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

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26 **Abstract**

27

28 In plants, carbon (C) molecules provide building blocks for biomass production, fuel for

29 energy and exert signalling roles to shape development and metabolism. Accordingly,

30 plant growth is well correlated with light interception and energy conversion through

31 photosynthesis. Because water deficits close stomata and thus reduce C entry, it has

32 been suspected that droughted plants are under C starvation and their growth under C

33 limitation. In this paper, these points are questioned by combining literature review with

34 experimental and modelling illustrations in various plant organs and species. First,

35 converging evidence is gathered from the literature that water deficit generally

36 increases C concentration in plant organs. The hypothesis is raised that this could be

37 due to organ expansion (as major C sink) being affected earlier and more intensively

38 than photosynthesis (C source) and metabolism. How such increase is likely to interact

39 with C signalling is not known. Hence, literature is reviewed on possible links between

40 C and stress signalling that could take part in this interaction. Finally, the possible

41 impact of water deficit induced C accumulation on growth is questioned for various sink

42 organs of several species by combining published as well as new experimental data or

43 data generated using a modelling approach. To this aim, robust correlations between C

44 availability and sink organ growth are reported in the absence of water deficit. Under

45 water deficit, relationships loosen or are modified suggesting a release of the influence

46 of C availability on sink organ growth. These results are interpreted as the signature of

47 a transition from source to sink growth limitation under water deficit.

48

49 Key words : C metabolism, water deficit, growth, C signalling, sugar, starch, sink

50 limitation, source limitation, model

51

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 a tight control over a wide range of processes including transcriptional, post-transcriptional and post-translational mechanisms (Koch *et al.,* 1996; Rolland *et al.,* 2006). At a much broader scale, biomass accumulation in a crop is a linear and remarkably stable function of intercepted light by the canopy and its transformation into dry 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 paper thus aims at questioning 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 raised 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.,*

77 2001), sunflower and sorghum (Turner *et al.,* 1978), lupin and eucalypt (Quick *et al.,*

78 1992), pine (Marron *et al.,* 2003), poplar (Bogeat-Triboulot *et al.,* 2007) or grapevine

79 (Cramer *et al.,* 2007). Carbohydrates also accumulate in stems (Bogeat-Triboulot *et al.,*

80 2007), flowers and fruits (Liu *et al.,* 2004; McLaughlin and Boyer, 2004; Mercier *et al.,*

81 2009), as well as in roots (Sharp *et al.,* 1990; Jiang and Huang, 2001; Freixes *et al.,*

82 2002). Accumulation occurs both after rapid osmotic shocks, *e.g.* using PEG or

83 mannitol (Zrenner and Stitt, 1991), and during slowly developing water deficits (Cramer

84 *et al.,* 2007; Hummel *et al.,* 2010).

85

86 Carbohydrates often accumulate in the form of abundant sugars such as hexoses and

87 sucrose (references above). However, a wider range of C-rich compounds may also

88 accumulate in response to soil water deficit. These include minor sugars such as

89 trehalose (Farías-Rodríguez *et al.,* 1998) or mannitol (Guicherd *et al.,* 1997), amino

90 acids (Morgan, 1992), in particular those with a high C/N ratio such as proline (Hare

91 and Cress, 1997) or pipecolic acid (Barnett and Naylor, 1966). Organic acids such as

92 malate (Franco *et al.,* 2006), fumarate (Hummel *et al.,* 2010) or citrate (Timpa *et al.,*

93 1986) also accumulate in response to water deficit in a range of species including

94 *Arabidopsis* (Hummel *et al.,* 2010). Quaternary ammonium compounds such as glycine

95 betaine, which accumulate in particular species or families (Ashraf and Foolad, 2007;

96 Gagneul *et al.,* 2007) may also be seen as C-rich compounds, as a quaternary

97 ammonium results from the substitution of 3 protons with 3 alkyl groups on an amine

98 residual (Rhodes and Hanson, 1993). Many of such compounds are considered as

99 'compatible solutes', as they can accumulate in large amounts without perturbing the

100 cell functions, and are thought to protect sub-cellular structures against deleterious

101 effects of cell water loss. This has triggered considerable research, in particular through

102 genetic engineering of pathways leading to these compounds in order to increase

103 drought tolerance (Bohnert and Jensen, 1996; Sakamoto and Murata, 2000;

104 Rathinasabapathi, 2000). However, because most of these compounds accumulate under

105 extreme stresses leading to desiccation, the relevance of this strategy has been

106 questioned for agricultural situations where it is not crop survival but crop productivity

107 that is critical (*e.g.* Tardieu, 1996; Serraj and Sinclair, 2002).

108

109 Structural C-rich compounds such as cellulose and lignin also accumulate under water

110 deficit. Indeed, water deficit accelerates lignification (Timpa *et al.,* 1986; Vincent *et al.,*

111 2005), decreases leaf intercellular air spaces (Hsiao and Acevedo, 1974), and increases

112 leaf thickness (Hummel *et al.,* 2010). All these responses contribute to the widely

113 reported increase in specific leaf mass occurring under water deficit (see Tardieu *et al.,*

114 1999 and references therein).

115

116 **Soil water deficits uncouple photosynthesis and growth while C**

117 **metabolism is often maintained or increased**

118

119 The increase in C concentration in organs of plants under water deficit must originate

120 from some uncoupling between C supply and demand. Accordingly, the photosynthesis

121 (C supply) of the Arabidopsis rosette is resilient to even severe water deficit while leaf

122 expansion (accounting for a major part of C demand) is strongly reduced by stress, as

123 quantitatively argued by Hummel *et al.* (2010). The maintenance of photosynthesis

124 under water deficit has been repeatedly reported (Boyer, 1970b; Quick *et al.,* 1992;

125 Bogeat-Triboulot *et al.,* 2007). The mesophylic component of CO_2 capture is

126 particularly resilient to water deficit (Kaiser, 1987; Cornic, 2000; Flexas and Medrano,

127 2002). For instance, Rubisco activity is maintained even when leaf relative water

128 content drops to 50% while stomata are already 75% closed (Kaiser 1987; Flexas *et al.,*

129 2006). In contrast, water deficit strongly reduces leaf or shoot expansion rates (Boyer,

130 1970a; Hsiao, 1973; Ben Haj Salah and Tardieu, 1997; Tardieu *et al.,* 1999, 2000).

131 Synthesis of published work on different plant species in which plant growth and

132 photosynthesis were measured under a range of water deficits is shown in Fig. 1. The

133 common feature in all species is that C demand (growth) always decays before C supply

134 (photosynthesis) is affected by water deficit. Though this analysis does not consider

135 other C demands such as respiration or root growth, it clearly illustrates the large

136 domain of water deficits in which C may be present in excess in the plant.

137

138 The impact of water deficit on C metabolism has been the matter of numerous studies.

139 They reports in some cases that enzymes involved show signs of down-regulation

140 (Chaves et al., 2009) but more often support the view of a maintained or increased

141 metabolic activity. For instance, sucrose cleaving enzymes increase their activity upon

142 water deficit in source leaves of cereals (Kim *et al.,* 2000), in pace with an increased

143 need for osmotic adjustment in these leaves (McCree *et al.,* 1984) and a higher C

144 demand by the seeds (Yang *et al.,* 2004). In the growing zone of maize leaves, the

145 activities of several enzymes involved in glycolysis and TCA cycle increase (Riccardi *et*

146 *al.,* 1998). In perennials such as poplar (Bogeat-Triboulot *et al.,* 2007) or grapevine

147 (Cramer *et al.,* 2007), the triggering of particular metabolic pathways including C

148 metabolism has been observed under moderate to severe water deficit. The activome of

149 *Arabidopsis* plants subjected to various levels of water stress has recently been

150 investigated by profiling a set of 30 enzymes from central C and N metabolism across

151 rosette development (Hummel *et al.,* 2010). In most cases, enzyme activities were

152 increased under water deficit, but these increases occurred slowly and were of low

153 magnitude, suggesting that even in plants facing a 75% drop in aerial biomass

154 production, there was no dramatic or specific reprogramming of metabolism.

155

156 While maintenance of C metabolism and increased C concentration in plant parts under

157 water deficit has been reported in most studies, there are also some cases where the

158 opposite is observed. This is notably the case when water deficit is so severe and

159 prolonged that photosynthesis becomes inhibited over a long period. An important co-

160 factor in that case is the elevated temperature, which is usually associated with water

161 deficit in nature and results in increased respiration, thereby negatively affecting the C

162 status. This extreme scenario has been proposed to be responsible, along with hydraulic

163 failure, for tree mortality under severe water deficit (McDowell *et al.,* 2008; McDowell

164 and Sevanto, 2010).

165

C signalling and possible interactions with stress

167

168 Besides their roles as bricks for structures and fuel for energy production, soluble C

169 compounds such as glucose and sucrose (Chiou and Bush, 1998; Laby *et al.,* 2000;

170 Moore *et al.,* 2003; Huang *et al.,* 2008), but also phosphorylated intermediates (*e.g.*

171 glucose-6-phosphate or trehalose-6-phosphate, Paul, 2007; Zhang *et al*., 2009), are

172 playing key signalling roles in the overall shaping of the metabolic and developmental

173 machinery, through both gene expression or post-translational regulations. Because

174 water deficits are likely to alter the concentration of these metabolites, it is important to

175 understand how C- and stress-signalling are integrated.

176

177   Original support for signalling roles of sugars came from single gene expression

178   analysis, pointing towards genes coding for enzymes directly involved in the utilisation

179   of C, such as sucrose synthase and invertase (Koch *et al.,* 1992; Ciereszko and

180   Kleczkowski, 2002). Later, microarrays have revealed that sugars influence the

181   expression of hundreds of genes involved in a wide range of processes (Contento *et al.,*

182   2004; Price *et al.,* 2004; Thimm *et al.,* 2004; Thum *et al.,* 2004; Bläsing *et al.,* 2005; Li

183   *et al.,* 2006). The cell cycle machinery is a key target of this control (Webster and Van't

184   Hof, 1969, Riou-Khamlichi *et al.,* 2000). Furthermore, ribosomal proteins and genes

185   involved in tRNA metabolism are among the functional categories that respond the

186   most consistently, at the transcriptional level, to fluctuations in the C resource (Thimm

187   *et al.,* 2004; Bläsing *et al.,* 2005; Osuna *et al.,* 2007). The fact that protein synthesis

188   represents a major sink for energy (Penning de Vries, 1975) strengthens the idea that a

189   tight link between C metabolism and protein synthesis is necessary to prevent acute C

190   starvation (Smith and Stitt, 2007), especially in growing tissues where most of the

191   protein synthesis contributes to building new biomass (Piques *et al.,* 2009). In line with

192   this, considerably more genes have been found to respond to low sugar than to high

193   sugar (Bläsing *et al.,* 2005). It has then been proposed that sugar sensing and signalling

194   enable the avoidance of acute C starvation under a wide range of environmental

195   conditions, thus maintaining the ability to grow under any circumstances (Smith and

196   Stitt, 2007). Strikingly, experiments that led to these conclusions were performed under

197   conditions where light (and thus C) was actually the only factor limiting growth, while

198   environmental stresses were absent.

199

200   How C- and (water) stress - signalling may interact is just beginning to be revealed.

201   Among the different sugar sensing systems proposed, the best known is a pathway

202 involving hexokinase (HXK1), which has been found to interact with abscisic acid

203 (ABA)-, ethylene-, auxin- and cytokinin-signalling pathways, suggesting a central role

204 in linking C-status to stress responses (Rolland *et al.*, 2006). Another glucose sensor,

205 which is located at the plasma membrane and coupled to a G protein complex, has

206 recently been found in *Arabidopsis* (Grigston *et al.*, 2008). G protein signalling is also

207 known to be involved in responses to various biotic and abiotic stresses (*e.g.* Nilson and

208 Assmann, 2010). A further pathway, which is thought to sense various sugars including

209 glucose-6-phosphate (Toroser *et al.*, 2000) and trehalose-6-phosphate (Schluepmann *et*

210 *al.*, 2004; Zhang *et al.*, 2009), involves SnRK1 protein kinases, which can act on both

211 gene expression and enzyme activity (Halford, 2006) and are also involved in hormone

212 (in particular ABA) signalling. Finally, while no sucrose receptor has been found so far

213 in plants, there is a sucrose-specific pathway, also involving SnRK1, leading to

214 translational control of a basic leucine zipper (bZIP) type transcription factor (Wiese *et*

215 *al.,* 2004), by which sucrose represses the expression of various enzymes including

216 proline dehydrogenase (Hanson *et al.,* 2008), which is also repressed under osmotic

217 stress (Yoshiba *et al.,* 1997) and induced upon rehydration (Oono *et al.,* 2003).

218

219 Together, these results are indicative of a variety of means by which C and stress

220 signalling could be integrated. Such a deep integration has been interpreted as resulting

221 from the fact that most stresses would negatively affect the overall C and energy status

222 of the plant (Baena-Gonzalez and Sheen, 2008). However, the analysis developed in the

223 former sections tends to contradict this interpretation. One hypothesis is that such

224 crosstalk could contribute to bypass critical signalling pathways, as an increase in sugar

225 availability provoked by water stress might otherwise be misleading. In line with this,

226 initial mutant screens revealing such shared signalling pathways have been conducted

227 with very high sugar concentrations (*e.g.* 6% w/v) ruling out the possibility that stressed

228 plants were C starved (Arenas-Huertero *et al*., 2000; Huijser *et al*., 2000; Laby *et al*.,

229 2000; Rook *et al*., 2001). Understanding the way water deficit modulates C-sensing and

230 –signalling therefore appears as an important topic towards the understanding of plant

231 performance under stressing conditions.

232

233 ## Water deficit differentially tunes the relationship between C

234 ## availability and growth in sink organs

235

236 Growth and development of sink organs is known to be at least partly under the control

237 of C availability. This has been repeatedly reported for roots (Aguirrezabal *et al.,* 1994;

238 Thaler and Pagès, 1996; Willaume and Pagès, 2006; Freixes *et al.,* 2002), young leaves

239 (Muller *et al.,* 2001; Granier and Tardieu, 1999), flowers (Guilioni *et al.,* 1997; Smith

240 and Stitt, 2007), fruits (Liu *et al.,* 2004; Borisjuk *et al.,* 2003; Wu *et al.,* 2005) or seeds

241 (Munier-Jolain and Ney, 1998; Munier-Jolain and Salon, 2003). Because water deficit

242 increases concentration of C and thus possibly C availability in plant tissues, it is

243 important to understand the consequences for organ growth. The analysis performed in

244 the next paragraphs is based on the occurrence of tight relationships between C

245 availability and the expansion or the development of different sink organs. The rationale

246 followed is to use the modification of these relationships as a diagnostic of an alteration

247 of C dependency of growth.

248

249 In roots, sucrose unloaded from the phloem is rapidly cleaved by invertase (Hellebust

250 and Forward, 1962; Giaquinta *et al.,* 1983) and/or by sucrose synthase (Martin *et al.,*

251 1993), which are highly abundant at the site of intense phloem unloading located in the

252   middle of the growing zone (Oparka *et al.,* 1994). This leads to very low sucrose

253   concentrations in the root zone showing rapid expansion (Sharp *et al.,* 1990; Muller *et*

254   *al.,* 1998). Concentrations of hexose released from sucrose in the growing zone are

255   therefore a good estimate of local C availability, as they depend on the balance between

256   C-inflow and -utilization. Following this rationale, hexose concentration was evaluated

257   in growing zones of single roots whose elongation rate had been measured during 24h

258   prior to sampling. In well-watered *Arabidopsis* plants exposed to various light

259   intensities or supplied with external sugars, quantitative relationships between root

260   elongation rate and hexoses concentration were found, for both primary and secondary

261   roots (Freixes *et al.,* 2002 and Fig. 2). Remarkably, these relationships were robust

262   enough to account for the variation among primary roots of different plants, as well as

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

264

265   When plants were subjected to a moderate (Ψ_{medium} = -0.3 MPa) or severe (Ψ_{medium} = -

266   0.5 MPa) water deficit by adding PEG in the root medium, root elongation rate was

267   reduced (Fig. 2). Furthermore, hexoses content increased dramatically in response to

268   stress, *i.e.* up to four times at the lowest water potential when compared to controls

269   supplied or not with sugars. Hence, the positive correlation between root elongation rate

270   and hexoses content loosened at moderate stress and totally vanished or became

271   negative at severe stress. This loosening can be interpreted as the result of root

272   elongation (and hence C utilization) being more reduced that C inflow. It is thus

273   indicative of some uncoupling between C availability and root elongation.

274

275   Growth of young, sink leaves is also highly sensitive to available C, whereas rapidly

276   expanding leaves grow more independently of C supply. For instance, 80% shading

277 strongly decreases expansion rates at early stages of leaf development, but has no effect

278 at later stages (Granier and Tardieu, 1999; Muller *et al.,* 2001). Moreover, C-

279 dependency of leaf growth is different between the day and the night. Grimmer and

280 Komor (1999) suggested that, leaf growth in Ricinus is sink-limited during the day but

281 source-limited at night. In Arabidopsis, the starchless mutant *pgm* shows a two-fold

282 reduction in leaf relative expansion rate (RER) at night as compared to the wild-type,

283 but there is only a little difference during the day (Wiese *et al.,* 2007).

284

285 In leaves, the amount of C available for growth is the result of the balance between net

286 photosynthesis, the accumulation of starch and various C-containing metabolites such as

287 organic acids during the day and their remobilisation at night, and C export to sink

288 organs (Kerr *et al.,* 1985; Hendrix and Huber, 1986). Starch turnover, defined as the

289 variation of starch content between the end of the day and the end of the night, provides

290 a good estimate of C availability, especially for night growth (Sulpice *et al.,* 2009).

291 Indeed, starch production proceeds at a stable rate throughout the photoperiod, and the

292 maximum concentration reached at the end of the day is well related to C availability

293 under a range of photoperiods (Gibon *et al.,* 2009), light intensities or CO_2 levels

294 (Sharkey *et al.,* 1985). In order to establish links between C availability and leaf growth,

295 a set of mutants affected in starch production or utilization (*pgm, sex1, mex1* and *dpe2*)

296 was used. Diurnal RER of well-watered plants (Fig. 3A) showed slight negative

297 correlations with starch turnover, which may be suggestive of a trade-off between

298 expansion and storage during the day (Walter *et al.,* 2002). The correlation was

299 moderately affected by water deficit (*i.e.* steeper slope and lower p-value) although no

300 significant difference was found between slopes. By contrast and as expected, well-

301 watered genotypes displayed a large variability in starch turnover, which was positively

302 related to leaf RER at night (Fig. 3B). This correlation was still significant at moderate

303 stress, but vanished at severe water deficit, indicating that severe water deficit released

304 the reliance of leaf expansion on C availability at night.

305

306 Flower set is highly sensitive to assimilate availability. In sunflower, tissue expansion in

307 the reproductive shoot apical meristem (capitulum) directly impacts crop productivity

308 because the number of initiated florets, a crucial component of grain yield (Cantagallo

309 and Hall, 2002), depends on the rate and duration of tissue expansion in the meristem

310 (Dosio *et al.*, 2006). The duration depends on the balance between the rate of centripetal

311 progression of the generative front where florets initiate and the expansion rate of the

312 central meristematic zone (Palmer and Steer, 1985 and Fig. 4A). A low value of the

313 expansion rate leads to accelerated meristem exhaustion, low number of initiated

314 primordia and low yield (Dosio *et al.*, 2006). In order to evaluate the dependency of

315 meristem expansion on C availability, sunflower plants were grown in field and

316 greenhouse, at high or low plant density (Dosio *et al.* 2006). Plants were also subjected

317 or not to a period of shading or of soil water deficit. Soluble sugar content was

318 measured in synchrony with capitulum expansion rate (Dosio *et al.*, 2010). In the

319 absence of soil water deficit, the changes in RER of the capitulum paralleled the

320 changes in soluble sugars induced by the treatments affecting light supply (Fig. 4B),

321 suggesting a strong role for C availability in the expanding activity of the capitulum

322 meristematic zone. When soil water deficit developed, soluble sugars accumulated in

323 the capitulum, while the rate of tissue expansion in the meristem decreased. The

324 maximum sugar concentrations were measured at the end of the late water deficit.

325 Remarkably, re-irrigation increased meristem expansion and decreased sugar content in

326 a way that corresponding points fit on the same relationship than in the absence of

327 stress. Taken together, these data suggest that water deficit altered the dependency of

328 meristem expansion on C availability.

329

330 Growth and development of fruits also strongly rely on continuous supply of

331 carbohydrates from source organs (Ho, 1988; Lebon *et al.,* 2008). Both fruit load and

332 leaf shading have a considerable impact on carbohydrate partitioning and fruit size

333 (Baldet *et al.,* 2002), possibly through the regulation of genes related to cell

334 proliferation at very early stages of flower development (Baldet *et al.,* 2006). C

335 starvation is also known to provoke the abortion of flowers or fruits at early stages of

336 their development (Boyle *et al.,* 1991; Guilioni *et al.,* 1997, 2003; Smith and Stitt,

337 2007). Interestingly, kernel abortion provoked by an extreme water stress in maize can

338 be strongly reduced when stems are infused with sucrose (McLaughlin and Boyer,

339 2004) suggesting that water deficit impairs phloem and thus sugar transport into the

340 ovaries (Makela *et al.,* 2005). In peach, water deficit decreases fruit growth whatever

341 the fruit load and thus C availability (Berman and DeJong, 1996).

342

343 Beyond these examples, only a few studies have questioned how water deficit modifies

344 the dependency of fruit growth on C. To address this question, a modelling approach

345 was performed, enabling the simulation of a wide range of environmental scenarios. The

346 model used was developed for peach fruits, and validated under various situations

347 (Fishman and Génard, 1998; Lescourret and Génard, 2005). This model (details can be

348 found as supplemental material) predicts dry matter accumulation as the balance

349 between phloem sugar unloading and fruit respiration, fresh matter (dry matter *plus*

350 water) accumulation, as the result of water fluxes driven by water potential gradients,

351 and volumetric fruit expansion by using the Lockhart (1965) equation which relates

352   tissue expansion to cell wall rheology and turgor. C availability was virtually affected,

353   by modifying sucrose concentrations in the phloem reaching the fruit (from 0.02 to 0.2

354   g g^{-1}). Then, to investigate the effects of water deficit, simulations were also run with

355   xylem water potential ranging from - 0.2 to - 2.8 MPa. Data of air temperature and

356   humidity corresponding to a natural climatic scenario were provided to the model.

357   Figures 5A and 5B give the simulated outputs for three sugar concentrations in the

358   phloem (low, intermediate and high) at three watering regimes (well-watered, moderate

359   and severe water deficit). For well-watered plants, final fruit fresh weight was around

360   250 g at high and intermediate sucrose concentrations, but was reduced to 90 g at low

361   sucrose (Fig. 5A) and fruit relative growth rate (RGR) computed from fresh weight

362   variations (Fig. 5B) reduced accordingly (Fig. 5B). This result fits well with

363   experimental data obtained by changing fruit load *vs*. leaf surface ratio in tomato (Ho,

364   1988), coffee (Vaast *et al.,* 2001) or peach (Berman and DeJong, 1996). In plants

365   subjected to a severe water deficit, the model also predicted that fruit expansion would

366   be reduced, resulting in a final fresh weight of 90, 60 and 20 g at high, intermediate or

367   low sucrose concentration in the phloem sap, respectively (Fig. 5A). However, while

368   water deficit had a negative influence on fruit RGR during the first 20 days of fruit

369   growth, RGR remained after this time essentially driven by the phloem sugar content,

370   independently of the xylem water potential (Fig. 5B). This was confirmed for a larger

371   range of sugar supply in Fig. 5C where the shape of the saturating relationship between

372   phloem sugar concentrations and fruit RGR (averaged during the rapid growth phase)

373   was only marginally altered, even at low xylem water potential. Strikingly, due to

374   higher fruit transpiration under well watered conditions (Fig 5E), RGR was not higher

375   than under moderate stress conditions (Fig. 5C), probably in relation with higher

376   cuticular conductance (Gibert et al., 2005). As observed for other organs, water deficit

377 strongly increased fruit sugar content, because passive concentration occurred due to

378 reduced fruit expansion. However, the slope of the relationship between RGR and sugar

379 content was not strongly altered, still indicating no interaction between water and sugar

380 availability (Fig. 5D). This result differs from those found in leaves, roots and

381 reproductive meristem. The reason for such a discrepancy is not known but could be

382 linked to the dominant role of sugars in fleshy fruits in which very high sugar

383 concentrations are essential contributors to lowering the osmotic potential and thus

384 maintaining high turgor. This role is likely to be shared among more actors in other

385 organs (Sharp *et al.,* 1990; Hummel *et al.,* 2010). For instance, in the maize root

386 growing zone, hexoses, together with K^+, strongly contribute to this role (Sharp et al.,

387 1990). By contrast, in the Arabidopsis rosette, other C rich compounds (mainly organic

388 acids and proline) contribute to more than 40% of osmotic adjustment whereas sugars

389 contribute to less than 10% (Hummel *et al.,* 2010).

390

391 Does this imply that fruit water relations do not interfere in the relationships between C

392 availability and growth ? Model outputs also suggest that at later stages of development,

393 rapidly expanding well-watered fruits showed strong fluctuations in RGR (Fig. 5B) due

394 to fruit shrinkage during days under high evaporative demand (days 131 and 139 in Fig

395 5B) and subsequent growth boost when air becomes wetter again, a situation commonly

396 observed in natural conditions (Johnson *et al.,* 2006). These natural climatic variations

397 were used to evaluate the effect of evaporative demand on the relationships between C

398 availability and fruit growth. From the well-watered situation, days were grouped

399 according to the mean vapour pressure deficit (VPD) occurring these days (either high

400 (> 1.25 kPa), intermediate (1.25 kPa > VPD > 0.75 kPa) or low (< 0.75 kPa)

401 evaporative demand) and fruit RGR were averaged for each of these groups.

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402   Remarkably, increasing VPD in well-watered plants reduced the slope of the

403   relationship between phloem or fruit sugar content and fruit RGR (Fig. 5F and 5G). In

404   the model, VPD reduces the amount of water in the fruit by increasing transpiration

405   according to a physical law describing the mass flow between air filled space of the fruit

406   and the ambient atmosphere. Fig. 5H shows to what extent high VPD increased

407   transpiration in the well-watered plants. However, it did not alter the fruit sugar content

408   (Fig. 5G). This fits with the view that increasing sink limitation, here by a purely

409   hydraulic process, leads to uncouple growth from C availability.

410

411   Significance of the relationships between C availability and

412   growth, and possible reasons for their modification under water

413   deficit

414

415   C is suspected to promote organ growth through a variety of mechanisms: (i) the supply

416   of energy to highly consuming meristematic regions (Bidel *et al*., 2000; Farrar and

417   Jones, 2000), (ii) the generation of turgor in expanding cells via the accumulation of

418   osmotically active C compounds (Sharp *et al*., 1990), (iii) the supply of C bricks to the

419   cell wall (Bret-Harte *et al*., 1991) and (iv) the triggering of developmental or metabolic

420   processes via C-signalling (Rolland *et al*., 2006). The positive relationships illustrated

421   in the previous section certainly integrate some if not all of these mechanisms. Their

422   loosening or more generally their modification may have at least two significations.

423   First, it is possible that bulk tissue concentrations may be less relevant as an estimate of

424   C availability under water deficit than under well watered conditions. Thus, the

425   importance of the vacuolar pool of C soluble compounds is likely to increase with water

426   deficit (Kim *et al.,* 2000) whereas the cytosolic sugars are probably more important for

427   sugar sensing or triggering energy production. Furthermore, growth and development

428   might be better related to sugar fluxes imported from the phloem and/or to sugar

429   gradients (Munier-Jollain and Salon, 2003; Borisjuk et al., 2003; Makela et al., 2005),

430   than to their concentrations (see Fig. 5). Another possibility is that water deficit mainly

431   reduces growth through C-independent mechanisms, thus uncoupling growth from C

432   availability. These C-independent mechanisms are likely to be related to water flux to

433   growing cells, that is reduced under soil water deficit (Tang and Boyer, 2002), or to

434   mechanical properties of growing cell walls possibly under the influence of hormones

435   or pH (Fan and Neumann, 2004). Accordingly, it was recently shown that the cell wall

436   loosening proteins expansins are intimately coupled, at the transcriptional level, with

437   local expansion in maize leaves (Muller *et al.,* 2007). This suggest that the modification

438   (loosening) of the relationship between C availability and growth can be interpreted as

439   the signature of the passage from a source (C based) growth limitation to a less- or non-

440   C based (*i.e.* sink) limitation.

441

Conclusion

443

444   When plants are facing soil water deficits, growth is reduced and C concentrations rise,

445   possibly due to organ expansion being affected earlier and more intensively than

446   photosynthesis and metabolism. This leads to increased concentrations in various C

447   molecular forms in several plant parts, ruling out the idea that a stress-induced energy

448   deprivation would be the usual cause of growth reduction under water deficit. Elevated

449   C concentrations under water deficit are also likely to interfere with C signalling in a

450   manner that will deserve further attention. Under well-watered conditions, tight

451   relationships linking C availability and growth illustrate the source-limitation of growth

452   in sink organs such as roots, leaves (at night), flowers and fruits. These relationships

453   probably reflect the different uses of C-compounds, *i.e.* as fuel for energy supply, bricks

454   for structure build-up, osmotica for turgor maintenance as well as signal molecules for

455   triggering developmental and metabolic programs. Under water deficit, these

456   relationships are modified suggesting that other mechanisms, possibly involving cell

457   wall rheology or water fluxes to growing cells, override the role of C and take the lead

458   on growth control.

Acknowledgments

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Figures legends

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 (1970a) and Tardieu *et al.* (1999) for sunflower and maize, Granier *et al.* (2006) and Hummel et al. (2010) for Arabidopsis. X-axis refer to the level of water deficit expressed as mid-day leaf water potential difference between stressed and non stressed plants (poplar), mid-day leaf water potential (maize, sunflower), pre-dawn water potential of the whole rosette (Arabidopsis). Y axis refers to both photosynthesis (A, plain lines) and organ growth (Growth, dotted line). Both are shown as % of 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 X and Y axis 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.

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

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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 Ψ_w = - 0.3MPa) or severe (black circle, solute Ψ_w = - 0.5MPa) water deficit induced by PEG that was poured at the surface of agar medium before sowing. Elongation of primary (A - left panel) or secondary (B - right panel) 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 loosened at moderate stress and totally vanished or became negative under severe stress. The statistical significance of this loss of correlation 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 lower than 10^{-3}, 10^{-2}, 0.05, 10^{-1}, or non significant, respectively.

Fig. 3. Relationship between starch turnover and day or night leaf expansion in *Arabidopsis thaliana*. Nine *Arabidopsis* genotypes (incl. 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 (predawn leaf Ψ_w = - 0.35 MPa), a moderate soil water deficit (predawn leaf Ψ_w = - 0.6 MPa), or a severe deficit (predawn leaf Ψ_w = - 1.1 MPa). Genotypes included two wild-types: 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 days after sowing, the plants displayed steady-state rates of leaf production and successive leaves showed comparable behaviour (Pantin, *et al.,* unpublished). Zenithal images of 8 plants were taken twice a day during 3 days, 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 relative expansion rates (RER) 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) relative expansion rate was plotted against starch turnover. A linear model was fitted to each dataset. Note that the positive correlation between night RER and starch turnover loosened under severe water deficit as shown by an analysis of covariance (ANCOVA) performed with the R software (R Development Core Team, 2008), 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 growth was dependent on the water potential ($p < 10^{-1}$). By contrast, no significant interaction was detected for the correlations for day growth indicating that water deficit did not alter

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relationships between starch turnover and day growth. Symbols '***', '**', '*', '.', and 'ns' indicate that the p-value of a Pearson's correlation test was lower than 10^{-3}, 10^{-2}, 0.05, 10^{-1}, or non significant, respectively.

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 sugars content (mg g^{-1} DW) 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 deficits using shading or varying crop density (open triangles) or to moderate soil water deficits (grey triangles) either from capitulum initiation to 1st floret initiation (upward triangles), or from 1st floret initiation to completion of floret initiation (downward triangles). Some plants exposed to soil water deficit were re-irrigated (grey circles). Circles, control plants in the greenhouse and in field plots; squares, isolated field plants. Bars, SE. Redrawn from Dosio *et al.* (2010).

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 pressures 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 the R software (R Development Core Team, 2008) and run from 80 to 149 days after bloom at a hourly time step. Climatic inputs are air vapour pressure deficit (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 hours. (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 a moderate water deficit (MWD, – 1.1 MPa), and dark symbols are for a 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 an intermediate sugar supply (0.13 g g^{-1}), and triangle for a

low sugar supply (0.04 g g^{-1}). (B): Daily evolution of fruit relative growth rate (RGR) during the same simulations. RGR was computed as the local slope of the natural logarithm of fresh weight as a function of time. Same symbols than 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 hold for well-watered plants, moderate water deficit and severe water deficit respectively. (F): Effect of phloem sugar concentration and evaporative demand (estimated through the vapour pressure deficit (VPD)) in well-watered peach plants on fruit RGR. Days were categorised according to average VPD. At all imposed phloem sugar concentration, 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.
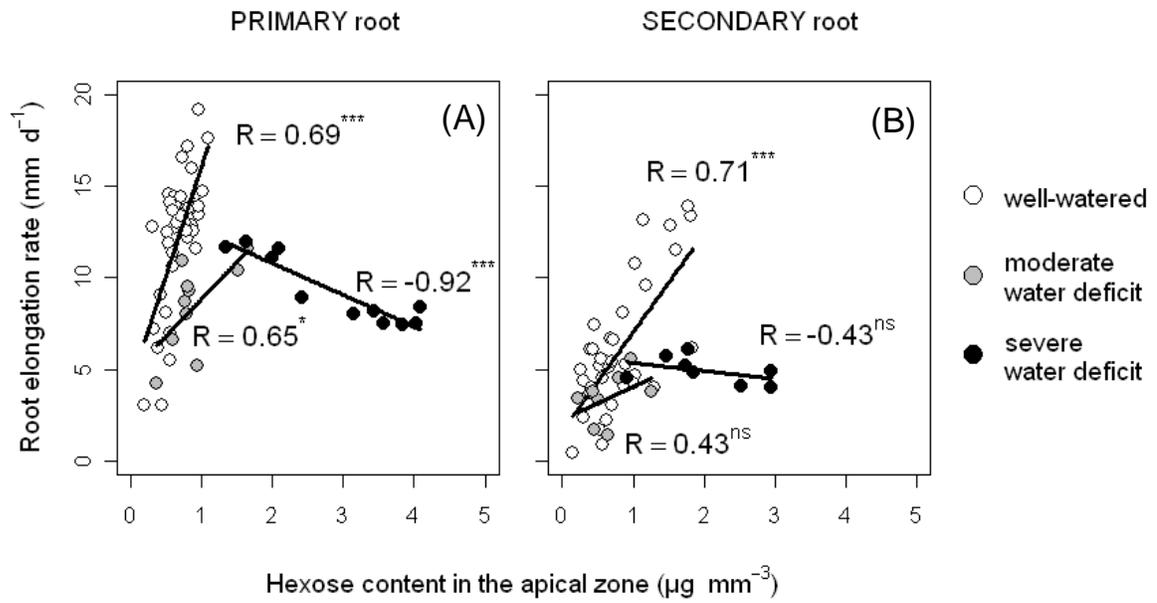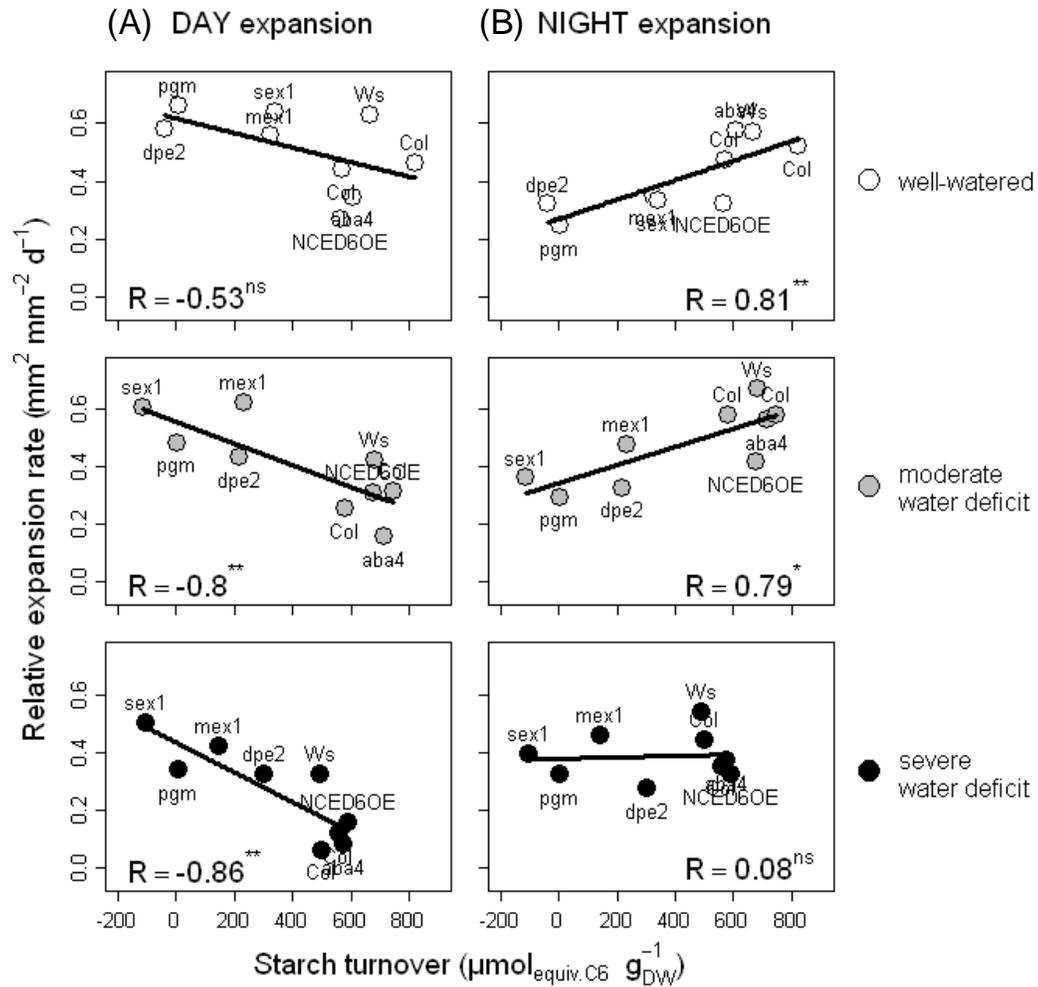
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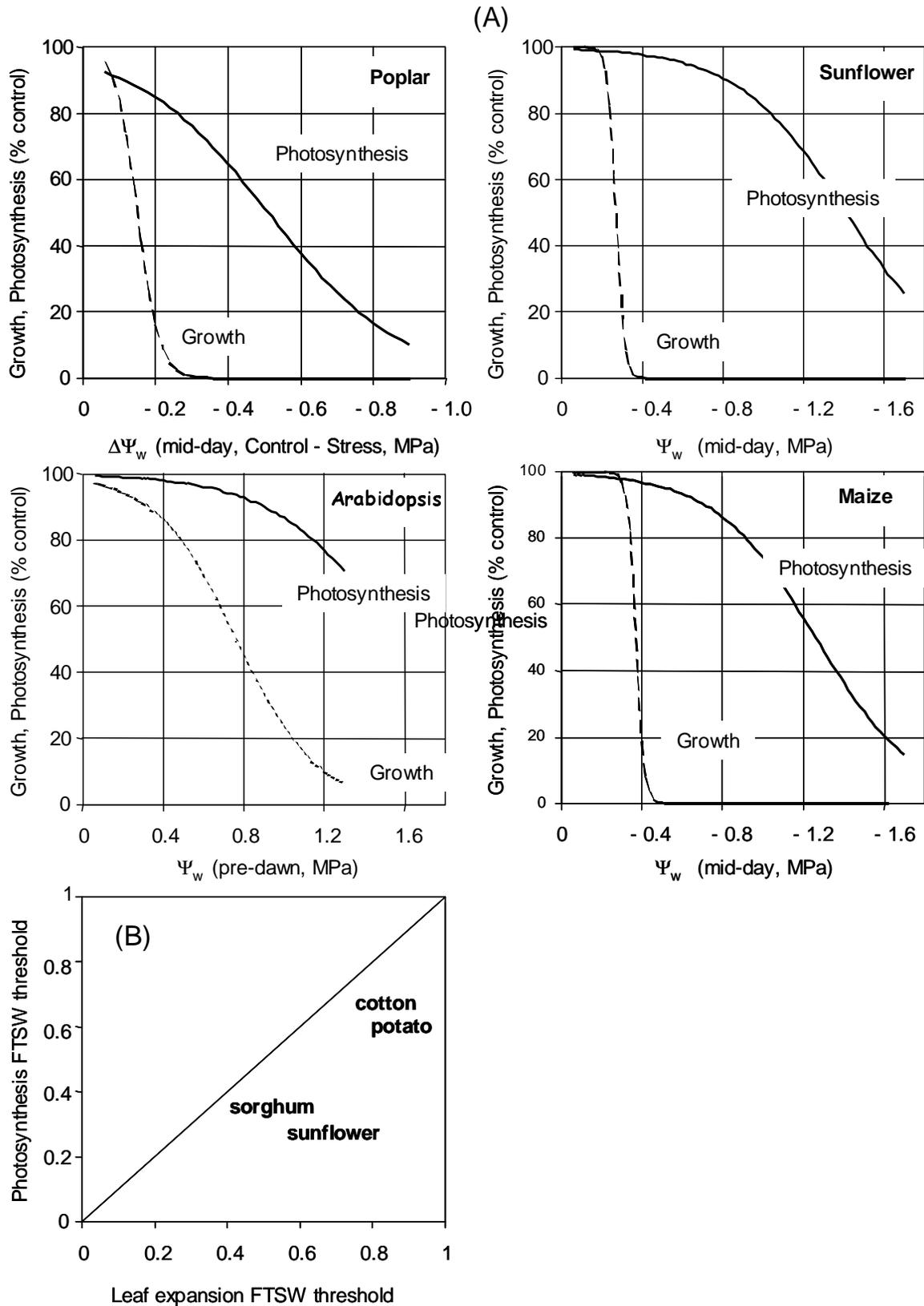
(A)

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