, 2001), although RL activation of the H+-ATPase pump was not confirmed by Taylor and Assmann 2001. Although the Calvin Benson cycle has been confirmed within GCs, it has also been suggested that GC photosynthesis is too low to support GC osmoregulation (Outlaw, 1989; Reckmann *et al.*, 1990). However, GC electron transport has been reported to be 80% of that of the mesophyll (Lawson *et al.*, 2002), and it has been suggested that the Calvin cycle is a major sink for the end products of electron transport (Lawson *et al.*, 2003). More recent studies have suggested that the GC behaviour acts more as sink than a source of photosynthate (Santelia and Lawson, 2016; Daloso *et al.*, 2017), and studies using transgenic plants with reduced Calvin cycle function in both mesophyll and guard cells showed no impact on g_s which remained (von Caemmerer *et al.*, 2004) or even greater than wild-type (Lawson *et al.*, 2008). Therefore, although the role of GC chloroplasts in stomatal behaviour remains unclear, they have been shown to be essential for stomatal function (Figure 1 CD).

In Arabidopsis mutants in which GC chlorophyll content was reduced, g_s was lower than wild-type (Azoulay-Shemer *et al.*, 2015), and Wang *et al.* (2014) showed that mutants completely lacking GC chloroplasts had reduced aperture most likely due to reduced ATP levels. Lower ATP levels in peels compared with intact leaves also suggested the

requirement of an additional mesophyll contribution for stomatal function (Wang *et al.*, 2014). A recent study by Lim *et al*. 2022 used ATP and NADPH sensor proteins to suggest that mitochondria are the main source of ATP and not GC photosynthesis for BL induced opening (linked to starch degradation), and demonstrated that GC chloroplasts import ATP (generated by mitochondria) from the cytosol via a NUCLEOTIDE TRANSPORTER 1 (NTT1). Mutants lacking NTTs (*ntt1*) had almost no starch in GC chloroplasts and showed impaired stomatal opening. However, isolated GC in wild-type plants illuminated with RL accumulated starch, which was blocked by DCMU, indicating that light driven photosynthesis electron transport in the GCs supports starch synthesis. Interestingly, isolated GCs of the Arabidopsis *stp1stp4* mutants that lack the plasma membrance monosaccharide-H+ symporters SUGAR TRANSPORT PROTEIN 1 and 4, (and therefore lack sugar import capabilities), starch build up (albeit lower than wild-type) with RL was clearly evident. However, when DCMU was applied, starch accumulation was blocked, clearly concluding that GC photosynthesis provided carbon precursors for starch accumulation and therefore contributes to starch synthesis in GCs.

Zhu *et al*. 2020 also confirmed earlier studies that GCs in epidermal peels directly respond to RL, which was inhibited by DCMU. As described above, the importance of GC chloroplasts has also been illustrated by Fuji *et al*. (2024) and shown to play a direct role in the phosphorylation of specific residues on the plasma membrane H^+-ATPase to facilitate RL and BL opening (Figure 2), with mesophyll photosynthesis also playing an important role. All these findings indicate a role for GC chloroplasts in stomatal function, either

through the supply of energy in the form of ATP, production of carbon skeletons for osmoregulation, or through sensing and signalling pathways.

Diurnal stomatal kinetics under fluctuating light with differing red and blue composition

Under natural conditions irradiance varies in both photon flux and spectrum composition on timescales ranging from seconds to hours, driving non steady-state g_s that can decouple g_s from A (McAusland *et al.*, 2016; Vialet-Chabrand *et al.*, 2017). Spectral composition also changes through the day as scattering and solar elevation alter biologically meaningful blue and red photon ratios (Kotilainen *et al.*, 2020) resulting in the relative contributions of BL and RL pathways to vary across the diel cycle.

In our illustrative dataset (Figure 3) recorded under a randomised "natural light" profile, a small BL fraction (10%) superimposed on RL (90%) advanced the morning rise in g_s and elevated the daytime plateau. Both treatments also showed a midday depression of g_s and a decline in g_s late in the diel period (Figure 3A, *ca*. 400 min). A tracks light intensity with frequent transient drops as flux decreases; however, A plateaus at a higher rate in the presence of RL+BL than RL alone (Figure 3B). Notably, A and g_s do not scale proportionally with the addition of BL, with greater increases in g_s than in A reducing instantaneous Wi (Figure 3C). Kinetic asymmetries matter because after a step increase in irradiance, slow stomatal opening transiently constrains A, whilst delayed closure following a decrease in irradiance wastes water and these effects depress diurnal W_i (McAusland *et al.*, 2016; Vialet-Chabrand *et al.*, 2017). A modest BL component increases g_s, diminishing A induction losses.

However, this greater g_s also raises water loss when stomatal closure lags (Lawson & Blatt, 2014; Zhen & Bugbee, 2020). BL contributes most to daily *A* when C_i is low (strong RL driven mesophyll drawdown), and when fluctuations are large and of long duration. Conversely, BL tends to penalise *Wi* when irradiance is lower, and/or fluctuations are of decreasing intensity, or when VPD is high and closure lags prevail.

Blue light effects are largely local to the illuminated surface (Zhen & Bugbee, 2020), meaning that adaxial to abaxial patterning and spatial heterogeneity can alter whole leaf spectral sensitivity across the day. Large area mapping shows that adaxial to abaxial asymmetries and patchiness in stomatal density can change the g_s/*A* covariance that emerges under mixed spectra (Fan *et al.*, 2025b). Notably, reducing surface specific stomatal density in cereals (i.e., wheat) can be compensated by enhanced BL sensitivity, thus preserving performance under RL+BL despite fewer pores (Fan *et al.*, 2025a). Taken together, these observations argue that the "value" of BL is diurnally contextual as BL is most productive when mesophyll demand is high and diffusional limitation dominates (raising *A* more than transpiration), but it can reduce *Wi* when rapid fluctuations and high VPD make closure delay the limiting factor. Methodologically, this means commonly practiced steady state step protocols or short induction assays (i.e., step changes in light intensity; Vialet-Chabrand *et al.* (2020) may be insufficient proxies to fully integrate diurnal responses of *A* and W_i. As BL:RL, light dynamics and plant physiology (circadian phase, hydration) vary over the day, the same plant measured at identical PPFD in morning vs afternoon can show different g_s–*A*– W_i relationships. These findings indicate that future studies on the impact of BL on g_s and *A* need validating under realistic fluctuating profiles that include diel changes in spectra

(Matthews *et al.*, 2018; Vialet-Chabrand *et al.*, 2017). This diurnal profile illustrates the significant potential to improve W_i by eliminating or dampening the BL response. As water loss is greater than improvements in carbon gain, minimising stomatal responses to BL and modifying light-gated channels like GtACR1 (Huang et al., 2021) or XXM2.0 (Huang et al., 2024) could greatly improve *Wi*, with limited impact on *A,* which has potential to improve yield in water limited environments. However, this approach would be species specific and more appropriate for crops with large stomatal BL responses such as rice and wheat (Vialet-Chabrand *et al.*, 2021), whilst crops with a more subtle BL response would be more diffusionally constrained relative to a potential savings in water.

Advancements in technology to aid understanding of stomatal RL and BL responses.

Recent advances in molecular and genomic techniques, such as CRISPR, as well as synthetic biology, have helped uncover the signalling pathways and mechanisms that influence stomatal behaviour under BL and RL. However, while these tools have been extensively used, the advancement in spatial transcriptomics and single cells omics will help our understanding of the cascade of regulatory changes in real time in response to various stimuli. As outlined above, advancements in phosphoproteome analysis have proven to be a powerful tool to fully elucidate the intricate details of signal transduction pathways and the phosphorylation targets of key proteins and these tools have been effectively used to demonstrate connections between RL and BL pathways (Hayashi *et al.*, 2024; Fuji *et al.*, 2024). Recently Pullen *et al*. 2025, described the proteome and

phosphoproteome of guard-cell enriched samples from open and closed stomata, which supported the suggestions that phosphorylation of endomembrane proteins contribute to the regulation of stomatal aperture. Exciting synthetic approaches have also been used to manipulate stomatal kinetic responses to light. Papanatsiou *et al*. (2019) used a synthetic BL gated K^+ channel, BLINK1, to modify ion flux in the guard cells and therefore stomatal kinetics, resulting in plants with improved photosynthesis and W_i. These novel approaches demonstrate the potential of using our knowledge of stomatal responses to RL and BL to manipulate these responses for improved crop performance.

While transgenic and -omics approaches provide mechanistic insights, exploring natural variation remains a powerful but underutilised tool to determine species specific RL and BL responses. There are a number of crop diversity panels that could be used to identify the underlying genes responsible for variation. To date there is no known GWAS study examining stomatal RL and BL responses, and this is most likely due to the lack of a high throughput method of measuring diversity panels. However, exploiting tools such as thermal imaging (Vialet-Chabrand and Lawson, 2019; 2020) has the potential to screen large numbers of plants simultaneously for both kinetic responses as well as overall magnitude of g_s changes. Thermal imaging paired with dynamic energy balance modelling has enabled time resolved maps of *gs* under fluctuating light and wind, providing a quantitative alternative to steady state thermal indices (Vialet-Chabrand and Lawson, 2019, 2020; Fan *et al.*, 2024). Taken together, our advancement in physiological tool development, omics approaches, and the development of large diversity panels opens up

many avenues to discover new targets for crop improvements based on stomatal responses to light.

Conclusions

Stomatal responses to RL and BL are connected and maybe not as separate as originally perceived, with several studies showing a dependence of RL for the BL response. Although clear differences in the magnitude of the BL response exist between species, the underlying reasons and mechanisms are not clear. Further work is needed to elucidate these, as understanding the mechanisms for this variation has the potential to deliver new targets for potential manipulation to improve W_i (see Outstanding Questions). Recent studies have also clearly demonstrated that GC photosynthesis is required for activation of specific residues on the plasma membrane H^+-ATPase that is required for both BL and RL stomatal opening. These findings reignite questions regarding the role of GC chloroplasts in stomatal function (see Outstanding Questions). Understanding both the independence and interplay between RL and BL responses in stomatal behaviour could reveal unexploited but critical targets for enhancing W_i and crop productivity.

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Advances box

- The guard cell (GC)-specific blue light (BL) response is species specific but not universal; the mechanisms responsible for the variation are unknown.
- Red light (RL) and BL stomatal responses are often defined as distinct pathways; however, there is increasing evidence of synergies between the two, with the BL component relying on RL.
- Both GC and mesophyll cell photosynthesis is required for activation of the plasma membrane H+-ATPase and important for stomatal function.
- Despite its known role in early morning GC opening, BL can have a greater impact later in the diel cycle.
- Emerging technologies and natural variation in BL responses provide opportunities to understand and manipulate BL signal transduction pathways, and breed crops with improved water-use efficiency (*Wi*).

Outstanding Questions

- What determines species specific differences in their response to BL?
- What drives diurnal variation in the magnitude of the BL response?
- Can we manipulate the specific BL to improve W_i? If so, how much can we reduce the BL response before other factors impact plant performance?
- How much does GC photosynthesis contribute to stomatal function and to which stimuli?
- What is/are the RL C_i-independent mesophyll signal(s) that co-ordinate A and g_s?

- How are the RL and BL pathways connected and what are the underlying key components?

- If one stomatal pathway was manipulated, would the other compensate?

- What differences are there in stomatal responses to RL and BL between the adaxial and abaxial leaf surfaces?

Figure legends

Figure 1- Signalling pathways for stomatal regulation. Signalling transduction pathways include red light-induced opening which can either begin within guard cell chloroplasts and/or mesophyll chloroplasts (A), red light induced and darkness induced closing (B), blue light-induced opening (C), and both red and blue light induced opening (D). Stomatal opening is indicated by the green dashed arrows, and stomatal closing is indicated by the grey dashed arrow. Black dashed arrows represent known signalling cascades, whilst blunt ended lines demonstrate signals that suppress ion channel activity. Purple dashed arrows and purple boxes represent hypothetical/unknown signalling cascades and functions. Cells and organelles are indicated within parentheses.

Figure 2 - Both red and blue light trigger phosphorylation of the plasma membrane H+-ATPase pump. Signalling pathways induced by blue light are indicated by the blue dashed arrows, and those induced by red light are indicated by the red dashed lines. Purple boxes represent hypothetical/unknown signalling cascades and functions. Cells and organelles are indicated within parentheses. Redrawn from Fuji *et al.*, 2024.

Figure 3 - Diurnal illustration of red and blue light effects under stochastic fluctuating irradiance. Time courses of (A) stomatal conductance to water vapor (g_s), (B) net CO_2 assimilation (A), and (C) instantaneous water-use efficiency (Wi) measured under a randomized, "natural-light" profile with either red + blue (90/10; cyan line) or red only (red line). Mean traces with light shading indicate variability across repeated runs (standard error) (unpublished data of M. Fan & T. Lawson).

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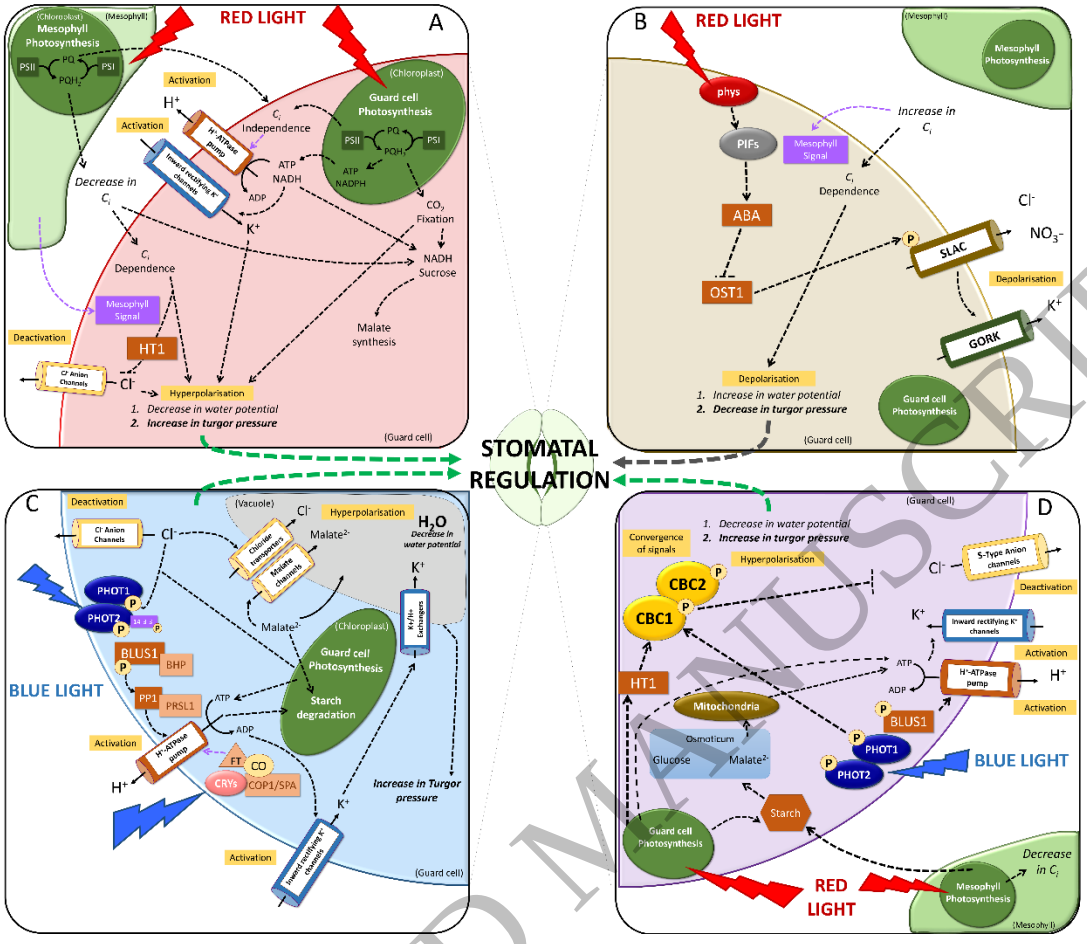

Figure 1
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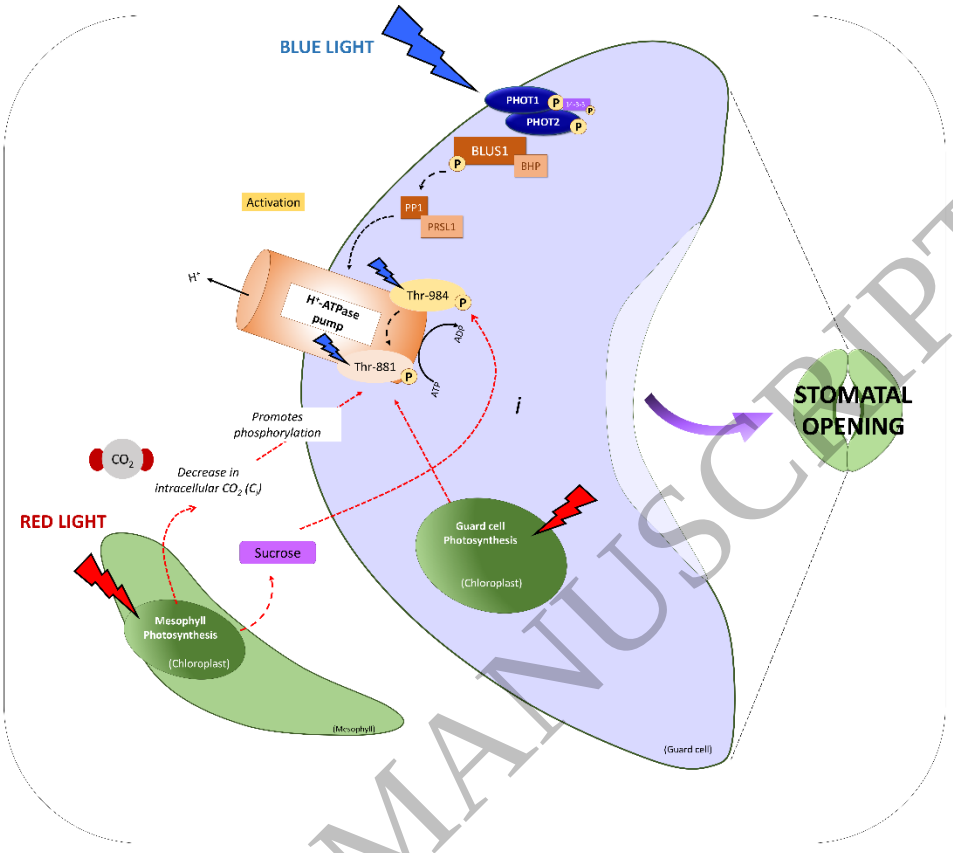

Figure 2
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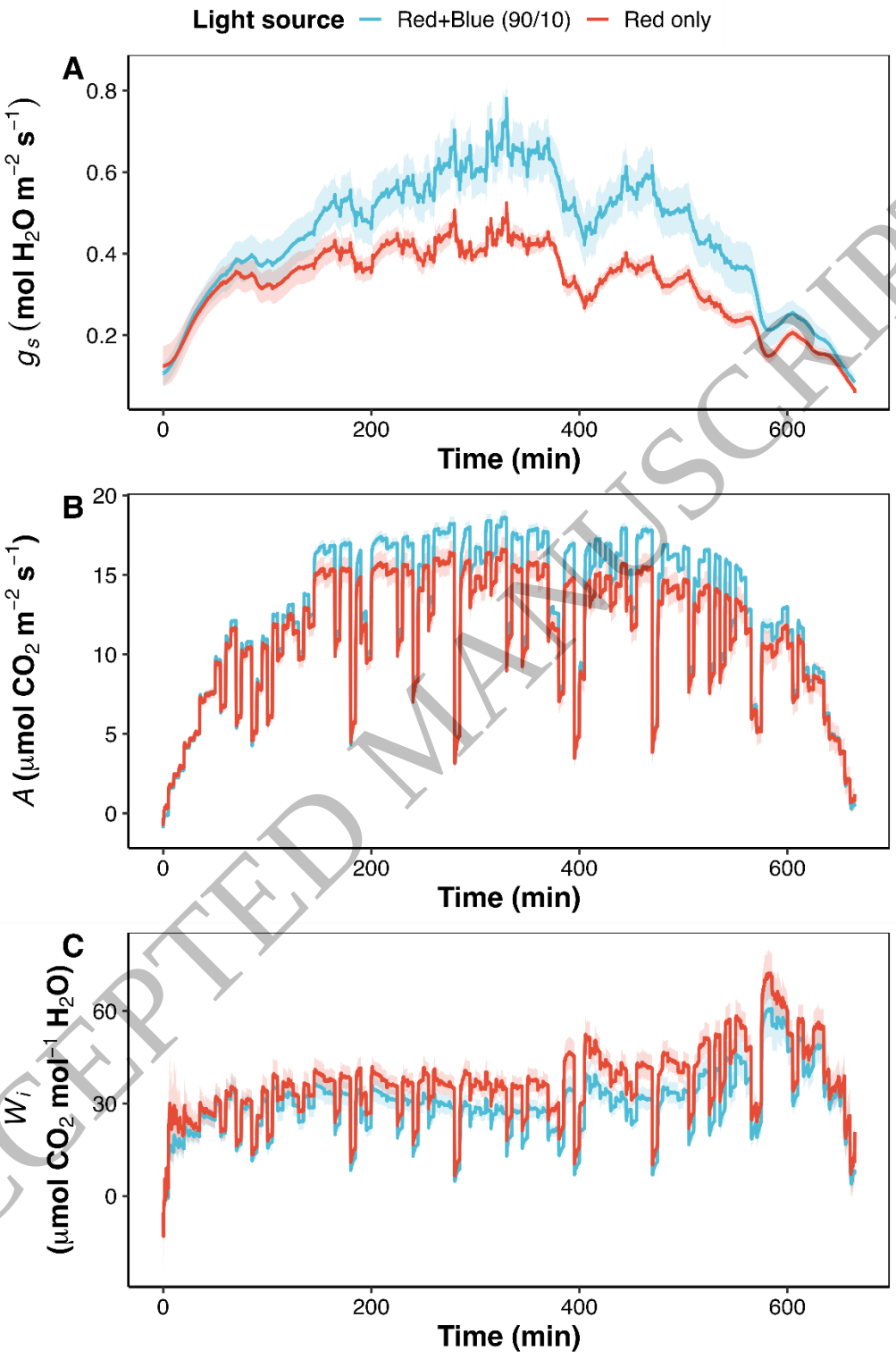

Figure 3
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