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Regulation of Sugar Uptake in Hypocotyls of Cotton[1]

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ABSTRACT

Uptake of sucrose and hexoses by hypocotyl segments of cotton (*Gossypium hirsutum* L.) was shown to be dependent upon sugar level in the tissue. The effect was not related to total sugar level inasmuch as a portion of previously accumulated sugar was without influence on uptake. That portion was presumed to be compartmentalized, most likely in vacuoles. Growth regulators modified the uptake pattern apparently through alterations in secondary metabolism. Uptake and incorporation were inhibited by rotenone and stimulated by light. The light effect was blocked by 3-(3,4-dichlorophenyl)-1,1-dimethylurea. A general model was presented for assimilate flux within sink tissues with free space as the conduit from phloem to carrier site.

Assimilate transport within sink tissues of higher plants may involve movement through the free space. We reported earlier (4) on the uptake of sugars from free space by cotton hypocotyl segments. The response to external concentrations of sucrose, glucose, and fructose and competition among these sugars was characterized, as was the dependence upon temperature, pH, and other variables. Uptake appeared to be an active carrier-mediated process, with separate carriers for sucrose and hexose. With aging, free space invertase appeared, allowing hydrolysis products of sucrose to compete for the hexose carrier.

The fate of the absorbed sugars appeared to play a regulatory role in uptake. Reported here is a series of experiments directed toward clarifying the role of end products and energy supply in controlling uptake.

MATERIALS AND METHODS

The materials and methods were the same as in the earlier experiments (4). Briefly, 1-mm segments of fully expanded young hypocotyls of cotton (*Gossypium hirsutum* L., 'Acala SJ2') were exposed to dilute labeled sugars (0.1 μM with a high specific radioactivity) in a background of unlabeled sugar of varied concentration. The segments were generally washed briefly (1 hr) in distilled H_2O and preincubated for 1 hr with unlabeled sugar before the labeled sugar was added. Uptake was linear with time during the 2-hr experimental uptake periods. The tissue was then washed in water to remove free space sugar and heated in ethanol to separate soluble and insoluble fractions. Scintillation counting was carried out on the total sample and separately on the soluble and insoluble fractions. Standard errors of means, being generally less than 6%, are not included with the data. Sugar characterization and analysis were by gas chromatography.

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RESULTS

Feedback. Preliminary experiments with fully grown plants showed that whole-plant manipulations which might reduce assimilate availability increased the rate of sugar uptake by stem tissue excised from these plants. It was observed that tissue from heavily fruited plants had a much higher rate of sugar uptake than did tissue from defruited plants. The point was investigated further by detopping cotton seedlings 1 day before sugar uptake was measured. Over a wide concentration range, uptake of both glucose and sucrose by hypocotyl segments was stimulated considerably by top removal (Fig. 1). Since invertase levels were not observed to be increased by whole-plant starvation, it is assumed that sucrose was taken up intact (4). Sugar levels in the tissue, measured at time of harvest, showed that detopping had reduced sucrose levels from 8 to 2 mM, and hexose from 1 or 2 mM to less than 0.1 mM.

Tissue starvation of that sort was easy to accomplish with whole-plant manipulations, but the assimilate relationships could not be controlled in any quantitative manner. That was achieved in excised tissues by overnight pretreatment of hypocotyl segments in either H_2O (starved condition) or 100 mM glucose, fructose, or sucrose before uptake experiments on the following day. Since a large amount of invertase was produced overnight, subsequent uptake of only labeled glucose and fructose was measured (Table I).

The least significant effect (glucose *versus* H_2O pretreatment before glucose uptake) and the most significant effect (sucrose *versus* H_2O pretreatment before fructose uptake) were further compared over a range of uptake concentrations (Fig. 2A). As before, glucose uptake differed little between glucose- and H_2O-pretreated tissue, whereas fructose uptake was strongly influenced. The soluble and insoluble fractions obtained with fructose uptake are distinguished in Figure 2B. Plotted in Lineweaver-Burk form (Fig. 2C), total uptake of fructose by starved tissue was linear, indicating that it conformed to a rectangular hyperbola. Uptake by sucrose-pretreated tissue was not linear across the entire concentration range. Higher concentrations (4–32 mM) gave the same K_m as starved tissue, though with a lower V_{max}. The multiphasic concentration response may indicate that several component processes affect fructose uptake and utilization.

The magnitude of the starvation effect varied, generally increasing with longer pretreatment periods (*in vivo* and *in vitro*). The least affected was usually glucose uptake with uptake of fructose (or of sucrose in freshly sliced tissue) affected more strongly. In all cases, starvation increased accumulation into both the soluble and insoluble fractions. Starvation was prevented least by glucose pretreatment, and most by sucrose pretreatment, in correlation with their rates of uptake (4): glucose was accumulated the most slowly, and sucrose, being a disaccharide, the most rapidly (expressed in hexose equivalents). That was especially true with overnight pretreatment, because the resulting invertase production allowed sucrose to be taken up by both hexose and sucrose carriers.

A sucrose concentration of less than 1 mM was sufficient to avoid starvation (Fig. 3). In this experiment, pretreatment and

uptake were both done in the dark to avoid any effects of photosynthetic sugar production. In other experiments, slightly higher concentrations (around 2 mM) were needed to avoid starvation. *In vivo* sugar concentrations in cotton seedlings were generally above that, both in the free space and in the tissue, and overnight incubation in <2 mM sucrose solutions elevated the tissue sucrose concentration to 15 or 20 mM.

The starvation effect could often be reversed by 5 hr of incubation in 100 mM sucrose (Fig. 4). In this experiment, the segments were preincubated overnight in 100 mM sucrose or H_2O. At time zero the next day, the treatments were switched. Thereafter, samples were taken at hourly intervals and incubated for 1 hr in 10 mM labeled fructose. Table II shows the initial and final sugar concentrations in the tissues. Although the sugar concentration in starved tissue (H_2O overnight) increased considerably after reversal, it was still less than with well fed (sucrose overnight) tissue. Since the starvation effect was reversed completely by the 5th hr, it is clear that sugar uptake cannot always be related to the total tissue sugar concentration. The results in Figures 1 to 4 and Table II strongly suggest feedback control of uptake. The simplest interpretation of those data is that only a portion of the cellular sugar (*e.g.* the nonvacuolar cytoplasmic fraction) was involved in such regulation. Since incorporation and soluble sugar accumulation both were strongly affected, uptake was clearly a general property of the whole tissue and was not limited to a specific tissue such as the phloem.

Reversal with sucrose indicates that sugar depletion was the principal consequence of starvation, although aging phenomena are not excluded. The possible role of aging was studied with hypocotyl segments pretreated for 2 days in water or 0.1 mM to 10 nM solutions of ABA, kinetin (Kn), GA_3, or 2,4-D, with and without 100 mM sucrose. Uptake of 10 mM glucose was then observed. Representative results obtained with those growth regulators at 10 μM are shown in Table III.

Pretreatment in H_2O and sucrose alone gave the highest and lowest uptake rates. The addition of growth regulators reduced the starvation enhancement while increasing uptake in well fed tissues. Assuming that sugar uptake into starved tissue was limited by carrier capacity and uptake into well fed tissue by utilization rate, it seems likely that the regulators affected utilization rather than uptake capacity. Short term applications of growth regulators had no effect on uptake.

Energy and Light. Sugar uptake was quite sensitive to inhibitors such as azide, 2,4-dinitrophenol, and rotenone. Figure 5 shows the effect of 10 μM rotenone on fructose uptake. In these summer-grown plants, K_m was unaffected while V_{max} was reduced by one-third. In winter-grown plants with a higher apparent K_m of uptake, rotenone also reduced K_m, though never below the K_m of summer-grown plants.

There are many reports that light energy can have a significant effect on active transport (5, 7), and light was also generally

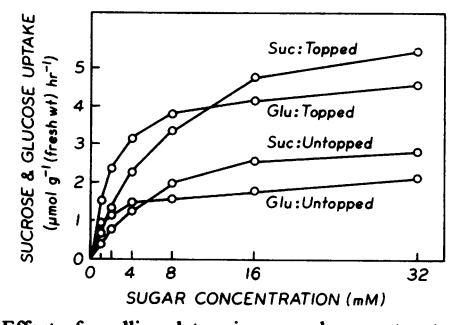

FIG. 1. Effect of seedling detopping on subsequent uptake of sucrose and glucose by hypocotyl segments. Curves labeled as (sugar taken up: pretreatment).

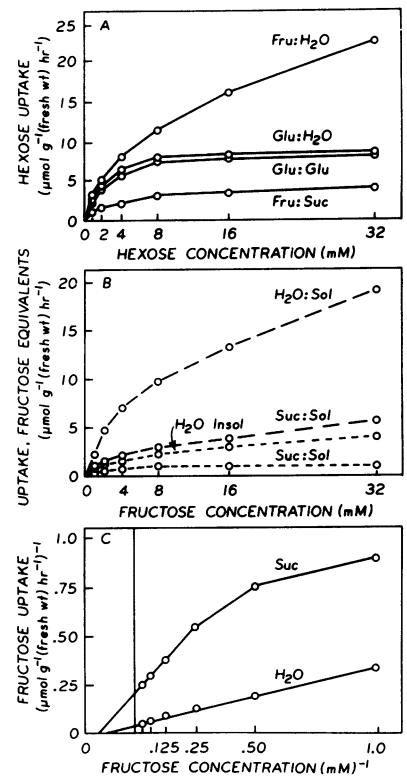

FIG. 2. A: Effect of overnight pretreatment in 100 mM glucose *versus* H_2O on subsequent total glucose uptake, and in 100 mM sucrose *versus* H_2O on fructose uptake by hypocotyl segments. Curves labeled as (sugar taken up:pretreatment). B: Effect of overnight pretreatment in 100 mM sucrose *versus* H_2O on subsequent fructose uptake and incorporation. Curves labeled as (pretreatment solution:soluble or insoluble fraction). C: Lineweaver-Burk plot of fructose uptake as affected by pretreatment in 100 mM sucrose *versus* H_2O (from Fig. 2B). For H_2O-pretreated tissue, V_{max} = 25 μmol g^{-1} (fresh weight) hr^{-1} and K_m = 7.1 mM.

Table I. The effects of overnight pretreatment in H_2O vs glucose, fructose or sucrose on subsequent uptake and incorporation of glucose and fructose

	Pretreatment solution, 100mM			
	Glucose	Fructose	Sucrose	H_2O
	μmol g^{-1} (fresh wt) hr^{-1}			
Glucose (10mM) uptake				
Soluble	12.6	9.2	9.8	12.8
Insoluble	1.8	1.7	1.7	2.0
Total	14.4	10.9	11.5	14.8
Fructose (10mM) uptake				
Soluble	15.4	13.4	12.8	21.8
Insoluble	2.0	2.0	1.9	2.6
Total	17.4	15.4	14.7	24.4

stimulating to sugar uptake and incorporation by cotton hypocotyls (Fig. 6). The experiment illustrated in Figure 6 was done by incubating hypocotyl segments in 60 mM sucrose in small black dishes covered with varying thicknesses of a neutral filter (white Plexiglas). Quartz-iodine lamps filtered to remove IR were used as a light source. Other experiments showed that glucose uptake was usually more affected by light than was sucrose uptake. The light effect was eliminated by the photosynthetic inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethyl urea) at 1 μM.

It was not convenient to shake the irradiated samples during those experiments, but a light *versus* dark effect was also apparent in experiments under standard uptake conditions (50-ml culture tube, shaken). If O_2 diffusion into the tissue was limiting to respiration, the light response might be due to a supply of photosynthetic O_2 to respiration. To test that hypothesis, uptake of 10 mM sucrose was measured in the dark for 2 hr under O_2-N_2 mixtures of 0, 10, 20, and 30% (v/v) O_2. Uptake and incorporation were reduced under anaerobic conditions, but treatments with 10 to 30% O_2 supported maximal uptake and incorporation. It is thus unlikely that the light stimulation of uptake resulted from the presence of photosynthetic O_2. Uptake approximated an ideal rectangular hyperbola more often in the dark or in the presence of rotenone than in the light in the absence of inhibitors.

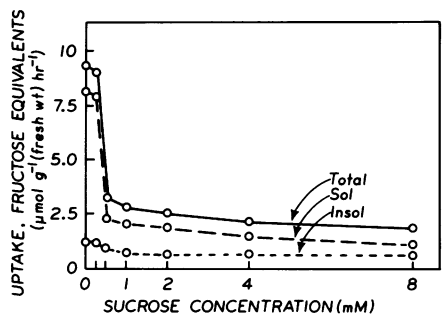

FIG. 3. Effect of overnight pretreatment in variable sucrose concentrations on subsequent uptake and incorporation of 10 mM fructose.

DISCUSSION

Membrane transport of sugar appears to play a key role in the source-sink relations of assimilate translocation. That may be quite important in determining growth patterns, since there is little doubt that assimilate is often strongly limiting to plant growth. Figure 7 is a generalized sink model for cotton stem tissue. Arrows represent enzyme- or carrier-mediated processes and so are potentially controllable. There is nothing unique to cotton in the processes illustrated, and it is possible that the system is quite generally applicable. A similar model (including several aspects of hormonal regulation) has been formulated to describe sink activity in sugarcane stem tissue (3). Key assumptions of our

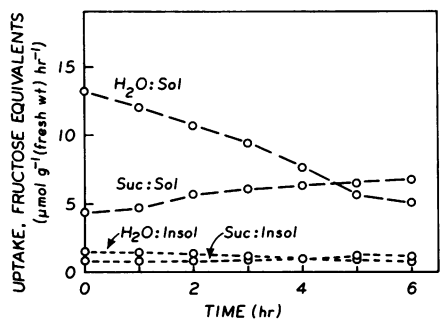

FIG. 4. Time course of reversal of pretreatment effect on uptake and incorporation of 10 mM fructose by hypocotyl segments. Segments were pretreated overnight in 100 mM sucrose or H_2O. Treatments were reversed at time zero. Samples were taken at hourly intervals thereafter and incubated in fructose. Curves labeled as (overnight pretreatment solution:soluble or insoluble fraction).

Table II. Sugar concentrations in cotton hypocotyl segments--after overnight incubation in H_2O and sucrose and later, 6 hr after the incubation solutions were reversed

SE around 8% of given values.

	Pretreatment solution	
	Sucrose, 100mM	H_2O
	mM	
Before reversal		
Glucose	21.8	2.0
Fructose	10.2	1.7
Sucrose	48.5	1.4
6 hr after reversal		
Glucose	18.2	12.2
Fructose	9.6	7.4
Sucrose	47.5	26.0

Table III. The effects of 2-day pretreatment in water or ABA, kinetin, GA_3 and 2,4-D, ± 100 mM sucrose, on subsequent uptake of 10 mM glucose by cotton hypocotyl segments

	Control	Hormone pretreatment, 10 μM			
		2,4-D	Kinetin	GA_3	ABA
		μmol g^{-1} (fresh wt) hr^{-1}			
+ Sucrose in pretreatment solution					
Soluble	2.0	2.2	3.4	2.8	3.2
Insoluble	0.3	0.4	0.5	0.4	0.4
Total	2.3	2.6	3.9	3.2	3.6
- Sucrose in pretreatment solution					
Soluble	7.6	6.2	4.0	6.6	7.0
Insoluble	1.3	1.0	0.5	1.3	1.2
Total	8.9	7.2	4.5	7.9	8.2

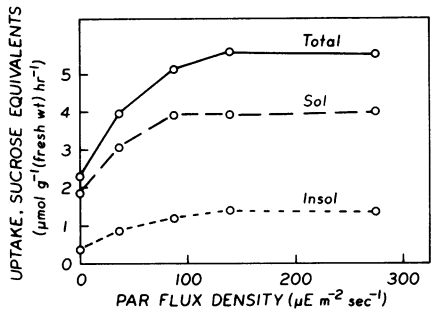

FIG. 5. Effect of light flux density on uptake and incorporation of 60 mM sucrose by hypocotyl segments.

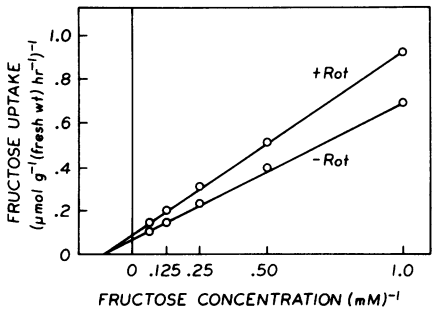

FIG. 6. Lineweaver-Burk plot of fructose uptake by cotton hypocotyl segments as affected by 10 μM rotenone. Apparent V_{max} μmol g^{-1} (fresh weight) hr^{-1} and K_m (mM) are: + rotenone, 10, 8; and − rotenone, 15, 8.

model are controlled phloem unloading into the apoplast, and active assimilate accumulation from the apoplast by growing cells. The possible significance of the apoplast in short distance assimilate transport has been recognized only recently. While the possibility of symplastic transport is not excluded, we have shown that cotton hypocotyl tissue can rapidly accumulate and utilize sugars from the free space, and that *in vivo* free space sugar levels were high enough to support the growth rates observed in the greenhouse (4).

In sink tissues of sugarcane (3), invertase has a key role in sucrose uptake. But in fresh cotton hypocotyl tissue where intercellular invertase was functionally absent, the kinetics of the sucrose carrier itself most likely determines the kinetics of uptake when assimilate levels are limiting. Intercellular invertase, however, would play a role in partitioning absorbed sucrose into metabolism or storage. Uptake rate *versus* substrate concentration was generally found to describe a rectangular hyperbola, a curve that can be characterized with the two parameters V_{max} and K_m. If K_m and V_{max} are observed to vary, then more fundamental parameters may be needed to describe uptake completely. The apparent K_m values of sugar uptake were considerably higher in winter-grown than in summer-grown plants suggesting that they were (at least as measured) complex variables. Certainly, V_{max} was highly sensitive to experimental manipulation.

Since *in vivo* free space sugar concentrations were usually low (in the range of the K_m or less), the uptake by hypocotyls may generally operate as a first-order process (uptake = k [S]) and the initial slope of the concentration response may be the most critical property of the carrier system. That would provide a high degree of substrate control of uptake. The "priority" of various tissues for assimilate could depend upon the differences in that slope among the tissues.

Many enzymes are regulated by some form of feedback control. Our results provide evidence of a similar phenomenon with sugar carriers. That is not surprising, considering that the maximum rate of sugar uptake was considerably in excess of the maximum rate of utilization. When storage capacity is saturated, uptake must be limited to the rate of utilization, making transport kinetics, in a sense, "transparent." The kinetic data of utilization, obviously, are integrative of a large number of separate processes (inversion, isomerization, phosphorylation, incorporation, etc.) and might result in a relatively amorphous concentration response for uptake. On the other hand, if one reaction, or parallel ones with similar K_m values, were dominant, then uptake might be described by Michaelis-Menten kinetics, having no relationship whatever to the nature of the carrier involved in transport. The use of nonmetabolizable sugar analogs has shown that utilization is not required for uptake, but that technique cannot be used in demonstrating the regulation of uptake by utilization.

The dry weight of a plant is composed largely of alcohol-insoluble compounds, and the rate of sugar incorporation into the insoluble pool will provide a more accurate relationship between

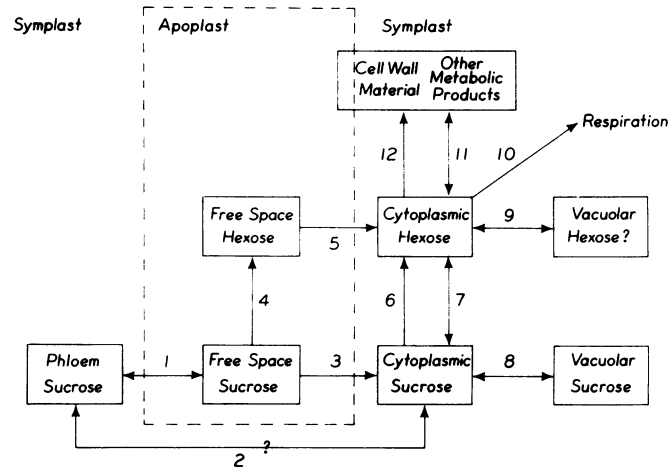

FIG. 7. Generalized sink model for cotton stem tissues.

1. Phloem unloading of sucrose. It is generally assumed that both phloem loading and unloading are highly specific, controlled processes. Little is known about unloading, but it is probably similar to phloem loading, which is carrier-mediated and active. However, since sugar concentrations in the phloem are generally higher than in the surrounding tissue, unloading need not be an active process. What sort of control mechanisms might operate are unknown, but unloading is almost certainly not controlled by simple diffusion gradients.

2. Symplastic route for short distance assimilate transport. The significance of this route is not known.

3. Sucrose uptake from the free space. An active, carrier-mediated process, probably the primary route of assimilate into cells. Appears to be regulated by internal sugar levels and is affected by energy availability.

4. Free space acid invertase. In other plants, invertase synthesis and activity are known to be affected by hormones and by specific invertase inhibitors. It has also been suggested that sucrose and hexose levels regulate invertase activity. In cotton stem tissue the levels are generally low, but can be increased greatly by excision and aging.

5. Hexose uptake from the free space. Glucose and fructose appear to share a common carrier. Uptake is active and apparently regulated by internal sugar levels and energy availability.

6 and 7. Cytoplasmic invertase and sucrose synthetase. Invertase inversion is irreversible since the energy of the glucose-fructose bond is not conserved. Sucrose synthetase conserves the energy (ATP is formed) and the process is reversible.

8. Sucrose storage in the vacuole. The majority of solutes in an expanding or mature parenchyma cell are in the vacuole, so this step may limit the rate of sugar accumulation by such cells. It is presumably carrier-mediated and acitve. The rate and amount of "storage" may be regulated by non-nutritional as well as nutritional factors.

9. Hexose storage in the vacuole. Hexoses generally comprise less than 5% of the sugar in cotton cells (by weight). Whether any are contained in the vacuole is not known.

10. Carbon lost as CO_2. Known to be controlled in a number of ways.

11. Formation of organic acids, amino acids, lipids etc.

12. Wall synthesis. Cell walls comprise the majority of the dry weight of vegetative matter, so this process is a principal factor in determining the rate of "growth" as measured by dry weight increase.

in vivo assimilate concentration and growth rate than does total rate of uptake, which also reflects the rate of sugar storage. The low K_m of incorporation and the high K_m and V_{max} of storage (4) are compatible with the idea that storage represents excess assimilate not immediately used in metabolism. The apparent requirement for continuous uptake to support incorporation (4) suggests that there may be more compartmentalization than is included in Figure 7. Other researchers have also observed that incorporation can be closely linked to uptake (1, 6).

Eaton (2) concluded that sugar levels in the cotton plant correlated more with environmental conditions than with the developmental state of the plant. They concluded that the "cut-out" in vegetative growth which occurs with intensive fruiting was not determined by competition for assimilate but by other factors, possibly hormonal. Sugar uptake rates were strongly affected by nutritional status, but need not correlate with the total concentration of sugar in the tissue (Fig. 4 and Table II). It is possible that the nutritional status is determined by a smaller (nonvacuolar cytoplasmic) pool of assimilate whose size is closely coupled with the rates of acquisition and assimilation, with a larger (vacuolar) pool being regulated by a number of other factors. The apparent dependence of incorporation on continuous assimilate importation could mean that the stored sugar content is largely irrelevant to the process of dry matter accumulation.

Even though *in vivo* sugar levels in cotton hypocotyl tissue never equaled those resulting from *in vitro* sugar incubation, the observed uptake rates by tissue from intact plants were considerably less than that of starved tissue from detopped plants (Fig. 1). It appears that as long as metabolic needs are met, uptake runs at significantly less than maximum capacity. That rate, however, was sufficient for storage to occur and presumably would have been reduced further if storage capacity became saturated.

A common factor in active transport is a dependence on energy availability. The sensitivity of sugar uptake to inhibitors indicates that it has a similar dependence (Fig. 5). Photosynthetic stimulation of sugar transport and utilization might be a strategy that would bias partitioning in favor of tissue in the light with interesting implications to the control of growth and development under field conditions.

While it is clear that sugar transport is a regulated process, it is not so obvious what specific internal conditions are responsible for this regulation. A more detailed knowledge of carbon metabolism and the molecular basis of feedback control is necessary to fully explain the effects of utilization on uptake. The nature of energy availability and coupling to transport is still unknown. Phloem unloading, and the possible significance of the symplastic route for short distance transport were not investigated, but may also be important factors in determining sink strength. Further investigations relating *in vitro* behavior to *in vivo* growth patterns are needed to demonstrate which regulatory processes are the most important in controlling sink strength *in vivo*.

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