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Leaf carbohydrate controls over *Arabidopsis* growth and response to elevated CO_2: an experimentally based model

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Abstract

• Transient starch production is thought to strongly control plant growth and response to elevated CO_2. We tested this hypothesis with an experimentally based mechanistic model in *Arabidopsis thaliana.*

• Experiments were conducted on wild-type (WT) *A. thaliana,* starch-excess (*sex1*) and starchless (*pgm*) mutants under ambient and elevated CO_2 conditions to determine parameters and validate the model.

• The model correctly predicted that mutant growth is approx. 20% of that in WT, and the absolute response of both mutants to elevated CO_2 is an order of magnitude lower than in WT. For *sex1*, direct starch unavailability explained the growth responses. For *pgm,* we demonstrated experimentally that maintenance respiration is proportional to leaf soluble sugar concentration, which gave the necessary feedback mechanism on modelled growth.

• Our study suggests that the effects of sugar-starch cycling on growth can be explained by simple allocation processes, and the maximum rate of leaf growth (sink capacity) exerts a strong control over the response to elevated CO_2 of herbaceous plants such as *A. thaliana.*

Keywords: acclimation, allocation model, assimilate allocation, elevated CO_2, leaf starch, leaf sucrose.

Introduction

The current atmospheric CO_2 concentration is suboptimal for C_3 photosynthesis, which suggests that any future increase should benefit plant growth. However, it is often observed that plants acclimate to elevated CO_2 conditions through a reduction in their photosynthetic activity, which is generally associated with an increase in soluble leaf carbohydrates and starch (Drake *et al.* 1997). This circumstantial evidence is substantiated by several studies showing that hexose accumulation or cycling within the cells can repress photosynthetic gene expression through a hexokinase-controlled signalling pathway (Krapp *et al.* 1993; Jang *et al.,* 1997; Moore *et al.,*1999). More recently, hydrolytic conversion of starch to sucrose has also been thought to contribute to this sugar-sensing pathway (Sharkey *et al.,* 2004). Other studies suggested that accumulation of large starch grains disrupt chloroplast integrity (Pritchard *et al.,* 1997) or hinder CO_2 diffusion from the intercellular space to the chloroplast stroma (Sawada *et al.,* 2001).

Both soluble sugar and starch metabolisms can affect photosynthesis. However, the role played by carbohydrates in mechanisms controlling plant acclimation to elevated CO_2 remains unclear, as their accumulation in photosynthetic tissues does not always correlate with photosynthetic acclimation to elevated CO_2 (Moore *et al.,* 1998; Sims *et al.,* 1998; Geiger *et al.,* 1999). The emerging paradigm is that of whole-plant-level control of photosynthesis through a signalling network that integrates the global source-sink status of the plant (Coruzzi & Zhou, 2001; Paul & Foyer, 2001). In this context, transient starch becomes functionally important as an overflow product for photosynthesis in plants with low sink capacity. Indeed, the sink-regulation hypothesis suggests that starch accumulation in leaves during the light period is enhanced when the photosynthetic production rate exceeds that of growth and transport processes. Grimmer & Komor (1999) report that carbon export from leaves of *Ricinus communis* L. during the light period was identical for plants exposed to 350 and 700 ppm CO_2, while night-time exports of 700-ppm plants doubled those of their 350-ppm counterparts. They hypothesized that *Ricinus* plants grown under ambient CO_2 conditions are sink-limited during daytime and source-limited at night. Starch accumulation would be just a symptom that sugar is available in excess of plant growth demand and phloem transport capacities.

Observations conducted on *Arabidopsis* plants affected in starch synthesis and breakdown rates suggest that it is not the starch content itself, but the ability to sustain a steady supply of soluble sugar, that is crucial for normal plant growth. The same phenotypic response of substantially reduced growth compared with the wild type (WT) was reported for both starch-excess (Caspar *et al.,* 1991) and starch-deficient mutants (Caspar *et al.,,* 1985; Schulze *et al.,* 1991; Sun *et al.,* 1999). The capacity of these mutants to benefit from elevated CO_2 conditions in terms of plant growth is also decreased compared with that of the WT. In *Arabidopsis* starch-deficient mutants, Sun *et al.* (1999) have shown that the growth response to atmospheric CO_2 enrichment was closely related to starch turnover. Moreover, they observed that, in the case of a starchless mutant — when the capacity of the plant to use starch as transient carbon storage is null — increased atmospheric CO_2 concentration did not stimulate growth at all. The photosynthetic response to elevated CO_2 of starch-deficient transgenic potatoes was also found to be positively correlated to their capacity to produce leaf starch (Ludewig *et al.*, 1998).

Arabidopsis mutants impaired in their starch metabolism display a sharply reduced growth rate and response to elevated CO_2 compared with their nonmutated counterpart. Starch contents of *A. thaliana* leaves display a pronounced diurnal pattern, where starch is converted back to sugar during the night to sustain maintenance and growth (Cheng *et al.,* 1998; Zeeman & ap Rees, 1999; Gibon *et al.,* 2004). If the main difference between ambient and elevated CO_2-grown plants happens during the night, as suggested by Grimmer & Komor (1999), then the ability of the plant to regulate a steady flux of available sugar during the night appears pivotal to normal growth and sustained response to elevated CO_2.

In the present study, we hypothesize that reduced sugar availability during the night in both starch-deficient and starch-excess mutants of *A. thaliana* is responsible for their lower growth rates and reduced responses to elevated CO_2 compared with the WT. To test this hypothesis, we developed an experimentally based model of the starch metabolism of *A. thalianaas* it relates to its growth pattern. The objective of the present research was to demonstrate that such a model can predict differential growth patterns between the WT and mutants based on single rate modifications of starch synthesis and breakdown in leaves.

Materials and Methods

Plant culture

Experiments were conducted on plants of *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia (referred to as WT) and two monogenic mutants: the *plastid phosphoglucomutase (pgm)* TC75 starchless mutant (Caspar *et al.,* 1985); and the *starch-in-excess 1 (sex1)*TC265 mutant (Caspar *et al., 1991).* The *pgm* mutant is unable to synthesize starch because of inactivation of the chloroplastic isozyme of the phosphoglucomutase, which converts glucose-6-phosphate into glucose-1-phosphate. The *sexl* mutant has an altered starch degradation rate because of its defect in the glucan water dikinase protein that functions as a regulator of starch mobilization by controlling the phosphate content of starch (Yu *et al.*, 2001). Seeds of these two mutant lines were kindly provided by C. R. Somerville (Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA, USA) and C. Dean (John Innes Centre, Norwich, Norfolk, UK), respectively.

Hydroponics culture as described by Tocquin *et al.* (2003) was used consistently for all sets of experiments. Briefly, seeds - first stratified for 3 d on wet filter paper at 2°C - were sown individually on 0.65% agar-containing seed holders. Eight seed holders were inserted in the cover of a 1-1 black plastic container filled with nutrient solution. The solution was renewed weekly from the third week of growth to prevent nutrient depletion. The composition of the nutrient solution was based on Hoagland's, with macronutrients diluted fourfold (Gibeaut *et al.*, 1997). Nitrogen was provided by $NO_3^-$and NH_4^+ in a constant ratio of 27 : 1. The N supply was adjusted at 3.5 mM NO_3^-

Plants were grown in phytotrons in 8-h short days. This photoperiod length was chosen to increase the duration of the vegetative stage and allow us to perform measurements over a longer period and with more leaf biomass. These experimental conditions chosen to facilitate data acquisition are not a requisite for observing contrasted growth responses between starch mutants and wild types, as demonstrated in 12-h-day studies (Zeeman *et al.,* 1998; Sun *et al.* 2002). The photon fluence rate was 120 µmol m^{-2} s^{-1} PAR (Very High Output fluorescent tubes, Sylvania, Zaventem, Belgium); temperature was 20°C (day : night) and relative humidity was 70%. CO_2 level in phytotrons was recorded and controlled by an infrared gas analyser (WMA-3, PPSystems, Hitchin, UK) equipped with a set-point controller (VISIREG$_+$, MCC, Issoudun, France). The atmospheric CO_2 concentration was either not controlled (ambient CO_2 conditions, ± 390 ppm) or set to 800 ppm (elevated CO_2 conditions).

Growth measurements

The total LS was measured three times a week, at 2- or 3-d intervals, on digital pictures of intact rosettes using image analysis software (SIGMASCAN PRO 4, SPSS Inc., Chicago, IL, USA), calibrated with a scale included in the picture.

The shoot fresh weight was recorded as the whole rosette biomass measured immediately after harvest, after drying the whole root system on filter paper. The shoot-to-root ratio was calculated on individual plants by simultaneous harvest of whole shoot and root parts, as described above.

Starch, sucrose and hexose determination

Approximately 100 mg liquid N_2-ground leaf material was extracted three times in 500 µl 80% (v/v) ethanol at 70°C. The ethanol-soluble fractions were pooled, dried under vacuum and dissolved in 250 µl distilled water. The ethanol-insoluble residue was dried briefly under vacuum and homogenized in 800 µl in 0.1 M citrate buffer pH 4.6. Starch was solubilized by 1 h autoclaving. Starch, sucrose and hexose were then assayed enzymatically in separate aliquots of samples (all enzymes from Roche Diagnostics, Vilvoorde, Belgium). This assay is based on the equimolar release of NADPH by the reaction of hexokinase/glucose-6-phosphate deshydrogenase on glucose in a reaction mixture containing 0.44 M triethanolamine pH 7.6, 5.8 mM $MgSO_4$, 0.3 mM $NADP^+$ and 0.3 mM ATP. NADPH is subsequently detected by its fluorescence at 445 nm (Jones, 1979) using a fluorimeter. In separate aliquots, fructose, sucrose and starch were first converted to glucose by the action of phosphoglucose isomerase, β-fructosidase/phosphoglucose isomerase and amyloglucosidase, respectively.

Gas-exchange measurements

The gas-exchange measurements were performed as described by Tocquin & Périlleux (2004) using a custom-made cuvette enclosing the whole rosette. The cuvette is attached to a differential CO_2/H_2O infrared gas analyser (Ciras-1, PPSystems) equipped with an automatic air supplier allowing control of the flow rate and the CO_2 and H_2O partial pressure of the air entering the cuvette.

The total LS, measured as described above, was used to calculate CO_2 assimilation per unit leaf area. Each data point is the mean of five measurements performed on three to five different plants. Unless specified, the CO_2 assimilation rate was measured under growing conditions.

The dark respiration rate in the dark and also in the light *(R_{day})* was measured according to Parsons *et al.* (1997) under controlled CO_2 concentration (400 ppm), close to ambient CO_2 level. According to Parsons *et al.* (1997), R_{day} is the minimum CO_2 production that occurs after the light is switched off and the release of CO_2 from photorespiration has ceased. In practice, we have determined that, in our experimental conditions, this minimum occurs 2 min after the light has been switched off. In these conditions, our whole-plant cuvette has been proven to give accurate measurements of small CO_2-exchange rates, minimizing technical errors that could arise with respiratory CO_2 measurements such as those reported by Jahnke (2001).

To calculate the maximum rate of carboxylation (V_{cmax}), CO_2 uptake was measured under saturating irradiance [$A_{sat'}$ ± 1000 µmol m^{-2} s^{-1} PAR] in response to variation in external CO_2 concentration. V_{cmax} was calculated by fitting model equations from von Caemmerer & Farquhar (1981) on curves obtained by plotting A_{sat} against calculated intercellular CO_2 by the least-squares method, using PHOTOSYN ASSISTANT (Dundee Scientific, Dundee, UK).

The model

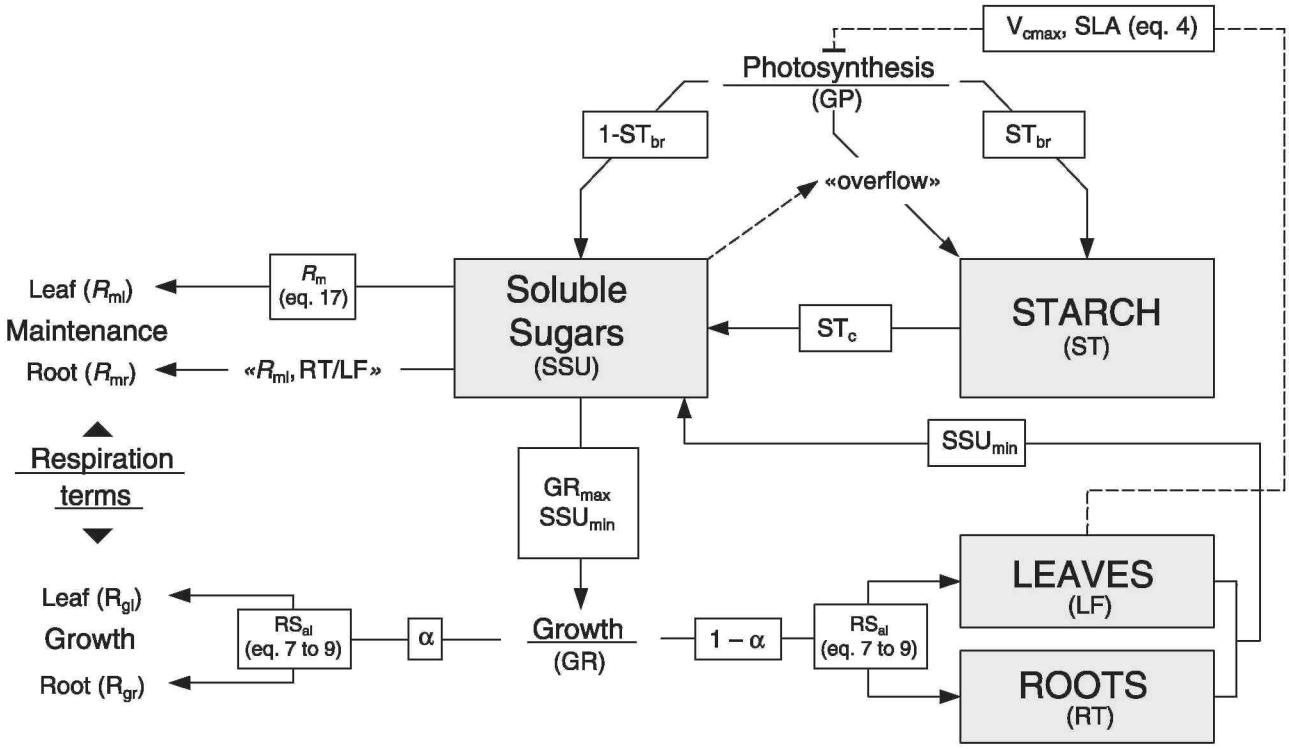
We developed a mechanistic allocation and growth model for *A. thaliana* that predicts the evolution over time of four reservoirs: sucrose, starch, leaf and root (Fig. 1). All reservoir units are in g C per plant. The model was encoded in FORTRAN 95, and uses a simple, explicit scheme where mathematical stability is ensured by taking short time steps (6 s). Figure 1 is a faithful representation of the logical succession of processes as described in the encoded model. Leaf photosynthesis is modelled with the equations of Farquhar *et al.* (1980). To run the basic version of this photosynthesis model, two leaf parameters are needed: the maximum rate of carboxylation (V_{cmax}) and the specific leaf area (SLA). All other photosynthesis parameters were identical to those of Farquhar *et al.* (1980), either in absolute value or in ratio, such as the maximum rate of electron transport *(J_{max})* which was taken as 2.1 times V_{cmax}.. The model was forced with environmental conditions in the growth chambers: irradiance, photoperiod, air CO_2 partial pressure and temperature.

The model simulates starch synthesis from photoassimi-lates during the light period and the conversion of starch into sugar during the dark period. In the model, we followed the hypothesis of Sun *et al.* (1999) that starch is produced at a programmed baseline rate when photosynthesis is low, and through an overflow mechanism when photosynthesis exceeds organ growth demand and respiratory costs. The fact that soluble sugar levels are quite stable in *Arabidopsis* leaves (Cheng *et al.,* 1998; Van der Kooij *et al,* 1999; Sun *et al,* 2002) suggests that baseline starch production (ST_{base}) is a function not of leaf sugar concentration, but rather of the rate of photoassimilate production. Starch is produced directly from photosynthesis, which does not involve intermediate sucrose stages (Geiger *et al.,* 2000). In agreement with Dewar *et al.* (1998), our modelling diagram reflects this point (Fig. 1):

$$ST_{base} = GP \times ST_{br} \qquad \text{Eqn 1}$$

where GP is gross photosynthesis and ST_{br} the baseline starch production coefficient (Fig. 1). A list of model variables and abbreviations is presented in Table 1.

Fig. 1*: Schematic representation of the model. Grey boxes, model pools; white boxes, model parameters; solid arrows, fluxes; dotted arrows, feedbacks. For abbreviations see Tables 1,2.*



When photosynthesis exceeds growth and maintenance demands, starch is produced through an overflow mechanism, as suggested by Eichelmann & Laisk (1994); Stitt (1996), which suggests that sink-limited growth conditions have been reached. Implementing this mechanism in the model requires determining the maximum growth demand. In practice, organ growth demand is difficult to measure because it includes above-and below-ground components and factors in the growth respiration costs. For this reason we chose to use the maximum leaf growth rate (GR , Fig. 1), which is a more easily measurable parameter and can be estimated from published leaf-growth studies (Van der Kooij & DeKok, 1996; Gibeaut *et al.,* 2001). In the model, organ growth demand is recomputed from GR_{max} as a function of the root-to-shoot allocation ratio (RS_{al}, Fig. 1) and the fraction of growth respiration (α, Fig. 1). Conversion of starch (ST) to sugar during the night was modelled as a linear decrease from the end-of-day to the end-of-night contents, according to:

$$d(ST)/dt = (ST_{maxl} \times ST_c)/t_{dp} \qquad \text{Eqn 2}$$

where ST_{maxl} is the maximum starch content at the end of the light period; ST_c is the proportion of ST_{maxl} that is

converted into soluble sugars during the dark period; and t_{dp} is the duration of the dark period. Literature data suggest that linear decrease is a better descriptor than first-order kinetics of night-time starch mobilization in the leaves of *A. thaliana* (Cheng *et al.,* 1998; Gibon *et al.,* 2004). In addition, a linear decrease is consistent with a preprogrammed starch consumption pattern, where the plant adapts its starch mobilization rate to the duration of the dark period (Gibon *et al.,,* 2004). The choice of starch degradation equation does not appear as a key hypothesis of the model, as preliminary sensitivity analyses conducted with the model indicated that final simulated plant surfaces differ by < 5% between linear decrease and fist order-kinetics scenarios (data not shown).

Table 1: *Abbreviations (for detail on model parameters see Table 2)*

Abbreviation	Meaning
α	Growth respiration coefficient
A_{gl}	Assimilates allocated to leaf growth
A_{gp}	Assimilates allocated to plant growth
A_n	Net assimilation
A_{sat}	Assimilation at saturating irradiance
GP	Gross photosynthesis
GR_{max}	Maximum leaf growth rate
LS	Leaf surface area
LF	Leaf biomass
R_{day}	Daytime leaf respiration
$R_{endnight}$	End-of-dark-period leaf respiration
R_{gl}	Leaf growth respiration
R_l	Total leaf respiration
R_{ml}	Leaf maintenance respiration
R_{mr}	Root maintenance respiration
RS_{al}	Root-to-shoot allocation ratio
RS_{bio}	Root-to-shoot biomass ratio
SLA	Specific leaf area
SSU	Leaf soluble sugar content
SSU_{min}	Minimum sugar in leaves
ST	Leaf starch content
ST_{base}	Baseline starch production
ST_{br}	Baseline starch production coefficient
ST_c	Proportion of night-time starch breakdown
ST_{maxl}	Maximum ST at the end of the light period
t_{dp}	Duration of dark period
V_{cmax}	Maximum rate of carboxylation

Soluble sugar concentration increases substantially in the leaves of the starchless *pgm* mutant during the light period, and drops sharply at the onset of the dark period (Caspar *et al.,* 1985). In parallel, the net photosynthetic assimilation of the *pgm* mutant is reduced compared with that of the WT (Sun *et al.,,* 1999). Several studies indicate that carbohydrate accumulation in cells produces negative feedbacks on photo-synthetic rates through repression of photosynthetic genes (Krapp *et al.,* 1993; Koch, 1996; Jang *et al.,* 1997) or a limitation in triose phosphate utilization, which inhibits P_i recycling (Sun *et al.,* 1999). Other studies report that photosynthetic electron transport, and total content and activity of Rubisco, are not significantly decreased by *pgm* mutations (Kofler *et al.,* 2000; Sun *et al.,* 2002), suggesting that the nature and magnitude of direct downregulation mechanisms remain somewhat elusive. Without excluding the existence of downregulation mechanisms, we suggest that the bulk of the reduction in net photosynthesis of the *pgm* mutant can equally be explained by a concomitant increase in the maintenance respiration rate, while the gross photosynthetic rate would remain mostly unaffected by increased soluble sugar concentrations. This hypothesis is not entirely new, as the dependence of maintenance respiration on soluble sugar, but not on nitrogen concentration, was demonstrated for dormant plants by Ogren (2000). This author suggests that increases in maintenance respiration in response to increases in soluble sugar concentrations are directly linked to the costs of maintaining concentration gradients. In our model, this hypothesis can easily be translated as:

$$R_{ml} = LS \times f(SSU) \quad \text{Eqn 3}$$

where R_{ml} is the total leaf maintenance respiration (g Cd^{-1} per plant); LS is the leaf surface area per plant (m^2 per plant); SSU is the total soluble sugar content of the leaf (g glucose-equivalent-C m^{-2} leaf); and *f* is an abbreviation for function. In the following parameter-determination section we experimentally confirm the existence of such a relationship, determine its nature and quantify its terms. Although this relationship is best demonstrated in the sugar-accumulating *pgm* mutant, we hypothesized here that its holds true across *Arabidopsis* lineages.

For lack of detailed experimental evidence, and for parsimony reasons, we considered the root maintenance respiration rate per unit biomass identical to that of shoots. Because leaf maintenance respiration depends on leaf soluble sugars, our parsimony hypothesis suggests that a similar process happens concomitantly in the roots through translocation processes. In that sense, the model representation (Fig. 1) suggests that a portion of soluble sugars from leaves is transferred to roots and immediately used to cover maintenance respiration costs of roots. This was done because root-soluble sugars are not explicitly modelled and are considered part of the root biomass, which is coherent with our root biomass measurements. We did not find sufficient information in the limited literature on translocation to roots in *Arabidopsis* WT and mutants to sustain a detailed mechanistic description of root soluble sugars. Neither did we have the opportunity to conduct high-frequency measurements of root soluble sugars and root respiration rates, preferably as pulse-chase experiments, in order to develop a full shoot—root translocation scheme in the model. Although, in reality, soluble sugar kinetics are not identical in roots and shoots of *Arabidopsis* (Zeeman & ap Rees, 1999), uncertainties about the exact nature of this relationship are unlikely to have major effects on the predictions of our model. Indeed, leaf-level processes largely dominate carbohydrate cycling in *Arabidopsis* because of its low sink capacity combined with its small response to sugar exports (Geiger *et al.,,* 2000).

Published experimental results indicate that the leaf soluble sugar content never reaches zero (Cheng *et al.,* 1998; Sun *et al.,* 1999), which required us to define a minimum leaf sugar concentration in the model (SSU_{min}; Fig. 1). We also created a venue in the model for the conversion of nonstarch metabolites from leaves and roots back to sugar, in order to cover maintenance respiration costs when sugar levels have reached their minimum concentration during the night and starch reserves are exhausted. This approach is warranted by experimental results with starchless mutants indicating that soluble sugar is consumed rapidly at the beginning of the dark period, while it never reaches zero at the end of the dark period (Caspar *et al.,* 1985). Thimm *et al.* (2004) have shown that the levels of hundreds of genes involved in amino acid, nucleic acid, lipid and cell-wall breakdown increase significantly at the end of the night in an *Arabidopsis* starchless mutant. These observations suggest that sources of energy other than starch can be mobilized during the dark period to cover maintenance respiration costs.

Parameters

Maximum rate of carboxylation V_{cmax} was measured on WT three times in each of three ambient CO_2 experiments conducted in our standard experimental conditions. Each of the nine measurements was itself the average of submeasure-ments conducted on three to five individual plants. The resulting average V_{cmax} was 18.8 (+4.9, n = 9) µmol CO_2 m^{-2} $leafs^{-1}$ at 20°C (Table 2).

Specific leaf area index SLA was obtained in a previous experiment that demonstrated the tight relationship between SLA (m^2 g^{-1} C) and the total leaf surface of the plant (LS, m^2) for *A. thaliana* across plant age, air CO_2 levels and nitrogen availability (P.T. and co-workers, unpublished data):

$$SLA = 0.155 + 0.229 \times \exp(-2315 \times LS) \ (r^2 = 0.85) \quad \text{Eqn 4}$$

Large potential starch accumulation in the *sex1* mutant suggests that SLA should be expressed per unit nonstarch leaf C, reflecting the fact that accumulated starch does not contribute to increases in leaf surface. Accordingly, leaf starch is decoupled from the photosynthesis-contributing leaf C in our model (Fig. 1). Leaf starch content of ambient CO_2 WT plants averaged 6% (+1%, n = 10) (data not shown). Therefore we divided the SLA values obtained from equation 4 by a factor of 0.94 in order to report SLA per unit nonstarch leaf C.

Table 2: *Model parameters*

Parameter	Symbol	Value	Units	Source
Baseline starch production coefficient	$s\tau_{br}$	0.125 (WT, sex1) 0 (*pgm*)	Dimensionless	This study
Growth respiration coefficient	α	0.195	Dimensionless	This study
Maintenance respiration	R_{ml}	equation 17	Dimensionless	This study
Maximum leaf growth rate	GR_{max}	0.41	$gg^{-1}d^{-1}$	Gibeaut *et al*. (2001), Van der Kooij & DeKok (1996)
Maximum rate of carboxylation at 20°C	$V_{cmax\ 20}$	18.8	µmol CO_2 m^{-2} s^{-1}	This study
Minimum sugar in leaves	ssu_{min}	0.05	g sucrose-C m^{-2}	This study
Proportion of night-time starch breakdown	$s\tau_c$	83 (WT)	Percentage	This study
		14 *(sex1)*	Percentage	Caspar *et al.* (1991)
Root-to-shoot allocation ratio	RS_{al}	equations 7-9	Dimensionless	This study
Specific leaf area	SLA	equation 4	m^2 $g\text{-}^1$ C	This study

Maximum growth rate of leaves GR_{max} was estimated on the basis of literature data. Our hypothesis that a GR_{max} exists at a given N status suggests that leaf growth reaches a given plateau despite increasing irradiance and air CO_2 concentration. According to our modelling hypothesis, this plateau corresponds to sufficient starch accumulation during the day so that maximum night growth can be attained. Van der Kooij *et al.* (1996) reported that under long days and high irradiance (12 h at 300 µmol m^{-2} s^{-1} PAR), WT leaf growth was insensitive to elevated CO_2 (350 vs 700 ppm) and averaged 0.42 d^{-1}. With slightly shorter days (10 h) and under 400 µmol m^{-2} s^{-1} PAR, Gibeaut *et al.* (2001) reported that the WT leaf growth responded little to elevated CO_2: 0.34 d^{-1} under 360 ppm CO_2 and 0.4 d^{-1} under 1000 ppm CO_2. As both these studies were conducted under N-availability conditions similar to ours, these results suggest that GR_{max} averages 0.41 d^{-1}.

Root-to-shoot allocation ratio RS_{al} is not a directly measurable quantity, but is intimately linked to the measurable root-to-shoot biomass ratio (RS_{bio}). Indeed, RS_{bio} corresponds to the ratio of the time integrals of the net amounts of growth assimilates per plant (A_{gp}) allocated to roots and shoots, respectively:

$$RS_{bio^t} = \left(\int_0^t \frac{A_{gp} \times RS_{al}}{RS_{al} + 1} dt \right) \Bigg/ \left(\int_0^t \frac{A_{gp}}{RS_{al} + 1} dt \right) \qquad \text{Eqn 5}$$

with the caveat that neither root nor shoot death occurred during the course of the experiment, which seems reasonable in our growing-phase experimental conditions. Equation 5 suggests that, if RS_{al} is constant through time, RS_{bio} is also constant through time and equal to RS_{al}. However, our experiments demonstrated that RS_{bio} is not constant in the early stages of *A. thaliana* development (Fig. 2). We therefore needed to back-calculate RS_{al} from measurements of RS_{bio}. Because A_{gp} changes with time and is not a measurable quantity we made the simplifying assumption that A_{gp} is directly proportional to the measurable leaf surface (LS). This assumption is valid only in constant environmental conditions such as ours, and suggests that the change in maintenance root respiration with changing RS_{bio} is minimal compared with A_{gp}. Under these conditions, equation 5 becomes:

$$RS_{bio^t} \approx \left(\int_0^t \frac{LS \times RS_{al}}{RS_{al} + 1} dt \right) \Bigg/ \left(\int_0^t \frac{LS}{RS_{al} + 1} dt \right) \qquad \text{Eqn 6}$$

We solved this equation numerically based on an experiment where LS and RS_{bio} of WT plants of different ages were measured (Fig. 2). To solve this equation, we used the *a priori* simple model that RS_{al} changes linearly with time from a minimum to a plateau value. Coherently with our model, RS_{bio} was expressed on a per nonstarch

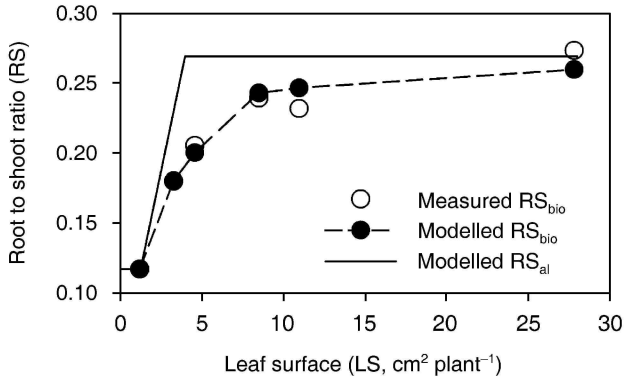
basis. The resulting equations were:

$$RS_{al} = 0.12,\ LS < 1.22 \times 10^{-4}\ m^{-2} \quad \text{Eqn 7}$$

$$RS_{al} = 0.0496 + 555 \times LS,\ 1.22 \times 10^{-4}\ m^2 < LS < 3.96 \times 10^{-4}\ m^{-2} \quad \text{Eqn 8}$$

$$RS_{al} = 0.27,\ LS > 3.96 \times 10^{-4}\ m^{-2} \quad \text{Eqn 9}$$

***Fig. 2** : Root-to-shoot allocation ratio (RS_{al} solid line) as a function of leaf surface area (LS) as described in equations 7-9, as derived numerically from equation 6 applied to measured root-to-shoot biomass ratio (RS_{bio}, open circles). The resulting modelled RS_{bio} (closed circles, dashed line) derived from the numerical integration of equation 6 is also represented.*



Baseline starch production coefficient ST_{br} was estimated on WT exposed to low light conditions in order to induce starch production at baseline levels. Gross photosynthesis (GP) in equation 1 was estimated as the sum of net leaf assimilation (A_n) and total respiration rate (R_l) measured at 2-h intervals throughout the 8-h light period on 6-wk-old plants grown at 120 PAR m^{-2} s^{-1} and exposed for 1 d at 20 and 50 µmol PAR m^{-2} s^{-1}. In the same experiment, we measured the increase in leaf starch content over the 8-h light period. Leaf starch accumulation during the light period was obtained from the difference between measurements conducted at the beginning and end, respectively, of the light period. Our results indicated that the conversion of assimilated sugars into starch was fairly similar at 20 and at 50 µmol PAR m^{-2} s^{-1}, which confirmed that a baseline level exists (Table 3). The resulting ST_{br} value was 0.125 (Table 2).

***Table 3:** Computation of the baseline starch production coefficient (ST_{br}) from wild-type (WT) Arabidopsis thaliana gross photosynthesis and net starch production (ST_{prod}) over an 8-h light period, at either 20 or 50 µmol PAR m^{-2} s^{-1}*

Irradiance (µmol PAR m^{-2} s^{-1})	Carbon fluxes (g C m^{-2} leaf 8-h^{-1})				
	A_n	R_l	GP	ST_{prod}	ST_{br}
20	0.092 (±0.046)	0.063 (±0.050)	0.155	0.017 (±0.002)	0.110
50	0.610 (±0.140)	0.122 (±0.047)	0.732	0.101 (±0.012)	0.138

Errors are SD for five measurements conducted every 2 h throughout the light period and reported on an 8-h basis. For abbreviations see Table 1.

Growth respiration coefficient α is the ratio of the growth respiration of the leaves (R_{gl}) to the total amount of assimilates allocated to leaf growth processes (A_{gl}):

$$\alpha = R_{gl}/A_{gl} \qquad \text{Eqn 10}$$

R_{gl}, which cannot be measured directly, is the difference between the total leaf respiration (R_l) and the maintenance respiration term (R_{ml}):

$$R_{gl} = R_l - R_{ml} \qquad \text{Eqn 11}$$

R_l represents the total respiration of the plant under active growth conditions, that is, during the light period (R_{day}). Our hypothesis that growth does not occur when starch reserves are exhausted at the end of the night period suggests that the respiration measured at that time ($R_{endnight}$) is equal to R_{ml} Therefore equation 11 becomes:

$$R_{gl} = R_{day} - R_{endnight} \qquad \text{Eqn 12}$$

Similarly to R_{gl}, A_{gl} cannot be measured directly. Nevertheless, A_{gl} can be estimated as follows during the daytime period:

$$A_{gl} = [GP \times (1 - ST_{br}) - R_{ml} - R_{mr}]/(1 + RS_{al}) \qquad \text{Eqn 13}$$

Starch production, assumed at its baseline level (ST_{br}), is discounted from GP because it is not a growth process *per se.* Similarly, maintenance respiration rates *(*R_{ml} and R_{mr}*)* need to be removed, as they are not involved with growth processes. The net assimilated C for growth processes then needs to be divided by ($1 + RS_{al}$), so that only the leaf component is taken into account. In equation 13, R_{mr} is unknown but can be linked to R_{ml} assuming proportionality to biomass:

$$R_{mr} = R_{ml} \times RS_{bio} \qquad \text{Eqn 14}$$

and

$$GP = A_n + R_l \qquad \text{Eqn 15}$$

Combining equations 10, 12-15, we obtain:

$$\alpha = [(R_{day} - R_{endnight}) \times (1 + RS_{al})]/[(A_n + R_{day}) \times (1 - ST_{br}) - R_{endnight} \times (1 + RS_{bio})] \qquad \text{Eqn 16}$$

Measurements of R_{day}, $R_{endnight}$ and A_n were conducted on 6-wk-old plants grown in our standard experimental conditions: R_{day}=0·95 μmol CO_2 m^{-2} s^{-1} (SD = 0.18, n = 20), $R_{endnight}$ = 0.47 μmol CO_2 m^{-2} s^{-1} (SD = 0.10, n= 5), A_n = 3.29 μmol CO_2 m^{-2} s^{-1} (SD = 0.43, n = 20). Parameters were: ST_{br} = 0.125 (Table 2); RS_{al} = 0.27 (Table 2); RS_{bio} = 0.25 (Fig. 2). These values combined in equation 16 yield α = 0.195, which is equal at two-digit precision to the 0.2 reference value generally used in models (Rasse *et al.,* 2001). In the model, OC for roots is assumed to be identical to that of shoots.

Starch-to-soluble sugar conversion Equation 2 requires an estimate of ST_c, the percentage of ST_{maxl} that is converted back to soluble sugars during the dark period. Measurements conducted on 8-h light-grown WT indicated that average end-of-light-period starch contents averaged 0.0392 g glucose-equivalent-C g^{-1} DW (±0.0070, n= 8), while end-of-dark-period starch contents averaged 0.0066 g glucose-equivalent-C g^{-1} DW (±0.0024, n = 8*).* Therefore the value of ST_c for WT was 83%.

Starch breakdown rate is the only difference in our model between WT and the *sex1* mutant. Several studies indicate that the *sexl* mutant is not an absolute starch-deficient mutant, but rather suffers from a much-reduced conversion rate of accumulated starch to soluble sugars (Caspar *et al.,* 1991; Trethewey & ap Rees, 1994; Zeeman & ap Rees, 1999). Based on the data presented by Caspar *et al.* (1991) for the *sex1* mutant, we estimated that ST_c approximates 14% of ST_{maxl} after 16 h night, as in our experimental conditions.

Relationship of R_{ml} to sugar content This relationship was established through concomitant measurements of total soluble sugar contents and leaf respiration rates in the *pgm* mutant. Measurements were conducted five times during a 1-d period. The first measurement was performed 1 h before the onset of the light period, at the end of the 16-h night period, and was followed by four other measurements every 2 h during the 8-h light period. At each time point, three to five individual plants were used for respiration measurements, and each rosette was harvested individually, sampled in two replicates and freeze-dried for subsequent sucrose measurements.

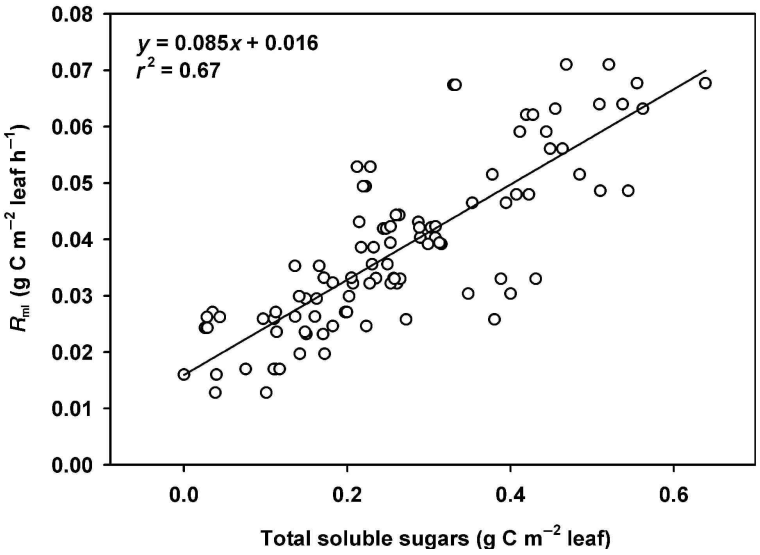
Respiration measurements compound maintenance and growth components, which called for a parallel estimate of R_{gl}. Given that OC had been determined previously, an actual measurement of the leaf growth rate is sufficient to derive R_{gl}. Here the *pgm* mutant presents the advantage, compared with WT, that night growth can be neglected given that (1) it is starchless, and (2) its soluble sugar content drops rapidly after the onset of the dark period (Caspar *et al.,* 1985). Therefore growth rates can be reported per hour of exposure to light, 8 h in our experimental conditions. Leaf surfaces of the *pgm* mutant were measured at the time of respiration measurements, 62 d after sowing (DAS) and were also evaluated for 18-d plants. Equation 4 was used to convert leaf surfaces into biomass C. We used a simple exponential growth model between the two dates to calculate the growth rate of *pgm* plant leaves at the time of the respiration measurements: 0.056 g C m^{-2} leaf h^{-1}. This net leaf growth value was multiplied by [α/(1 - α)] to yield R_{gl} : 0.0136 g C m^{-2} leaf h^{-1}. This R_{gl} estimate was then subtracted exclusively from our daytime R_1 measurements. We obtained an excellent relationship between estimated R_{ml} rates (g C m^{-2} h^{-1}) and total soluble sugar content per unit leaf area (SSU, in g C m^{-2}; Fig. 3):

$$R_{ml} = 0.085 \times SSU + 0.016 \ (r^2 = 0.67, P < 0.01) \qquad \text{Eqn 17}$$

This experiment confirms our hypothesis that high soluble sugar contents increase maintenance respiration rates, which could account to some extent for the apparent reduction in net photosynthetic rate reported for starchless and starch-deficient mutants (Caspar *et al.,* 1985; Sun *et al.,* 1999).

Minimum sucrose content in leaves Minimum sucrose content in the leaves of *A. thaliana* was taken as the lowest night-time value obtained in the experiment conducted to determine the R_{ml}-SSU relationship. This value averaged 0.05 g sugar C m^{-2} leaf.

Fig. 3: *Relationship between rate of leaf maintenance respiration (R_{ml}) and total soluble sugar content (SSU) of leaves of the Arabidopsis pgm mutant.*



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Simulation settings

Simulations could not be started at the time of planting because emergence-related processes were not taken into account in our allocation model. Therefore model simulations were started at 18 DAS and initialization values were those of measured plant surfaces at that date. Plants at 18 DAS averaged < 3% of their final size at 42 DAS. Simulations were conducted with all measured parameters (Table 2) and the model was not calibrated.

Model validation and sensitivity analyses

The allocation model faithfully captured the substantial growth difference observed between WT, on the one hand, *and pgm* and *sexl* mutants, on the other (Fig. 4). Measured surfaces of *pgm* and *sexl* plants grown in ambient CO_2 conditions were not significantly different from one another (Fig. 4a), and averaged only 18% of that of the WT. The model predicted this value to be 21%, with little difference between *pgm* (18.5%) and *sexl* (23.0%). The model also correctly predicted that the relative growth of both mutants compared with WT decreased from 18 to 25 DAS and seemed to stabilize afterwards.

The model produced satisfying results when applied to the growth of *Arabidopsis* WT and mutants exposed to elevated CO_2 conditions (Fig. 4b). The model correctly predicted the large measured response to elevated CO_2 of the WT compared with the mutants. The main discrepancy between measured values and modelled ones was for WT at the end of the measurement period, when the model overestimated the growth response under elevated CO_2 conditions. By that time, data indicate that the WT was no longer growing exponentially, which could not be predicted by our allocation model because it does not simulate phenology. The model also seemed slightly to overestimate the response of the *pgm* mutant to elevated CO_2 conditions.

Data indicate that *sexl* and *pgm* mutations resulted in similar growth curves under our experimental conditions. This absence of substantial difference between mutants was quite correctly predicted by our model. A sensitivity analysis indicated that simulated plant growth increases with increasing ST_c values (Fig. 5), which is in agreement with the respective growth behaviours of WT (ST_c = 83%) and the *sexl* mutant (ST_c = 14%). The sensitivity analysis required that biomass at 18 DAS be initialized at identical values for WT and *sexl,* which explains that simulated surfaces are not identical in Figs 4, 5. In addition, the sensitivity analysis was conducted at 36 DAS because the data suggested that growth was departing from exponential phase conditions towards the end of the experiment. The model predicts that the growth of a total starch-excess mutant (ST_c = 0%) would be extremely impaired and that its response to elevated CO_2 would be null. On the other hand, the model predicts that the growth and elevated CO_2 response of a putative mutant that could convert 100% of its leaf starch during the night would not be much enhanced compared with the WT. Most importantly, the model indicates that the ability of the plant to use its leaf starch reserves during the night directly conditions its ability to benefit from elevated CO_2 conditions. This is confirmed by the experimental results, indicating that average plant size stimulation by elevated CO_2 between 32 and 42 DAS was 95% for WT and only 51% for *sex1*.

A second sensitivity analysis indicated that, under ambient CO_2 conditions, increases in GR_{max} beyond our estimated value of 0.41 d^{-1} will result in little additional growth (Fig. 6). Under elevated CO_2 conditions, the model predicts that substantial biomass gains would result from increases in GR_{max}. As a result, the model predicts that the stimulation of *Arabidopsis* growth by elevated CO_2 is sensitive to small changes in GR_{max} around our estimated value of 0.41 d^{-1}.

Finally, the model proved insensitive to small changes in ST_{br} because photosynthesis consistently exceeded plant growth demand in our constant experimental conditions, which necessarily triggered the overflow pathway of starch synthesis (simulation not shown).

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Fig. 4: *Measured (symbols) vs modelled (lines) plant surface growth of the Arabidopsis WT (open circles, solid lines); pgm (closed circles, dashed lines); and sex1 mutants (open squares, dotted lines) grown under (a) ambient and (b) elevated CO_2.*

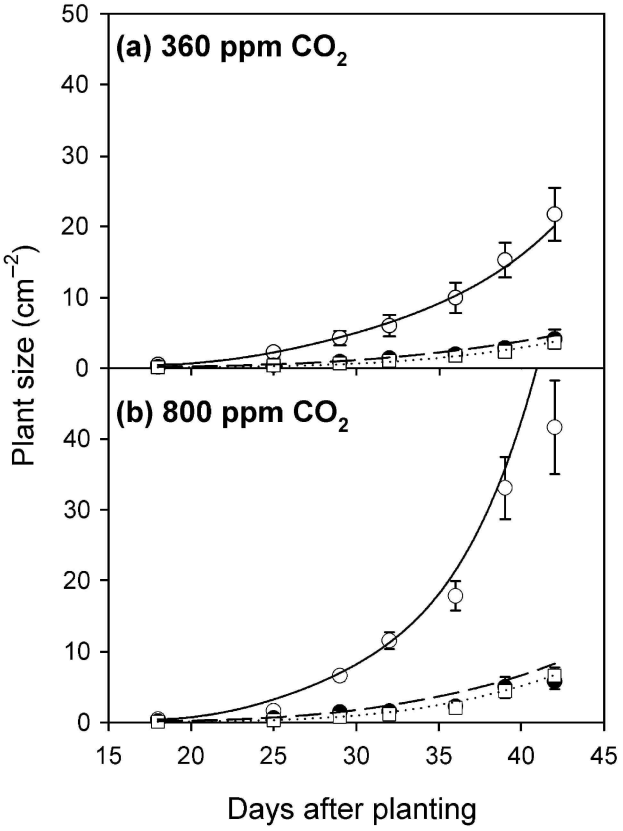


Fig. 5: *Sensitivity analyses of the starch-to-sucrose conversion rate (ST_C; Table 2) on the simulated growth of Arabidopsis thaliana in ambient and elevated CO_2 conditions, (a) Surface growth of 36-d-old plants grown under ambient and elevated CO_2 conditions, (b) Stimulation computed as 100 x (elevated - ambient)/ambient. Vertical dashed lines, ST_C values of WT and sex1.*

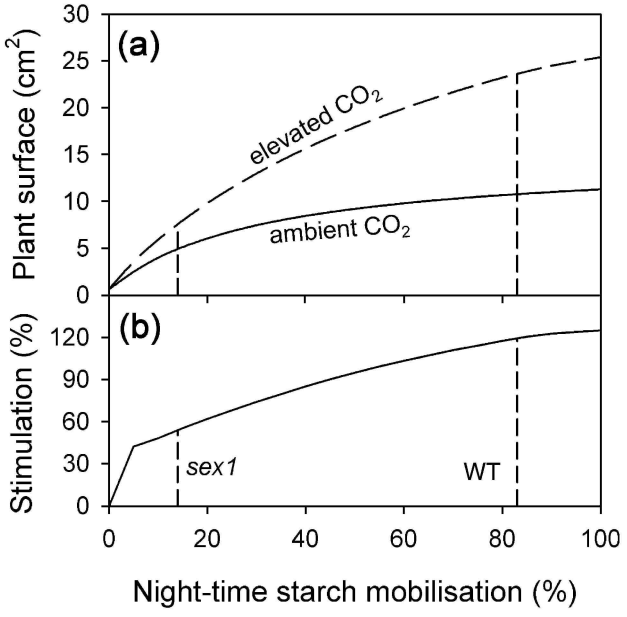
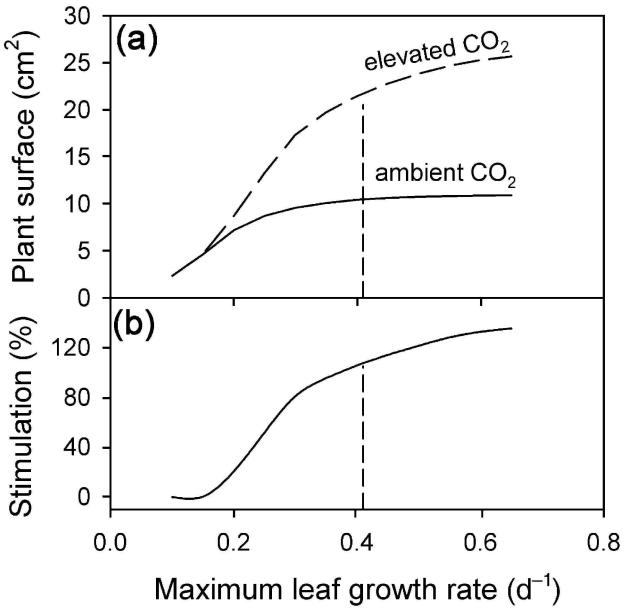


Fig. 6: *Sensitivity analyses of the maximum leaf growth rate (GR_{max}; Table 2) on the simulated growth of Arabidopsis thaliana in ambient and elevated CO_2 conditions, (a) Surface growth of 36-d-old plants grown under ambient and elevated CO_2. (b) Stimulation computed as 100 x (elevated - ambient)/ambient. Vertical dashed lines, GR_{max} values as determined in this study.*



Discussion

Plant carbohydrate allocation is known to exert a major regulatory role on growth response to elevated CO_2 (Stitt, 1991; Makino & Mae, 1999). In the present study, we showed that single-parameter modifications of the starch synthesis and breakdown equations had profound effects on both the simulated growth of *A. thaliana* and its response to elevated CO_2 (Figs 4-6). A reduction in ST_c was sufficient to model the reduced growth of *sexl* compared with WT, which is in agreement with both our measured values (Fig. 4) and the literature (Caspar *et al.,* 1991; Zeeman & ap Rees, 1999). Similarly, forcing the sugar-to-starch conversion to zero in the model produced realistic *pgm :* WT growth ratios compared with measured values (Fig. 4). This finding concurs with several studies reporting reduced growth of starchless vs WT (Schulze *et al.,* 1991). Our results were obtained with a noncalibrated model containing only parameters that were either directly measured or computed from indirect measurements and literature data (Fig. 1; Table 2).

Our experimentally based model was possible with *A. thaliana* because of mutant availability, and because this species presents fairly simple starch and sugar cycles, which are mostly controlled by activities occurring within the leaves. *Arabidopsis* preferentially transforms excess photosynthates into leaf carbohydrates rather than increasing exports to sink organs such as roots (Geiger *et al.,* 2000). In contrast, allocation models that have tried to incorporate multiple storage and structural sink terms require numerous nonmeasurable parameters left to calibration exercises (Escobar-Gutierrez *et al.,* 1998; Bonato *et al.,* 1999; Yang & Midmore, 2005). In plants with larger nonleaf sink capacity than *Arabidopsis,* translocation might play a more substantial role. This potentially explains why woody plants with large storage capacity respond more to elevated CO_2 than their herbaceous counterparts, as reported by Nowak *et al.* (2004). This does not mean, however, that the mechanisms described in our *Arabidopsis* model are not relevant to more complex plant architectures. Not only are the principles inherently similar, but within-leaf allocation processes remain potentially crucial in larger-storage plants because sugar export from leaves can be transport-rate limited, as reported by Grimmer & Komor (1999).

Starch and sugar pools in plant leaves are regulated by complex enzymatic machinery (Zeeman *et al.,* 2004; Lloyd *et al.,* 2005). Sugar and starch accumulation in leaves can have direct effects on photosynthesis through physiological mechanisms (Pritchard *et al.,* 1997), biochemical feedbacks (for review see Paul & Foyer, 2001) and gene-expression control (Krapp *et al.,* 1993; Jang *et al.,* 1997; Moore *et al.,* 1999). The interplay of these putative controllers under elevated CO_2 conditions appears highly complex and uncertain. Here we argue that, in addition to potential biochemical feedbacks, the response of starch mutants to elevated CO_2 might be controlled essentially by their capacity to supply a constant rate of soluble sugars that matches optimal growth demand. This capacity is affected very differently in *sex1* and *pgm* mutants, and we need to consider the two cases

separately.

For the *sex1* starch-excess mutant, our model does not invoke any special relationship beyond the obvious fact that a portion of assimilates is sequestered in excessive starch reserves and therefore is not readily available for growth. Implemented in the model, this simple relationship was sufficient to explain our experimental results of a much reduced growth response of *sex1* vs that of WT under both ambient and elevated CO_2 conditions.

In the *pgm* starch-deficient mutant, all assimilates remain as growth-available soluble sugars. This calls for a feedback mechanism of soluble sugar content on growth in order to explain the reduced growth response *of pgm* vs that of the WT under both ambient and elevated CO_2 conditions. Literature information suggests that an increase in soluble sugar concentration can directly downregulate photosynthesis in *pgm* mutants of *Arabidopsis* (Sun *et al.,* 1999). However, both the magnitude and the mechanism of downregulation remain uncertain. Sun *et al.* (2002) reported that genotypes of starch mutants and wild types of *Arabidopsis* were similar in total activity and Rubisco content in response to elevated CO_2 conditions. Kofler *et al.* (2000) reported no significant decrease in photosynthetic electron transport in *pgm* mutants compared with WT. These studies exemplify that the response mechanism remains elusive, which prevented us from integrating direct downregulation of photosynthesis into our model. Our experimental results indicate that maintenance respiration correlates positively with soluble sugar concentration, which provided us with the needed quantifiable feedback of soluble sugar concentration on *pgm* plant growth. This does not mean that direct downregulation does not happen, rather it suggests that the simple relationship linking soluble sugar concentration to maintenance respiration is a good mathematical approximation of the global contribution of both mechanisms (increased maintenance respiration and downregulation of photosynthesis) to the apparent reduction of CO_2 assimilation and growth rate observed in starchless mutants. The exact importance of each remains to be determined, but our results emphasize the need for further evaluation of the role played by maintenance respiration in response to elevated CO_2, at least in sink-limited conditions.

While several studies reported a link between maintenance respiration and tissue N concentration (Ryan, 1991; Ryan *et al.,* 1996), it is clear that N concentration is only a proxy for biochemical processes, which are likely to include soluble sugar production. Overall, maintenance respiration remains a poorly understood process. Hirai *et al.* (2002) reported large differences in maintenance respiration rates among rice species, independent of N concentrations. The initial response of maintenance respiration to temperature changes is large, but subsequent acclimation occurs (Wythers *et al.,* 2005). Most importantly, our results agree with those of Ogren (2000), who reported that maintenance respiration correlates with soluble sugar but not with nitrogen concentration in dormant plants. This author suggests that increases in maintenance respiration in response to increases in soluble sugar concentrations are directly linked to the costs of maintaining concentration gradients.

In our study, the simulated increase in maintenance respiration associated with increased sugar concentration substantially affected the *pgm* mutant only, and not the WT and *sexl* mutant. A sensitivity analysis indicates that the simulated plant sizes for WT and *sexl* remained nearly unaffected (between 2 and 6%) using this relationship (equation 17) vs considering a fixed basal maintenance respiration value (data not shown). The model therefore suggests that no negative feedback (whether through downregulation or increased respiration) of elevated CO_2 on WT growth needs to be invoked to explain the difference in WT growth curves of Fig. 4a vs b. This absence of significant photosynthetic downregulation of *Arabidopsis* WT growth in response to elevated CO_2 conditions under our experimental conditions was demonstrated in a parallel study (Tocquin *et al,* 2006).

In its present version, the model demonstrates that simple modifications of leaf starch production and degradation rates (themselves the result of complex enzymatic interplay) are bound to carry enormous effects on growth of leaf sink-limited plants and of their response to elevated CO_2 conditions. It is certain that the present model is a simplification of the complexity of biochemical regulation. In addition to direct effects on photosynthesis, it is probable that soluble sugar availability modifies allocation to roots, although this effect is likely to be minor in *Arabidopsis,* as suggested by Geiger *et al.* (2000). It is also probable that the baseline-and-overflow starch production mechanism is smoothed and fine-tuned through biochemical regulation. As research progresses, we hope the present model will serve as a basis to probe the potential effects of these biochemical fine-tuning mechanisms on *Arabidopsis* growth and response to elevated CO_2.

The maximum rate of leaf growth (GR_{max}) appeared as the most crucial allocation parameter affecting *Arabidopsis* response to elevated CO_2, while the model suggests a substantial safety margin in the proportion of starch breakdown (ST_c) before effects on the response to elevated CO_2 occur (Fig. 6 vs Fig. 5). In the present study, conducted in constant environmental conditions, starch synthesis was entirely controlled by GR_{max} because the baseline starch production rate (ST_{base}) was always surpassed and therefore did not modify the simulation

results (Fig. 1). Our simulations suggest that small changes in GR_{max} have a dramatic impact on the response to elevated CO_2 (Fig. 6). The existence of a maximum relative growth rate is an important assumption of sugar—starch allocation models (Escobar-Gutiérrez *et al,* 1998). Without this sink-limited approach, the plant could use its photosynthetic sugar for immediate growth, and would have little advantage to invest in starch storage except for covering maintenance respiration costs during the night.

Plants of the C_3 photosynthetic pathway respond positively to short-term increases in atmospheric CO_2 concentration because of increased carboxylation efficiency. Nevertheless, in the long term this response varies considerably in magnitude among species (Curtis & Wang, 1998; Nowak *et al.,* 2004). Neither resource-based nor plant functional type models provide a comprehensive explanation for this variability (Nowak *et al.,* 2004). While a common photosynthetic machinery is shared among C_3 species, the pattern of starch accumulation varies substantially among species (Zeeman *et al.,* 2004 and references therein). Notwithstanding environmental stress factors and downregulation of photosynthesis as major controls over this variability (Drake & Rasse, 2003; Rasse *et al.,* 2005), our study confirms that regulation of diurnal starch and sugar cycles in leaves can substantially control herbaceous plant response to elevated CO_2.

Downregulation of photosynthesis in response to elevated CO_2 conditions has been reported in numerous studies (Cheng *et al.,* 1998; Makino & Mae, 1999; Bae & Sicher, 2004 and references therein), although exceptions exist (Sun *et al.,* 2002; Zhao *et al.,* 2004). A decrease in Rubisco enzyme concentration is often associated with a decrease in whole leaf N content (dePury & Farquhar, 1997). A similar enzymatic downregulation associated with N availability is likely to exist for GR_{max}. At this point we can only speculate as to which of these two enzymatic systems is most affected by downregulation processes. Interestingly, increased enzymatic sucrose synthesis, potentially at the expense of starch, has been shown to stimulate plant yields independently of modifications in photosynthetic activity (Sharkey *et al.,* 2004). In a recent study, Bae & Sicher (2004) reported that the expression of six soluble proteins was modified in *Arabidopsis* plants subjected to elevated CO_2 conditions. Of these six proteins, three were growth-related while only one was photosynthesis-related. These results support our model analysis (Fig. 6) that the photosynthetically induced stimulation of plant growth by elevated CO_2 can be restricted by a downregulation of GR_{max} independently from downregulation of V_{cmax}.

In conclusion, the model demonstrates that simple modifications of starch synthesis and breakdown rates in *Arabidopsis* are bound to generate large differences in the photosynthetically induced growth stimulation by elevated CO_2. Our results suggest that the night-time starch-to-sugar conversion rate, and even more so the maximum rate of leaf growth, which is a major component of the sink capacity of herbaceous plants, are key parameters needed in comprehensive mechanistic models that aim to predict plant responses to elevated CO_2 conditions.

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