Radiotracer studies using ^{18}FDG and $^{11}CO_2$ as an opportunity for gaining new insights into cellulose synthesis in plants

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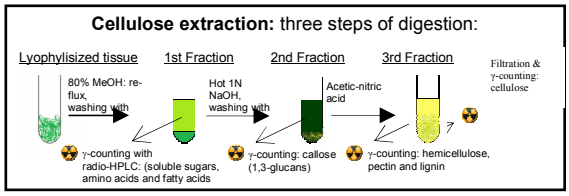
Motivation: Developing new technologies enabling the measurement of cellulose rates of formation in plants gives many opportunities for basic research to identify ways in which plant sugars can be manipulated for higher throughput into cell-wall cellulose, a key component of biomass that can be readily converted to biofuel.

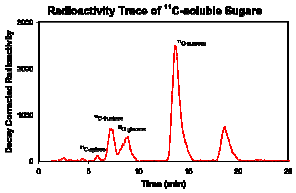
Materials and Approach: Studies were conducted using *Nicotiana tabacum* L (cv Samsun) grown to the 9-leaf stage under a 16/8 light cycle (400 µmol/m^2 s). Two studies were conducted testing the efficacy for using short-lived radioisotopes to measure cellulose rates of formation:
(i) study 1 looked at leaf age, comparing cellulose mass and rates of formation in young apex leaves with older mature leaves;
study 2 looked at the effects of administering a 150 µM solution of the herbicide, isoxaben (ISX). ISX inhibits cellulose production although the mechanism of action is unknown. $^{11}CO_2$ fed to leaves, is rapidly fixed by the Calvin-Benson Cycle making ^{11}C-sucrose and ^{11}C-starch. ^{11}C-Sucrose can be degraded to ^{11}C-glucose by sucrose synthase where a cellulose synthesizing enzyme stitches the glucose molecules together in a 1,4-glucan change.
We also used ^{18}FDG, 2-[^{18}F]-fluoro-2-deoxyglucose, a radioactive glucose analog that can be taken up by the roots and transported to leaves where it is used by the plant as a glucose substitute for cell-wall synthesis. The combination of using $^{11}CO_2$ and ^{18}FDG tracers gave new insight into the role sugars play in making cellulose.
The scheme below outlines 3 extraction steps that were used to rapidly break down plant tissue into cell-wall components. A combination of radio-HPLC analysis and gamma counting gave information of plant carbon partitioning into metabolic pathways:



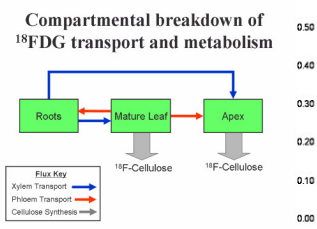
FDG to the roots

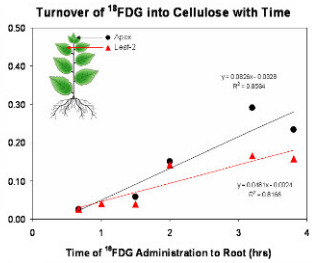
HPLC of first extraction

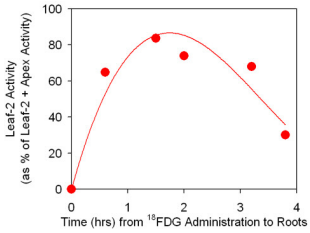
$^{11}CO_2$ to the leaves

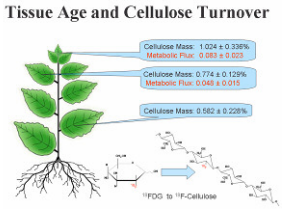


The fragments of the HPLC could be identified as fructose, glucose, sucrose and trehalose.







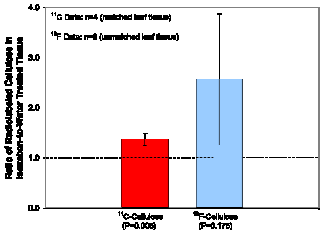


Metabolic flux analysis requires an understanding of the physical transport of tracer into, and out of target tissue over time. Here the amount of ^{18}FDG in leaf-2 changes over time because sugar can reload into the phloem and export out.

Cellulose mass presented as % gram fresh wt. decreases with leaf age. The rates of cellulose formation as measured by the %^{18}F-cellulose were also found to decrease with leaf age validating the tracer approach.

Isoxaben

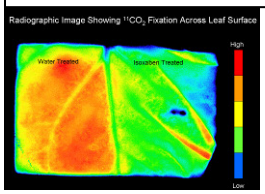
Red: ^{11}C data
Blue: ^{18}FDG data



ISX increases metabolic flux of ^{11}C and ^{18}F into cellulose relative to water (control) treatment. ISX decreases radiolabeled sugar specific activities, but for different reasons. For glucose, the ^{11}C pool decreases, but the ^{12}C pool remains unchanged. For sucrose, the ^{11}C pool is unchanged, but the ^{12}C pool increases:

Comparative Ratios of ISX-to-Water Treated Tissue within the Same Leaf

Soluble Sugar	Ratio of Specific Activities in ISX-to-Water	Ratio of [^{11}C]-Sugar Activity in ISX-to-Water	Ratio of [^{12}C]-Sugar Mass in ISX-to-Water	Number of Sample Replicates
Glucose	0.59 ± 0.17	0.57 ± 0.16	0.96 ± 0.11	2
Sucrose	0.58 ± 0.16	0.86 ± 0.13	1.49 ± 0.19	3



ISX decreases photosynthetic activity by 30% ± 0.06 (n=4), relative to controls.

Summary: We believe the effects of ISX are two-fold:
(i) involving metabolic reprogramming of plant carbon flux between starch and sugar production; and
(ii) inhibiting the sucrose synthase enzyme from degrading plant sucrose to glucose - essentially starving the plant of needed glucose for cell-wall cellulose synthesis.

Acknowledgements: Frank Rösch (Johannes Gutenberg Universität Mainz), Deutscher Akademischer Austauschdienst, Michael Schueller, David Alexoff and Richard Ferrieri