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REVIEW PAPER

Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs

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Abstract

In plants, carbon (C) molecules provide building blocks for biomass production, fuel for energy, and exert signalling roles to shape development and metabolism. Accordingly, plant growth is well correlated with light interception and energy conversion through photosynthesis. Because water deficits close stomata and thus reduce C entry, it has been hypothesised that droughted plants are under C starvation and their growth under C limitation. In this review, these points are questioned by combining literature review with experimental and modelling illustrations in various plant organs and species. First, converging evidence is gathered from the literature that water deficit generally increases C concentration in plant organs. The hypothesis is raised that this could be due to organ expansion (as a major C sink) being affected earlier and more intensively than photosynthesis (C source) and metabolism. How such an increase is likely to interact with C signalling is not known. Hence, the literature is reviewed for possible links between C and stress signalling that could take part in this interaction. Finally, the possible impact of water deficit-induced C accumulation on growth is questioned for various sink organs of several species by combining published as well as new experimental data or data generated using a modelling approach. To this aim, robust correlations between C availability and sink organ growth are reported in the absence of water deficit. Under water deficit, relationships weaken or are modified suggesting release of the influence of C availability on sink organ growth. These results are interpreted as the signature of a transition from source to sink growth limitation under water deficit.

Key words: C metabolism, C signalling, growth, model, sink limitation, source limitation, starch, sugar, water deficit.

Introduction

Plant growth and carbon (C) metabolism are intimately connected, as carbohydrates generated by photosynthesis provide building blocks and energy for the production and maintenance of biomass. Furthermore, carbohydrates are known to exert tight control over a wide range of processes including transcriptional, post-transcriptional and post-translational mechanisms (Koch, 1996; Rolland *et al.*, 2006). On a much broader scale, biomass accumulation in a crop is a linear and remarkably stable function of light intercepted by the canopy and its transformation into dry

Abbreviations: ABA, abscisic acid; bZIP, basic leucine zipper; HXK1, hexokinase; PEG, polyethylene glycol; RER, relative expansion rate; RGR, relative growth rate; VPD, vapour pressure deficit.

matter through photosynthesis (Monteith, 1965), which implies that plant growth relies on C fluxes. Because water deficit induces stomatal closure and thus reduces photosynthesis, it has been suggested that it negatively affects plant C status by impairing C metabolism (e.g. Chaves *et al.*, 2009), ultimately promoting growth failure due to C starvation (Boyle *et al.*, 1991). This article thus aims to question the impact of water deficits on the C status of plant organs and the consequences of these alterations on C signalling and sink organ growth that, in the absence of stress, strongly depends on C supply.

Soil water deficit leads to C accumulation

Contrary to the prediction made that water deficit would induce C starvation, literature converges to support the conclusion that C compounds most often accumulate in organs resulting in increased C concentrations. Such accumulation under water deficit has been reported in several species, various plant parts, and for different (i.e. soluble or structural) C forms. Soluble carbohydrate concentrations increase under water deficit in the leaves of maize (Kim *et al.*, 2000), cotton (Timpa *et al.*, 1986), barley (Teulat *et al.*, 2001), eucalyptus and sorghum (Turner *et al.*, 1978), lupin and eucalyptus (Quick *et al.*, 1992), pine (Marron *et al.*, 2003), poplar (Bogeat-Triboulot *et al.*, 2007), and grapevine (Cramer *et al.*, 2007). Carbohydrates also accumulate in stems (Bogeat-Triboulot *et al.*, 2007), flowers, and fruits (Liu *et al.*, 2004; McLaughlin and Boyer, 2004; Mercier *et al.*, 2009), as well as in roots (Sharp *et al.*, 1990; Jiang and Huang, 2001). Accumulation occurs both after rapid osmotic shocks, e.g. using polyethylene glycol (PEG) or mannitol (Zrenner and Stitt, 1991), and during slowly developing water deficit (Cramer *et al.*, 2007; Hummel *et al.*, 2010).

Carbohydrates often accumulate in the form of abundant sugars such as hexoses and sucrose (references above). However, a wider range of C-rich compounds may also accumulate in response to soil water deficit. These include minor sugars such as trehalose (Farías-Rodríguez *et al.*, 1998) or mannitol (Guicherd *et al.*, 1997), amino acids (Morgan, 1992), in particular those with a high C/N ratio such as proline (Hare and Cress, 1997), or pipecolic acid (Barnett and Naylor, 1966). Organic acids such as malate (Franco *et al.*, 2006), fumarate (Hummel *et al.*, 2010), or citrate (Timpa *et al.*, 1986) also accumulate in response to water deficit in a range of species including *Arabidopsis* (Hummel *et al.*, 2010). Quaternary ammonium compounds such as glycine betaine, which accumulate in particular species or families (Ashraf and Foolad, 2007; Gagneul *et al.*, 2007), may also be seen as C-rich compounds, as a quaternary ammonium results from the substitution of three protons with three alkyl groups on an amine residue (Rhodes and Hanson, 1993). Many such compounds are considered to be 'compatible solutes', as they can accumulate in large amounts without perturbing cell functions, and are thought to protect subcellular structures against the deleterious effects of cell water loss. This has motivated considerable research, in particular the genetic engineering of pathways producing these compounds in order to increase drought tolerance (Bohnert and Jensen, 1996; Rathinasabapathi, 2000; Sakamoto and Murata, 2000). However, because most of these compounds accumulate under extreme stress leading to desiccation, the relevance of this strategy has been questioned for agricultural situations where it is not crop survival but crop productivity that is critical (e.g. Tardieu, 1996; Serraj and Sinclair, 2002).

Structural C-rich compounds such as cellulose and lignin also accumulate under water deficit. Indeed, water deficit accelerates lignification (Timpa *et al.*, 1986; Vincent *et al.*, 2005), decreases leaf intercellular air spaces (Hsiao and Acevedo, 1974), and increases leaf thickness (Hummel *et al.*, 2010). All these responses contribute to the widely reported increase in specific leaf mass occurring under water deficit (see Tardieu *et al.*, 1999 and references therein).

Soil water deficit uncouples photosynthesis and growth while C metabolism is often maintained or increased

The increase in C concentration in organs of plants under water deficit must originate from some uncoupling between C supply and demand. Accordingly, the photosynthesis (C supply) of the *Arabidopsis* rosette is resilient to even severe water deficit while leaf expansion (accounting for a major part of C demand) is strongly reduced by stress, as quantitatively argued by Hummel *et al.* (2010). The maintenance of photosynthesis under water deficit has been repeatedly reported (Boyer, 1970*b*; Quick *et al.*, 1992; Bogeat-Triboulot *et al.*, 2007). The mesophylic component of CO_2 capture is particularly resilient to water deficit (Kaiser, 1987; Cornic, 2000; Flexas and Medrano, 2002). For instance, Rubisco activity is maintained even when leaf relative water content drops to 50% while stomata are already 75% closed (Kaiser 1987; Flexas *et al.*, 2006). In contrast, water deficit strongly reduces leaf or shoot expansion rates (Boyer, 1970*a*; Hsiao, 1973; Ben Haj Salah and Tardieu, 1997; Tardieu *et al.*, 1999, 2000). Analysis of published work on different plant species in which plant growth and photosynthesis were measured under a range of water deficits is shown in Fig. 1. The common feature in all species is that C demand (growth) always decays before C supply (photosynthesis) is affected by water deficit. Though this analysis does not consider other C demands such as respiration or root growth, it clearly illustrates the large domain of water deficits in which C may be present in excess in the plant.

The impact of water deficit on C metabolism has been the matter of numerous studies. They report in some cases that the enzymes involved show signs of down-regulation (Chaves *et al.*, 2009) but more often support the view of maintained or increased metabolic activity. For instance, sucrose cleaving enzymes increase their activity upon water

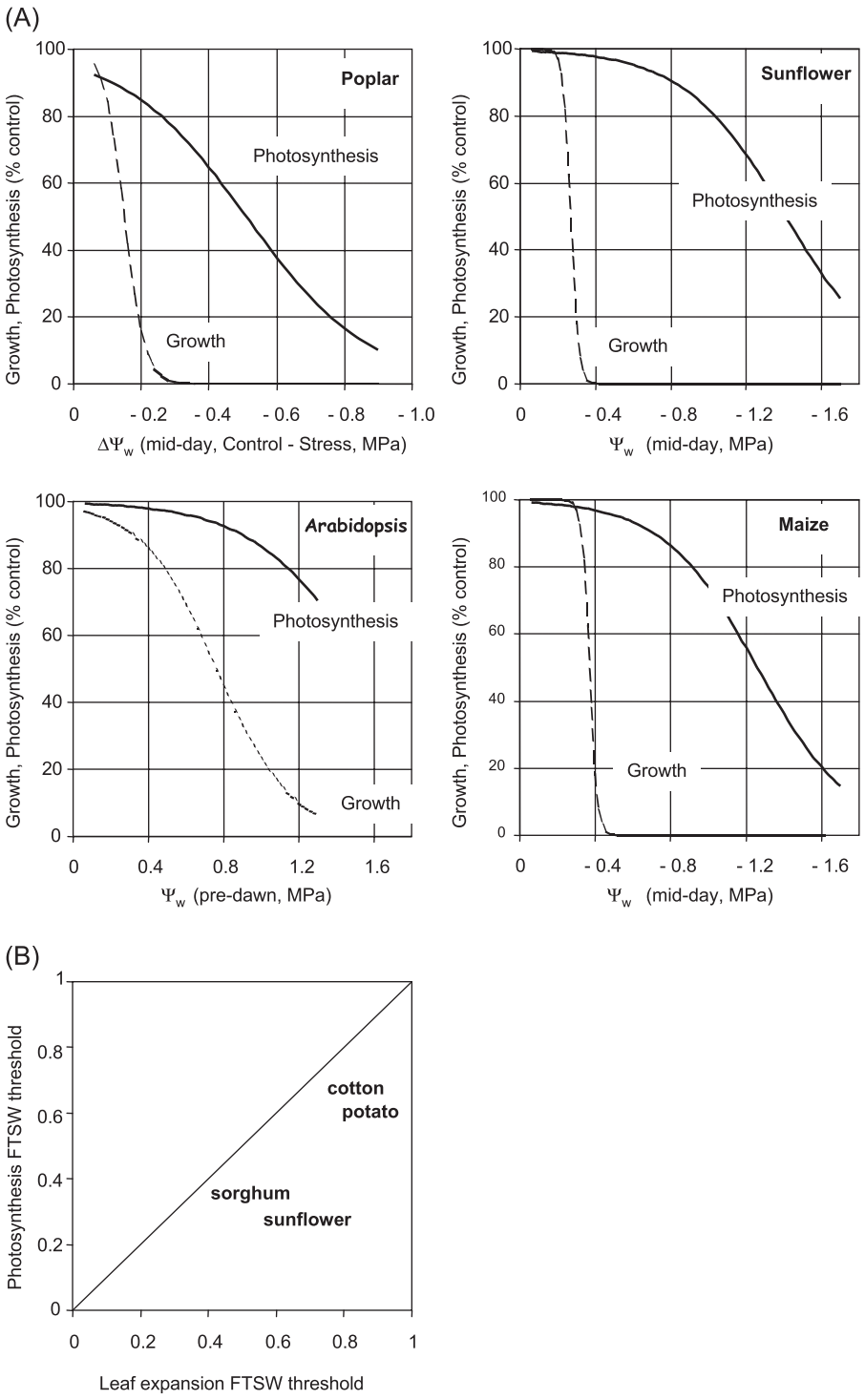

Fig. 1. Differential sensitivity of shoot growth and photosynthesis to soil water deficit. (A) Data originate from Bogeat-Triboullot *et al.* (2007) for poplar, Boyer (1970*a*) and Tardieu *et al.* (1999) for sunflower and maize, and Granier *et al.* (2006) and Hummel *et al.* (2010) for *Arabidopsis*. The *x*-axis refers to the level of water deficit expressed as midday leaf water potential difference between stressed and non-stressed plants (poplar), midday leaf water potential (maize, sunflower), pre-dawn water potential of the whole rosette (*Arabidopsis*). The *y* axis refers to both photosynthesis (A, plain lines) and organ growth (Growth, dashed line). Both are shown as a percentage of the well-watered control. Growth is expressed as shoot height increase in poplar, rosette size at bolting in *Arabidopsis*, and leaf elongation rate during day periods in maize and sunflower. In all cases, sigmoidal curves $\{y=100/[1/(1+\exp(-(\psi-\psi 0)/b]\}$ were adjusted to the data or available published curve. Note that the *x* and *y* axes do not always refer to the same variables. This figure should thus not be used to compare species sensitivities to soil water deficit. (B) Data originate from a survey performed by Sadras and Milroy (1996). They modelled leaf expansion and photosynthesis response as a function of water deficit using a bilinear model with a threshold defined as the fraction of available soil water (FTSW) at which each of these variables start to decline. Original references are found in Sadras and Milroy (1996). Only those examples from which both variables were measured in the same study by the same group are reported.

deficit in source leaves of cereals (Kim *et al.*, 2000), in pace with an increased need for osmotic adjustment in these leaves (McCree *et al.*, 1984) and a higher C demand by the seeds (Yang *et al.*, 2004). In the growing zone of maize leaves, the activities of several enzymes involved in glycolysis and TCA cycle increase (Riccardi *et al.*, 1998). In perennials such as poplar (Bogeat-Triboulot *et al.*, 2007) or grapevine (Cramer *et al.*, 2007), the triggering of particular metabolic pathways including C metabolism has been observed under moderate to severe water deficit. The activome of *Arabidopsis* plants subjected to various levels of water stress has recently been investigated by profiling a set of 30 enzymes from central C and N metabolism across rosette development (Hummel *et al.*, 2010). In most cases, enzyme activities were increased under water deficit, but these increases occurred slowly and were of low magnitude, suggesting that even in plants facing a 75% drop in aerial biomass production, there was no dramatic or specific reprogramming of metabolism.

While maintenance of C metabolism and increased C concentration in plant parts under water deficit have been reported in most studies, there are also some cases where the opposite is observed. This is notably the case when water deficit is so severe and prolonged that photosynthesis becomes inhibited over a long period. An important co-factor in that case is elevated temperature, which is usually associated with water deficit in nature and results in increased respiration, thereby negatively affecting the C status. This extreme scenario has been proposed to be responsible, along with hydraulic failure, for tree mortality under severe water deficit (McDowell *et al.*, 2008; McDowell and Sevanto, 2010).

C signalling and possible interactions with stress

Besides their roles as bricks for structure and fuel for energy production, soluble C compounds such as glucose and sucrose (Chiou and Bush, 1998; Laby *et al.*, 2000; Moore *et al.*, 2003; Huang *et al.*, 2008), but also phosphorylated intermediates (e.g. glucose-6-phosphate or trehalose-6-phosphate; Paul, 2007; Zhang *et al.*, 2009), play key signalling roles in the overall shaping of the metabolic and developmental machinery, through both gene expression and post-translational regulation. Because water deficits are likely to alter the concentration of these metabolites, it is important to understand how C and stress signalling are integrated.

Original support for signalling roles of sugars came from single gene expression analysis, pointing towards genes coding for enzymes directly involved in the utilization of C, such as sucrose synthase and invertase (Koch *et al.*, 1992; Ciereszko and Kleczkowski, 2002). Later, microarrays revealed that sugars influence the expression of hundreds of genes involved in a wide range of processes (Contento *et al.*, 2004; Price *et al.*, 2004; Thimm *et al.*, 2004; Thum *et al.*, 2004; Bläsing *et al.*, 2005; Li *et al.*, 2006). The cell cycle machinery is a key target of this control (Webster and Van't Hof, 1969; Riou-Khamlichi *et al.*, 2000). Furthermore, ribosomal proteins and genes involved in tRNA metabolism are among the functional categories that respond the most consistently, at the transcriptional level, to fluctuations in the C resource (Thimm *et al.*, 2004; Bläsing *et al.*, 2005; Osuna *et al.*, 2007). The fact that protein synthesis represents a major sink for energy (Penning de Vries, 1975) strengthens the idea that a tight link between C metabolism and protein synthesis is necessary to prevent acute C starvation (Smith and Stitt, 2007), especially in growing tissues where most of the protein synthesis contributes to building new biomass (Piques *et al.*, 2009). In line with this, considerably more genes have been found to respond to low sugar than to high sugar (Bläsing *et al.*, 2005). It has then been proposed that sugar sensing and signalling enable the avoidance of acute C starvation under a wide range of environmental conditions, thus maintaining the ability to grow under any circumstances (Smith and Stitt, 2007). Strikingly, experiments that led to these conclusions were performed under conditions where light (and thus C) was actually the only factor limiting growth, while environmental stresses were absent.

How C and (water) stress signalling may interact is just beginning to be revealed. Among the different sugar sensing systems proposed, the best known is a pathway involving hexokinase (HXK1), which has been found to interact with abscisic acid (ABA)-, ethylene-, auxin-, and cytokinin-signalling pathways, suggesting a central role in linking C status to stress responses (Rolland *et al.*, 2006). Another glucose sensor, which is located at the plasma membrane and coupled to a G-protein complex, has recently been found in *Arabidopsis* (Grigston *et al.*, 2008). G-protein signalling is also known to be involved in responses to various biotic and abiotic stresses (e.g. Nilson and Assmann, 2010). A further pathway, which is thought to sense various sugars including glucose-6-phosphate (Toroser *et al.*, 2000) and trehalose-6-phosphate (Schluepmann *et al.*, 2004; Zhang *et al.*, 2009), involves SnRK1 protein kinases, which can act on both gene expression and enzyme activity (Halford, 2006), and are also involved in hormone (in particular ABA) signalling. Finally, while no sucrose receptor has been found so far in plants, there is a sucrose-specific pathway, also involving SnRK1, leading to translational control of a basic leucine zipper (bZIP)-type transcription factor (Wiese *et al.*, 2004), by which sucrose represses the expression of various enzymes including proline dehydrogenase (Hanson *et al.*, 2008), which is also repressed under osmotic stress (Yoshiba *et al.*, 1997) and induced upon rehydration (Oono *et al.*, 2003).

Together, these results are indicative of a variety of means by which C and stress signalling could be integrated. Such a deep integration has been interpreted as resulting from the fact that most stresses would negatively affect the overall C and energy status of the plant (Baena-Gonzalez and Sheen, 2008). However, the analysis developed in the former sections tends to contradict this interpretation. One hypothesis is that such cross-talk could contribute to the bypass of critical signalling pathways, as an increase in

sugar availability provoked by water stress might otherwise be misleading. In line with this, initial mutant screens revealing such shared signalling pathways have been conducted with very high sugar concentrations (e.g. 6% w/v) ruling out the possibility that stressed plants were C starved (Arenas-Huerteroet *al.*, 2000; Huijser *et al.*, 2000; Laby *et al.*, 2000; Rook *et al.*, 2001). Understanding the way water deficit modulates C sensing and signalling therefore appears to be an important topic towards the understanding of plant performance under stressing conditions.

Water deficit differentially tunes the relationship between C availability and growth in sink organs

Growth and development of sink organs is known to be at least partly under the control of C availability. This has been repeatedly reported for roots (Aguirrezabal *et al.*, 1994; Thaler and Pagès, 1996; Freixes *et al.*, 2002; Willaume and Pagès, 2006), young leaves (Granier and Tardieu, 1999; Muller *et al.*, 2001), flowers (Guilioni *et al.*, 1997; Smith and Stitt, 2007), fruits (Borisjuk *et al.*, 2003; Liu *et al.*, 2004; Wu *et al.*, 2005), and seeds (Munier-Jolain and Ney, 1998; Munier-Jolain and Salon, 2003). Because water deficit increases C concentration and thus possibly C availability in plant tissues, it is important to understand the consequences for organ growth. The analysis performed in the next paragraphs is based on the occurrence of tight relationships between C availability and the expansion or the development of different sink organs. The rationale followed is to use the modification of these relationships as diagnostic of an alteration in the C dependence of growth.

In roots, sucrose unloaded from the phloem is rapidly cleaved by invertase (Hellebust and Forward, 1962; Giaquinta *et al.*, 1983) and/or by sucrose synthase (Martin *et al.*, 1993), which are highly abundant at the site of intense phloem unloading located in the middle of the growing zone (Oparka *et al.*, 1994). This leads to very low sucrose concentrations in the root zone showing rapid expansion (Sharp *et al.*, 1990; Muller *et al.*, 1998). Concentrations of hexose released from sucrose in the growing zone are therefore a good estimate of local C availability, as they depend on the balance between C inflow and utilization. Following this rationale, hexose concentration was evaluated in growing zones of single roots whose elongation rate had been measured during 24 h prior to sampling. In well-watered *Arabidopsis* plants exposed to various light intensities or supplied with external sugars, quantitative relationships between root elongation rate and hexose concentration were found for both primary and secondary roots (Freixes *et al.*, 2002 and Fig. 2). Remarkably, these relationships were robust enough to account for the variation between primary roots of different plants, as well

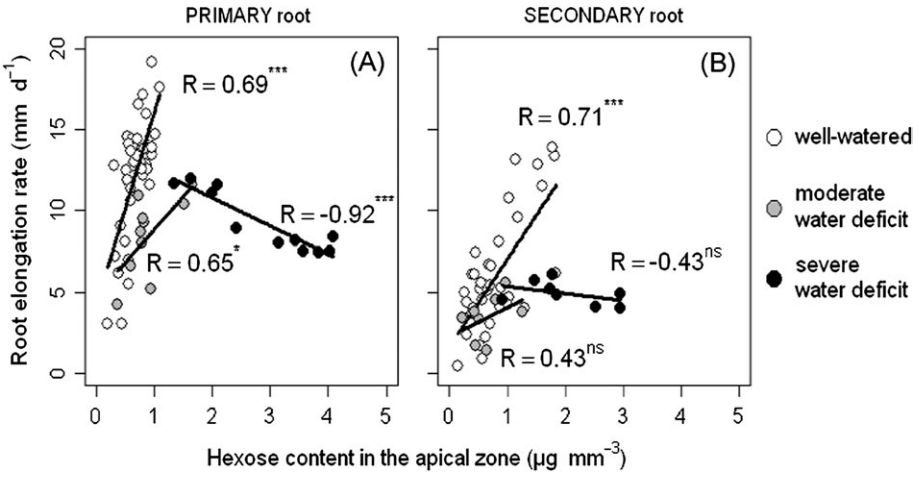

Fig. 2. Relationship between hexose content and elongation rate in primary and secondary roots in *Arabidopsis thaliana*. Plants were grown in agar in Petri plates at various light levels (5–20 mol m^{-2} d^{-1}) and were supplied with different sucrose concentrations in the root medium (0, 0.5, or 2% w/v) as in Freixes *et al.* (2002). Plants were grown under well-watered conditions (open circle) or under moderate (grey circle, solute $\psi_w = -0.3$MPa) or severe (black circle, solute $\psi_w = -0.5$MPa) water deficit induced by PEG, which was poured on to the surface of the agar medium before sowing. Elongation of primary (A) or secondary (B) roots was monitored during three consecutive days. The 3-mm apical region of individual primary and secondary roots encompassing the growing zone was harvested and soluble sugar content was determined as in Freixes *et al.* (2002). Results were normalized using sample volume. A linear model was fitted to each dataset. Note that the positive correlation found between root elongation rate and hexose content weakened at moderate stress and totally vanished or became negative under severe stress. The statistical significance of correlation changes was given by an analysis of covariance (ANCOVA) performed with the R software (R Development Core Team, 2008), using the water potential as factor and the apical hexose content as continuous variable. The interaction term between water potential and hexose content was high enough ($P < 10^{-8}$ and $P < 10^{-4}$ for the primary and secondary roots, respectively) to indicate that the effect of sugar content on growth was dependent on the water potential. Symbols ***, **, *, ·, and 'ns' indicate that the P value of a Pearson's correlation test was $<10^{-3}$, $<10^{-2}$, <0.05, $<10^{-1}$, or non-significant, respectively.

as for the variation between secondary roots of the same plant (Freixes *et al.*, 2002).

When plants were subjected to a moderate (ψ_{medium}=–0.3 MPa) or severe (ψ_{medium}=–0.5 MPa) water deficit by adding PEG in the root medium, root elongation rate was reduced (Fig. 2). Furthermore, hexose content increased dramatically in response to stress, i.e. up to four times at the lowest water potential when compared with controls supplied or not with sugars. Hence, the positive correlation between root elongation rate and hexose content weakened at moderate stress and totally vanished or became negative at severe stress. This accumulation can be interpreted as the result of root elongation (and hence C utilization) being more reduced than C inflow. It is thus indicative of some uncoupling between C availability and root elongation.

Growth of young, sink leaves is also highly sensitive to available C, whereas rapidly expanding leaves grow more independently of C supply. For instance, 80% shading strongly decreases expansion rates at early stages of leaf development, but has no effect at later stages (Granier and Tardieu, 1999; Muller *et al.*, 2001). Moreover, C dependence of leaf growth is different between the day and the night. Grimmer and Komor (1999) suggested that leaf growth in *Ricinus* is sink limited during the day but source limited at night. In *Arabidopsis*, the starchless mutant *pgm* shows a 2-fold reduction in leaf relative expansion rate (RER) at night as compared with the wildtype, but there is only a little difference during the day (Wiese *et al.*, 2007).

In leaves, the amount of C available for growth is the result of the balance between net photosynthesis, the accumulation of starch and various C-containing metabolites such as organic acids during the day and their remobilization at night, and C export to sink organs (Kerr *et al.*, 1985; Hendrix and Huber, 1986). Starch turnover, defined as the variation in starch content between the end of the day and the end of the night, provides a good estimate of C availability, especially for night growth (Sulpice *et al.*, 2009). Indeed, starch production proceeds at a stable rate throughout the photoperiod, and the maximum concentration reached at the end of the day is well related to C availability under a range of photoperiods (Gibon *et al.*, 2009), light intensities, or CO_2 levels (Sharkey *et al.*, 1985). In order to establish links between C availability and leaf growth, a set of mutants affected in starch production or utilization (*pgm*, *sex1*, *mex1*, and *dpe2*) was used. Diurnal RER of well-watered plants (Fig. 3A) showed slight negative correlation with starch turnover, which may be suggestive of a trade-off between expansion and storage during the day (Walter *et al.*, 2002). The correlation was moderately affected by water deficit (i.e. steeper slope and lower *p*-value) although no significant difference was found between slopes. In contrast and as expected, well-watered genotypes displayed large variability in starch turnover, which was positively related to leaf RER at night (Fig. 3B). This correlation was still significant at moderate stress, but vanished at severe water deficit, indicating that severe water deficit released the reliance of leaf expansion on C availability at night.

Flower set is highly sensitive to assimilate availability. In sunflower, tissue expansion in the reproductive shoot apical meristem (capitulum) directly impacts crop productivity because the number of initiated florets, a crucial component of grain yield (Cantagallo and Hall, 2002), depends on the rate and duration of tissue expansion in the meristem (Dosio *et al.*, 2006). The duration depends on the balance between the rate of centripetal progression of the generative front where florets initiate and the expansion rate of the central meristematic zone (Palmer and Steer, 1985 and Fig. 4A). A low value of the expansion rate leads to accelerated meristem exhaustion, a low number of initiated primordia, and low yield (Dosio *et al.*, 2006). In order to evaluate the dependency of meristem expansion on C availability, sunflower plants were grown in field and greenhouse, at high or low plant density (Dosio *et al.*, 2006). Plants were also subjected or not to a period of shading or of soil water deficit. Soluble sugar content was measured in synchrony with capitulum expansion rate (Dosio *et al.*, 2011). In the absence of soil water deficit, the changes in RER of the capitulum paralleled the changes in soluble sugars induced by the treatments affecting light supply (Fig. 4B), suggesting a strong role for C availability in the expanding activity of the capitulum meristematic zone. When soil water deficit developed, soluble sugars accumulated in the capitulum, while the rate of tissue expansion in the meristem decreased. Maximum sugar concentrations were measured at the end of the late water deficit. Remarkably, re-irrigation increased meristem expansion and decreased sugar content in such a way that corresponding points fit on the same relationship as in the absence of stress. Taken together, these data suggest that water deficit altered the dependence of meristem expansion on C availability.

Growth and development of fruits also rely strongly on a continuous supply of carbohydrates from source organs (Ho, 1988; Lebon *et al.*, 2008). Both fruit load and leaf shading have a considerable impact on carbohydrate partitioning and fruit size (Baldet *et al.*, 2002), possibly through the regulation of genes related to cell proliferation at very early stages of flower development (Baldet *et al.*, 2006). C starvation is also known to provoke the abortion of flowers or fruits at early stages of their development (Boyle *et al.*, 1991; Guilioni *et al.*, 1997, 2003; Smith and Stitt, 2007). Interestingly, kernel abortion provoked by extreme water stress in maize can be strongly reduced when stems are infused with sucrose (McLaughlin and Boyer, 2004) suggesting that water deficit impairs phloem and thus sugar transport into the ovaries (Makela *et al.*, 2005). In peach, water deficit decreases fruit growth whatever the fruit load and thus C availability (Berman and DeJong, 1996).

Beyond these examples, only a few studies have questioned how water deficit modifies the dependence of fruit growth on C. To address this question, a modelling approach was used, enabling the simulation of a wide range of environmental scenarios. The model used was developed for peach fruits, and validated under various situations

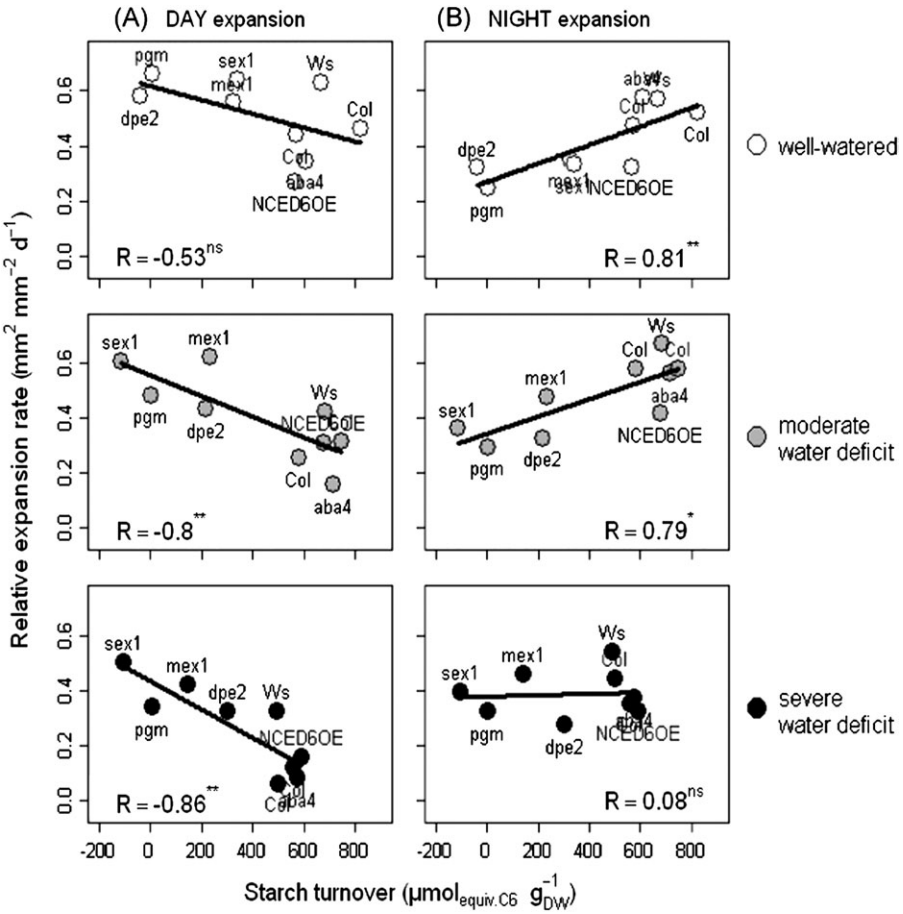

Fig. 3. Relationship between starch turnover and day or night leaf expansion in *A. thaliana*. Nine *Arabidopsis* genotypes (including accessions and mutants) were grown at three levels of soil water content as in Hummel *et al.*(2010). In brief, pot-grown plants were irrigated to a target soil water content corresponding to a well-watered situation (pre-dawn leaf $\psi_w = -0.35$ MPa), moderate soil water deficit (pre-dawn leaf $\psi_w = -0.6$ MPa), or severe deficit (pre-dawn leaf $\psi_w = -1.1$ MPa). Genotypes included two wildtypes: Col-0 (replicated twice) and Ws; four starch-related mutants: *pgm* (Caspar *et al.*, 1985), *mex1* (Niittylä *et al.*, 2004), *sex1* (Caspar *et al.*, 1991), and *dpe2* (Chia *et al.*, 2004; Lu and Sharkey, 2004); and two ABA-related genotypes: *aba4* KO mutant (North *et al.*, 2007) and *NCED6* overexpressing line (Lefebvre *et al.*, 2006). At 45 d after sowing, the plants displayed steady-state rates of leaf production and successive leaves showed comparable behaviour (F. Pantin, T. Simonneau, B. Muller *et al.*, unpublished). Zenithal images of eight plants were taken twice a day for 3 d, at the end of both the dark and the light period. A semi-automated program developed on ImageJ software (http://rsb.info.nih.gov/ij/) was used to extract the area of individual leaves. Day and night RERs were computed from several individual leaves and averaged to obtain a single representative value. Four samples of actively growing leaves were then harvested at the end of day and at the end of night for evaluation of starch turnover. The day (A, left panels) or night (B, right panels) RER was plotted against starch turnover. A linear model was fitted to each dataset. Note that the positive correlation between night RER and starch turnover weakened under severe water deficit as shown by an ANCOVA performed with the R software, using the water potential as factor and the starch turnover as continuous variable. The interaction term between water potential and starch turnover indicated that the effect of starch turnover on night RER was dependent on the water potential ($P < 10^{-1}$). In contrast, no significant interaction was detected for the correlations for day RER indicating that water deficit did not alter relationships between starch turnover and day RER. Symbols ***, **, *, ·, and 'ns' indicate that the *P*-value of a Pearson's correlation test was $<10^{-3}$, $<10^{-2}$, <0.05, $<10^{-1}$, or non-significant, respectively.

(Fishman and Génard, 1998; Lescourret and Génard, 2005). This model (details can be found as Supplementary data, available at *JXB* online) predicts dry matter accumulation as the balance between phloem sugar unloading and fruit respiration, fresh matter (dry matter plus water) accumulation, as the result of water fluxes driven by water potential gradients, and volumetric fruit expansion by using the Lockhart (1965) equation, which relates tissue expansion to cell wall rheology and turgor. C availability was virtually affected, by modifying sucrose concentrations in the phloem reaching the fruit (from 0.02 to 0.2 g g^{-1}). Then, to investigate the effects of water deficit, simulations were also run with xylem water potential ranging from –0.2 to –2.8 MPa. Data of air temperature and humidity corresponding to a natural climatic scenario were provided to the model. Figure 5A, B gives the simulated outputs for three

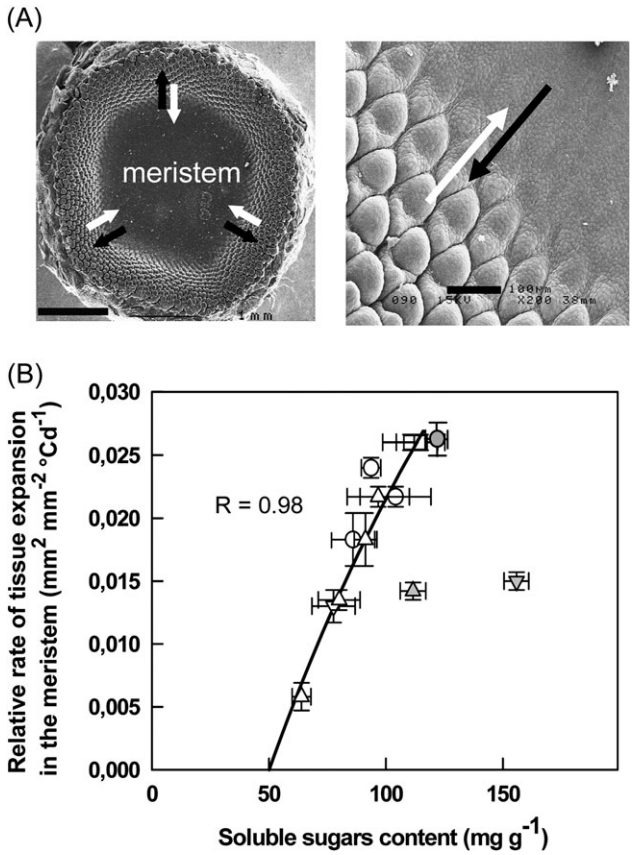

Fig. 4. Relationship between soluble sugar content and tissue expansion in the meristem of sunflower capitulum. (A) Top view of a sunflower capitulum during floret initiation (left; scale bar, 1mm) and detailed view of newly initiated primordia at meristem rim (right; bar, 100 μm). The apparent growth of the meristem of sunflower capitulum results from the opposite effects of two processes (arrows). The expansion of the inner meristem itself (black arrows) increases meristem size while floret primordia tend to fill the expanding field of tissue with individual flowers (white arrows). The rate of tissue expansion in the meristem is thus calculated from the time courses of meristem area, primordium area, and floret number (Dosio *et al.*, 2006). (B) Relative rate of tissue expansion in the meristem as a function of soluble sugar content (mg g^{-1} dry weight) in the capitulum for plants grown in different environmental conditions, in which light (open symbols) or soil water (grey symbols) were altered. Plants were subjected to light deficit using shading or varying crop density (open triangles) or to moderate soil water deficit (grey triangles) either from capitulum initiation to first floret initiation (upward triangles), or from first floret initiation to completion of floret initiation (downward triangles). Some plants exposed to soil water deficit were re-irrigated (grey circle). Circles, control plants in the greenhouse and in field plots; squares, isolated field plants. Bars, SE. Redrawn from Dosio *et al.* (2011).

sugar concentrations in the phloem (low, intermediate, and high) at three watering regimes (well-watered, moderate, and severe water deficit). For well-watered plants, final fruit fresh weight was ~250 g at high and intermediate sucrose concentrations, but was reduced to 90 g at low sucrose (Fig. 5A) and fruit relative growth rate (RGR) computed from fresh weight variations (Fig. 5B) reduced accordingly (Fig. 5B). This result fits well with experimental data obtained by changing the fruit load to leaf surface ratio in tomato (Ho, 1988), coffee (Vaast *et al.*, 2001), and peach (Berman and DeJong, 1996). In plants subjected to severe water deficit, the model also predicted that fruit expansion would be reduced, resulting in a final fresh weight of 90, 60, and 20 g at high, intermediate, or low sucrose concentration in the phloem sap, respectively (Fig. 5A). However, while water deficit had a negative influence on fruit RGR during the first 20d of fruit growth, RGR remained after this time essentially driven by the phloem sugar content, independently of the xylem water potential (Fig. 5B). This was confirmed for a larger range of sugar supply in Fig. 5C where the shape of the saturating relationship between phloem sugar concentrations and fruit RGR (averaged during the rapid growth phase) was only marginally altered, even at low xylem water potential. Strikingly, due to higher fruit transpiration under well-watered conditions (Fig 5E), RGR was not higher than under moderate stress conditions (Fig. 5C), probably in relation to higher cuticular conductance (Gibert *et al.*, 2005). As observed for other organs, water deficit strongly increased fruit sugar content, because passive concentration occurred due to reduced fruit expansion. However, the slope of the relationship between RGR and sugar content was not strongly altered, still indicating no interaction between water and sugar availability (Fig. 5D). This result differs from those found in leaves, roots, and reproductive meristem. The reason for such a discrepancy is not known but could be linked to the dominant role of sugars in fleshy fruits in which very high sugar concentrations are essential contributors to lowering the osmotic potential and thus maintaining high turgor. This role is likely to be shared among more actors in other organs (Sharp *et al.*, 1990; Hummel *et al.*, 2010). For instance, in the maize root growing zone, hexoses, together with K^+, strongly contribute to this role (Sharp *et al.*, 1990). In contrast, in the *Arabidopsis* rosette, other C-rich compounds (mainly organic acids and proline) contribute to > 40% of osmotic adjustment whereas sugars contribute to < 10% (Hummel *et al.*, 2010).

Does this imply that fruit water relations do not interfere in the relationships between C availability and growth ? Model outputs also suggest that at later stages of development, rapidly expanding well-watered fruits showed strong fluctuations in RGR (Fig. 5B) due to fruit shrinkage during days under high evaporative demand (Days 131 and 139 in Fig 5B) and subsequent growth boost when the air becomes wetter again, a situation commonly observed in natural conditions (Johnson *et al.*, 2006). These natural climatic variations were used to evaluate the effect of evaporative demand on the relationships between C availability and fruit growth. From the well-watered situation, days were grouped according to the mean vapour pressure deficit (VPD) occurring during those days [either high (>1.25 kPa), intermediate (1.25 kPa>VPD>0.75 kPa), or

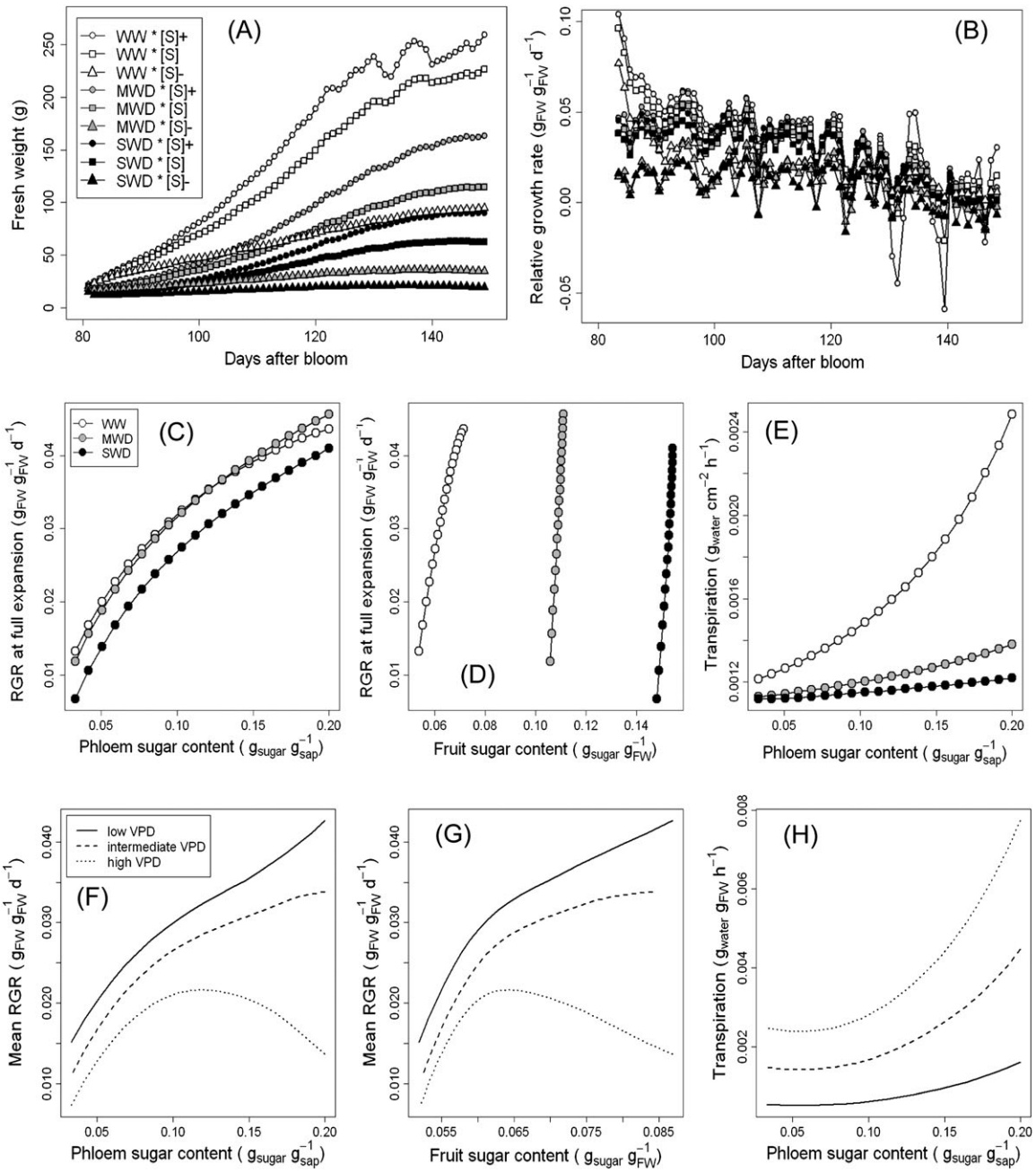

Fig. 5. Simulations of peach fruit growth under a wide range of phloem sugar concentrations and xylem water potentials. A biophysical model designed to simulate the transport of water and sugar into fruit (Fishman and Génard, 1998; Lescourret and Génard, 2005) was used with minor modifications to assess the effects of phloem sugar concentration and xylem water potential on peach fruit growth. The model is based on the representation of plastic fruit growth as a function of turgor pressure using the Lockhart (1965) equation. The fruit is considered as one compartment separated from xylem or phloem tissue by a membrane; flows across this membrane are described by thermodynamic equations, involving differences in hydrostatic and osmotic pressure on both sides of the membrane, and properties of the membrane towards water (hydraulic conductivity) and solutes (reflection coefficient and permeability). The total uptake of carbon from phloem is the sum of contributions due to mass flow, passive diffusion, and active transport. The virtual experiment was implemented with R software and run from 80 to 149 d after bloom in hourly time-steps. Climatic inputs are air VPD, which strongly impacted fruit transpiration and air temperature obtained from a representative natural dataset. Plant inputs are phloem sugar concentration and xylem water potential; in each simulation, they follow a sinusoidal function with fixed extremes in a period of 24 h. (A) Daily evolution of peach fresh weight for three xylem water potentials × three phloem sugar concentrations. Open symbols represent well-watered plants (WW, daily averaged xylem water potential of –0.4 MPa), grey symbols are for moderate water deficit (MWD, –1.1 MPa), and dark symbols are for severe deficit (SWD, –1.8 MPa). Circles are for a high sugar supply (daily averaged phloem concentration of 0.18 g g^{-1}), squares for intermediate sugar supply (0.13 g g^{-1}), and triangle for low sugar supply (0.04 g g^{-1}). (B) Daily evolution of fruit RGR during the same simulations. RGR was computed as the local slope of the natural logarithm of fresh weight as a function of

low (<0.75 kPa) evaporative demand] and fruit RGR was averaged for each of these groups. Remarkably, increasing VPD in well-watered plants reduced the slope of the relationship between phloem or fruit sugar content and fruit RGR (Fig. 5F, G). In the model, VPD reduces the amount of water in the fruit by increasing transpiration according to a physical law describing the mass flow between the air-filled space of the fruit and the ambient atmosphere. Figure 5H shows to what extent high VPD increased transpiration in the well-watered plants. However, it did not alter the fruit sugar content (Fig. 5G). This fits with the view that increasing sink limitation, here by a purely hydraulic process, leads to uncoupling of growth from C availability.

Significance of the relationships between C availability and growth, and possible reasons for their modification under water deficit

C is hypothesised to promote organ growth through a variety of mechanisms: (i) the supply of energy to highly consuming meristematic regions (Bidel *et al.*, 2000; Farrar and Jones, 2000); (ii) the generation of turgor in expanding cells via the accumulation of osmotically active C compounds (Sharp *et al.*, 1990); (iii) the supply of C bricks to the cell wall (Bret-Harte *et al.*, 1991); and (iv) the triggering of developmental or metabolic processes via C signalling (Rolland *et al.*, 2006). The positive relationships illustrated in the previous section certainly integrate some if not all of these mechanisms. Their weakening or more generally their modification may have at least two significations. First, it is possible that bulk tissue concentrations may be less relevant as an estimate of C availability under water deficit than under well-watered conditions. Thus, the importance of the vacuolar pool of C-soluble compounds is likely to increase with water deficit (Kim *et al.*, 2000) whereas the cytosolic sugars are probably more important for sugar sensing or triggering energy production. Furthermore, growth and development might be better related to sugar fluxes imported from the phloem and/or to sugar gradients (Borisjuk *et al.*, 2003; Munier-Jollain and Salon, 2003; Makela *et al.*, 2005), than to their concentrations (see Fig. 5). Another possibility is that water deficit mainly reduces growth through C-independent mechanisms, thus uncoupling growth from C availability. These C-independent mechanisms are likely to be related to water flux to growing cells, which is reduced under soil water deficit (Tang and Boyer, 2002), or to mechanical properties of growing cell walls possibly under the influence of hormones or pH (Fan and Neumann, 2004). Accordingly, it was recently shown that the cell wall loosening proteins expansins are intimately coupled, at the transcriptional level, with local expansion in maize leaves (Muller *et al.*, 2007). This suggests that the modification (weakening) of the relationship between C availability and growth can be interpreted as the signature of the passage from source (C-based) growth limitation to less- or non-C-based (i.e. sink) limitation.

Conclusion

When plants are facing soil water deficit, growth is reduced and C concentrations rise, possibly due to organ expansion being affected earlier and more intensively than photosynthesis and metabolism. This leads to increased concentrations in various C molecular forms in several plant parts, ruling out the idea that stress-induced energy deprivation would be the usual cause of growth reduction under water deficit. Elevated C concentrations under water deficit are also likely to interfere with C signalling in a manner that will deserve further attention. Under well-watered conditions, tight relationships linking C availability and growth illustrate the source limitation of growth in sink organs such as roots, leaves (at night), flowers, and fruits. These relationships probably reflect the different uses of C compounds, i.e. as fuel for energy supply, bricks for structure build-up, osmotica for turgor maintenance as well as signal molecules for triggering developmental and metabolic programmes. Under water deficit, these relationships are modified, suggesting that other mechanisms, possibly involving cell wall rheology or water fluxes to growing cells, override the role of C and take the lead on growth control.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary information: rationale and main equations from the biophysical model of fruit growth (Fishman and Génard, 1998) used to construct Fig. 5.

time. Same symbols as in (A). (C) Effect of phloem sugar content on fruit RGR during rapid expansion (averaged between Days 110 and 115) at three selected xylem water potentials. (D) Relationship between fruit sugar concentration and RGR at full expansion in the same conditions as in (C). (E) Effect of phloem sugar content on fruit transpiration for the same set of conditions as in (C). In (C), (D), and (E), open, grey, and dark circles represent well-watered plants, moderate water deficit, and severe water deficit, respectively. (F) Effect of phloem sugar concentration and evaporative demand (estimated through the VPD) in well-watered peach plants on fruit RGR. Days were categorized according to average VPD. At all imposed phloem sugar concentrations, RGR was averaged for each VPD level. Solid line for low evaporative demand (0.75 kPa>VPD), dashed line for intermediate evaporative demand (1.25 kPa>VPD>0.75 kPa), dotted line for high evaporative demand (VPD>1.25 kPa). (G) Effect of fruit sugar concentration and VPD on fruit RGR in well-watered peach plants. Same conditions as in (F). (H) Effect of phloem sugar concentration and VPD on fruit transpiration. Same conditions as in (F).

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