Cell wall thickness and composition are involved in photosynthetic limitation

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Abstract

The key role of cell walls in setting mesophyll conductance to CO₂ (gₘ) and, consequently, photosynthesis is reviewed. First, the theoretical properties of cell walls that can affect gₘ are presented. Then, we focus on cell wall thickness (T_cw) reviewing empirical evidence showing that T_cw varies strongly among species and phylogenetic groups in a way that correlates with gₘ and photosynthesis; that is, the thicker the mesophyll cell walls, the lower the gₘ and photosynthesis. Potential interplays of gₘ, T_cw, dehydration tolerance, and hydraulic properties of leaves are also discussed. Dynamic variations of T_cw in response to the environment and their implications in the regulation of photosynthesis are discussed, and recent evidence suggesting an influence of cell wall composition on gₘ is presented. We then propose a hypothetical mechanism for the influence of cell walls on photosynthesis, combining the effects of thickness and composition, particularly pectins. Finally, we discuss the prospects for using biotechnology for enhancing photosynthesis by altering cell wall-related genes.

Keywords: Cell wall composition, cell wall thickness, cellulose, CO₂ diffusion, hemicellulose, land plants, leaf anatomy, mesophyll conductance, pectin.
Introduction

Cell walls (CWs) have a well recognized importance in plant physiology and biotechnology. As such, they are intensively studied from different perspectives. A reflection of their importance is that different aspects of CW biology have been repeatedly reviewed and updated over the last 70 years, for example in the Annual Review series (Frey–Wysling, 1950; Albersheim et al., 1969; Cleland, 1971; Northcote, 1972; Cassab and Varner, 1988; Popper et al., 2011; Wolf et al., 2012; Anderson and Kieber, 2020) as well as in many other journals, including the Journal of Experimental Botany (e.g. Sarkar et al., 2009; Carpita and McCann, 2015; Sorek and Turner, 2016). Interestingly, among all these reviews, the word ‘photosynthesis’ is only mentioned by Sarkar et al. (2009), to say that photosynthesis influences CWs, which contrasts with the more recent view that CWs can be crucial in regulating photosynthesis. Hence, the global aim of this review is to highlight the CW as a mechanistic limitation on photosynthesis.

Photosynthesis is an essential plant process, and improving crop photosynthesis is a major goal (Zhu et al., 2010; Ray et al., 2013; Ort et al., 2015) that up to now has been addressed mostly by attempting to modify either stomata (Drake et al., 2016; de Boer et al., 2016; Lawson and Vialet-Chabrand, 2019; de Sousa et al., 2020), Rubisco and Calvin cycle enzymes (Price et al., 2013; Schlüter and Weber, 2016; South et al., 2019), or dynamic sun–shade aspects (Kromdijk et al., 2016). These attempts reflect a long-standing view of the scientific community in which photosynthesis is mainly limited by either stomatal closure or restricted biochemical and photosynthetic activity. However, it is now widely recognized that mesophyll conductance ($g_{\text{m}}$)—the ease of diffusion of $\text{CO}_2$ from substomatal cavities to Rubisco active sites inside chloroplast stroma—plays a similarly important role to those of stomata and photo–biochemistry (Gago et al., 2020a). Indeed, $g_{\text{m}}$ often shows the most consistent limitation of photosynthesis across the terrestrial plant phylogeny (Gago et al., 2019; Flexas and Carriquí, 2020).

Mesophyll conductance limits photosynthesis to an extent similar to that exerted by the well-known stomatal and biochemical limitations, which results in a globally significant correlation between net photosynthesis ($A_n$) and $g_{\text{m}}$ (Fig. 1). Because of this, based on standard photosynthesis modeling (Farquhar et al., 1980), it has been estimated that only small improvements (typically $10–30\%$) in photosynthesis can be expected from alleviating a single limitation alone. Larger improvements require two or more limitations to be alleviated simultaneously (Flexas, 2016; Gago et al., 2019). The optimal combination would improve mesophyll conductance and photo–biochemistry without altering stomatal limitations, achieving simultaneous increases in photosynthesis and photosynthetic water use efficiency (Flexas, 2016; Flexas et al., 2016). Consequently, $g_{\text{m}}$ is a significant target—alone or in combination with other components of photosynthesis—for enhancing crop photosynthesis using biotechnological approaches (Flexas et al., 2016; Bailey-Serres et al., 2019).

Although the role of $g_{\text{m}}$ in determining $A_n$ is now widely recognized within the scientific community, limitations arise from lack of knowledge of the mechanistic basis of $g_{\text{m}}$. Mesophyll conductance is a composite conductance of an intercellular gas phase ($g_{\text{in}}$) and a liquid phase ($g_{\text{liq}}$):

$$g_{\text{m}} = \frac{1}{g_{\text{in}}} + \frac{RT_j}{H \times g_{\text{liq}}}$$

(1)

where $H/(RT_j)$ is the dimensionless form of Henry’s law constant, needed to convert $g_{\text{liq}}$ to a gas phase equivalent conductance, as $g_{\text{in}}$ is defined as a gas–phase conductance (Niinemets and Reichstein, 2003; Tøsens et al., 2012a). At the same time, $g_{\text{liq}}$ is a composite conductance of the apoplastic and cellular components of the $\text{CO}_2$ pathway comprised from the mesophyll CW surface to the carboxylation site in the chloroplast. Thus, $g_{\text{liq}}$ can be expressed on a leaf projected surface area basis by scaling it by the chloroplast surface area exposed to intercellular airspaces per unit of leaf area ($S_i/S$):

$$g_{\text{liq}} = \frac{S_i}{S} \times \frac{1}{r_{\text{cw}} + r_{\text{pl}} + r_{\text{cyt}} + r_{\text{en}} + r_{\text{st}}}$$

(2)

where $r_{\text{cw}}$, $r_{\text{pl}}$, $r_{\text{cyt}}$, $r_{\text{en}}$ and $r_{\text{st}}$ account for the CW, plasma membrane, cytosol membrane, chloroplast membrane, and stroma conductances, respectively. Concomitantly, each partial conductance is determined by several anatomical properties and/or biochemical properties. While biochemical components, probably explaining the observed rapid variations in response to varying environment (Flexas et al., 2007; Vrábl et al., 2009; Douthe et al., 2012), are still a matter of debate (Gu and Sun, 2014; Théroux–Rancourt and Gilbert, 2017; Carriquí et al., 2019a; Shrestha et al., 2019; Evans, 2021), there is ample
agreement that leaf anatomical properties are important drivers of $g_m$ variability, at least at the interspecific level (Evans et al., 2009; Terashima et al., 2011; Tomás et al., 2013; Peguero-Pina et al., 2017; Veromann-Jürgenson et al., 2017, 2020; Carriquí et al., 2019b; Gago et al., 2019). Previous studies have highlighted the importance of anatomical properties, such as mesophyll porosity (Earles et al., 2018; Lundgren et al., 2019; Baillie and Fleming, 2020) or the chloroplast distribution, in particular the S/S (Ren et al., 2019), in determining $g_m$. Although not negligible, the contribution of mesophyll air space porosity to the overall $g_m$ has been shown to be much lower than that of S/S (Han et al., 2018; Carriquí et al., 2020; Hu et al., 2020), except perhaps in succulent leaves (Earles et al., 2018). Still, mesophyll porosity may have indirect important effects as apparently it determines the formation of functional stomata during leaf development (Lundgren et al., 2019; Baillie and Fleming, 2020).

Besides CW thickness ($T_{cw}$) and associated traits [e.g. porosity ($p$) or tortuosity ($\tau$)], S/S has been generally suggested to be the most limiting anatomical factor for $g_m$ (Tosens et al., 2016; Peguero-Pina et al., 2017; Carriquí et al., 2019b; Ren et al., 2019; Veromann-Jürgenson et al., 2020). However, a dominant role for $T_{cw}$ in $g_m$ has also been described, and the anatomical limitation analysis of $g_m$ introduced by Tomás et al. (2013) has shown that, in fact, CW limitations are frequently equal or greater than S/S and that together these constitute the primary anatomical factors for setting the maximum $g_m$ that a species can achieve (Tomás et al., 2013; Tosens et al., 2016; Carriquí et al., 2019a,b; Veromann-Jürgenson et al., 2020). In addition to thickness, recent evidence points to an effect of CW composition on photosynthesis (Weraduwage et al., 2016, 2018; Ellsworth et al., 2018; Clemente-Moreno et al., 2019; Carriquí et al., 2020; Roig-Oliver et al., 2020b,c; Evans, 2021).

The aim of this review is to focus on CWs, highlighting their importance in setting $g_m$ and, hence, photosynthesis. For this, we first conceptualize which are the CW properties expected to affect $g_m$. Then, we review the available empirical evidence of $T_{cw}$ and CW composition effects on $g_m$ both within and between species. Afterwards, we propose a hypothetical mechanism for the influence of CWs on $g_m$ and photosynthesis, and we speculate on potential CW-mediated trade-offs between photosynthesis, leaf water relations, and hydraulic characteristics. Based on all the evidence, we briefly discuss prospects for improving photosynthesis by CW manipulation. It is important to highlight that all concepts reviewed here account mostly for C$_3$ plants. Until very recently, it was technically difficult to estimate $g_m$ in C$_4$ plants, limiting an understanding of the role of CWs and other anatomical components in this group (Tosens et al., 2016; Cousins et al., 2020; Pathare et al., 2020).

**Cell wall properties affecting $g_m$**

The plant CW is a complex biological structure with multiple functions, including maintaining cell structural integrity, providing flexibility for cell division and expansion, and acting as a barrier for pathogens and protecting cells from the environment (Anderson and Kieber, 2020). Depending on the plant group, cell type, and developmental stage, the CW exhibits variable composition and structural complexity (Sarkar et al., 2009; Popper et al., 2011; Maron, 2019; Yokoyama, 2020). In particular, photosynthetic cells possess a primary CW mainly composed of a variable proportion and content of cellulose, hemicellulose, pectin, structural proteins, and some additional components (Cosgrove, 2005; Sarkar et al., 2009; Cosgrove and Jarvis, 2012). The universal presence of the CW of photosynthetic cells of all species constitutes a barrier that hinders CO$_2$ diffusion (Evans et al., 2009; Terashima et al., 2011; Tholen and Zhu, 2011).

In the journey of atmospheric CO$_2$ molecules through the photosynthetic organs to the carboxylation sites inside chloroplast stroma, CWs are the first resistance in unistratose organs (e.g. most moss phyllids and filmy fern fronds), and the second in multistratose organs with intercellular air spaces, that CO$_2$ molecules must face (Hanson et al., 2014; Flexas et al., 2018; Carriquí et al., 2019b). However, the role of CWs in limiting photosynthesis has often been neglected (e.g. Sims and Pearcy, 1989; Ellsworth and Reich, 1993; Ninemets, 1999) for several reasons. For instance, the CW represents only a tiny fraction of the apparent CO$_2$ pathway length of most photosynthetic organs since its thickness ranges between 0.1 µm and 4 µm in land plants (Han et al., 2016; Carriquí et al., 2019b; Coe et al., 2019; Sugiura et al., 2020), while the mesophyll can be up to several millimetres or even centimetres thick (Earles et al., 2018; Carriquí et al., 2020; Herrera, 2020). Moreover, CW pore size, ranging between 30 Å and 50 Å (3–5 nm), is sufficient to allow the free crossing of CO$_2$ molecules, which have a kinetic diameter of only 3.3 Å (Carpita et al., 1979). Additionally, CW properties were considered to be quite static concerning their influence on photosynthesis (Evans et al., 2009). However, there is now broad consensus that the physicochemical properties of the CW constitute a key determinant of $A_p$ and $g_m$ (Flexas et al., 2018; Gago et al., 2020a,b; Sugiura et al., 2020; Evans, 2021).

$g_{cw}$, the inverse of CW resistance ($r_{cw}$), is determined, as in the other components of the CO$_2$ pathway, by four main physicochemical properties: diffusivity ($D$, 1.79×10$^{-9}$ m$^2$s$^{-1}$ at 25 °C), porosity ($p$, expressed in m$^{-3}$), apparent diffusion path length ($\Delta L$, expressed in m), and tortuosity ($\tau$, expressed in m$^{-1}$), which are inter-related as follows (Evans et al., 2009; Terashima et al., 2011):

$$g_{cw} = \frac{1}{r_{cw}} = \frac{D \times p}{\Delta L \times \tau}$$

Diffusion of CO$_2$ in CW occurs in the liquid phase (Rondeau-Mouro et al., 2008), for which $D$ is at least 10$^4$ times lower than in the intercellular gas phase (Terashima et al., 2011). In turn, $D$ is also determined by the atmospheric pressure, Henry’s law.
constant (which accounts for the equilibrium air–water partition coefficient), and the combination of pH and \([\text{CO}_2]\), which modifies the diffusion coefficient by influencing the fraction of \(\text{CO}_2\) that crosses the CW in the form of bicarbonate. However, considering the slightly acidic pH in the apoplast, the influence of the conversion of \(\text{CO}_2\) to bicarbonate on \(\text{CO}_2\) diffusivity might be small (Niinemets and Reichstein, 2003; Terashima et al., 2011; Tholen and Zhu, 2011; Xiao and Zhu, 2017). Finally, \(D\) is perhaps also affected by carbonic anhydrases, as their presence has been reported by Chen et al. (2009) in the CW apoplast of rice calli. Certainly, further studies about the effects of \(\text{pH}\) and the presence of carbonic anhydrases are still required.

The other three properties determining \(g_{cw}\) largely depend on the amount, structure, organization, and interaction between CW components. Very briefly, CWs are composed of cellulose microfibrils to which hemicellulosic polysaccharides link, and all of this is embedded in a pectin matrix, forming a tangled web (Cosgrove, 2005; Anderson and Kieber, 2020; Yokoyama, 2020). As the CW assembly determines several properties, such as \(p\) and \(\tau\), at the same time, it influences \(g_{cw}\) in a very complex (as further discussed in the following sections) and dynamic way, given the constant remodelling and reconstruction to which the wall is subjected (Sarkar et al., 2009; Bellincampi et al., 2014; Houston et al., 2016; Clemente-Moreno et al., 2019; Maron, 2019; Zhang et al., 2019).

\(\Delta L\) is equivalent to \(T_{cw}\) (Evans et al., 2009; Tosens et al., 2012b; Tomás et al., 2013), and \(g_{cw}\) has been reported to be lower at larger \(T_{cw}\), and therefore \(\Delta L\) (Evans et al., 2009; Carriquí et al., 2015; Peguero-Pina et al., 2017; Sugiuira et al., 2020). This has been further confirmed by the analytical models based on anatomical traits (Tosens et al., 2012b; Tomás et al., 2013; Xiao and Zhu, 2017), that—although also based on several assumptions that are debatable, such as the actual value for wall porosity—predict \(g_{m}\) quite accurately (Tosens and Laanisto, 2018; Flexas and Carriquí, 2020) and emphasize the importance of \(T_{cw}\) to \(g_{m}\) limitation (Veromann-Jürgenson et al., 2017, 2020; Han et al., 2018; Carriquí et al., 2019b). For its ease of measurement, \(\Delta L\) is the most characterized CW property, with hundreds of measurements in different species and conditions. Although never proved experimentally, potentially \(\Delta L\) could be associated with CW composition.

\(p\) is determined by the quantity of wall pores of sufficient size for the passage of \(\text{CO}_2\). On the other hand, \(\tau\) is related to path lengthening due to non-linearity in the diffusion path that \(\text{CO}_2\) molecules must face due to the cellulose and hemicellulose microfibril entanglement (Burgert and Dunlop, 2011). These two properties are the least known and the most difficult parameters to determine due to their close intercorrelation. In fact, there are still no direct measurements for these properties and, in most approximations of \(\text{CO}_2\) diffusion through walls, both are usually combined as a single parameter, \(p/\tau\) (i.e. effective porosity) (Nobel, 2004; Terashima et al., 2006; Evans et al., 2009; Tosens et al., 2012b; Tomás et al., 2013). Based on simple physicochemical estimations, it was traditionally considered that effective porosity was large enough for \(g_{cw}\) not limiting \(g_{m}\) and photosynthesis (Nobel, 2004). Later, Terashima et al. (2006), considering also the relationship between \(g_{m}\) and the mesophyll anatomical traits, and studies on wall permeability to water on algae (Gutknecht, 1967) with extremely thick walls (Okuda et al., 1997), suggested that \(p/\tau\) might be actually limiting \(g_{m}\) and inversely proportional to \(T_{cw}\) (Evans et al., 2009), based on microscopy images showing pore size in onion (McCann et al., 1990), also suggested the possibility that \(p/\tau\) may be limiting \(g_{m}\) under certain conditions. Evans (2021) proposed that the important variability of \(g_{m}/S_{c}/S\) for a given \(T_{cw}\) (see Fig. 2E) could be largely explained by differences in \(p/\tau\) and membrane permeability. Thus, most analytical models of \(g_{m}\) have considered a variable \(p/\tau\) closely related to \(T_{cw}\) (Tosens et al., 2012b, 2016; Tomás et al., 2013; Veromann-Jürgenson et al., 2017, 2020), although Carriquí et al. (2020) proposed that this might not be the case in species with large \(T_{cw}\). The underlying problem is that the assumption of one porosity or another can radically change the conclusions of the study (Evans, 2021). Nowadays, despite advances in tools to quantify \(p/\tau\) (Liu et al., 2019), complicated interactions among CW basic components and physical microenvironments such as polysaccharides, wall proteins, and apoplastic pH, a precise understanding of how these parameters interact and affect \(\text{CO}_2\) diffusion (see ‘Effects of cell wall composition on \(A_{n}\) and \(g_{m}\)’ for further details) remains elusive.

### Relationships among mesophyll conductance, cell wall thickness, and leaf water relations and hydraulics across species and phylogenetic groups

Historically, studies linking photosynthesis, \(g_{m}\), and \(T_{cw}\) have been mostly dedicated to angiosperms (Flexas et al., 2012). However, in recent years, measurements of a significant number of previously understudied gymnosperms (Veromann-Jürgenson et al., 2017, 2020; Carriquí et al., 2020) ferns and fern allies (Carriquí et al., 2015; Tosens et al., 2016), and mosses and liverworts (Carriquí et al., 2019b; Coe et al., 2019) have allowed the exploration of the relationships between \(A_{n}\), \(g_{m}\), and its anatomical drivers from a phylogenetic and evolutionary perspective. Photosynthetic capacity, \(A_{n}\), and its underlying parameters [stomatal conductance (\(g_{s}\)), \(g_{m}\), and biochemical capacity] display a progressive phylogenetic increase from bryophytes to angiosperms (Gago et al., 2019). A similar increasing tendency is observed for \(S_{c}/S\), while a decreasing tendency is observed for \(T_{cw}\) (Gago et al., 2019). When all plant groups are pooled together, \(T_{cw}\) strongly correlates with \(g_{m}\) and \(A_{n}\) (\(P<0.001\) Fig. 2A, B). On the other hand, a weaker but equally significant correlation between \(g_{m}\) and \(S_{c}/S\) is observed (Fig. 2C), despite the correlation between \(S_{c}/S\) and \(T_{cw}\) (Fig. 2D). In addition, the \(g_{m}/S_{c}\) is significantly decreased with increasing \(T_{cw}\).
Fig. 2. Relationships among photosynthesis and anatomical traits in C₃ plants. Lines represent linear SMA fittings (y=a x+b) on data pooled across all phylogenetic groups. (A, y=4.110 x–4.504) Net assimilation rate (Aₙ) and cell wall thickness (Tcw) (303 entries from 204 species); (B, y = 0.067 x–0.140) Mesophyll conductance to CO₂ diffusion (gₘ) and Tcw (280 entries from 183 species); (C, y = 0.026 x–0.148) gₘ and chloroplast surface area exposed to intercellular air spaces per leaf area (S/S) (327 entries from 201 species); (D, y=3.032 x–0.750) S/S and Tcw (293 entries from 195 species); (E, y=0.005 x–0.008) Mesophyll conductance expressed on a chloroplast surface area basis (gₘ S/S) and Tcw (273 entries from 178 species). SMA fittings were performed using the function sma() in the smatr R package. Data and references are available at Dataset S1 at Dryad.

While the correlations showed here may not necessarily implicate causality, they agree with the observations based on mechanistic gₘ limitation analysis showing that S/S and Tcw are indeed the two strongest anatomical limitations to the maximum gₘ achievable by a given species or genotype (Tomás et al., 2013; Tosens et al., 2016; Carriqué et al., 2019a, b; Gago et al., 2019; Verommann-Jurgenson et al., 2020). Tcw and S/S may vary independently and compensate for each other to achieve substantial gₘ in some cases, as described for Mediterranean Quercus species (Peguero-Pina et al., 2017) and, to an extreme extent, in resurrection plants (Fernández-Marín et al., 2020; Nadal et al., 2021). These hardy species have been found to achieve similar or greater photosynthesis than their non-resurrection counterparts by breaking the often-reported trade-off between S/S and Tcw, so that they have a much larger S/S concomitant with larger Tcw than non-resurrection species. However, it has to be considered that, while Tcw can be easily determined from TEM images as a 2D trait, the 3D estimation of S/S is critical (Théroux-Rancourt et al., 2017; Earles et al., 2019; Harwood et al., 2020). Indeed, the S/S values Dataset S1 available at the Dryad Digital Repository, https://doi.org/10.5061/dryad.qbzk18gs, were derived from 2D images with broad assumptions regarding cell and chloroplast shapes. In this sense, the methodological issues in estimating S/S could perhaps partly contribute to the variation of gₘ–S/S relationship between and within groups.

There is strong support for the concept that an increased photosynthetic rate was likely to be an important adaptive process contributing to the successful angiosperm radiation (Brodribb and Feild, 2010). The observed trajectory of increasing gₘ due largely to decreasing Tcw (Gago et al., 2019) fits well into this broader narrative, possibly explaining one of the primary limitations that delayed a transition from an ancestral low photosynthetic poikilohydric state (observed in most extant bryophytes), to a derived homeohydric high photosynthetic condition in the vascular plant lineage. Among the diverse morphological innovations that occurred during land plant diversification, such as stomata development, the hydrophobic cuticle, and the hydraulic system (Kenrick and Crane, 1997), evolution in Tcw has received little attention. This allows us to hypothesize and speculate about some evolutionary aspects that may deserve future experimental attention.

Concerning the general decline of Tcw along the phylogeny, from bryophytes to angiosperms, one interesting possibility is that a large Tcw may have been a fundamental constraint on the evolution of high Aₙ, and that Tcw could not be reduced until homeohydric features such as stomatal regulation of gas exchange and efficient vasculature had evolved. An explanation for this may be that desiccation tolerance (DT) requires thick CWs to prevent tissue damage during dehydration. DT is a prerequisite for terrestrial species with low vascular efficiency to carry out photosynthesis at humidities below 100% (Brodribb et al., 2020). These plants can survive dehydration to <30% relative water content or water potentials < –100 MPa for extended periods (Alpert and Oliver, 2002; Gaff and Oliver, 2013). Bryophytes constitute the group of land plants with the highest percentage of studied desiccation-tolerant species, although desiccation-tolerant ferns and lycophytes are also common (Oliver et al., 2005; Vitt et al., 2014; Fernández-Marín et al., 2016). There is evidence that species exposed to frequent changes in cell volume appear to require thick CWs with high elasticity preventing damaging leaf deformation upon dehydration/rehydration (Balsamo et al., 2003). The occurrence of...
thick CWs in angiosperms that have recently evolved DT (as a homoplasy with DT in bryophytes) provides strong support for the idea that thick CWs are a prerequisite for DT (Perera-Castro et al., 2020). If this is the case, it may be that a transition from DT and poikilohydry was a critical step to developing high $A_n$, because the thin CWs required for high $A_n$ are only effective where cell hydration is maintained within narrow limits. Assuming that this connection between $T_{cw}$ and DT is constitutive, it is easy to envisage an evolutionary sequence in land plants whereby evolution in water relations ( stomatal regulation of transpiration and efficient water transport) enabled a shift away from DT, enabling a decline in $T_{cw}$ and an increase in $g_{m}$ and $A_n$.

Homeohydry in most seed plants may enable cell $T_{cw}$ to be much lower (and $g_{m}$ to be much higher) than in early branching land plant clades, but it also adds an important water transport limitation on $A_n$. Evidence of this is clearly seen in the coordination between hydraulic conductance ($K_{wad}$) and $A_n$ among land plants (Brodribb et al., 2007; Flexas et al., 2013; Xiong et al., 2017; Lu et al., 2019; Xiong and Nadal, 2020), since water loss and CO2 uptake share a common path at the leaf surface. The observed coordination between water supply and $A_n$ across land plants broadly agrees well with the theoretical resource allocation in leaves based on optimality theory (Deans et al., 2020), and can be observed in patterns of leaf vein evolution across the land plant phylogeny (Boyce et al., 2009; Brodribb and Feild, 2010). Efficient water supply to leaves can enable high rates of water and CO2 exchange across the epidermis enabling high $A_n$, but this could only happen with a parallel evolution of stomata on the leaf surface (Brodribb et al., 2017). Early vascular plants are characterized by large and relatively immobile guard cells, but evolution in guard cell size and shape occurred in parallel with leaf vascular systems such that modern angiosperms possess very dense arrays of small, highly mobile stomata (Franks and Beerling, 2009; de Boer et al., 2012) and very dense leaf venation (Roddy, 2019), which together provide sufficient $g_{m}$ to enable angiosperm leaves to profit from the high $g_{m}$ that is allowed by low $T_{cw}$.

Dynamic changes in cell wall thickness and their effects on mesophyll conductance and photosynthesis

Photosynthetic CW thickness has been considered as a relatively static parameter with only interspecific variations (Evans et al., 2009). However, recent studies demonstrate that $T_{cw}$ changes very dynamically even in mature leaves (Table S1 at Dryad). The responses of $g_{m}$ during acclimation to environmental changes can be explained by the variation of anatomical traits (e.g. $T_{cw}$ and/or $S_{/S}$), although physiological factors such as aquaporins and carbonic anhydrases can also be involved (Flexas et al., 2006; Perez-Martin et al., 2014; Groszmann et al., 2017; Momayyezi et al., 2020; Evans, 2021). In general, $g_{m}$ variation in response to internal and external factors is not so tightly coupled with either $T_{cw}$ or $S_{/S}$ as are the differences in the maximum $g_{m}$ among species, seen in the previous section.

$T_{cw}$ has been reported to change during leaf development (Niinemets et al., 2012). After leaf emergence, $T_{cw}$ increases, even after full expansion (Miyazawa and Terashima, 2001; Miyazawa et al., 2003; Tosens et al., 2012a; Borniego et al., 2020; Carriqui et al., 2021), and then it may remain stable or decrease with ageing in some species (Hanba et al., 2001; Saito et al., 2006). Effects of the age-dependent changes in $T_{cw}$ on $g_{m}$ and $A_n$ vary greatly depending on growth environment, leaf life span, and plant species. In some trees, $g_{m}$ increases regardless of the increase in $T_{cw}$ possibly because $S_{/S}$ also increases during ontogeny (Hanba et al., 2001; Miyazawa and Terashima, 2001; Tosens et al., 2012a). On the other hand, some herbaceous plants show a marked decrease in $g_{m}$ during ontogeny. For example, in tomato and soybean, $T_{cw}$ increases by 40–80% in leaves 10–14 d after full expansion, while $S_{/S}$ remains virtually unchanged, and $g_{m}$ decreases (Berghuijs et al., 2015; Sugiuira et al., 2020). An observed decrease in $g_{m}$ in bean during leaf ontogeny (Miyazawa et al., 2003) was due more to the decrease in $S_{/S}$ than to any change in $T_{cw}$ (Sugiuira et al., 2020).

$T_{cw}$ can also change according to light environment (Syvertsen et al., 1995; Oguchi et al., 2003; Gratani et al., 2006; Fini et al., 2016), although this might depend on species-specific light requirements. Generally, plants grown under high light conditions tend to show higher $g_{m}$ without changing $T_{cw}$, which could be explained by an increase in $S_{/S}$ (Syvertsen et al., 1995; Hanba et al., 2002; Tosens et al., 2012a; Peguero-Pina et al., 2016). However, Fini et al. (2016) reported that three woody species of the Oleaceae family with different light requirements changed $g_{m}$ and $T_{cw}$ in a different manner when grown under 30% or 100% of full sunlight. While sun-requiring species increased $g_{m}$ despite an increase in $T_{cw}$, shade-tolerant species decreased $g_{m}$ despite unchanged $T_{cw}$ and $S_{/S}$. Whether light requirement is involved in the predominance of anatomical or biochemical components of $g_{m}$ needs further confirmation.

The effect of CO2 concentration during growth in leaf anatomy and $g_{m}$ has been less frequently assessed. Teng et al. (2006) reported an increase in $T_{cw}$, cellulose, and pectin in Arabidopsis plants grown at elevated CO2. Unfortunately, $g_{m}$ was not determined in that study. However, Mizokami et al. (2019) did not observe a significant variation of $g_{m}$ despite the increase of $T_{cw}$ in response to high [CO2] in this species. Using free-air CO2 enrichment (FACE) technology, rice developed leaves with thicker CWs and decreased $g_{m}$, while in wheat higher [CO2] resulted in unchanged $T_{cw}$ and $g_{m}$ (Zhu et al., 2012). Another factor affecting $T_{cw}$ is the sink–source balance, which varies interspecifically and depends greatly on environmental conditions. Sugiuira et al. (2017) showed that the amount of CW materials accumulated in leaves was higher for radish plants grown under lower nitrogen and higher CO2 conditions (i.e. lower sink), while it was lower in radish with
larger hypocotyls (i.e. higher sink activity). Furthermore, soybean and bean showed coordinated increases in $T_{cw}$ and decreases in $g_{m}$ and $A_{n}$ after a substantial decrease in sink activity by defoliation, which supports the idea that photosynthesis may be down-regulated by anatomical changes in response to changes in the sink–source ratio (Sugiura et al., 2020).

In some studies, $g_{m}$ decreases in response to long-term drought together with a general decrease in $S_{l}/S$ and a slight increase in $T_{cw}$ (Miyazawa et al., 2008; Tosens et al., 2012a; Galmés et al., 2013; Tomás et al., 2014; Han et al., 2016; Ouyang et al., 2017; Du et al., 2019). However, intercultural and interspecific differences in the response of $S_{l}/S$ and $T_{cw}$ to water stress have been observed. Ouyang et al. (2017) reported that many cultivars of rice showed drought-induced increases in $T_{cw}$ and decreases in $g_{m}$ without changes in $S_{l}/S$. Instead, wheat showed an increase in $S_{l}/S$ under drought conditions (Ouyang et al., 2017).

**Effects of cell wall composition on $g_{m}$ and $A_{n}$**

Changes in CW components and their biochemical properties can modify CW thickness, conformation, and complexity, which could affect the CO$_2$ diffusion by physicochemical interactions through the CW (Le Gall et al., 2015; Houston et al., 2016; Clemente-Moreno et al., 2019). Only recently, researchers have begun to study the role of CW composition on $g_{m}$ using genetic approaches. Although some studies testing mutants with changes in their CW composition have reported reductions in the photosynthetic rate (Zhang et al., 2020) and/or anatomical changes that alter mesophyll architecture and, potentially, leaf CO$_2$ diffusion (Weraduwage et al., 2016), to the best of our knowledge only two studies have directly addressed the effect of CW composition on $g_{m}$. Firstly, Ellsworth et al. (2018) described a strong $g_{m}$ reduction (83%) in mutants with disrupted synthesis and accumulation of the hemicelluloses (1,3- and (1,4)-linked β-glucosyl polysaccharides, implying photosynthesis rate reductions of ~30–40%. This allowed them to propose that CW mixed-linkage glucans content improves p/$\tau$ in rice. However, a significant part of the $g_{m}$ reduction with the mutation was due to pleiotropic effects affecting other anatomical traits (Evans, 2021), since at least 23% of the $g_{m}$ constraint was caused by a reduction of $S_{l}/S$. However, other reduced parameters such as lower leaf mass area (LMA), leaf thickness, and $T_{cw}$ could facilitate higher $g_{m}$, implying that $g_{m}$ was at least partially constrained by altered CW composition. More recently, Roig-Oliver et al. (2020a) reported a $g_{m}$ reduction related to diminished galacturonic acid amounts in Arabidopsis mutants with alterations in pectin acetyl- and methylesterases, although whether these mutations affected other key anatomical traits determining $g_{m}$ was not assessed.

Besides genetic approaches, interspecific comparisons may also help in disentangling the role of CW components on $g_{m}$. Recently, Carríquí et al. (2020) performed an interspecific study in which seven conifer species were evaluated under non-stress conditions. The advantage of using conifers is that they lie on the quasi-flat region of the $g_{m}$ to $T_{cw}$ exponential decay, where variations of $g_{m}$ are less dependent on variations of $T_{cw}$ (Fig. 2). Although no significant correlation between CW components and $A_{n}$ was found, probably because $g_{m}$ was not the main limitation to photosynthesis in these species, hemicellulose and cellulose contents were negatively correlated with $g_{m}$. Also, while pectin content itself was not related to $g_{m}$, the pectin to cellulose and hemicellulose ratio strongly correlated with $g_{m}$.

In another recent study in rice (Ye et al., 2020), genotypes with higher LMA also presented greater leaf thickness and $T_{cw}$ and lower $g_{m}$. In this study, the amount of celluloses, hemicelluloses, and pectins also scaled positively with LMA and $T_{cw}$, while the pectin to cellulose and hemicellulose ratio declined (Ye et al., 2020). Hence, in rice, co-variation of $T_{cw}$ and CW composition makes it difficult to establish whether variation in $g_{m}$ is due only to $T_{cw}$, composition, or an interaction of both.

Additional evidence for a possible effect of CW composition on $g_{m}$ and $A_{n}$ arises from studies of photosynthesis response to abiotic stress. In tobacco exposed to salinity and drought, pectins showed a significant negative relationship with both $A_{n}$ and $g_{m}$, whilst the hemicellulose/pectin ratio correlated positively with both $g_{m}$ and $A_{n}$ in contrast to the cellulose/hemicellulose ratio, which correlated negatively with $g_{m}$. Interestingly, these changes were associated with the activity of antioxidant apoplastic enzymes linked to CW alterations as well as with specific CW primary metabolites such as galactose, glucosamine, and hydroxycinnamate, which were exclusively correlated with $g_{m}$ and not with other photosynthetic traits (Clemente-Moreno et al., 2019). In grapevines subjected to stresses including high and low temperature and water stress, it was observed that stress-induced variations in cellulose concentrations were negatively related to changes in $g_{m}$ and $A_{n}$ (Roig-Oliver et al., 2020b). In sunflower plants acclimated to different water availability conditions, lignin accumulation was significantly associated with $g_{m}$ reductions (Roig-Oliver et al., 2020a). Interestingly, the same study showed that the proportion between cellulose and hemicelluloses to pectins was positively correlated with changes in $T_{cw}$. Altogether, these results indicate that abiotic stresses provoke species-dependent changes in CW composition. These affect the different main CW compounds, so that the relative proportions between them vary, probably altering the physicochemical CW environment and affecting CO$_2$ diffusion. In addition, minor CW compounds have also been suggested to affect photosynthesis. For instance, in *Triticale*, increased amounts of CW-bound phenolics, especially ferulic acid, resulted in increased photosynthesis (Hura et al., 2009, 2012), whilst in sunflower coumarate content in CWs was negatively correlated with $g_{m}$ (Roig-Oliver et al., 2020a).
Hypothetical mechanism for the influence of cell walls on photosynthesis

As discussed in the previous sections, both $T_{cw}$ and chemical composition (which in turn also potentially determines $T_{cw}$) appear to be involved in setting and perhaps regulating $g_m$. However, the empirical evidence concerning both factors is limited. The correlation between $g_m$ and $T_{cw}$ is strong regardless of the phylogenetic groups considered (Fig. 2A), as might be expected due to the central position of $T_{cw}$ in the theoretical equation for $g_m$. For CW composition, instead, data are still scarce and discrepancies appear between studies. These apparent discrepancies are likely to reflect, on one hand, the different and complementary roles of thickness and composition in setting $g_m$, and, on the other, that the system is complicated and some CW components (e.g. pectins) play a variety of roles in the physicochemical properties defining diffusive conductance of the CW ($g_{cw}$, a major component of $g_m$). As a result, these may potentially act in opposite directions and/or have pleiotropic effects.

What is the position of CW components in the equation of $g_{cw}$? We suggest that CW composition—with a major role for pectins and/or the proportion between pectins and other compounds—determines $p$ and, very probably, $\tau$, yet in a complex manner (Fig. 3). If so, CW composition (even a single compound) may have effects on both the numerator and the denominator of the $g_m$ equation, and in a non-proportional way, meaning that strong simple correlations between $g_m$ and the concentration of any single CW component is unlikely.

Among principal CW compounds, pectins are often considered the master regulators of $p$ (Ochoa-Villareal et al., 2012; Bidhendi and Geitmann, 2016; Cosgrove, 2016; Novakovic et al., 2018). While the general view is that pectins decrease porosity, we argue that $p/\tau$ could instead be positively related to an increased fraction of pectins, based on experiments in which pectin degradation results in larger molecules capable of traversing the wall (Baron-Epel et al., 1988; Fleischer et al., 1999). This is because pectins exhibit hydrocolloid properties and can bind several times their own volume of water (Panchev et al., 2010; Schiraldi et al., 2012). Because CO$_2$ is a very small molecule as compared with average CW pore sizes (Carpita et al., 1979), slight reductions of $p$ may not affect its diffusion significantly. However, as CO$_2$ diffuses in solution, it might be the hydrophilic fraction of the pore which affects its diffusion. In this sense, pectins may increase what could be called the ‘effective porosity to water and CO$_2$’. Regardless of these speculative thoughts about the effect of pectin abundance on $p$, there is no doubt that increased pectin content modifies $p$ due to interactions with other molecules (Fig. 3).

![Diagram](https://academic.oup.com/jxb/article/72/11/3971/6199837)
The action of pectin-remodelling enzymes (PREs) promotes changes in pectin physicochemical properties, which modify the pectin matrix status as well as pectin interaction with other CW components, thus determining CW characteristics (Pelloux et al., 2007; de Souza and Pauly, 2015). In particular, the degree of pectin methylesterification is also an important feature that affects $p$. However, paradoxically, de-esterification has been related to both increased and decreased $p$ and CW stiffness (Bidhendi and Geitmann, 2016). Apparently, the effect depends on how pectin de-esterification occurs. Basically, there are two mechanisms of de-esterification, blockwise and non-blockwise. Blockwise de-esterification results in a continuous region of de-esterified pectin, which allows better cross-linking of pectins with Ca$^{2+}$ bonds, resulting in gelation, which increases wall stiffness and, presumably, decreases $p$. On the contrary, non-blockwise de-esterification pectin methylesterases (PMEs) act discontinuously, resulting in reduced cross-linking of pectins with Ca$^{2+}$ and also facilitating pectin degradation by polygalacturonases, hence resulting in wall softening and increased porosity (Bidhendi and Geitmann, 2016). The occurrence of blockwise or non-blockwise de-esterification depends on which specific PMEs are involved, which in turn depends on pH, initial stage of methylesterification, and cation concentration (Osorio et al., 2008). This implies that $p$ may not even be constant through the CW, since all these parameters can change from one side to another in the apoplast. For instance, a pH gradient may occur from slightly acidic apoplast to weakly alkaline symplast, thus through the CW pathway $\text{HCO}_3^-$ will tend to increase in concentration assuming that there is an equilibrium between $\text{CO}_2$ aqueous and $\text{HCO}_3^-$ (Evans et al., 2009). Apoplast redox status and the activity of several antioxidant apoplast enzymes such as peroxidases (POXs) varies depending on stress conditions (O’Brien et al., 2012; Tenhaken, 2015). Under stress, apoplast water pH tends to alkalinize (Geilfus, 2017), reducing the pH gradient with the symplast, and at equilibrium $[\text{HCO}_3^-]/[\text{CO}_2]$, the anion $\text{HCO}_3^-$ will increase in concentration relative to $\text{CO}_2$ along the apoplast and CW.

Besides methylesterification, other factors have been related to $p$. For instance, interaction of pectins with ion metals such as aluminium or copper is known to reduce $p$ and affect the hydraulic conductivity of the pectic matrix, and pectin dimerization and branching have also been suggested to affect $p$ somehow (Bidhendi and Geitmann, 2016). In addition, the apoplast redox status will be modified by the role of reactive oxygen species (ROS) and antioxidant apoplastic enzymes, which are also involved in modifying CW chemistry, especially in response to stress (Tenhaken, 2015). Aplastic ROS and their related enzymes are linked to different CW modification processes such as cell elongation, lignin and suberin formation, and cross-linking of CW components directly influencing the cell stiffening/loosening properties (Schmidt et al., 2014). The large complexity of mechanisms potentially involved in pectin-dependent regulation of $p$ may explain why correlations with $g_m$ are not as clear as those observed between $g_m$ and $T_{cw}$, but a hypothetical understanding of the action of these compounds will help in elucidating the precise mechanisms in future studies.

Concerning $\tau$, this might be mostly related to the specific arrangement of cellulose and hemicellulose fibrils, which may strongly depend on hemicelluloses and pectins because these bind covalently among them (Burgert and Dunlop, 2011). It may also depend on phenolic compounds and lignins, because these also form covalent bounds with hemicelluloses (Burgert and Dunlop, 2011; Anderson and Kieber, 2020) and increase CW hydrophobicity (Niklas et al., 2017), which might be relevant as $\text{CO}_2$ diffuses dissolved in water. Indeed, lignins have been shown to negatively correlate with $g_m$ in one study (Roig-Oliver et al., 2020a). Additionally, the role of structural proteins can also be an extra factor to be evaluated as, depending on species, they can range from 1% to 10% of the total CW, modifying CW properties in response to abiotic and biotic stress (Olmos et al., 2017) which may also affect $\text{CO}_2$ diffusion through the CW.

It is worth saying that the physical equation and terms that we use in this section (conductance, porosity, and tortuosity) rely on the assumption that $\text{CO}_2$ moves across CWs in a simple, non-facilitated diffusion manner—following Fick’s first law. However, this may not necessarily be the case, and chemical interactions between $\text{CO}_2$ molecules—or water molecules where $\text{CO}_2$ is dissolved—and wall components may eventually occur inside the CW pores (Terashima et al., 2011). If so, the actual $\tau$ would be a combination of the ‘physical’ $\tau$ (i.e. the twisting of the path length due to the non-linear position of the pores across the fibre matrix) and the ‘chemical’ $\tau$ (i.e. any kind of chemical interactions between $\text{CO}_2$ and CW compounds that could slow $\text{CO}_2$ diffusion). Rondeau-Mouro et al. (2008) reported that the aqueous phase diffusion in Arabidopsis CW residues (i.e. CWs in which pectins, proteins, and starch have been removed mechanically) approximates that in free water. For this reason, for several years, chemical $\tau$, also referred to $\tau_f$ [i.e. a dimensionless factor that accounts for the reduction of $D$ compared with free water (Weisiger, 1998)], has been considered negligible in studies in which mesophyll conductance was modelled (Tosens et al., 2012a, 2016; Tomás et al., 2013; Carriquí et al., 2019b). However, phenolic compounds, one of the potential components of this chemical $\tau$, have been shown to negatively correlate with $g_m$ in Roig-Oliver et al. (2020a) and with $A_n$ in a multispecies comparison (Sumbelé et al., 2012), and, in general, any other compound capable of establishing molecular interactions such as transient van der Waals bonds with diffusing water and/or $\text{CO}_2$ itself may significantly reduce chemical $\tau$; that is, increase $\tau_f$.

Such potential chemical interactions between water, $\text{CO}_2$, and CW composition have not been investigated in vivo in plants, but they are known and used in industrial procedures related to phenol transformation, cellulose-derived materials,
Prospects for improving photosynthesis by cell wall manipulation

Up until recently, most of the attempts to improve photosynthesis through anatomical changes have been focused on stomatal traits such as size, shape, and density (Gago et al., 2020b, and references therein), mesophyll cell density, distribution, and cell to cell contact (Masle et al., 2005; Takai et al., 2013; Lehmeier et al., 2017), and S/ S (Tholen et al., 2008; Li et al., 2013; He et al., 2017). This topic has been reviewed by Tholen et al. (2012) and Ren et al. (2019). However, biotechnological attempts to specifically study the relationship between CW composition and gm (and, thus, An) have been scarce.

Based on the evidence discussed in the sections above, the most obvious ways to increase gcw would be achieving lower Tcw and increasing p/τ. Unfortunately, the genes regulating these properties are unknown, and changing a single gene can have important pleiotropic consequences. The genes regulating the metabolic pathways of the major CW compounds and enzymatic activities related to their synthesis and degradation would directly affect leaf expansion, growth, cell density, and cell to cell contact (Tenhaken, 2015). However, knowledge of how those alterations could also affect CO2 diffusion through differential physicochemical interactions in the CW matrix remains unclear (Clemente-Moreno et al., 2019). Molecular strategies focused on altering any of these components could have huge consequences on the highly coordinated network to sustain CW functionality. For instance, virus-induced gene silencing (VIGS) of CESA1 and CESA2 (genes encoding enzymes catalysing cellulose synthesis) in Nicotiana benthamiana showed strong reductions of cellulose, but this alteration promoted PME gene regulation, avoiding CW weakness by a reduction in the PME degree accompanied by an increase in Ca2+ cross-linkages (Burton et al., 2000; Weraduwage et al., 2018).

Because of the pivotal role of the cell in plant physiology, any alteration in CW genetics would have dramatic effects on the plant’s anatomy and performance (Weraduwage et al., 2018), making it more difficult to use CW mutants to establish specific cause–effect relationships between cell composition affecting gcw. So, the question emerges: how can we disentangle the CW changes that affect general plant physiological status (and also their photosynthetical capacity) from those specifically altering CO2 diffusion?

p, τ, and intrinsic physicochemical properties interacting with the CO2 diffusion will mostly depend on the matrix network developed by the major polysaccharide compounds, glycoproteins, and phenolic compounds, defining both the final CW conformation and general leaf anatomy (thus, affecting gm) (Ren et al., 2019). Reductions in cellulose content will drive strong effects in the CW and weaken it. In order to strengthen the weakened CW, increased levels of pectins (esterified and unesterified, with increased Ca2+–mediated cross-linkage with xyloglucan and extensins), reduced levels of arabino-galactan proteins (AGPs), and changes in extensins have been observed (Burton et al., 2000; Méliña et al., 2009, and references therein). Additionally, these changes in CW profile can be accompanied by general mesophyll rearrangements—Tcw and cell elongation—as observed in the cellulose-defective mutants AtCesA7 in Arabidopsis (Zhong et al., 2003), that could also affect gm (Evans et al., 2009; Evans, 2021).

Despite the key role in CW modifications described for pectins (Tenhaken, 2015), just a few studies focused on how altering PEs, including PMEs and pectin acetyl-esters, could affect photosynthesis. Weraduwage et al. (2016) showed that Arabidopsis mutants with reduced and overexpressed levels of PME showed minor differences in their An. Contrarily, Roig-Oliver et al. (2020c) tested other Arabidopsis mutants with alterations in pectin acetyl and methyl-esters, and showed that reductions in both An and gm were paralleled by a diminished pectin amount.

In summary, the CW is a highly coordinated molecular and physiological network that performs an essential role in cells, and any change in its composition dynamically activates a response to compensate and sustain their main functionality from genes to enzymatic activity (Burton et al., 2000; Weraduwage et al., 2018). These changes will affect not just its intrinsic composition, but also a general rearrangement of leaf anatomy that will also affect leaf gas exchange and plant growth (Weraduwage et al., 2016, 2018). Indeed, most studies show that relationships with gm are significantly stronger when considered as ratios among all the major compounds rather than with any single compound, indicating a complex interaction that could affect p/τ (Clemente-Moreno et al., 2019; Carriqui et al., 2020; Roig-Oliver et al., 2020b).

Before altering CW CO2 diffusion properties using biotechnological approaches becomes feasible, further efforts are required to elucidate how the different interactions between CW compounds, including structural proteins, can affect gcw. Unfortunately, CW mutants often display strong phenotypes due to the essential role of the CW in plant physiology. Specific CW-inducible transformants become highly desirable to avoid dramatic phenotypes that can alter the whole-plant functioning, complicating specific examination of the effect of CW composition on gm and An. In parallel, additional technologies should be employed to further characterize CW properties, including immunolocation assays to determine the position and distribution of their main components, precise CW characterization from its main monomers to its principal components and their conformations, and ultrastructural
p determination, among others, in combination with gas exchange measurements.

Concluding remarks

In addition to their multiple and recognized functions in plant cells, there is now ample evidence for a role for CWs in $g_{\text{mm}}$ and photosynthesis. It has been shown that a phylogenetic trend exists from thick CWs and low photosynthetic capacities in bryophytes to thin walls and high photosynthesis in angiosperms. Besides thickness, an emerging role of CW composition in $g_{\text{mm}}$ is highlighted. While the role of thickness is well understood, further studies are required to better understand the mechanisms by which changes in composition may affect CO$_2$ diffusion. We hypothesize that these effects might be multiple and complex, complicating efforts to disentangle the particular role of each compound. Yet with current perspectives, we can hypothesize a major role for pectins and/or the pectin to cellulose plus hemicellulose ratio. More genetic approaches are needed to investigate the possibility of improving crop photosynthesis by means of CW manipulation. While already presenting thin CWs, which may preclude improvement, the possibility of improving crop photosynthesis by manipulating CW composition has yet to be evaluated. In addition, such studies should also evaluate the possibility that CW-related improvement of photosynthesis could have detrimental effects on plant water relations, plant tolerance to water stress, and/or any of the other fundamental functions that CWs have in plant cells.

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Author contributions


Data availability

Dataset included in the figures of this review are available at the Dryad Data Repository: https://doi.org/10.5061/dryad.qbzkhh18gs (Flexas et al., 2021). Dataset S1. Photosynthetic and anatomical data compilation from previously published papers.

References

Balsamo RA, Bauer AM, Davis SD, Rice BM. 2003. Leaf biomechanics, morphology, and anatomy of the deciduous mesophyte Prunus serrulata (Rosaceae) and the evergreen sclerophyllous shrub Heteromeles arbutifolia (Rosaceae). American Journal of Botany 90, 72–77.
Brodribb TJ, Feild TS, Jordan GJ. 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. Plant Physiology 144, 1890–1898.
Carpita N, Sabularese D, Montezinos D, Delmer DP. 1979. Determination of the other fundamental functions that CWs have in plant cells.

...


Deans RM, Brodribb TJ, Busch FA, Farquhar GD. 2020. Optimization can provide the fundamental link between leaf photosynthesis, gas exchange and water relations. Nature Plants 6, 1116–1125.


Maron L. 2019. Rethinking our models of the plant cell wall. The Plant Journal 100, 1099–1100.


