Coming of leaf age: control of growth by hydraulics and metabolics during leaf ontogeny

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Review

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Summary

Leaf growth is the central process facilitating energy capture and plant performance. This is also one of the most sensitive processes to a wide range of abiotic stresses. Because hydraulics and metabolics are two major determinants of expansive growth (volumetric increase) and structural growth (dry matter increase), we review the interaction nodes between water and carbon. We detail the crosstalks between water and carbon transports, including the dual role of stomata and aquaporins in regulating water and carbon fluxes, the coupling between phloem and xylem, the interactions between leaf water relations and photosynthetic capacity, the links between Lockhart's hydromechanical model and carbon metabolism, and the central regulatory role of abscisic acid. Then, we argue that during leaf ontogeny, these interactions change dramatically because of uncoupled modifications between several anatomical and physiological features of the leaf. We conclude that the control of leaf growth switches from a metabolic to a hydromechanical limitation during the course of leaf ontogeny. Finally, we illustrate how taking leaf ontogeny into account provides insights into the mechanisms underlying leaf growth responses to abiotic stresses that affect water and carbon relations, such as elevated CO_2, low light, high temperature and drought.

I. Leaf growth: volume, structures, water and carbon

Leaf growth consists of two components: an increase in volume – the expansive growth – and an increase in dry matter – the structural growth. Leaf growth, at timescales of minutes to days is strongly related to temperature (Parent & Tardieu, 2012; Eqn 1 in Fig. 1) thus defining a potential growth that must be confronted by major constraints. Indeed, the irreversible increase in the size of leaf cells during their expansion requires a massive influx of water into the vacuoles. It also demands a substantial supply of carbon skeletons to build new structures (e.g. cellulose and hemicellulose in the new cell walls) and additional photosynthesis products to

fuel the various energy-consuming mechanisms (e.g. cell cycle, protein synthesis, functioning of pumps and channels to upload solute in vacuoles and generate turgor). From this, the availability of water and carbon have often been considered the main limitations of leaf growth, classically referred to as the hydraulic and the metabolic control, respectively (Kriedemann, 1986; Dale, 1988; Walter *et al.*, 2009). These views are validated by experimental evidence and models.

1. Water relations and wall rheology provide the biophysical basis of expansive growth

Cell expansion relies on a tight coupling between water fluxes and cell wall rheological modifications (Cosgrove, 1986), as described in the Lockhart model (Lockhart, 1965; Eqn 5 in Fig. 1). Three steps must be satisfied in a coordinated manner for growth to be sustained (Moulia & Fournier, 2009; Eqn 5 in Figs 1, 2): (1) cell turgor pressure transmits tensional stresses in the cell wall, which stretches irreversibly when a yield threshold pressure is exceeded; (2) this deformation is accompanied by a passive water flow governed by the water potential gradient between the cell and the water source; and (3) this gradient is actively maintained by adjustments of the cell osmotic potential, which in turn generates cell turgor pressure. Originally developed and validated on single giant algal cells, the Lockhart model is supported by experimental data in several plant species at the leaf scale (e.g. Bunce, 1977; Bouchabké *et al.*, 2006; Ehlert *et al.*, 2009, 2011; Zhang *et al.*, 2011). It accounts for the reduction of leaf expansion rate under soil water deficit when water potential gradients towards growing cells are less steep. It also accounts for a lowered leaf growth during high transpiration periods, when expansive growth competes for water with transpiration, although only 1–2% of transpiration water is used for growth (Fricke, 2002). Accordingly, expansive growth in both dicots and monocots is classically reported as being lower during the day than during the night, when stomata are closed (Ben-Haj-Salah & Tardieu, 1996; Pantin *et al.*, 2011).

2. Carbon metabolism supplies the material and the energy to achieve structural growth

Stomatal opening in the light gives access to atmospheric CO_2 for photosynthesis whose products are essential for growth, providing both the building bricks (e.g. cell wall cellulose and hemicellulose) and the energy required for structural growth (Dale, 1985; Fig. 2). Consistent with this, accumulation of plant biomass is strongly related to intercepted radiation and cumulated photosynthesis, as originally formalized by Monteith (1977; Eqn 2a in Fig. 1). Moreover, in many instances, leaf growth rate or shoot biomass correlate well with either net photosynthesis, activity of carbon metabolism enzymes, or level of carbon metabolites (maize, Rocher *et al.*, 1989; tobacco Fichtner *et al.*, 1993; Arabidopsis, Cross *et al.*, 2006, Sulpice *et al.*, 2009, 2010). During nights, carbohydrate availability in leaves is buffered by transitory storage compounds, among which starch predominates in most species (Stitt & Zeeman, 2012). Starch turnover is also controlled by the circadian clock and it could represent a crucial node in the regulation of growth (Sulpice *et al.*, 2009; Graf *et al.*, 2010). Accordingly, mutations in the starch metabolism have drastic effects on leaf growth at night, while normal leaf expansion is maintained during the day (Wiese *et al.*, 2007; Pantin *et al.*, 2011). In addition to their roles as substrate for metabolism, carbohydrates and sugars also play intricate signalling roles over a broad range of developmental processes and stress responses (Rolland *et al.*, 2006). This multiplicity of roles, as well as the difficulty of measuring local carbohydrate availability (as opposed to water status for example), explain why, beyond correlations (Freixes *et al.*, 2002; Cross *et al.*, 2006; Muller *et al.*, 2011), there is no available mathematical formalism relating growth to carbon availability at a fine time-scale. Therefore, it is tempting to question if and how carbon metabolism may fit into already established formalisms, notably the Lockhart biophysical model.

II. Coupling water and carbon limitations through the Lockhart model?

The Lockhart model offers several bridges between cell hydromechanics and carbon metabolism. Although rarely exploited, these couplings include osmotic adjustment, wall properties and turgor sensing.

1. Osmotic adjustment relies on organic compounds

In the Lockhart model, osmotic potential plays a central role by generating turgor pressure, which ultimately stretches cell walls (Eqn 5 in Fig. 1). Osmotic potential arises from a passive or active accumulation of solutes within the cell, which creates a difference in solute concentration between the inside and the outside of the cell. Obeying thermodynamics, a passive water flow then occurs into the growing cell where osmotic potential is lower. This coupling is generally at the core of models simulating fruit growth, where sugars are the main contributors to osmotic potential (Fishman & Génard, 1998; Martre *et al.*, 2011; Muller *et al.*, 2011). In leaves, although inorganic osmotica such as nitrate and potassium are classically reported as being the main contributors to the baseline osmotic potential, osmotic adjustment in response to water status fluctuations may strongly rely on organic compounds (Turner *et al.*, 1978). In Arabidopsis leaves, variations of organic acids, proline, other amino acids and sugars represent > 50% of the osmotic adjustment under severe water deficit (Hummel *et al.*, 2010).

2. Carbon control over wall properties

Several lines of evidence suggest that the cell wall, which is at the core of the Lockhart analysis, should not be restricted to two fixed parameters but envisioned as a carbon-modulated compartment within a hydromechanical framework. Carbon availability could modulate wall properties at two levels: directly as a substrate responsible for the adjustment of wall thickness and indirectly by modulating the enzymes controlling wall mechanics. At first sight, the Lockhart model does not tell much about the deposition of wall material during cell expansion because it assumes that the rate of

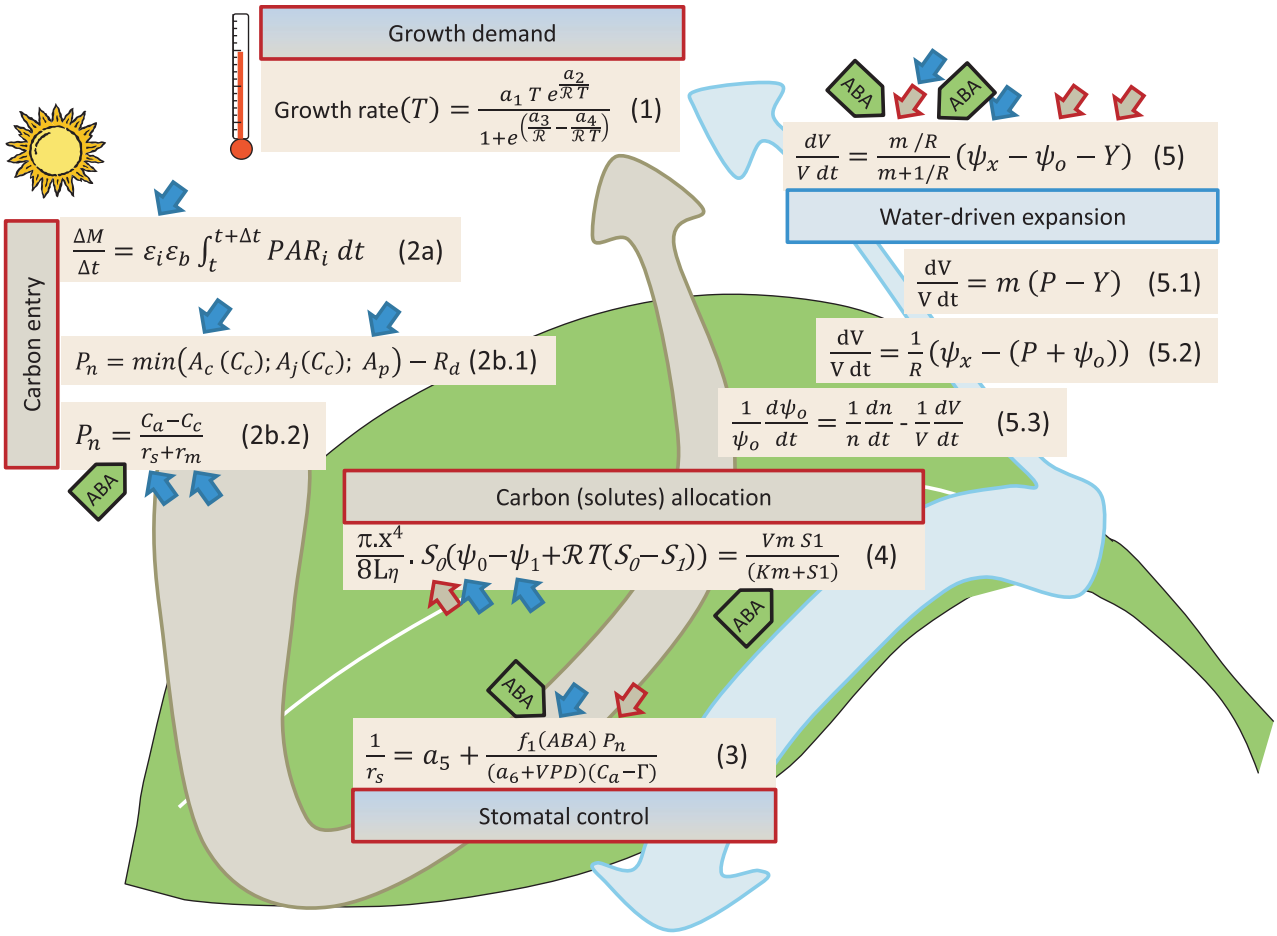

Fig. 1 Synoptic view of the main representative equations used for modelling leaf growth response to climatic fluctuations highlighting where carbon and water limitations interact. (1) Temperature (T) determines the potential (being nonlimited by other factors) growth rate of organs. An augmented Boltzmann–Arrhenius equation accounts for the increase in growth rate at suboptimal temperatures with enthalpy of activation a_2, and the decrease at supra-optimal temperatures with enthalpy and entropy of inactivation a_3 and a_4 (Parent *et al.*, 2010). Parameter a_1 is a scaling coefficient. This potential growth rate is achieved when both the demands of water for expansive growth and carbon for structural growth are satisfied. (2) Carbon entry in the plant is governed by incident photosynthetically active radiation (PAR_i) and is formalized in two possible ways. In Monteith (1977) Eqn 2(a), any change in leaf growth rate has further influence on light interception through radiation use efficiency (RUE or ε_i), then intercepted light is converted into new biomass (ΔM) with biological efficiency ε_b. Initially written for nonlimiting water conditions, this equation can incorporate water limitations effects on ε_b. A more detailed approach couples net photosynthesis rate (P_n) and gas diffusion. The most widely used model for P_n has been proposed by Farquhar *et al.* (1980) as a balance between the rates of respiration (R_d), carboxylation and oxygenation. In this model, carboxylation rate may be limited by three different biochemical reactions depending on the CO_2 concentration (C_c) at the carboxylation sites in the chloroplasts (2b.1). Thus, the carboxylation rate is the minimum of A_c when limited by RuBisCO carboxylase activity, A_j when limited by ribulose biphosphate regeneration rate or A_p when limited by triose phosphate use. Temperature influences all the terms of this model, light determines A_j, and water deficit has been shown to reduce A_c and A_j. Stomata and leaf mesophyll oppose resistances (r_s and r_m, respectively) to gas diffusion thereby lowering C_c below the available CO_2 concentration (C_a) at the leaf surface (2b.2). (3) Because stomata respond to light, air vapour pressure deficit (VPD) and soil water deficit, they are considered as the main coordinators between carbon gains and water losses by the plant. Several models of stomatal resistance (e.g. Leuning, 1995) explicitly formalize this interrelation between r_s (and hence water losses) and P_n as a function of VPD and direct (hydraulic) or indirect (via drought-induced abscisic acid) effects of soil water deficit (f_1 is a function of ABA that increases r_s; a_5 and a_6 are empirical parameters). By contrast, r_m, which probably responds to water deficit in parallel with r_s, can partially uncouple water losses from carbon acquisition because the mesophyll pathway only concerns CO_2 diffusion. (4) Organic solute fluxes from source (total osmolyte concentration S_0) to sink (concentration S_1) are convective and obey to the Münch law following a hydrostatic gradient, here shown as both a water potential and a solute concentration gradient between source and sink. The flux is weighted by resistance from the Poiseuille law. At equilibrium, this flux equals the utilization that can be modelled following a Michaelis–Menten formalism (with constants V_m and K_m) as proposed in Minchin *et al.* (1993). (5) In the Lockhart model (1965), expansive growth ($1/V \cdot dV/dt$) is considered as a change in water volume driven by the difference between water potential in the xylem source (Ψ_x) and osmotic potential (Ψ_o) in the growing cells (the more osmolytes in the growing cells, the more negative the osmotic potential and hence the steeper the gradient for growth). Moreover, water entry into the growing cells has to overcome the extensibility m of the cell walls and a yield threshold Y (that can be modified by carbon supply), and the hydraulic resistance R on the path from xylem to growing cells. Eqn 5 is the solution of two equations that detail the role of turgor (P) in growing cells, both exerting forces on the wall for cell expansion (5.1) and limiting water entry from the xylem source (5.2). A third eqn (5.3) expresses the changes in Ψ_o caused by the dilution by water influx as the cell expands, which should be counterbalanced by an increase in osmolarity (n), that is, osmotic adjustment in the growing cells. Small arrows denote possible limitations for each modelled process because of low availabilities of carbon (brown) and water (blue). This shows how water limitations can alter carbon flow, and reversely how carbon limitation can limit water flow. Green arrows also points to multiple processes where ABA can influence carbon and water flows (wide light-brown and light-blue arrows, respectively).

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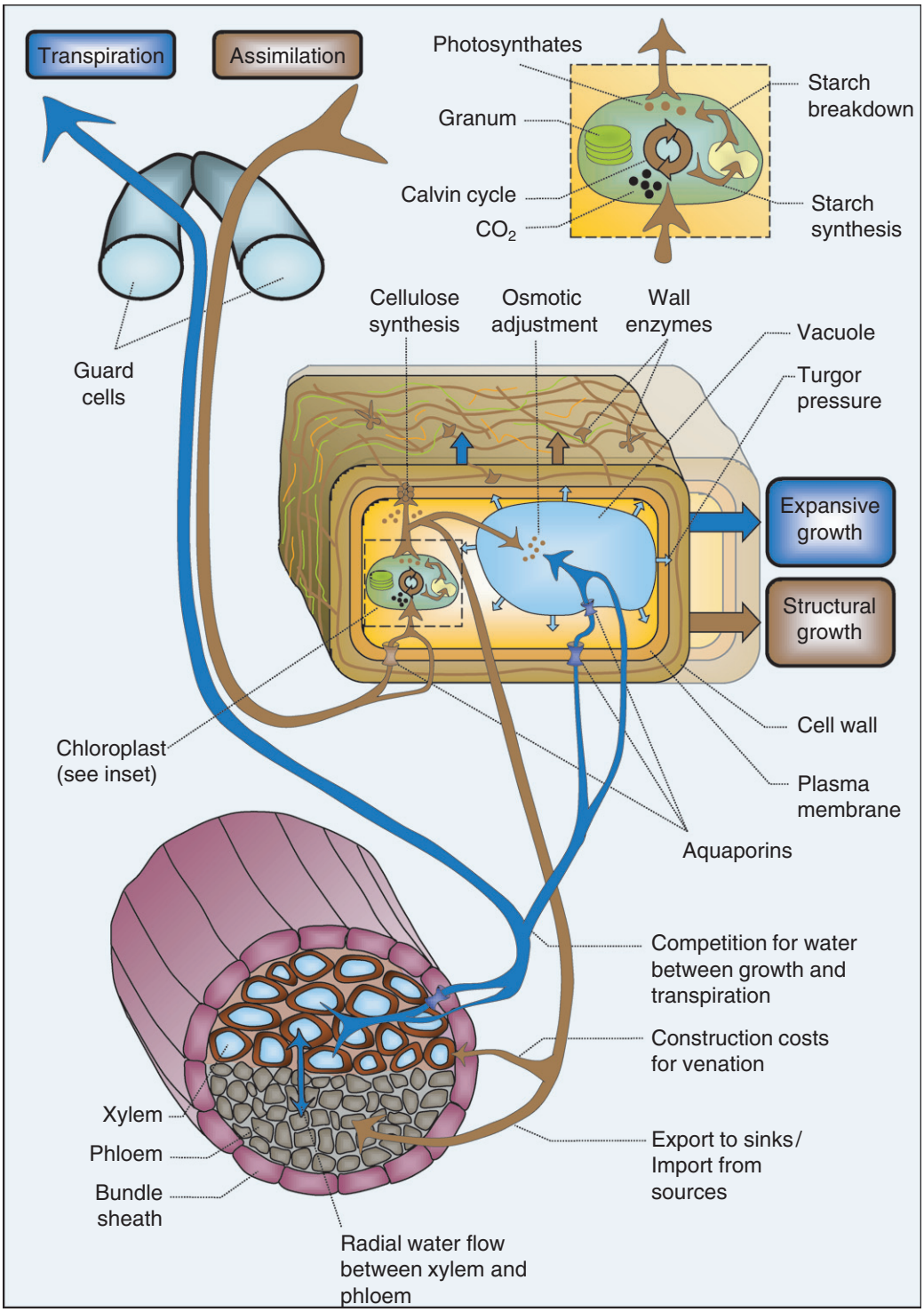

Fig. 2 Control of leaf growth: interaction between carbon metabolism, hydraulics and mechanics. The growth of a leaf cell is both expansive (increment in water volume) and structural (increment in dry matter). Expansive growth requires the cell turgor pressure to stretch the cell walls according to an irreversible deformation, during which wall rheology is actively controlled by enzymes such as expansins or endoglucanases, accompanied by a volumetric water flow. Adjustments of the cell osmotic potential, for example by importing photosynthates into the vacuole, is necessary to attract water. Extracted from the soil, water flows through the xylem and then follows an apoplastic path or a cell-to-cell path, the latter involving aquaporins. There is a competition between the volumetric water flow required for leaf growth and the process of transpiration, namely water evaporation predominantly through stomata. Stomatal opening is required for carbon assimilation through photosynthesis. Some aquaporin isoforms allow for the acceleration of CO_2 transport to the carboxylation sites. This assimilation allows new material (cellulose, hemicelluloses, pectins) to be synthesized and incorporated into the cell walls. Carbon availability is buffered by transitory starch, synthesized during the day and degraded during the night. Depending on the status of the growing leaf, sugars are also imported from sources or exported to sinks. Some of the photosynthates are diverted to construct the venation network. Compartments and fluxes are representational only and not to scale.

deposition matches the rate of expansion-induced thinning. Although wall deposition and expansion are uncoupled under several circumstances, such as during hypocotyl elongation (Cosgrove, 2005), this coupling assumption accounts for the growth of cell tips such as pollen tubes (Dumais *et al.*, 2006). This assumption implicitly requires that carbon availability crosstalks with wall material deposition, for example to prevent excessive thinning of cell walls during periods of low carbon (Hanba *et al.*, 2002). A coupling between expansion and cell wall deposition could be the result of a tight regulation of the molecular actors involved in cell wall relaxation and synthesis. Indeed, cell wall extensibility is controlled by several proteins and enzymes such as expansins, endoglucanases, peroxidases and xyloglucan endotransglycolases/hydrolases (Cosgrove, 2005). Their function has been incorporated in a modified version of the Lockhart model, where the activity of loosening enzymes appears as a parameter that represents the rate at which load-bearing hemicellulose molecules linking cellulose microfibrils are cut (Passioura & Fry, 1992). In Arabidopsis and maize leaves, the expression or activity of these proteins is tightly associated with growth changes (Cho & Cosgrove, 2000; Muller *et al.*, 2007) is affected by water deficit (Muller *et al.*, 2007; Harb *et al.*, 2010), and also responds to elevated CO_2 in different species (Ranasinghe & Taylor, 1996; Ferris *et al.*, 2001; Ainsworth *et al.*, 2006) suggesting that these environmental influences on growth could be represented by changes in extensibility parameters in the Lockhart model. Finally, cellulose synthases, subordinated to the availability of their carbon substrate, are possibly feedback-regulated by the activity of enzymes responsible for cell wall loosening (Somerville, 2006). This may provide a molecular convergence in the control of wall metabolism and wall loosening.

3. Making use of turgor to control wall metabolism and mechanics

Another candidate for the coupling between carbon availability, wall metabolism, wall mechanics and the hydraulic control of growth could be turgor itself. In *Chara corallina* cells, turgor acts as the mechanical force to facilitate incorporation of large polymers into the wall (Proseus & Boyer, 2005). In this alga, a turgor-sensitive step in the process of wall synthesis has been identified as the 'pectate cycle' (Proseus & Boyer, 2007). In the tightening wall, calcium crosslinks the pectate polymers in the matrix. A threshold of turgor pressure is necessary to distort the bonds between pectate and calcium. When supplied to the wall matrix, newly synthesized pectate chelates the calcium released from the distorted bond, allowing wall loosening and irreversible expansion. The pectate then integrates the wall by developing crosslinks with the existing molecules in the matrix, while the free calcium enters the wall and retightens the structure. The complete model thus reconciles wall expansion, wall deposition and wall mechanical status through turgor sensitivity (Proseus & Boyer, 2007). Furthermore, the combined action of the pectate cycle and turgor on deposition of new materials in the cell wall also accounts for the so-called 'stored growth', that is, the above-normal flush of growth observed when turgor recovers after a period of turgor loss and stopped growth (Proseus & Boyer, 2008). Such interactions between turgor and wall metabolism could inspire plant modellers because stored growth is also observed in leaves, especially during recovery from water deficit (Hsiao *et al.*, 1970).

Finally, it has also been proposed that cell expansion and carbohydrate metabolism in Arabidopsis is under the control of a cell wall sensing machinery able to detect changes in turgor pressure, and to feedback on turgor through the regulation of solute metabolism. First, inhibition of cellulose biosynthesis affects the cell wall integrity and modifies the expression of genes involved in mechanoperception, possibly through changes in turgor pressure, thereby triggering the same cascade as described upon hypo-osmotic shock (Hamann *et al.*, 2009). Second, inhibition of cellulose biosynthesis also causes transcriptional, enzymatic and metabolic changes in the central metabolism, which are relieved upon osmotic stress, suggesting that a turgor-sensitive mechanism tunes carbohydrate metabolism (Wormit *et al.*, 2012). Third, the expression and activity of vacuolar invertase, an enzyme that contributes to osmotic adjustment, are dependent on the functioning of a wall-associated kinase involved in cell wall sensing and cell expansion (Kohorn *et al.*, 2006).

Leaf rheology is thus at the crossroads of hydraulics and metabolics, and it is tempting to subordinate the Lockhart parameters to carbon metabolism. Interestingly, a conceptual model was drawn in line with this view > 25 yr ago (Hsiao *et al.*, 1985). Nevertheless, formalisms describing this complex subordination are still lacking, partly because rheological properties respond to numerous other stimuli, including pH (Thompson, 2001), reactive oxygen species (Liszkay *et al.*, 2003), or ABA (Cramer *et al.*, 1998). Because ABA affects wall rheology, tunes the fluxes of water and carbon and mediates growth responses to various stresses, its central role in orchestrating hydraulics and metabolic interactions is analysed in the next section.

III. ABA signalling pathway as a hub to coordinate water and carbon relations

Abscisic acid accumulates in plants under water deficit and affects leaf growth in multiple, sometimes contradictory, direct and indirect manners (Tardieu *et al.*, 2010). Part of this complexity arises from the involvement of ABA in both carbon and water fluxes. It regulates stomata and aquaporins, which in turn control both water loss from the leaf and incoming of carbon dioxide. In addition, ABA signalling is known to interfere with sugar sensing, with hexokinase (HXK) being the central pivot of this interaction (Rolland *et al.*, 2006). Other kinases such as the family of sucrose nonfermenting-1-related protein kinases (SnRKs) also connect ABA signalling with primary metabolism through sugar phosphate intermediates (notably trehalose-6-phosphate) and transcription factors (Delatte *et al.*, 2011; Ma *et al.*, 2011), thus coordinating metabolic and stress signalling (Hey *et al.*, 2010). Abscisic acid also regulates carbon metabolism at the enzymatic level, both transcriptionally and post-transcriptionally (Trouverie *et al.*, 2004; Zhu *et al.*, 2011). It inhibits cell (Wang *et al.*, 1998) and plastid (Galpaz *et al.*, 2008) division. Thus, ABA affects both expansive and structural growth.

1. Stomata integrate hydraulic and metabolic cues via ABA signalling

As a major determinant of stomatal closure, especially under water deficit, ABA supervises the trade-off between transpiration and assimilation (Eqn 3 in Figs 1, 2). Hence, at first sight, ABA promotes expansive growth by saving leaf water and reducing xylem tension, but likely alters structural growth by limiting CO_2 entry. It also interferes with stomatal responses to atmospheric CO_2 and byproducts of photosynthesis and could therefore play a more subtle role in optimizing the trade-off between water loss and carbon fixation. Experimental data show an interaction between ABA and CO_2 in the control of stomatal conductance, for example, in cocklebur (*Xanthium strumarium*; Raschke, 1975), in Arabidopsis (Leymarie *et al.*, 1998) or in various tree species (Aasamaa & Sõber, 2011). Furthermore, guard cell sensitivity to CO_2 depends on air relative humidity and vice versa (Bunce, 1996; Talbott *et al.*, 2003), while stomatal response to humidity is partly mediated by ABA, as shown in Arabidopsis (Xie *et al.*, 2006; Okamoto *et al.*, 2009). At the molecular level, ABA and CO_2 interact to regulate stomatal conductance in a network that is just starting to be revealed. Both ABA and elevated CO_2 would act at an undefined convergence point where Ca^{2+} sensitivity is primed to trigger stomatal closure (Kim *et al.*, 2010). Although rarely cited, sugars could also interact with ABA to control stomata. Indeed, carbon metabolism is involved in the control of night-time stomatal conductance, for example, in Arabidopsis (Lascève *et al.*, 1997) or in *Vicia faba* (Easlon & Richards, 2009). Furthermore, starch, sucrose, hexoses and malate are convenient sources of energy and osmotica required for stomatal movements (Vavasseur & Raghavendra, 2005; Penfield *et al.*, 2012). Thus, connections can be hypothesized between products of photosynthesis and the HXK and SnRKs network. Overall, the complex scheme of ABA, CO_2 and sugar signalling pathways promises exciting perspectives to elucidate the way by which stomata arbitrate between water and carbon fluxes.

2. Aquaporins: internal modulators of water and carbon fluxes

In addition to stomata, aquaporins are another major target for ABA to regulate both water and carbon fluxes. Abscisic acid partly mediates aquaporin regulation in response to environmental stresses (Maurel *et al.*, 2008) by modulating their gene expression and their protein abundance or activity. This affects plant water relations in various organs, via, for example, an increase in root hydraulic conductivity in maize (Parent *et al.*, 2009) or tomato (Thompson *et al.*, 2007), a decrease in leaf hydraulic conductance (Shatil-Cohen *et al.*, 2011) or an increase in leaf protoplast water permeability in Arabidopsis (Morillon & Chrispeels, 2001), although the last result was attributed to an indirect effect of ABA on transpiration rate. Not only are aquaporins water channels, but they also transport CO_2 and some organic solutes (Maurel *et al.*, 2008; Fig. 2). They have a significant role in photosynthesis through mesophyll conductance to CO_2 in tobacco or Arabidopsis (Flexas *et al.*, 2006; Heckwolf *et al.*, 2011), as well as in carbohydrate routing in Arabidopsis (Ma *et al.*, 2004). Furthermore, they may be involved in the interaction between water and sugar transport required for the fruit development as shown in tomato (Chen *et al.*, 2001). Thus, any change in aquaporin regulation mediated by ABA (or any other stimuli) could alter both carbon and water fluxes.

IV. Leaf venation: just a two-way pipe network?

We have emphasized the role of ABA in orchestrating the influence of water and carbon on leaf growth. Abscisic acid is highly mobile in the plant: local synthesis intermixes with a flow from roots to leaves via the xylem and a backflow from source leaves to sink organs via the phloem (Hartung *et al.*, 2002). However, the central role of vascular networks in the interaction between hydraulic and metabolic control of growth goes well beyond ABA redistribution only.

1. Xylem and phloem are hydraulically coupled

Xylem conduits bring water and nutrients from roots to leaves while phloem conduits bring photosynthates from source leaves to sink organs. Interestingly, xylem and phloem conduits are hydraulically connected (Hölttä *et al.*, 2006; Fig. 2). Active loading of organic solutes in phloem conduits of source leaves decreases the phloem osmotic potential and attracts water from the surrounding tissues, including xylem as a major source of water. This ultimately gives rise to turgor pressure in sieve elements of source leaves, which represents the hydrostatic force that triggers phloem flow (Fig. 1, Eqn 4). Consequently, the phloem flow is sensitive to changes in water potential in the xylem of the source leaves and any increase in transpiration rate decreases the phloem assimilate export to sink organs through a purely hydraulic mechanism (Lacointe & Minchin, 2008). This central role of xylem and phloem in long-distance redistribution is likely to have driven leaf venation to organize in a network that optimizes the use of water and carbon resources.

2. A cost–benefit approach of leaf venation

Remarkably, across species, leaf area is negatively correlated with the density of major veins and positively correlated with major vein diameter (Sack *et al.*, 2012), two central leaf traits that govern the leaf hydraulic efficiency (Sack & Frole, 2006). Venation properties and leaf size become of primary importance under adverse hydraulic conditions, where low xylem lumen diameter, high major vein density and small leaf area confer greater tolerance (Blackman *et al.*, 2010; Scoffoni *et al.*, 2011). Interestingly, drought-tolerant species are also shade-tolerant in dry habitats, because the safety strategy they develop (slow growth, low photosynthesis, low risk of xylem cavitation) makes them better competitors in low-resource environments (Markesteijn *et al.*, 2011). Indeed, the dual role of stomata implies that high photosynthesis partly depends on high transpiration, which requires high water supply capacity to minimize the risk of high xylem tension. Accordingly, leaf hydraulic conductance is coordi-

nated with its photosynthetic capacity across a wide range of species (Sack & Holbrook, 2006). Notably, the course of angiosperm diversification was marked by a surge in leaf vein density and photosynthetic capacity (Boyce *et al.*, 2009; Beerling & Franks, 2010; Brodribb & Feild, 2010; Feild *et al.*, 2011). More precisely, the ability of the vein network to fill leaf space governs the photosynthesis rate by modulating either the photosynthate export capacity (Raven, 1994) or the leaf hydraulic efficiency (Brodribb *et al.*, 2007). However, space-filling represents a massive investment because lignified tissues are net carbon sinks that do not contribute directly to photosynthesis (Fig. 2). The ability of plants to manage these construction costs in relation to their benefits has been a determining factor in their evolution (Beerling & Franks, 2010; Pittermann *et al.*, 2012). In leaves, hydraulic efficiency relative to construction cost is increased by vein hierarchy, tapering and density (McKown *et al.*, 2010). A recent study in *Nothofagus cunninghamii* also shows that the development of veins and stomata is coordinated to optimize the carbon investment in leaf venation (Brodribb & Jordan, 2011); similarly, along grass blades, an acropetal increase in stomatal conductance is balanced by an acropetal decrease in the distance from vascular bundles to stomatal pores, allowing a developmental increase in photosynthesis without a dramatic drop in water potential between vessels and evaporation sites (Ocheltree *et al.*, 2012). This stresses the need for an ontogenetic vision of the way hydraulics and metabolics interact in the control of leaf growth.

V. Leaf ontogeny orchestrates the actors involved in the control of leaf growth

Despite some species-specific and environment-dependent variations on the relative timing of each developmental event, a general, species-independent sequence of morphological, anatomical and physiological changes during leaf ontogeny emerges from the literature, as shown for dicots in Fig. 2. A similar pattern could certainly be depicted in monocots along a spatial gradient. Leaf area expands in a sigmoidal way, with an early exponential growth, a marked increase and a deceleration followed by a plateau (Fig. 3). The absolute expansion rate peaks at the inflection point of the curve, when the leaf reaches 50% final area; however, the relative expansion rate, that is, the area accumulated per unit area and per unit of time, is at its highest in the earliest stages and decreases afterwards (Fig. 3). This pattern holds especially in dicot leaves where the growth is not restricted to a basal zone of stable size for several days as in monocots, although a basipetal gradient of increasing growth is generally observed in dicots (Granier & Tardieu, 1998; Walter *et al.*, 2009; Kuchen *et al.*, 2012). Similarly, the relative cell division rate is at its highest in the early stages of leaf development, and decreases sharply before the relative leaf expansion rate starts declining, for example, before 10% final leaf area (Fig. 3), with a basipetal progression of the cell cycle arrest front (Granier & Tardieu, 2009; Kazama *et al.*, 2010; Andriankaja *et al.*, 2012). By contrast, the relative cell expansion rate peaks shortly after the relative cell division rate starts declining (Fig. 3). Thus, cell division precedes cell expansion but these processes largely overlap (Kriedemann, 1986; Granier & Tardieu, 2009). Leaf thickness increases in pace with leaf area, with a rapid thickening occurring at *c.* 20% of final leaf area and corresponding to the volumetric expansion of the palisade cells and the development of intercellular spaces in the spongy parenchyma (Tichá, 1985; Wuyts *et al.*, 2010, 2012; Fig. 3). The increase in dry mass lags slightly after leaf area, so that leaf mass per area increases during leaf ontogeny after a short period of stability or decrease (Tichá *et al.*, 1985; Gratani & Bonito, 2009; Fig. 3). This general trend provides the framework to obtain deeper insights into the developmental pattern of the metabolic and hydromechanical control of leaf growth.

Water and carbon relations of the expanding leaf were reviewed 25 yr ago (Dale, 1985; Šesták, 1985; Barlow, 1986; Kriedemann, 1986). Considerable advances have been made since then, but a synthesis of these works is currently lacking. Using a genetic and environmental approach on Arabidopsis, we have recently shown that the control of leaf expansion is predominantly metabolic in the early stages, while hydraulic influence establishes as the leaf develops (Pantin *et al.*, 2011). Immediately after leaf emergence, drops in leaf expansion have been observed during night-time, likely resulting from limited carbon availability in the starch pool stored on the previous day. In line with this, these early drops were exacerbated in a set of mutants affected in starch metabolism. Later, drops in leaf expansion occurred during the day, when water movement into the growing cells is limited by transpiration. Consistent with this, these diurnal drops were amplified and occurred earlier during ontogeny in a set of mutants affected in the stomatal control of transpiration, as well as in plants exposed to low soil water potential or high atmospheric vapour pressure deficit. The day/night pattern of leaf turgor and starch content in leaves of contrasting ages further highlighted that the Arabidopsis leaf experiences a developmental switch from a metabolic to a hydraulic control of growth (Pantin *et al.*, 2011). We illustrate thereafter how developmental changes in water and carbon relations could define an ontogeny-dependent control of leaf growth in different plant species.

1. A demonstration by example: insights from CAM species

Plants fitted with the crassulacean acid metabolism (CAM) magnify the interaction between ontogeny, hydraulics and metabolics on the control of leaf growth. Crassulacean acid metabolism emerged several times during evolution in response to hydraulic-selective pressures (Borland *et al.*, 2011). This mode of photosynthesis is mainly characterized by a time lag between light harvested during the day and stomatal uptake of CO_2 at night, when evaporative demand lowers. This mechanism enables a substantial water economy by avoiding excessive water loss by transpiration during the day. In some species, either environmental or developmental conditions are able to trigger a metabolism switch from C_3 to CAM to optimize carbon and water balance with regard to plant growth (Walter *et al.*, 2008; Borland *et al.*, 2011). In facultative CAM species, leaves operate routinely in C_3 mode and induce CAM under adverse environmental conditions, especially drought stress. In other species, CAM is induced by leaf age. Remarkably, this metabolic switch translates into a switch in nycthemeral leaf

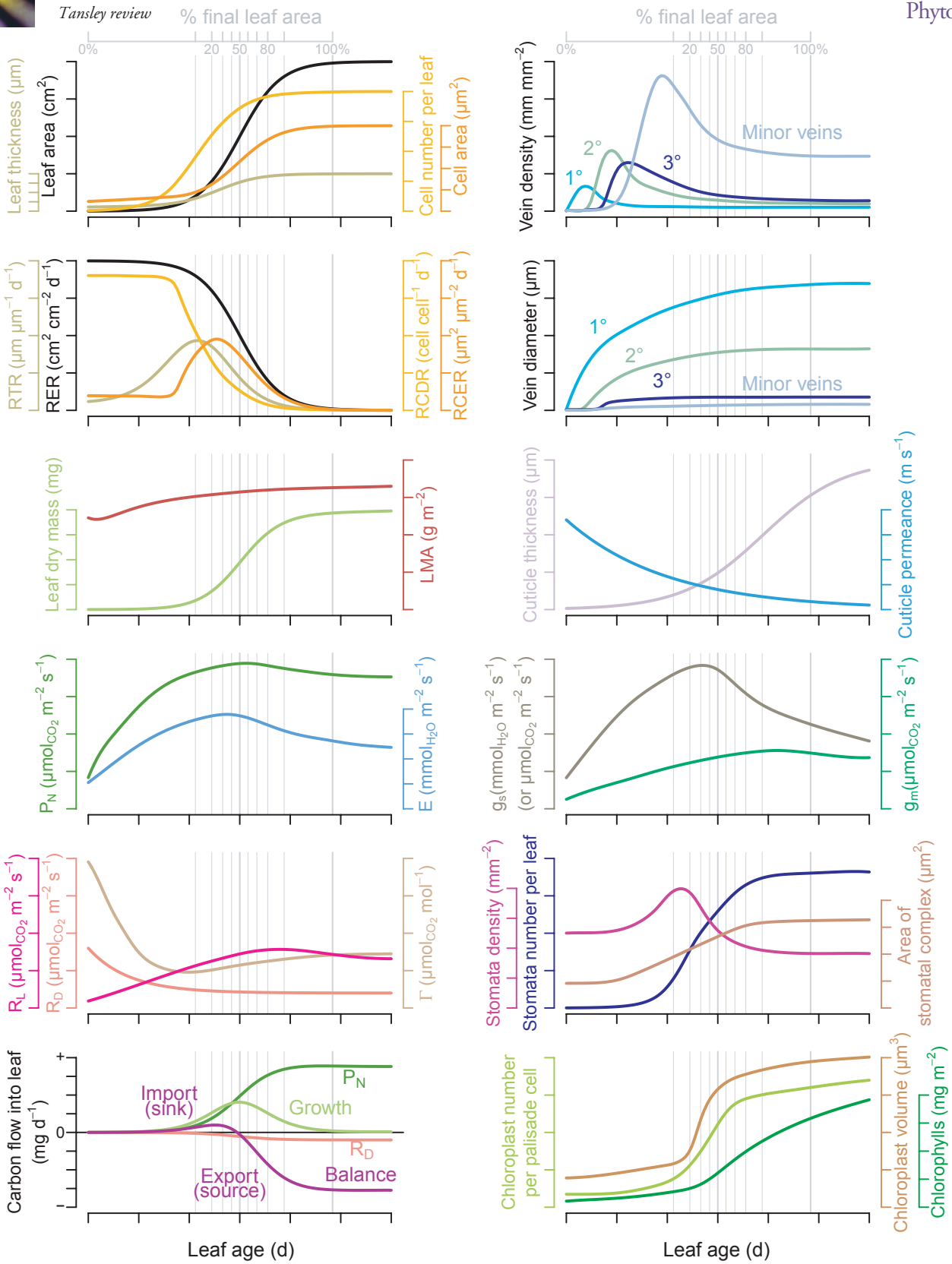

Fig. 3 Timeline of anatomical, developmental and physiological events occurring during leaf ontogeny. These curves were drawn to synthesize data on a wide range of species obtained from the references cited in the text, especially key reviews, meta-analysis or monograph (Šesták, 1985; Kriedemann, 1986; Turgeon, 1989; Granier & Tardieu, 2009; Sack *et al.*, 2012). This timeline does not represent processes related to senescence. See Section V 'Leaf ontogeny orchestrates the actors involved in the control of leaf growth' for more details. RER, relative leaf expansion rate; RTR, relative leaf thickening rate; RCDR, relative cell division rate; RCER, relative cell expansion rate; LMA, leaf mass per unit leaf area; P_N, net photosynthesis; E, transpiration; R_D, dark (night) respiration; R_L, light (photo)respiration; Γ, CO_2 compensation point; 1°, 2°, 3°, veins of the first, second, and third order; g_s, stomatal conductance for H_2O or CO_2; g_m, mesophyll conductance for CO_2.

growth patterns. For example, when the switch from C_3 to CAM is induced either by drought stress in *Clusia minor* or by ontogeny in *Clusia alata*, leaf growth switches from a higher leaf growth rate at night to a higher leaf growth rate during the day (Walter *et al.*, 2008), following changes in water relations induced by movements of stomata. As the timing of stomata opening in CAM species is first an adaptation to arid environments, as in *C. minor* under water stress, it is tempting to speculate that the developmental induction of CAM in *C. alata* arises from the ontogenetic emergence of hydraulic constraints, while the C_3 mode would be used when juvenile to favour efficient carbon gain and support structural growth. Interestingly, in leaves of *Mesembryanthemum crystallinum*, the day/night abundance pattern of some aquaporin isoforms coordinates with the developmentally triggered CAM, so that the cell water permeability balances the nycthemeral changes in osmotic potential induced by CAM (Vera-Estrella *et al.*, 2012). These results obtained in facultative CAM are consistent with the conclusion of Pantin *et al.* (2011) in Arabidopsis that the control of leaf growth switches from metabolics to hydraulics during the course of its ontogeny.

2. Leaf carbon balance and the sink-to-source transition

That the importance of carbon in the control of leaf growth would decrease during leaf ontogeny makes sense in the light of the sink-to-source transition, that is, the moment when a growing leaf becomes a net carbon exporter. This positive carbon balance is the result of both an increase in carbon supply through photosynthesis and a decrease in carbon demand by growth and respiration (Turgeon, 1989). In monocots, the growing zone is virtually nonphotosynthetic and the source behaviour is acquired with maturation, resulting in a basipetal progression of the carbon balance from net export (tip) to net import (base) on the same leaf. This was recently depicted at the molecular level (Li *et al.*, 2010; Majeran *et al.*, 2010; Pick *et al.*, 2011), showing that the basal growing zone is characterized by transcripts and proteins associated with the establishment of the photosynthetic machinery and structural growth such as photoreceptors, chlorophyll precursors and secondary cell wall biosynthetic enzymes, while in the distal region the cellular machinery is strongly devoted to starch metabolism and photosynthesis reactions. In dicots, the sink-to-source transition occurs when the leaf reaches 30–60% of its final size (Turgeon, 1989; Fig. 3). This transition is accompanied by changes in central metabolism, enzymatic machinery, phloem structure and other anatomical traits to favour CO_2 net assimilation and carbohydrate export. Notably, according to the thorough monograph of Šesták (1985) encompassing a wide range of species, net photosynthesis per unit area or mass increases strongly during early expansion and peaks at 25–100% final leaf area, with a stronger and earlier decrease in annuals than in grasses or evergreens. Conversely, dark respiration decreases dramatically during the early stages of leaf development. Photorespiration follows roughly the trend of net photosynthesis while the CO_2 compensation point decreases dramatically during early ontogeny. This general pattern is concomitant with a gradual increase in mesophyll conductance to CO_2, an initial increase in stomatal conductance, as well as a later increase in chloroplast number and volume, and in pigments such as chlorophylls and carotenoids (Šesták, 1985; Fig. 3). Finally, during leaf expansion, the carboxylase activity of RuBisCO or phosphoenolpyruvate carboxylase (PEPc) as well as the amount of these enzymes parallel the ontogenetic changes in activities of photochemical reactions and in net photosynthesis (Šesták, 1985). Accordingly, transcripts related to photosynthesis increase at the end of the proliferating phase and remain high in mature leaves in Arabidopsis (Skirycz *et al.*, 2010; Andriankaja *et al.*, 2012). This ontogenetic sequence of events is well-coordinated so that the diffusional and biochemical limitations to photosynthesis share similar proportion throughout leaf expansion (Grassi & Magnani, 2005).

It is generally observed that carbohydrate supply affects cell division but not cell expansion, because the carbon requirement for leaf expansion is remarkably low compared with photosynthetic capacity of the leaf itself (Kriedemann, 1986). Moreover, under low carbon availability leaves are able to maintain expansive growth at the expense of dry matter deposition per unit area (Tardieu *et al.*, 1999). It makes sense that exceptions to this rule are likely to be very young leaves, where metabolic needs are maximized compared with photosynthetic capacity (Muller *et al.*, 2001; Pantin *et al.*, 2011). Any environmental, genetic or developmental perturbation that lowers carbon availability in the leaf also makes leaf expansion tightly dependent on carbon supply, for example, very low light or starch mutants in Arabidopsis (Wiese *et al.*, 2007; Pantin *et al.*, 2011), or a competition for carbon with other sinks in soybean (Wenkert *et al.*, 1978). Hence, the young leaf growth rate strongly depends on local carbohydrate availability, as in many other sink organs (Muller *et al.*, 2011). It is interesting to consider that sinks partly control photosynthesis of source leaves: a decrease in sink demand for assimilates leads to carbohydrate accumulation in phloem-loading sites of source leaves, which triggers a downregulation of photosynthesis (Fig. 4). The sink regulation of photosynthesis in source leaves occurs at several levels. For example, synthesis of endproducts, such as sucrose and starch, exerts a short-term metabolic feedback on photosynthesis, while carbohydrate contents interfere with the expression of photosynthetic genes (Paul & Foyer, 2001). In some experiments where sinks are removed from source leaves, photosynthesis is downregulated before carbohydrates accumulate, suggesting that photosynthesis is regulated by changes in sugar and starch turnover rather than assimilate contents (Nebauer *et al.*, 2011; in *Citrus*). An alternative hypothesis is that ABA accumulates in the source leaf following sink removal and triggers stomatal closure (Setter *et al.*, 1981; in soybean). Indeed, source leaves continuously feed young leaves with ABA via the phloem (Cornish & Zeevaart, 1984; in cocklebur). This suggests that source and sink leaves cross-talk to regulate not only photosynthesis but also stomatal conductance and water relations (Fig. 4). In line with this view, the gradual transition from sink to source during leaf ontogeny is thought to occur concurrently with the establishment of a hydraulic limitation (Pantin *et al.*, 2011). How hydraulics could establish during the course of leaf ontogeny is discussed in the next paragraphs.

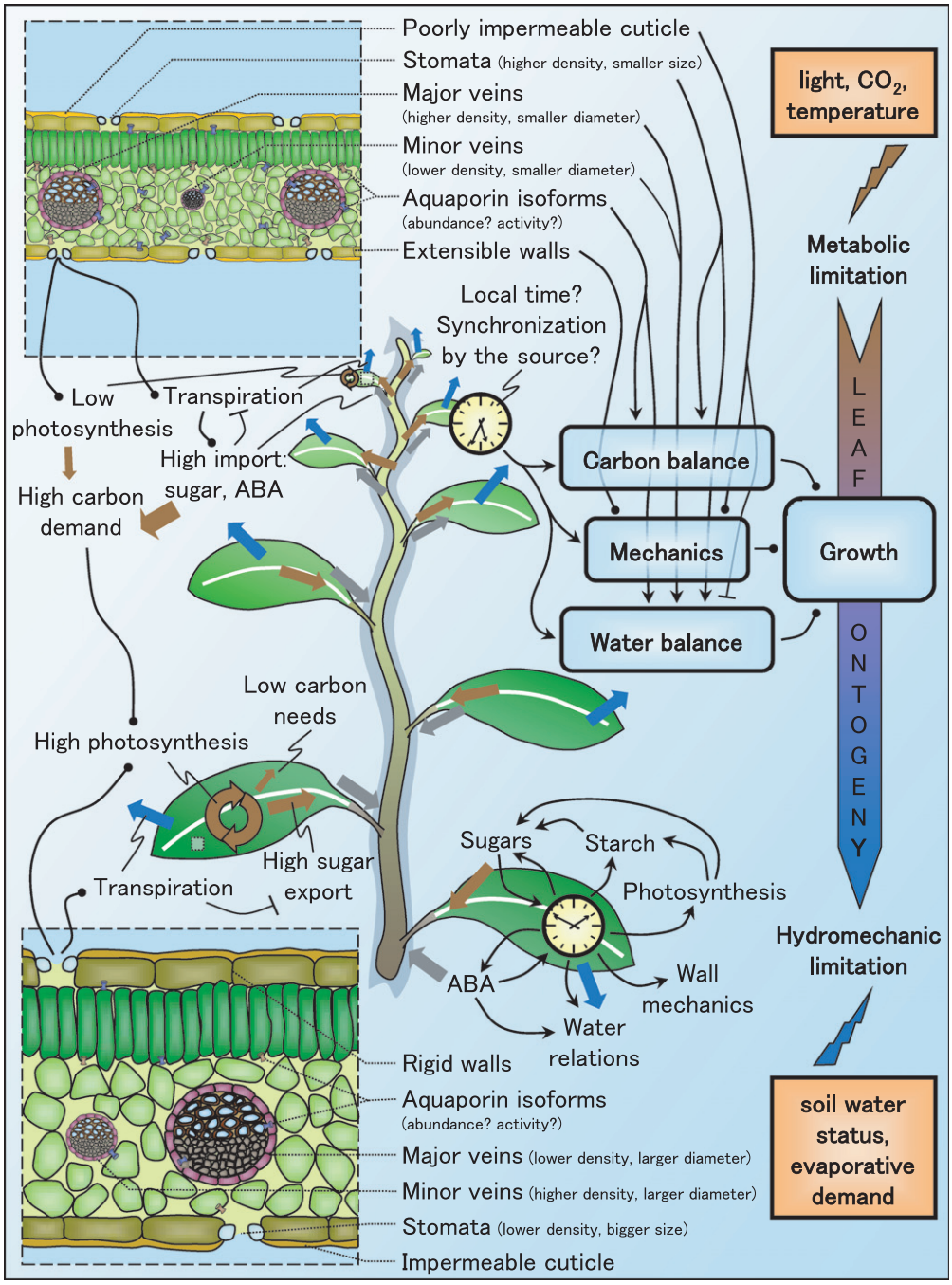

Fig. 4 Ontogenetic orchestration of the control of leaf growth. A growing shoot (here of a dicot plant) encompasses leaves of contrasting developmental stages. Basal, source leaves have a high photosynthesis and low carbon needs for maintenance; distal, sink leaves have a low photosynthetic capacity and high carbon requirements for respiration and growth (wall and cellular content synthesis). Sink demand for carbon stimulates source photosynthesis. Source leaves feed sink leaves with sugars (brown arrows) and with ABA (grey arrows) through the phloem. Transpiration (blue arrows) decreases the export and increases the import because of the hydraulic coupling between the xylem and the phloem. However, negative feedback is likely to occur in sink leaves where the imported ABA (from the phloem as well as the xylem) could tend to close stomata. The circadian clock interplays with the actors involved in the control of leaf growth: the clock interacts with sugars and ABA and more, generally, modulates carbon and water relations as well as wall rheology. Whether the clock of young leaves could differ from the clock of older leaves or be synchronized by imported products such as sugars or ABA is not known. The upper and lower insets on the left show a typical cross-section of a young leaf and a fully expanded leaf, respectively. In the young leaves, cell walls (as well as the cuticle) are more extensible than in older leaves, providing tissue mechanics fitted for leaf growth. Furthermore, the water balance is likely to be affected by a less impermeable cuticle. Ontogenetic changes of stomata, aquaporins and venation network have been described, but how these changes affect water and carbon balances remains unclear, partly because of the various magnitudes and directions of these changes. Overall, the control of leaf growth is characterized by an ontogenetic switch from a metabolic limitation to a hydromechanical limitation. Accordingly, young leaves are more sensitive to environmental factors affecting carbon status (light, CO_2, and putatively temperature), while older leaves are more sensitive to environmental factors affecting water status (soil and air water potentials). Lines headed with a point, a bar or an arrow represent promotion, inhibition or an equivocal/unknown influence, respectively. Compartments and fluxes are representational only and not to scale.

3. Ontogenetic changes in leaf venation and aquaporins accompany leaf expansion

Water flux per leaf increases in pace with development of leaf area. Thus, as a first approximation, leaf venation should closely follow leaf expansion for water supply to match transpiration. Accordingly, vascular networks are subjected to ontogenetic changes (Fig. 3). In most dicots, venation is constructed according to a hierarchical order during which the 1° vein or midvein differentiates acropetally, followed by the 2° veins which expand towards leaf margins and define the boundaries of the network; the 3° and minor veins finally confer the reticulate pattern (reviewed in Sack *et al.*, 2012). This pattern is tightly coordinated with cell division and mesophyll differentiation (Scarpella *et al.*, 2004; Kang *et al.*, 2007). As illustrated in Arabidopsis, the ontogenetic dynamics of the different vein orders overlap each other, with a peak in vein density as procambium forms followed by a dilution owing to leaf expansion, although the density of minor veins get stabilized during late expansion through a maintained initiation; meanwhile, each vein increases in diameter, especially in the two first orders (reviewed in Sack *et al.*, 2012; Fig. 3). This general developmental pattern accounts for the global scaling relationships between leaf size and traits of major veins (Sack *et al.*, 2012). By contrast, some species-specific variations in the formation of minor veins, for example, a decrease in minor vein density during late expansion (on five Solanaceous species, Gupta, 1961; on *Trifolium repens*, Denne, 1966), uncouple leaf size from the density of minor veins across species. If the ontogenetic pattern in vein density is well-described, knowledge about its functioning is far more sporadic. Leaf hydraulic conductance decreases during the expansion phase of the horse-chestnut leaf (Nardini *et al.*, 2010) while it increases in poplar (Aasamaa *et al.*, 2005), but data on the very early stages are lacking in both studies.

The temporal framework observed in dicots translates into a spatial framework in monocots. Thus, in tall fescue, axial hydraulic conductivity increases throughout the growing zone and decreases as soon as the leaf is exposed to light in relation to xylem maturation (Martre *et al.*, 2001). Interestingly, the capacity of the extra-xylem pathway to transport water could coordinate with the xylem properties. In barley, the hydraulic conductivity of epidermis and mesophyll protoplasts is higher in growing than in non-growing tissues (Volkov *et al.*, 2007). Concomitant changes in aquaporins could also be involved: in monocots, differential patterns in expression of several aquaporin isoforms are observed among developmental stages of a leaf, suggesting specific roles yet to be discovered for some isoforms during leaf ontogeny (Wei *et al.*, 2007; Hachez *et al.*, 2008; Besse *et al.*, 2011; Fig. 4).

4. Adjustment of water efflux: stomata and cuticle

If water supply is actively adjusted during leaf ontogeny, water efflux from either stomata or cuticle also shows a dynamic developmental pattern. From a hydraulic viewpoint, transpiration competes with expansive growth for water (Figs 1, 2). However, possible benefits of transpiration for the growing leaf could be that high transpiration flux carries more nutrients (Cramer *et al.*, 2008) and favours phloem assimilate import (Lacointe & Minchin, 2008; Fig. 4). Thus, the possible benefit for leaf growth of a given transpiration pattern should be interpreted carefully. The general ontogenetic trend for transpiration shows a pattern similar to net photosynthesis and follows the changes in stomatal conductance, with an early increase and a peak at 25–100% final leaf area (Constable & Rawson, 1980; Tichá *et al.*, 1985; Fig. 3). Although generally well-coordinated, the peaks in net photosynthesis and stomatal conductance can be partly uncoupled (Čatský *et al.*, 1985). Moreover, contrary to what is observed across species in mature leaves, there is no clear developmental relationship between stomatal conductance and stomatal density, which peaks before stomatal conductance at 10–60% of final leaf area, before a rapid dilution owing to leaf expansion (Tichá, 1985; Fig. 3). This suggests that stomatal functioning is acquired with leaf ontogeny. In line with this, the ontogenetic decrease in stomatal density is balanced by an increase in the size of stomatal complex and the area of stomatal pore (Tichá, 1985; Kheibarshekan Asl *et al.*, 2011; Fig. 3), as well as an increase in K^+ in guard cells (Pappas *et al.*, 1988). Thus, mature leaves have generally a higher stomatal conductance than young leaves where the stomata are typically more dense but occluded or underdeveloped (Snider *et al.*, 2009). Finally, the boundary layer conductance may also vary dramatically during the course of leaf ontogeny because of changes in size, exposure and surface roughness of the leaf, but the huge differences in these variables between species as well as the scarcity of available data prevent drawing conclusions about a general trend.

If plants actively regulate their water balance through stomatal movements, the leaf cuticle acts as the major barrier to passive water loss (Kerstiens, 2006). Dramatic changes in cuticle composition and thickness occur during leaf ontogeny. In monocots, where the growing zone is enclosed by sheaths of older leaves, cutin deposition parallels cell elongation, while the spatiotemporal pattern of cuticular wax deposition is regulated to anticipate the moment when epidermal cells are exposed to the atmosphere, as in barley (Richardson *et al.*, 2007) or maize (Hachez *et al.*, 2008). In dicots, where leaf expansion occurs while tissues are exposed to the atmosphere, the cuticle thickens throughout leaf development because synthesis and deposition of both cutin and waxes are well coordinated with cell expansion, while wax composition changes continually, as seen in a broad range of species (Hauke & Schreiber, 1998). In both monocots and dicots, cuticle thickening as well as accumulation of waxes during the course of leaf expansion coincides with a decrease in cuticular permeance (Hauke & Schreiber, 1998; Richardson *et al.*, 2007; Fig. 3). In line with this, the response to osmotic stress of genes involved in cuticle biosynthesis is differentially modulated by leaf developmental stage in Arabidopsis (Skirycz *et al.*, 2010). Thus, the dynamic behaviour of the cuticle throughout leaf ontogeny is likely to influence water relations of the growing leaf.

5. Coordination between leaf rheology and leaf developmental program

The ontogenetic changes in water balance result in ontogenetic changes in leaf turgor, as illustrated in maize (Bouchabké *et al.*,

2006) or Arabidopsis (Pantin *et al.*, 2011). However, a direct conclusion on the role of turgor in relation to growth is difficult because leaf rheology also changes as the leaf ages (Fig. 4). In *Robinia pseudoacacia*, turgor increases during the course of leaf expansion but extensibility decreases while yield threshold increases, leading to a developmental decrease in leaf growth rate (Zhang *et al.*, 2011). At the molecular level, genes associated with wall properties are differentially expressed in Arabidopsis leaves of contrasting ages (Cho & Cosgrove, 2000; Skirycz *et al.*, 2010). Similarly, in maize, developmental expression patterns of expansins correlate with growth rates of leaf segments of different ages whatever the experimental condition, suggesting that expansins are downstream, unspecific targets of a range of developmental, environmental and genetic sources of variation (Muller *et al.*, 2007). In barley, plasma membrane H^+–ATPase activity is enhanced in the elongating zone of the leaf, suggesting that the apoplast is locally acidified to facilitate turgor-induced cell wall deformation (Visnovitz *et al.*, 2012). Moreover, genes and proteins associated with wall synthesis are differentially regulated along the developmental gradient of maize leaves (Li *et al.*, 2010; Majeran *et al.*, 2010), while in aspen leaves, metabolic markers of cell wall maturation relate to the sink-to-source transition (Jeong *et al.*, 2004). Finally, the cuticle itself strengthens and stiffens during leaf ontogeny and may gradually constrain expansion (Takahashi *et al.*, 2012). These results support the view that leaf rheology is tightly adjusted during the course of leaf ontogeny to coordinate its growth rate with its developmental programme, including the dynamics of its hydraulic and metabolic limitations.

6. Leaf ontogeny reprograms the circadian clock

The ontogenetic gradients in leaf growth patterns arising from these developmental changes in hydromechanical and metabolic limitations imply that several growth rhythms coexist in plants where leaves of several developmental stages are present. This brings into question the role of the circadian clock in the central regulation of such a system. Experiments on Arabidopsis hypocotyls (Nusinow *et al.*, 2011), roots (Yazdanbakhsh *et al.*, 2011) or leaves (Poiré *et al.*, 2010) strongly support the view that the clock is a major actor in the control of growth rhythms. The clock tunes the transcription of genes involved in photosynthesis, carbon metabolism, water influx, wall mechanics (Harmer *et al.*, 2000), ABA metabolism (Fukushima *et al.*, 2009) and ABA response (Mizuno & Yamashino, 2008). We propose that the clock orchestrates the actors of leaf growth in a differential manner throughout leaf ontogeny by anticipating metabolic and hydraulic constraints (Fig. 4). In support of this hypothesis, genes related to the circadian photoperiod are differentially expressed along the developmental gradient of the maize leaf (Li *et al.*, 2010). Stomatal conductance and photosynthesis (Arabidopsis, Dodd *et al.*, 2005), leaf starch breakdown (Arabidopsis, Graf *et al.*, 2010), leaf hydraulic conductivity (sunflower, Nardini *et al.*, 2005), aquaporin expression, hydraulic conductivity and water distribution in roots (*Lotus japonicus*, Henzler *et al.*, 1999; Arabidopsis, Takase *et al.*, 2011), all have been shown to oscillate with the clock. Interestingly, the shoot clock synchronizes the root clock (a simplified version of the shoot clock) by a photosynthesis-related signal (James *et al.*, 2008). Although very speculative, it is tempting to generalize this relationship to other sink organs such as young leaves. Because changes in carbon and water relations occur during leaf ontogeny (see sections V.2 'Leaf carbon balance and the sink-to-source transition' to V.4 'Adjustment of water efflux: stomata and cuticle') and because the clock itself is regulated by sugars (Dalchau *et al.*, 2011) and ABA (Legnaioli *et al.*, 2009), both sugars and ABA could modulate the influence of the clock on leaf growth throughout leaf development. Furthermore, both sugars and ABA allow communication between leaves of contrasting ages because both compounds move through the phloem (Fig. 4). Finally, the involvement of the mobile flowering regulator FT in circadian movements of stomata and its expression in vasculature (Hubbard & Webb, 2011) also make it a putative candidate able to mediate differential coordination of water and carbon fluxes by the clock during leaf ontogeny.

VI. The growing leaf in a changing world

The partially uncoupled changes in carbon and water relations experienced by the growing leaf as it develops make it differentially sensitive to environmental stresses throughout its ontogeny. Furthermore, young and mature leaves cross-talk to adjust their respective response to environmental stresses. For example, systemic signals from mature leaves exposed to elevated CO_2, shading or low humidity trigger decreases in stomatal density of new, nonexposed leaves in Arabidopsis or poplar (Lake *et al.*, 2001; Coupe *et al.*, 2006; Miyazawa *et al.*, 2006), in a mechanism putatively involving sugar signalling, ABA, and transpiration rate itself (Lake *et al.*, 2002; Lake & Woodward, 2008). We show in this section how the effects on leaf growth of those environmental stresses challenging water or carbon status are conditional on leaf ontogeny.

1. Young leaves strongly rely on light and CO_2: sink sensitivity to carbon starvation

Because structural growth of young leaves requires a net import of carbon before the sink-to-source transition (see section V.2 'Leaf carbon balance and the sink-to-source transition'), environmental inputs affecting carbon balance have stronger impacts at early stages of leaf ontogeny (Fig. 4). The sensitivity of young leaves to assimilate supply is illustrated in shading experiments where young leaves exhibit a marked reduction of growth, unlike older leaves (Granier & Tardieu, 1999; Tardieu *et al.*, 1999; Muller *et al.*, 2001; Cookson & Granier, 2006). Consistent with this, leaves of several species grown under high CO_2 grow faster, especially during the early exponential growth phase (Kriedemann & Wong, 1984; Ferris *et al.*, 2001) and effects are visible at the transcriptional level (Ainsworth *et al.*, 2006; in soybean). In line with this, leaves acclimatize to elevated CO_2 early in their ontogeny. For example, when sugarcane or grain sorghum plants are subjected to high CO_2, photosynthesis and central metabolism are upregulated early in leaf ontogeny and decline to levels comparable to those in control conditions at later stages (Vu *et al.*,

2006; Prasad *et al.*, 2009). Photosynthesis of tobacco leaves exposed to elevated CO_2 reaches a maximum while leaves are still expanding and declines with leaf ageing earlier than in control plants, accounting for the downregulation of photosynthesis generally observed in mature leaves after long-term exposure to high CO_2 concentrations (Miller *et al.*, 1997). The effect of high CO_2 may also be dependent upon the capacity of the source leaf to export assimilates at higher rates. In *Ricinus communis*, the phloem loading system of source leaves is saturated during the day under either ambient or elevated CO_2 (sink limitation), while the export rate at night is reduced in plants under ambient CO_2 (source limitation) (Grimmer & Komor, 1999). The benefit of elevated CO_2 to leaf growth may not be mediated by a sole substrate effect. Indeed, wall extensibility at elevated CO_2 is increased in the young leaves but not in older leaves of poplar (Taylor *et al.*, 2003). Finally, considering that high temperature can induce carbon starvation (Vasseur *et al.*, 2011), elevated CO_2 and supra-optimal temperature are expected to have opposite effects on the growth of young leaves. This point deserves particular interest in a world where both atmospheric CO_2 and temperature are continuously rising.

2. A crosstalk between leaves favours drought tolerance in the young leaf

It is commonplace to observe that when exposed to severe water deficit, young leaves start to wilt after older leaves and keep the capacity to boost their expansion rate when water is resupplied (Rawson & Turner, 1982; Fig. 4). Young leaves also accumulate more ABA than older leaves because of high import from source leaves combined with a low catabolism but, paradoxically, their stomatal conductance remains high and their water potential therefore becomes very low (Jordan *et al.*, 1975; Raschke & Zeevaart, 1976; Sivakumaran & Hall, 1978; Cornish & Zeevaart, 1984). That the leaf response to ABA would be impaired in young leaves is consistent with a preferential transcription in mature leaves of the genes related to ABA signalling, as shown in Arabidopsis under osmotic stress (Skirycz *et al.*, 2010). This ontogenetic gradient in leaf response to ABA could be related to acclimatization mechanisms, as some events in leaf history, such as early exposure to stress or ABA, modify the current ability of growth and stomata to respond to changes in leaf water status (Atkinson *et al.*, 1989; Pospíšilová, 1996; Fanourakis *et al.*, 2011). How could turgor be maintained in young leaves under water stress, despite a high stomatal conductance and a low water potential? We propose that maintaining transpiration only in the young, small leaves would favour solute translocation and osmotic adjustment therein, while minimizing water loss from the whole plant. Beyond these hydraulic relationships between source and sink leaves, mechanisms facilitating stress recovery and preventing ABA-induced senescence in the young leaves (Sivakumaran & Hall, 1978; Cornish & Zeevaart, 1984; Atkinson *et al.*, 1989; Lee *et al.*, 2011) could also reconcile ABA insensitivity and drought tolerance. From the above rationale, we raise the hypothesis that these cross-talks between young and older leaves confer an adaptive advantage to young leaves under water stress.

Given the negative effect of water deficit on photosynthesis, the conclusion that young leaves are more drought-tolerant than older leaves may seem contradictory with the fact that young leaves are more sensitive to carbon starvation (see section VI.1 'Young leaves strongly rely on light and CO_2: sink sensitivity to carbon starvation'). However, because drought affects growth long before photosynthesis, plants are generally not carbon-limited at the beginning of soil drying and growth becomes uncoupled from carbohydrate availability in sink organs (Muller *et al.*, 2011). Sugars in excess can be diverted to contribute to osmotic adjustment at low cost (Hummel *et al.*, 2010), with a priority to young leaves that may result, for example, from differential activity of invertases between source and sink leaves (Kim *et al.*, 2000; Luquet *et al.*, 2008). Accordingly, expanding leaves under water stress develop source features earlier in their ontogeny than leaves of well-watered plants (Schurr *et al.*, 2000). This time-lag of sensitivity to drought between growth and photosynthesis releases young leaves from their strong dependence on carbon availability, solving the apparent paradox that young leaves are more drought-tolerant than adult leaves, although they are more susceptible to carbon supply.

VII. Conclusion

Leaf growth can be envisioned as a dual increment in water volume and in dry matter, two processes tightly coupled with the expansive growth and the structural growth. Analyses of the determinisms of water relations, tissue rheology and carbon partitioning at various scales have shown several interaction nodes between each other. First, transport systems for water and carbon within the plant share some key components: stomata arbitrate the trade-off between carbon gain and water loss; the aquaporin family comprises isoforms able to facilitate the transport of water or CO_2; and the xylem is hydraulically coupled with the phloem. Second, the leaf water transport capacity is coordinated with its photosynthetic capacity, a property that can be considered to be the result of an evolutionary driven trade-off between the space-filling property of leaf venation and the carbon construction costs of veins. This translates into a crosstalk in the development of veins and stomata. Third, the Lockhart hydromechanical model accounting for volumetric growth has potentially interesting connections with carbon metabolism, including osmotic adjustment and wall biochemistry. Fourth, the drought hormone ABA has a central position in regulating water and carbon fluxes, by acting on stomata and aquaporins, flowing in every part of the plant through both xylem and phloem, and interfering with sugar sensing.

Acknowledging this complexity sheds light on the controversial nature of the debate about the main limitation of leaf growth. Certainly related to this is the active debate on the relative contribution of hydraulic and metabolic limitations to tree survival under severe drought episodes (McDowell, 2011). Here, we argue that leaf ontogeny has to be taken into account to study the influence on leaf growth of hydraulics, mechanics and metabolics. Source–sink relationships provide a cornerstone to address ontogenetic changes in leaf carbon balance. Water relations are likely to be ontogenetically modulated through functional changes in xylem

network, stomata and aquaporins. Leaf rheology is modified during the course of leaf ontogeny through changes in biochemical properties of the walls, driven by the activity of key enzymes and proteins. The circadian clock, which exerts control over carbon partitioning, water relations and wall mechanics, could display some asynchrony between the young, sink leaves and the older, source leaves. The regulation of long-distance transport of sugars and ABA is a central question to tackle because these compounds interplay with numerous elemental processes involved in either expansive or structural leaf growth. Overall, we defend the view that the control of leaf growth switches from a metabolic limitation to a hydromechanical limitation during the course of leaf ontogeny. Finally, we believe that considering leaf ontogeny will greatly improve our knowledge of the mechanisms underlying leaf growth responses to the abiotic stresses that affect water and carbon status, such as those forecasted for crops and wild plants in our changing world.

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