Supply and demand: sink regulation of sugar accumulation in sugarcane

Running title: Source-sink regulation in sugarcane

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Supply and demand: sink regulation of sugar accumulation in sugarcane

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Abstract

Sugarcane (\textit{Saccharum} spp. hybrids) accumulates sucrose to high concentrations and, as a result, has been the focus of extensive research into the biochemistry and physiology of sucrose accumulation. Despite this, the relationship between source leaf photosynthetic activity and sucrose accumulation in the culm sink is not well understood. The observations that photosynthetic activity declines during culm maturation in commercial cultivars and that high-sucrose accumulating noble ancestral genotypes (\textit{Saccharum officinarum} L.) photosynthesize at rates two-thirds of those of low-sucrose ancestors (\textit{Saccharum spontaneum} L.) indicate that source-sink communication may play a pivotal role in determining sucrose yield. Although maturation of the culm results in a decreased demand for sucrose, recent evidence from partial leaf shading, defoliation and transgenic studies indicates that sugarcane cultivars are capable of further increases in sugar content. Furthermore, sugarcane leaves appear to retain the capacity to increase the supply of assimilate to culm tissues under conditions of increased assimilate demand. The relationship between source and sink tissues in sugarcane should be viewed within a supply-demand paradigm; an often neglected conceptual approach in the study of this crop. Uncoupling of the signaling pathways that mediate negative feedback between source and sink tissues may result in improved leaf assimilation rates and, consequently, lead to increased sugarcane sucrose yields.
Introduction

All higher plants can be described as an integrated organization of photosynthetic carbon sources and non-photosynthetic carbon consuming sinks such as growth and respiration.

Sugarcane represents a unique source-sink system for two reasons: 1) storage of assimilate at exceptionally high concentrations is in the form of sucrose which is an osmotically active solute whereas most plants store insoluble polysaccharides such as starch; and 2) storage occurs in the stalk (culm) parenchyma tissue and not in terminal sink organs. Other plants that also store high concentrations of sucrose include sugar beet (Beta vulgaris L.) which stores in the root (Giaquinta, 1979) and sweet sorghum (Sorghum bicolor L.) which is closely related to sugarcane and stores sucrose in the culm (Hoffmann-Thoma, 1996). Sugarcane is one of the world’s oldest and most important cash crops, accounting for 70% of sugar produced worldwide (Lakshmanan et al., 2005) and is an increasingly important source for biofuel production (Dias de Oliveira et al., 2005). In order to increase sucrose yields in this crop, sugar industries around the world have encouraged the development and utilisation of new molecular techniques to improve our understanding of the fate of sucrose in the culm and the mechanisms that regulate sucrose accumulation there (Lakshmanan et al., 2005; Brumbley et al., 2008; Snyman et al., 2008). This has lead to the identification of several molecular markers and putative metabolic control points for increased culm sucrose accumulation that are currently being utilized in concert with conventional sugarcane breeding programs to improve crop performance (Snyman et al., 2008). However, despite this investment of considerable effort worldwide, little or no progress has been made in improving sucrose accumulation in the culm. This may be because conventional breeding has already maximised sucrose accumulation in culm tissue (Grof & Campbell, 2001). Accumulation of sucrose may also be limited by the narrow genetic base of germplasm currently used in sugarcane breeding programs (Jackson, 2005). However, several reports indicate that while current sugarcane cultivars appear to accumulate sucrose to maximum levels, the potential exists for substantial further gains without the loss of other favourable characteristics such as stress tolerance, pest and disease resistance (Bull & Glasziou 1963; Jackson, 2005; Moore et al., 1997; Wu & Birch, 2007).

Several physiological processes may limit the extent to which sucrose accumulates in sugarcane: 1) leaf photosynthetic rates and carbon partitioning into pools other than culm storage; 2) phloem loading in the leaf and unloading in the culm (e.g. Lalonde et al., 2003;
Walsh et al., 2005); 3) culm metabolism, including membrane transport into storage vacuoles, and sucrose partitioning, turnover and re-mobilisation (Uys et al., 2007); and 4) developmental constraints, such as duration and timing of maturation (Moore et al., 1997). However, an additional constraint to crop yields may result from the ‘sensitivity’ of source photosynthetic activity to sink demand (e.g. McCormick et al., 2006, 2008a,b,c,d). Strategies to increase sucrose concentrations in sugarcane via transgenic manipulation require a broader understanding of the processes involved in sucrose accumulation, including the possibility that culm sucrose accumulation may be regulated by sink (including storage) demand (Watt et al., 2005).

Most efforts to manipulate sugarcane sucrose concentrations by transgenesis have targeted single genes encoding putative rate-limiting sucrolytic enzymes in the culm. These include soluble acid invertase (EC 3.2.1.26) (Botha et al., 2001), neutral invertase (Rossouw et al., 2007), pyrophosphate-dependent phosphofructokinase (EC 2.7.1.90) (Groenewald & Botha, 2007) and a yeast invertase carrying various leader sequences for targeting to the apoplast, cytosol or vacuole compartments (Ma et al., 2000). Despite successful transformation, transgenic plants did not result in a significant increase in overall culm sucrose accumulation (Botha et al., 2001; Groenewald & Botha, 2007). Here we suggest that attempts to increase sucrose content of sugarcane by the transgenic manipulation of sucrose metabolism enzymes, whether singly or in tandem, must take cognisance of regulatory feedback mechanisms known to exist in other plants (Paul & Foyer, 2001; Paul & Pellny, 2003; Rolland et al., 2006; Paul, 2007) and which have been proposed for sugarcane (McCormick et al., 2006). To-date, attempts to increase sucrose content in the sugarcane culm through the modification of carbon flux and partitioning have ignored this potential regulatory feedback between the culm and leaf.

A conceptual source-sink model
In plants, the relationship between leaves and sink tissues can be thought of as being similar to a factory production line (Fig. 1). The requirements of mass conservation dictate that in a closed system production by the supply must be regulated by consumption of the intermediate to prevent either depletion or accumulation of the intermediate (Hofmeyr, 1998; Hofmeyr & Cornish-Bowden, 2000). Using metabolic control theory, these authors established the principle that control of flux through a metabolic system is not only regulated by demand, but also by the sensitivity of supply to demand (Fig. 1A). Studies in yeast have indicated that the
control of flux through glycolysis is shared among many enzymes in the metabolic system (Schaaf et al., 1989; Fell, 1996), but the uptake of glucose into the cell is a crucial point for flux regulation (Galazzo & Bailey, 1990; Bisson et al., 1993). The control of glycolytic flux ultimately rests with the demand for the ATP that is produced by glycolysis and utilised elsewhere (Hofmeyr, 1998). In plants, the leaves are the ‘source’ carbohydrate supply while ‘sink’ demand includes growth, respiration and storage. In sugarcane, the import and immobilisation of sucrose in vacuolar storage in culm parenchyma cells may act as a strong additional “demand” component. This storage may contribute to maintaining a high sink demand for sucrose (Fig. 1B), resulting in the extraordinary sucrose yields observed in sugarcane. In this model, changes in sink activity (i.e. both storage and immediate utilisation for growth and respiration) may result in adjustments in the rates of symplastic and apoplastic phloem loading and flow processes (Lalonde et al., 2003). This could, in turn, modify the sucrose pool in the leaf and elicit feedback signaling in the source photosynthetic tissue, thus regulating photosynthesis.

Using metabolic control analysis, flux control can be quantitatively determined for individual enzyme-catalyzed reactions in a plant system based on the elasticity of the enzyme (Giersch et al., 1990) or an entire metabolic pathway (Sweetlove et al., 1998), where elasticity is defined as the change in reaction flux in response to changes in concentration of a given metabolite. In a supply-demand system, the flux and concentration of any linking intermediate metabolite are co-regulated by the supply and demand processes (Hofmeyr & Cornish-Bowden, 2000). These authors showed that the steady-state behaviour of the linking metabolite is controlled by the gradients of the slopes of supply and demand (Fig. 2A); which, in kinetic terms, indicate the elasticity of each process. However, the functions of flux- and concentration-control are mutually exclusive, in that if one of the two processes increasingly regulates flux of the linking metabolite it loses control over the magnitude in variation of the concentration of the metabolite to the other (Hofmeyr & Cornish-Bowden, 2000). For instance, if the elasticity of demand is decreased to zero (i.e. the demand becomes saturated with the metabolite), the demand process has complete control over flux, while supply has none. However, the elasticity of supply then completely determines the variation in concentration of the metabolite in the system (Hofmeyr & Cornish-Bowden, 2000). Similarly, if sucrose in the phloem is considered as the intermediate linking the source (supply) and sink (demand) tissues in sugarcane, the functions of flux- and concentration-control exercised by sucrose must be shared between source and sink activities. For example, changes in sink activity will result in
feedback control of the flux of sucrose through the overall system (Fig. 2). In sugarcane, because it accumulates sucrose in the culms, it is likely that the sink demand pathway of maturing cane is typically saturated and exerts little control over the concentration of sucrose. However, reduced sink demand will lead to the accumulation of sucrose in the phloem and consequently regulate sucrose production at the source, although the actual concentration of sucrose is largely controlled by the source photosynthetic supply (Fig. 2A). Source supply can only meet sink demand within a specific range of sucrose concentrations, during which allosteric feedback leads to a significant reduction in source photosynthetic activity (Fig. 2, grey zones). A reduced inhibition of source photosynthesis by sucrose and associated hexoses would allow for an extended range of sucrose concentrations in which supply can meet demand (Fig. 2B). Thus, we propose that in order to increase sucrose concentration in sugarcane, a reduction in the elasticity of the supply is likely to be required, such that supply is less sensitive to inhibition by its sucrose product.

Source-sink coordination in C3 and C4 plants

In C3 plants, there is good evidence of source photosynthetic responses to sink requirements (e.g. Basu et al., 1999; Iglesias et al., 2002; Franck et al., 2006); with this relationship being mediated by several sugar-sensitive feedback systems (Rolland et al., 2006; Paul, 2007). Sugar-induced feedback inhibition of photosynthesis plays a central role in co-ordinating the regulation of photosynthesis by such factors as light, tissue type, and developmental stage (von Schaewen et al., 1990; Krapp et al., 1993; Sheen, 1994). Accumulation of photo-assimilate in source leaves, induced by sugar feeding to excised leaves or cold-girdling techniques, has been shown to lead to a feedback regulation of leaf sucrose metabolism and photosynthetic activity in spinach (Spinacia oleracea L.), tobacco (Nicotiana tabacum L.) and potato (Solanum tuberosum L.) (Krapp et al., 1993; Krapp & Stitt, 1995). Similarly, transgenic disruption in the ‘product export pathway’, through the over-expression of a yeast-derived invertase in the cell walls of tobacco leaves, impaired phloem loading leading to an increase in leaf sugar concentrations and consequent photosynthetic inhibition (Stitt et al., 1991). Potato is a convenient research plant to demonstrate the influence of storage tissue removal, since the sink tubers are easily excised; a manipulation which results in decreased photosynthetic rates (Basu et al., 1999). Top-down metabolic control analyses have provided strong evidence that sucrose is the sole metabolite sensed between the source and sink tissues in potato plants (Sweetlove et al., 1998; Sweetlove & Hill, 2000). Although the control of carbon flux from source to sink tissues appears to be largely vested in the reactions of the leaves in potato (Sweetlove & Hill,
Sweetlove et al. (1998) predicted that a sink-dependent change in the apoplastic leaf sucrose pool (i.e. an increase or decrease in sink activity) would likely lead to changes in source photosynthetic capacity.

Reduced ‘sensitivity’ to source sugar accumulation has been demonstrated in several species. For example, photosynthetic rates in C₄ Flaveria bidentis (L.) and C₃ tobacco remained unaffected when sucrose concentrations were increased 2 to 3 fold through sugar feeding (Furbank et al., 1997). Spinach and rye grass (Lolium perenne L.) can accumulate a greater than 50-fold increase above normal leaf sucrose concentrations before photosynthesis or growth become limited (Housley & Pollock, 1985; Goldschmidt & Huber, 1992; Krapp & Stitt, 1995). Conversely, tomato (Lycopersicon esculentum L.) and Arabidopsis thaliana (L.) are highly sensitive to leaf sucrose accumulation and are characterized by severe photoinhibition and growth retardation (Goldschmidt & Huber, 1992; Furbank et al., 1997). However, several Arabidopsis mutants have been identified as insensitive to high glucose (gin; Zhou et al., 1998) or sucrose (sig; Pego et al., 2000), or both (sis; Laby et al., 2000), indicating that inhibition by sugars can be uncoupled from feedback mechanisms regulating growth and development.

**Source-sink coordination in sugarcane**

In sugarcane, co-ordination between source leaf photosynthetic rates and culm sink sucrose accumulation has been known for sometime. For example, Saccharum spontaneum (L.), a low sucrose accumulator, has a 30% higher photosynthetic rate compared to higher sucrose accumulating Saccharum spp. (L.) hybrids (Irvine, 1975). Several studies have demonstrated large differences in leaf photosynthetic rates related to the maturity of the culm, with young plants typically assimilating at significantly higher rates compared to older plants (Hartt & Burr, 1967; Bull & Tovey, 1974; Amaya et al., 1995; Allison et al., 1997). Nevertheless, partial defoliation of sugarcane plants produced no significant change in culm sucrose concentration compared with control plants (Gutiérrez-Miceli et al., 2004), which indicated that the remaining intact leaves were capable of maintaining the required supply of carbon based on the demand from sink tissues.

Past studies have also provided good physiological evidence for end-product repression of photosynthesis in sugarcane (Alexander, 1973). Hartt (1963) demonstrated a correlation between the inhibition of photosynthetic activity and the extent to which sucrose translocation was restricted by simulated wind injury of leaves (e.g. breakage of the midrib or laminae). In
subsequent detached leaf experiments, an extended photosynthetic assimilation period resulted in an increase in leaf sucrose and hexose concentrations and a parallel decrease in photosynthetic rates, suggesting an inhibition of photosynthesis by photoassimilate accumulation (Hartt, 1963; Waldron et al., 1967). More recently, sugarcane leaf shading studies have shown that increased sink demand for carbon resulted in increased rates of photosynthesis and sucrose export in source tissues (McCormick et al., 2006), suggesting that photosynthetic rates are typically limited by culm requirements. A decline in photosynthetic rates following leaf sugar accumulation in excised and intact, cold-girdled sugarcane leaves (McCormick et al., 2008c; McCormick et al., 2008d), has further corroborated the existence of sugar-sensing systems in sugarcane. Together, these studies indicate that, although local sugar-sensing mechanisms are active at the source, the principal intermediate linking the supply and demand pathway is the phloem transport product, sucrose. Nevertheless, the relationship between source photosynthetic rates and sink demand in sugarcane is not well documented, and several important questions remain to be answered: 1) How does phloem sucrose concentration influence leaf sugar pools to achieve regulation of leaf photosynthesis? 2) How does the source leaf sense changes in leaf tissue sugars? 3) How do these signaling mechanisms differ between cultivars and with varying environmental conditions? The paradigm of an integrated supply and demand system (Minchin & Lacointe, 2004) should be used in order to understand sucrose accumulation and to enable transgenic manipulation.

Sugarcane is characterized by a relatively high accumulation of leaf sucrose during the day (ca. 10 mg g$^{-1}$ FW) (Du et al., 2000; Grof et al., 1998; McCormick et al., 2008c), and high sucrose concentrations in the culm sink. Furthermore, prior to the onset of senescence eight-month-old sugarcane leaves have been shown to retain the ability to accumulate and export sucrose at similar rates to two-month-old leaves under increased sink demand (McCormick et al., 2008b). Viewing these observations in terms of supply-demand principles (Hofmeyr & Cornish-Bowden, 2000) raises the possibility that sugarcane leaves have a decreased sensitivity to sugar-feedback mechanisms, which may have been inadvertently selected for in the quest for varieties that accumulate large quantities of culm sucrose (Jackson, 2005). Thus the accumulation of sucrose in the culm could be dictated by the relative sensitivity of sugar-sensing systems in the source leaf metabolism and the consequent realised photosynthetic rate. Moreover, sugarcane leaves appear to have a strong capacity for adapting to increased demand from culm sinks indicating that photosynthesis of the leaves is seldom maximised (McCormick et al. 2006). For example, limitation of source supply through partial shading or defoliation
treatments resulted in 48% and 51% increases in photosynthetic rates in the remaining source leaves, respectively (McCormick et al., 2006; McCormick et al., 2008b).

Transgenic sugarcane producing the sucrose isomer isomaltulose exhibited a 50 to 80% increase in overall sugar content per unit fresh weight (Wu & Birch, 2007). As a consequence of increased total sugar accumulation, leaf photosynthetic rates were significantly increased and leaf senescence was delayed by 15 to 20 d (Wu & Birch, 2007). This study corroborates the innate capacity of source leaves to augment supply under conditions of increased sink demand, and furthermore, indicates that culm tissues of current cultivars do indeed have the capacity to store more sugar in the culm (Wu & Birch, 2007). This is contrary to the notion that osmotic constraints limit increased culm sucrose accumulation (Moore, 1995) and that increased sugar yields will arise only from further increases in overall cane yield (Jackson, 2005). Thus, the successful manipulation of sucrose yield in sugarcane may not necessitate an increase in culm demand, as has been previously attempted (Ma et al. 2000; Botha et al., 2001; Groenewald & Botha, 2007), but rather an uncoupling of the signal pathways that mediate negative feedback between source and sink tissues.

Increased culm sucrose accumulation may thus require further suppression of signaling mechanisms of sugarcane leaves that are possibly already partially sugar-insensitive, to lift the limit on leaf photosynthesis. Sugarcane leaves have demonstrated the capacity to respond to sustained demand under typical agricultural conditions (McCormick et al., 2006; Wu & Birch, 2007), even in mature leaves approaching senescence (McCormick et al., 2008b). In sugarcane, photosynthetic activity and leaf hexose and sucrose concentrations appear closely linked to the rate of sucrose export from the leaf (McCormick et al., 2006). In partially shaded plants decreasing leaf hexose concentrations were correlated with increasing photosynthetic activity (McCormick et al., 2006). Conversely, increased sucrose and hexose accumulation in cold-girdled sugarcane leaves resulted in a 66% and 55% decrease in leaf assimilation and electron transport rates over time, respectively (McCormick et al., 2008c). These studies provide evidence of a direct role for sucrose and hexose in modulating leaf photosynthetic rates, as also observed in C3 species (Krapp et al., 1993; Chiou & Bush, 1998; Gibson, 2005), which suggests that these sugars are key signal molecules in regulating the carbon supply between source and sink tissues in sugarcane.

**Potential targets for transgenic manipulation**
It is likely that sucrose accumulation in the sugarcane culm suppresses leaf photosynthesis through differential expression of genes relating to the sugar-sensing mechanisms that are known from work on C₃ plants. In C₄ sugarcane leaves, there are several points during the export of sucrose from photosynthetic source cells (i.e. the mesophyll and bundle sheath) to the phloem sieve elements where sugar sensing/signaling could potentially occur. There is no evidence of symplastic plasmodesmatal connections between the bundle sheath and the conducting cells of the phloem in sugarcane leaves (Robinson-Beers & Evert, 1991), which indicates that sucrose is actively loaded into phloem sieve elements from the apoplasm (Rae et al., 2005). Thus sucrose symporters that are known to be directly involved in apoplastic phloem loading, such as SUT1 and SUT-like homologues (Kühn et al., 1997; Stadler & Sauer 1996; Reinders et al., 2006), are likely to be important in signaling extracellular sugar status (Lalonde et al., 1999). Furthermore, several intracellular sugar sensing mechanisms exist in the cytosol that may play a key role in mediating source photosynthetic rates in sugarcane leaves, including hexokinase (HXK; EC 2.7.1.1) (Moore et al., 2003) and HXK-independent mechanisms, such as trehalose metabolism (Eastmond et al., 2003).

Trehalose 6-phosphate (T6P) has been identified as an important regulator of carbohydrate metabolism, involved in nearly all aspects of plant development (Eastmond et al., 2003; Paul, 2007; Ramon & Rolland, 2007). In Arabidopsis, T6P is essential for modulating efficient carbohydrate usage during growth (Schluepmann et al., 2003). Furthermore, increases in the concentration of the T6P in transgenic tobacco due to over-expression of E. coli trehalose 6-phosphate synthase (TPS; EC 2.4.1.15) have resulted in a 40% improvement in photosynthetic assimilation per unit leaf area (Pellny et al., 2004). Recently, a potential signaling role for the T6P has been identified in sugarcane leaves using a microarray technique (McCormick et al., 2008c). In these experiments, a doubling in leaf sugar concentration resulted in an 80% decrease in maximum photosynthetic rate and a 14-fold decrease in the ratio of TPS to trehalose 6-phosphate phosphatase (TPP; EC 5.3.1.1) expression. In addition, an average five-fold increase in genes related to sugar trans-membrane transport was observed. From these results TPS, TPP and the sugar transporters seem to be good candidates for transgenic manipulation to achieve increased sucrose accumulation in sugarcane (McCormick et al., 2008c). The recent identification of several vacuole-specific sucrose transporters in barley (Hordeum vulgare L.) and Arabidopsis (Endler et al., 2006) may provide an additional transgenic approach to reduce the effect of sugar-based feedback on photosynthetic activity by increased loading of sucrose into the vacuole during the light period.
Conclusion

Photosynthetic rates in sugarcane leaves are determined by the demand for carbon from sink tissues. Several genes and gene networks have been identified that are potentially involved in regulating sugar-based feedback signals that limit photosynthetic rates, leading to an eventual decline in leaf carbon assimilation and the onset of leaf senescence in sugarcane. Future research aimed at improving sugarcane sucrose accumulation should examine the role of these leaf sugar-sensing systems for potential transgenic manipulation of sugarcane sucrose accumulation.

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References


**Figure captions**

**Fig. 1. A)** A simplified model of a metabolic supply-demand system modified from Hofmeyr (1998). The rate of product formation is regulated by feedback inhibition of the supply process (dashed line) exercised through the concentration of the intermediate, which is in turn determined by the balance between supply and demand. **B)** In plants, demand is likely to be the consequence of metabolic activities such as growth, respiration and storage, particularly in high carbon accumulating species such as sugarcane. Feedback inhibition is indicated as a function of the sensitivity of supply to the concentration of the intermediate, in this case, sucrose.

**Fig. 2. A)** The postulated steady-state behaviour of a supply-demand system operating in sugarcane, modified from a generalised steady-state behaviour of a supply-demand metabolic system described by Hofmeyr & Cornish-Bowden (2000). Lines indicate source supply (solid) and three different sink demand activities (dashed lines), resulting in different steady-state concentrations of sucrose (points 1 to 3). If demand is high (1), flux of sucrose through the system increases but the concentration decreases, while if demand is low (3), flux decreases but sucrose concentration increases. The rate of the supply process is inhibited above a certain concentration of sucrose leading to inhibition of supply, indicated by the range of sucrose concentrations in the grey zone. **B)** In order to decrease the inhibition of photosynthesis by sucrose, the elasticity (i.e. sensitivity) of the photosynthetic system must be decreased, resulting in an increase in overall sucrose concentration, indicated by a shift in the range of sucrose concentrations to the right (grey zone) and also a decrease in the slope of the supply function (wider grey zone).