

# A research model for carbon-partitioning in sugarcane

*Yellow Canopy Syndrome (YCS), first observed in 2012, is an undiagnosed condition affecting Australian sugarcane. It causes mid-canopy leaves to turn yellow, decreasing crop sugar yields. Dr Frederik Botha oversees YCS research for Sugar Research Australia (SRA). Focused on gene expression and protein and metabolite levels, this research seeks molecular targets to improve genetic tolerance to YCS and enhance sugarcane productivity in general. In particular, a model of how sucrose build-up regulates leaf metabolism through feedback control has been developed. Understanding what elevates leaf sucrose levels as YCS develops could provide insights into mechanisms underpinning sugarcane diseases and physiological disorders.*

Commercial sugarcane (a hybrid of *Saccharum officinarum* and *S. spontaneum*) produces a higher biomass yield than the other major world crops, rice, wheat and maize. However, sugarcane yields worldwide have not improved significantly over the past three decades. Good crop yields depend on ensuring that, at each stage of plant growth, the supply of assimilates from the 'source' (leaves) to the 'sink' (growing or filling tissues) is optimal. Although sugarcane is one of the most efficient crops in converting solar energy into biomass, commercial yields remain half that of experimental potential.

There are several reasons for inefficient conversion of solar energy into biomass. Of particular interest in sugarcane are reduced photosynthetic rates in the leaves and slowed biomass gain in the culms due to feedback control of the plant's metabolism by high levels of sucrose and other sugars in the leaves. It is difficult to experimentally manipulate sugar levels without changing light input or damaging leaf and culm tissues. Since in YCS leaf sucrose exceeds normal physiological levels, discovering what causes this could give clues to improving productivity.

Sugarcane turns yellow for various reasons that can now be distinguished from YCS, including herbicide application, nutrition and known diseases. Indications are that the syndrome is a combination of abiotic and biotic factors leading

to a physiological disorder. Dr Botha and colleagues have found that YCS is especially associated with altered carbon-partitioning in the leaf. Disruption of the sink-source relationship causes sugars to accumulate in leaves, and when sugar exceeds a critical level it induces senescence. High levels of sucrose in sugarcane leaves are therefore an indicator of compromised crop health.

## THE SOURCE-SINK SYSTEM

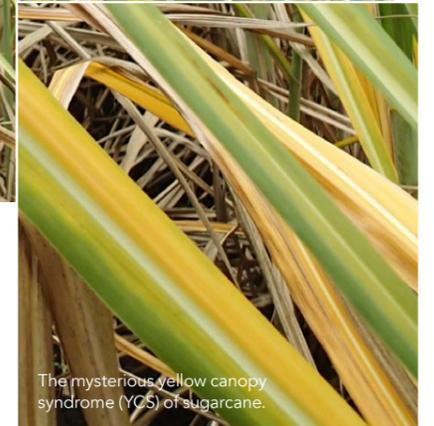
How well a plant grows depends on acquiring raw material (carbon fixation and mineral uptake), distributing this through plant organs and coping with environmental stresses. The process known as carbon-partitioning is critical for distributing the energy captured by plants through photosynthesis. In C4 plants like sugarcane, CO<sub>2</sub> is converted into four-carbon sugar compounds. These then enter into chemical reactions that take place in chloroplasts, the plant cell organelles conducting photosynthesis.

Carbon fixed during photosynthesis and converted into sugar in 'source' cells is distributed to 'sink' cells. Phloem is the tissue that transports the soluble organic compounds (mainly sucrose), made during photosynthesis and known as photosynthates, to wherever they are needed in the plant. The sugars are imported into sink tissues for consumption (providing energy for plant functions) or storage. Some stored sugars provide structural biomass as cellulose, hemicelluloses and lignin.

Sucrose synthesis in source tissue, its translocation and its partitioning between storage, respiration and biosynthesis are



A field of sugarcane affected by yellow canopy syndrome.



The mysterious yellow canopy syndrome (YCS) of sugarcane.

systemically coordinated in plants. Not only is sucrose the primary product of photosynthesis and the building block for biomass accumulation but it also serves as a sensitive metabolic switch controlling photosynthesis and carbon-partitioning in the plant. A model for the biochemical process of carbon-partitioning in sugarcane is being developed through research on YCS.

Sugarcane has a unique source-sink system. Stem-sinks store photosynthates as soluble sucrose, which can reach exceptionally high concentrations in commercial sugarcane varieties. Most other plant stems store carbon as insoluble polysaccharides (such as starch or cellulose) with low concentrations of sucrose. In many plants, sucrose is stored (after conversion to insoluble starch) in terminal sink organs such as tubers, grains or fruits, rather than in the stem. Valuable sucrose from sugarcane culms is extracted and purified for use in the food industry or fermented to produce ethanol.

During development, sucrose synthesised in sugarcane leaves is translocated via phloem to internodes (the stem sections that run between leaf-carrying nodes), the storage sink. Sucrose accumulates inside and outside the cell membranes,

in the symplast and apoplast respectively. Immature sugarcane tissues partition carbon into protein and fibre, whereas mature culms mainly partition it to sucrose storage. During maturation of commercial sugarcane cultivars, leaf photosynthetic activity decreases, as culm sucrose content increases. Thus, sink regulation of source capacity is taking place.

## SUCROSE ACCUMULATION IN SUGARCANE

In YCS, leaf yellowing occurs in the late stage of sucrose accumulation, senescence is induced and tissue death begins. Normal diurnal changes of sucrose concentrations (low in the morning and high at the end of the day) are absent in YCS affected plants, even

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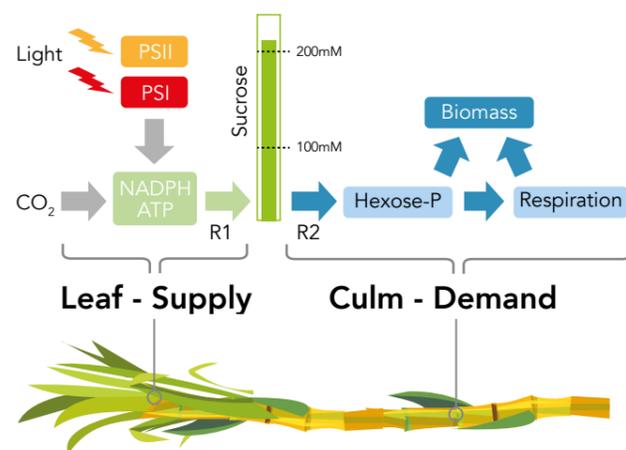
before yellowing. So, significant metabolic changes occur well before visual signs. Studies at SRA reveal that these changes include an increase in soluble sugars, a decrease in photosynthetic rate, decreased internal leaf CO<sub>2</sub>, decreased conductance through stomata (pores in leaves and stems for gas exchange), uncoupling of the photosynthetic electron transport (PET) chain and altered carbon-partitioning.

The excessive increase in sucrose suggests disruption of phloem transport. Sugar is loaded into the phloem but not exported from the leaf, since the highest levels are found in the midrib and sheath. Expression levels of genes for sucrose transporters and SWEET protein (not previously characterised in sugarcane) are also greatest in these plant parts. The sucrose accumulation could be caused by physical blockage of the phloem (for which there is currently no evidence) or arise because the sink is not using transported sugar fast enough which creates an

overflow into the surrounding leaf blade, midrib, dewlap and sheath. Increased sucrose also leads to elevated glucose, fructose and trehalose, sugars that play major roles in metabolic signalling. Furthermore, sucrose synthesis slows down which probably leads to a lowering of available inorganic phosphate (Pi) within chloroplasts. A feedback signalling mechanism involving sucrose in the symplast could result from chronic cellular

### INTERMEDIATE SUCROSE

- Over reduction of the PET
- Transcription and translation of the major components of PSII down regulated
- Physiological 'fitness' of PET reduced and photosynthetic reaction centres inactivated (more heat production and less reductant and ATP produced)
- Water splitting around PSII decreased as a result of changes in gene expression
- Cyclic electron flow upregulated to protect PSII
- Carbon partitioned to the phenylpropanoid pathway to act as antioxidants
- Carbon partitioned to  $\beta$  glucan to reduce sucrose accumulation
- Stomata partially closed to reduce photosynthesis
- Changes in both nuclear and plastid gene expression



### HIGH SUCROSE

- Accelerated senescence initiated
- Chloroplast and total protein breakdown stimulated
- Major upregulation of components of the ubiquitin pathway
- Accumulation of amino acids and amines
- Down regulation of the C4 and C3 carbon fixation pathways
- Increase in sugars associated with cell wall degradation
- Major changes in both nuclear and plastid gene expression
- Heat shock and other stress proteins accumulate
- Up regulation of chlorophyll breakdown
- Yellowing of the leaf evident
- Respiration maintained to facilitate senescence process
- Carotenoid retention

Leaf health is determined by the sucrose level in the photosynthetic mesophyll and bundle sheath cells. The sucrose level is determined by the difference between production (R1) and utilisation in the culm (R2). Daily fluctuation between 20 and 100 mM is normal as a result of variation in photosynthetic rate. Between 100 and 200mM sucrose (intermediate levels) a series of events are triggered that are aimed at protection of the photosynthetic electron transport chain, reduction in carbon fixation and creation of an alternative sink for the reduced carbon. Prolonged levels above 200mM (high) triggers accelerated senescence, collapse of the electron transport system, chlorophyll breakdown and cell death.

Pi limitation. Research shows that raised sucrose also alters gene expression of key photosynthetic proteins in leaf cells.

From the model developed so far, YCS symptoms appear to be caused by down-regulation of photosynthesis through Pi limitation leading to chronic inability to export reductant away from the PET chain during cellular sugar accumulation. Down-regulation of genes encoding Photosystem (PS) II and I, cytochrome and CP12 (an essential regulatory protein) results in decreased synthesis of these proteins, which then limits photosynthesis.

### ADVANCING GENETIC STUDIES OF SUGARCANE

The sugarcane genome has only recently been mapped, owing to sugarcane's complexity: high polyploidy (more than two-paired sets of chromosomes); aneuploidy (varied numbers of chromosomes); bispecific origin of chromosomes; and structural differences and interspecific chromosome recombinants. A reference genome is now available for researchers. DNA sequencing, development of gene-expression technologies and improved genetic/genomics resources for *Saccharum* are enabling the regulatory networks of carbon-partitioning to be further elucidated.

Metabolome (low-molecular-weight metabolites produced during

metabolism) and transcriptome (messenger RNA molecules expressed from the genes) analyses of the metabolic pathways in the leaves and sink tissues of sugarcane are helping researchers to identify reactions that lead to YCS. Comparing leaf transcriptomes of symptomatic and asymptomatic plants confirms that a complex network of changes in gene expression underpin the observed changes in the metabolome.

Fluorescence and gene expression data from YCS studies indicate that PS II is the sensitive process/component, linked to reduced electron flow producing reduced co-enzyme. The early change in photosynthetic rate is accompanied by changes in the expression of

## A model for the biochemical process of carbon-partitioning in sugarcane is being developed through research on YCS.

phosphoenolpyruvate carboxylase (PEPC). NADP-malic dehydrogenase expression is more sensitive to the accumulation of sucrose than are NAD-malic dehydrogenase and PEPC. This demonstrates that chloroplast metabolism is down-regulated when sucrose levels rise.

Furthermore, genes in the shikimate and phenylpropanoid metabolic pathways are upregulated in early response to

elevated sucrose. This increases caffeoyl-quinic acids and quinate, compounds that provide antioxidants to buffer free radical production in the chloroplast as a result of decreased electron flow to the terminal electron acceptors of PS I. Upregulation of the phenylpropanoid pathway probably shifts carbon-partitioning towards lignins, flavonoids and anthocyanins.

In the early stages of sucrose accumulation, several other changes also occur: significant levels of metabolites indicative of microorganisms that associate with injured tissue, especially where there are significant available carbohydrates; significant increases in caffeoyl/chlorogenic type compounds indicative of wounding and activation of plant defence systems; and increases in amino acids and metabolites indicative of stress metabolism and of disruption of the electron transport system, which is dependent on fast turnover of protein components.

A genomic approach is now being pursued for YCS in sugarcane, using next-generation RNA sequencing to compare and analyse genetic data for affected and unaffected plants from diverse field locations. Genetic explorations of how different tissue samples express different proteins, continues to provide clues to the cause of YCS and to understanding sugarcane metabolism in general.



# Behind the Research

## Dr Frederik (Frikkie) Botha

E: [fbotha@sugarresearch.com.au](mailto:fbotha@sugarresearch.com.au) T: +61 488400074 / +61 7 33313318 W: <https://sugarresearch.com.au/team/dr-frikkie-botha/>

### Research Objectives

Dr Botha's work examines leaf sucrose levels in sugarcane, among other plants, and their impact on overall plant health.

### Detail

50 Meiers Road  
Indooroopilly  
Queensland 4078  
Australia

### Bio

Frederik (Frikkie) Botha is the Executive Manager Strategic Initiatives at Sugar Research Australia and Honorary Professor at the University of Queensland, Australia. His research focus is on the genetic and molecular control of carbon partitioning in the culm and leaves of sugarcane, which is the driver of biomass composition and yield. The research aims to understand the control of carbon partitioning between the cell wall components, respiration and sucrose accumulation in the culm and the impact of this on sink strength. An early switch to sucrose accumulation reduces biomass accumulation and reduces sink strength. The limited capacity to buffer leaf sucrose through partition of carbon to starch requires maintenance of a strong sink demand to prevent induction of premature senescence in the canopy.

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### Co Authors

- Annelie Marquardt (SRA) Sugar Research Australia
- Gerard Scalia (SRA) Sugar Research Australia
- Kate Wathen-Dunn (SRA) Sugar Research Australia
- Robert Henry (UQ) Queensland Alliance for Agriculture and Food Innovation, The University of Queensland



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### Personal Response

#### What impact do you hope this research will have over the next five years?

Conventional and genetic manipulation studies have shown that accumulation of sucrose leads to biomass penalties consistent with sucrose feedback control on photosynthesis. We need a better understanding of why sucrose accumulates in the leaves of sugarcane during stress and what the impact of this is on leaf metabolism and crop yield. This will contribute to the finding of management solutions for physiological disorders and biotic stress that lead to sucrose accumulation. However, more importantly it could lead to genetic targets that provide an opportunity to break out of the current yield plateau that has frustrated sugarcane breeders for the past three decades. "